

Evaluating the effect of GnRH on reproductive performance in nulliparous Assaf ewes

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Abstract

This study aimed to evaluate the effect of short-term progesterone-based oestrus synchronization protocol with or without gonadotropin-releasing hormone (GnRH) administration in nulliparous Assaf ewes at the end of the breeding season. Nulliparous ewes ($n = 117$) were randomly divided into three groups. Ewes in Group 1 received an intravaginal sponge and were injected with GnRH at the time of sponge insertion. On the day of sponge removal, all ewes received d-cloprostenol and 500 IU equine chorionic gonadotropin (eCG). In Group 2, the same treatment was received except for GnRH injection. Group 3 was designed as a control group, and no hormonal treatments were administered. The rams were joined into the herd for one ram for four ewes on day 8. The oestrus rates were similar in synchronization groups, 93.8% in Group 1 and 95.5% in Group 2, but higher than the control group (56.5%; $P < 0.05$). The pregnancy rates on day 25 were higher in the synchronization groups than in the control group ($P < 0.001$). The fertility rate was significantly higher in the synchronization groups than in the control group ($P < 0.05$). The fecundity and prolificacy rates tended to be higher in Group 2. These results suggest that nulliparous Assaf ewes can be synchronized successfully at the end of the season with short-term progesterone-based protocol. However, exogenous GnRH administration at the beginning of the protocol does not tend to improve fertility rates.

Fertility, ovine, short-term progesterone, synchronization

Animal-source foods (ASFs) are excellent aliments that can provide high-quality and rich nutrients for humans. Animal-source foods are richer in high quality protein, vitamin A, vitamin D3, calcium, iron, zinc, folic acid, and essential fatty acids compared to plant-based foods. While there is an effort to change nutritional intake for humans from ASFs to plant-based due to the industry's effects on climate change, food security or other factors, ASFs are essential, particularly for children under five years old and in low-income populations (Adesogan et al. 2020). Therefore, livestock production sustainability is critical in global food production's milk and meat requirements. One of the key points is reproductive management and ensuring appropriate fertility indices of those animals (Capper 2013).

Small ruminants are the major mainstay of ASFs in developing and transition countries. However, sheep and goats are short-day, seasonally polyoestrous animal species, and their breeding season is limited in the calendar year. Hence, the oestrous synchronization and breeding control help to improve reproductive performance. Reproductive management and oestrus synchronization of sheep can allow for taking advantage of regular meat and milk production in a scheduled program and support sustainability (Whitley and Jackson 2004; Santos-Jimenez et al. 2022). The common oestrus synchronization protocols are based on progestogens, prostaglandin F2 α (PGF2 α), equine chorionic gonadotropin (eCG), and melatonin during the breeding and non-breeding seasons. The use of gonadotropin-releasing hormone (GnRH) for synchronizing oestrus and ovulation in sheep is still

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a grey area because there is no agreement on the advantage of GnRH administration during synchronization protocols for pregnancy rates in different sheep breeds (Cavalcanti et al. 2012; Kaya et al. 2013; Takçı et al. 2024; Año-Perello et al. 2020; Kuru et al. 2022). In ruminants, GnRH can be used at the beginning of the progesterone-based protocols, a few days after progesterone removal, or following artificial insemination. However, some of the studies revealed that GnRH administrations at different times of protocols affect pregnancy rates positively, while other studies stated that the pregnancy rates were not affected by GnRH administrations in sheep (Baki Acar et al. 2013; Habeeb and Kutzler 2021; Cizmeci et al. 2022).

The growth rate and the first lambing age of nulliparous ewes are also important for sheep productivity. Sheep breeders prefer maximum prolificacy for flock productivity. Increased reproductive efficiency in the flock positively affects economic indicators, annual milk and meat production, and farm profitability (Ajafar et al. 2022). Studies on the enhancement of reproductive productivity of nulliparous ewes are still ongoing. The reproductive management efficiency of nulliparous sheep still needs to be defined (Baki Acar et al. 2013; Santos-Jimenez et al. 2022; Rosales-Nieto et al. 2024). Based on this uncertainty, this study aims to determine the efficacy of GnRH administration at the beginning of oestrus synchronization protocol in nulliparous Assaf sheep at the end of the breeding season.

Materials and Methods

Location

The study was carried out at the end of the breeding season (from the beginning of December to the end of the parturition period) on a commercial farm in Niğde province (latitude of 37°58'0.00"N and longitude of 34°40'60.00"E, in the northern hemisphere), Türkiye.

Animal management

The institutional Ethics Committee of Niğde Ömer Halisdemir University, Niğde, Türkiye provided the ethical clearance (approval number: 31936052/050.99-312590-2023/01) for the conduction of this study. A total of 117 nulliparous Assaf ewes, aged between 7 and 9 months, weighing 46–54 kg, were used in the study. The animals' mean body condition score was evaluated according to Ferguson et al. (1994) on a 1–5 scale, where 1 = emaciated and 5 = obese.

Feed

The ewes were fed dry hay and corn silage supplemented with a 650 g/d commercial mixture (14% crude protein, 2,750 Kcal/ME, 3.50% fat; yearling lamb fattening feed, Kocayusuf Yem Fabrikası, Mersin, Türkiye) and had free access to shade, water, and mineral blocks. The ewes were then housed in indoor shelters at night, and the entire flock was maintained under natural lighting conditions. Adult, intact, and fertile (according to farm records) rams (n = 20) were housed separately from the ewes.

Experimental design

Nulliparous ewes were randomly divided into three groups. Ewes in Group 1 (GnRH + progesterone; n = 49) received an intravaginal sponge containing 60 mg of medroxyprogesterone acetate (Esponjavet®, HIPRA, İstanbul, Türkiye) and all were injected with GnRH intramuscularly (4 µg of busereline acetate, Bustral®, SANTAVET, İstanbul, Türkiye) at the time of sponge insertion (day 0). On the day of sponge removal (day 7), all ewes received 75 µg d-cloprostenol (Gestavet Prost®, HIPRA) and 500 IU eCG (Oviser®, HIPRA) intramuscularly. The rams were joined into the herd at one ram per four ewes on day 8. The onset of oestrus in ewes was noted based on symptoms such as frequent urination, restlessness, searching for the male, vulvar oedema and hyperaemia, vaginal mucus, and immobility on mounting. In Group 2 (progesterone; n = 45), the same treatment was received except for GnRH injection on day 0; rams were introduced on day 8. Group 3 (control; n = 23) was designed as a control group, where no hormonal treatments were administered, and the rams were joined at the same time as with Groups 1 and 2 (Fig. 1).

Oestrus signs and mating were monitored with video recordings for three days. Pregnancies were confirmed on day 25 by transrectal ultrasonography (WED-3000V, HASVET, Türkiye) and on day 45 by transabdominal ultrasonography. Oestrus response, pregnancy rates, embryonic death, abortions, parturition rates, twinning rates, fecundity, prolificacy, and fertility rates were noted and defined in below. Vaginal discharge characteristics after sponge removal were observed and scored according to Martinez-Ros et al. (2018). The vaginal discharge was evaluated for its characteristics (odour, amount, texture, and colour) and scored (0—negligible or no discharge;

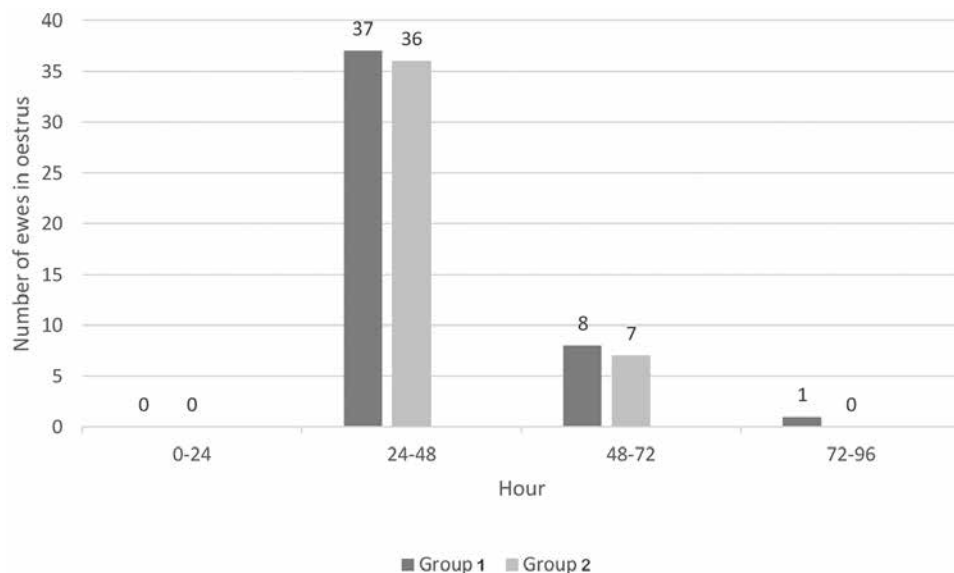


Fig. 1. The distribution of the onset of oestrus in synchronization groups.

1—some amount but clear discharge; 2—abundant amounts and haemorrhagic or purulent). Lamb survival rates on the 28th day were also recorded.

Oestrus response = Number of ewes in oestrus / Number of total ewes $\times$ 100

Pregnancy rate = Pregnant ewe number / Mated ewe number $\times$ 100

Parturition rate = Number of ewes lambbed / Number of ewes pregnant $\times$ 100

Twinning rate = Number of ewes having twin lambs / Number of ewes lambbed $\times$ 100

Fecundity = Number of lambs born per ewe mated

Prolificacy = Number of lambs born per ewe lambbed

Fertility = Number of ewes lambbed

Blood sampling and progesterone assay

Blood samples were collected at the beginning of the study (day 0) from all ewes and after intravaginal sponge removal (day 7) from synchronization groups. The jugular blood samples were centrifuged at $2000 \times g$ for 15 min and stored at -80°C until they were assayed for progesterone determination.

Progesterone was determined by using a chemiluminescent microparticle immunoassay (CMIA) kit from Architect Progesterone (Abbott Laboratories, IL 60064, USA), according to the manufacturer's guidance. The intra- and inter-assay coefficients of variation for all analyses were below 5%.

Statistical analyses

Statistical analyses were performed using the SPSS software package (version 25.0; IBM Corp., Armonk, NY, USA). Normality of continuous variables was assessed using the Shapiro-Wilk test, and homogeneity of variances was evaluated using Levene's test. For comparisons between the groups, continuous variables such as oestrus duration and pregnancy length were analysed using one-way ANOVA when parametric assumptions were met, or the Kruskal-Wallis test when assumptions were violated. *Post hoc* pairwise comparisons were performed using Bonferroni correction for ANOVA or Dunn's test for Kruskal-Wallis where significant differences were observed. Categorical variables, including pregnancy rate, oestrus response, embryonic death, abortion, parturition rate, twinning rate, fertility, lamb sex distribution, and vaginal discharge score, as well as lamb survival rate, were analysed using chi-square test. Body condition scores, being ordinal, were evaluated using Kruskal-Wallis test.

The distribution of oestrus onset, measured repeatedly over time, was analysed using a repeated measures approach suitable for non-parametric data to account for within-subject correlations. Serum progesterone concentrations measured on Day 0 and Day 7 satisfied normality and homogeneity assumptions and were analysed using a two-way mixed ANOVA, with 'time' (Day 0 and Day 7) as the within-subject factor and 'treatment group' (Group 1, Group 2, and Control) as the between-subject factor. Pairwise comparisons were adjusted using Bonferroni correction when significant effects were observed. All data are presented as mean $\pm$ standard error of the mean (SEM), and differences were considered significant at $P < 0.05$.

Results

The animals' mean body condition scores were 2.86 ± 0.06 in Group 1, 2.67 ± 0.06 in Group 2, and 2.83 ± 0.10 in Group 3. The mean concentrations of serum progesterone in Group 1, Group 2, and Control on Day 0 were 1.98 ± 0.32 , 1.75 ± 0.32 , and 3.09 ± 0.73 ng/ml, respectively. In Groups 1 and 2, mean progesterone concentrations were 3.29 ± 0.45 and 3.19 ± 0.47 ng/ml, respectively, on Day 7 following progesterone sponge removal. The progesterone concentrations between groups did not differ significantly ($P > 0.05$). Reproductive performance results of nulliparous Assaf ewes are presented in Table 1.

Vaginal discharge characteristics were compared between synchronization groups. The results are presented in Table 2. The vaginal discharge scores were significantly different between score 1 and score 2 in the synchronization groups ($P < 0.05$). The score 1 rate was significantly higher in Group 2. In contrast, in Group 1, score 2 was significantly higher, and the vaginal discharge amount and characteristics were more severe than in Group 2. However, the vaginal discharge characteristics and scores did not affect embryonic mortality and pregnancy rates significantly ($P > 0.05$).

Table 1. Reproductive performance results of the studied nulliparous Assaf ewes.

Indicator	Group 1	Group 2	Control	<i>P</i> value	
Oestrus response (%)	93.87 ^a (46/49)	95.55 ^a (43/45)	56.52 ^b (13/23)	0.001	
Duration of oestrus (h; mean ± SEM)	18.99 ± 2.13	18.88 ± 2.00	-	0.987	
Onset of oestrus (h; mean ± SEM)	42.79 ± 1.77	39.81 ± 1.71	-	0.544	
Pregnancy rate on day 25 (%)	79.59 ^a (39/49)	93.33 ^a (42/45)	52.17 ^b (12/23)	0.001	
Pregnancy rate on day 45 (%)	63.26 ^{ab} (31/49)	82.22 ^a (37/45)	52.17 ^b (12/23)	0.026	
Embryonic mortality rate (%)	20.51 (8/39)	11.90 (5/42)	0.0 (0/12)	0.178	
Abortion rate (%)	0.0 ^a (0/31)	16.21 ^{ab} (6/37)	30 ^b (4/12)	0.008	
Pregnancy duration (mean ± SEM)	146.10 ± 4.93	146.79 ± 3.99	-	0.771	
Progesterone concentration on day 0 (ng/ml; mean ± SEM)	1.98 ± 0.32	1.75 ± 0.32	3.09 ± 0.73	0.535	
Progesterone concentration	3.29 ± 0.45	3.19 ± 0.47	-	0.656	
Parturition rate (%)	79.48 (31/39)	73.80 (31/42)	75.0 (8/12)	0.641	
Twin rate (%)	35.48 (11/31)	41.93 (13/31)	25.0 (2/8)	0.659	
Triplet rate (%)	3.22 (1/31)	3.22 (1/31)	0.0 (0/8)	0.877	
Pluriparous rate (%)	38.70 (12/31)	45.16 (14/31)	25.0 (2/8)	0.577	
Sex distribution of lambs (%)	Female	52.27 (23/44)	54.35 (25/46)	50.0 (5/10)	0.062
	Male	47.73 (21/44)	47.73 (21/44)	50.0 (5/10)	

^{a,b}Different superscripts within a row differ significantly ($P < 0.05$).

Table 2. Vaginal discharge characteristics and pregnancy rates in the studied nulliparous Assaf ewes according to vaginal discharge score (VDS) in Group 1 and Group 2.

VDS	Score rates			Embryonic mortality rates			Pregnancy rates		
	Group 1	Group 2	<i>P</i> value	Group 1	Group 2	<i>P</i> value	Group 1	Group 2	<i>P</i> value
0	0.0 (2/45)	4.44 (29/45)	0.138 (0/0)	0.0 (0/2)	0.0 (4/26)	-* (0/0)	0.0 (2/2)	100.0 (26/29)	-*
1	44.89 ^a (22/49)	64.44 ^b (29/45)	0.001	33.33 (6/18)	15.38 (4/26)	0.167	81.81 (18/22)	89.65 (26/29)	0.425
2	55.10 ^a (27/49)	31.11 ^b (14/45)	0.002	9.52 (2/21)	7.14 (1/14)	0.058 (21/27)	77.77 (14/14)	100.0	0.059

*Comparison could not be performed due to the lack of data in Group 1.

^{a,b}Different superscripts within a row differ significantly ($P < 0.05$).

Results of reproductive performances are presented in Table 1. The oestrous rates were similar in synchronization groups, 93.8% in Group 1 and 95.5% in Group 2 ($P > 0.05$) but higher than the control group (56.5%; $P < 0.05$). The oestrus duration and oestrus onset were also non-significant in hormone treatment groups ($P > 0.05$). The distributions of the onset of oestrus in synchronization groups are shown in Fig. 1. The pregnancy rates on Day 25 were higher in the synchronization groups than in the control group ($P < 0.001$). However, pregnancy examination on day 45 revealed eight (20.5%) embryonic losses in Group 1 and five (11.9%) losses in Group 2. In contrast, no embryonic loss was observed in the control group. There was a significant difference in abortion rates between the groups ($P < 0.05$). While there were four abortions in the control group and six abortions in Group 2, there were no abortions in Group 1.

The fertility, fecundity, and prolificacy values are shown in Table 3. The fertility rate was significantly higher in the synchronization groups than in the control group ($P < 0.05$). The highest fecundity and prolificacy rates were found in Group 2 ($P > 0.05$). The singleton, twin, and triplet rates and the sex of lambs were found non-significant in all groups ($P > 0.05$). The survival rates of lambs between days 0 to 28 were significantly lower in Group 1 ($P < 0.05$) than in the other two groups. The survival rates of lambs between days 0 to 28 are presented in Table 4.

Table 3. Fertility, fecundity, and prolificacy of the studied nulliparous Assaf ewes.

Indicator	Group 1	Group 2	Group 3	<i>P</i> value
Fertility (%)	63.26 ^a (31/49)	68.88 ^a (31/45)	34.78 ^b (8/23)	0.021
Fecundity	0.95	1.06	0.76	0.772
Prolificacy	1.41	1.48	1.25	0.948

^{a,b}Different superscripts within a row differ significantly ($P < 0.05$).

Table 4. Survival rates of lambs between days 0 to 28.

Group	Number of lambs born	Number of lambs alive on day 28	Survival rates %	<i>P</i> value
Group 1	44	34	77.3 ^b	0.032
Group 2	46	43	93.5 ^a	
Control	10	10	100.0 ^a	

^{a,b}Different superscripts within a column differ significantly ($P < 0.05$).

Discussion

GnRH administration at the time of progesterone insertion revealed a similar oestrus response and pregnancy rates with the classical progesterone-based synchronization group in nulliparous Assaf ewes. It was hypothesized that the GnRH administration at the beginning of the protocol might support fertility by eliminating large follicles and emerging new follicular wave in nulliparous ewes in this study. Año-Perello et al. (2020) reported that GnRH treatment in progesterone-based synchronization protocols increases oestrus response and reproductive efficiency in ewes by improving follicular turnover. Exogenous GnRH injection increases serum progesterone concentration, decreases oestradiol concentration, and increases metabolic process by increasing the level of total cholesterol, protein, glucose, and pentose in the blood of sheep. This metabolic effect improves ovarian and uterine activity (Skliarov et al. 2021). On the contrary, results showed that the GnRH administration did not positively affect oestrus response and other reproductive performances in nulliparous Assaf ewes at the end of the breeding

season. Several factors, such as breed, season, stage of the oestrous cycle, body condition score, and environmental factors affect the reproductive production response to different synchronization protocols in ewes (Titi et al. 2010; Skliarov et al. 2021; Bottino et al. 2024). The difference in GnRH administration effects between this study and others might be due to the abovementioned factors.

The oestrous response of both treatment groups was above ninety percent in this study. The pregnancy and fertility rates, fecundity, and prolificacy results were also satisfactory. The oestrus response of this study was lower (95.5% vs 100%) than the result reported by Hajibemani et al. (2023) performed in nulliparous Ghezel ewes synchronized with intravaginal sponge insertion and eCG injection protocol. However, the fecundity (1.06 vs 1.02) and pregnancy (93.3% vs 85.4%) rates in this study were higher than in the nulliparous Ghezel ewes. Similarly, the oestrous response, fecundity, and prolificacy results in this study were higher than the results of Cosentino et al. (2021) performed in nulliparous Corriedale ewes synchronized with progestogen-based protocol and GnRH injection 36 h after sponge removal; the results of Metodiev and Raicheva (2014) in nulliparous Synthetic Population Bulgarian Milk ewes synchronized with classic sponge-eCG protocol; the results of Santos-Jimenez et al. (2022) in multiparous Segurena ewes synchronized with GnRH, progesterone-loaded silicon-based devices (CIDRs) and GnRH protocol. The oestrous response and fertility results revealed that both synchronization protocols used in the present study were successful in nulliparous Assaf ewes at the end of the season compared to the previous studies.

Vaginal discharge characteristics were evaluated and scored in this study. Progestogen-impregnated intravaginal sponges negatively affect vaginal pH, microbiota, and immunity, and cause blockage of vaginal secretions. Various amounts and characteristics of vaginal discharge have been observed following progestogen-based synchronization protocols (Hameed et al. 2021; Güner and Kısadere 2022). Martinez-Ros et al. (2018) reported that CIDRs cause less vaginal mucus discharge than sponges in ewes. Moreover, they state that the duration of intravaginal sponge insertion affects the amount and characteristics of vaginal mucus discharge; a short-term sponge insertion causes less severe vaginal discharge. In the present study, short-term progestogen sponges were inserted (for seven days) into Assaf ewes. The vaginal discharge score 2 was significantly higher in Group 1 (GnRH + P4). This result suggested that GnRH administration at the time of sponge insertion supported progesterone concentrations via luteinization of Graafian follicles. The serum progesterone concentrations on day 7 showed that the results in Group 1 were a little higher than in Group 2 (3.29 ± 0.45 ng/ml vs 3.19 ± 0.47 ng/ml), even though the difference was non-significant. The vaginal discharge scores did not affect pregnancy and fertility rates in this study, a finding similar to the results of Martinez-Ros et al. (2018) and Doğan and Kutlu (2022).

In conclusion, small ruminant production is essential for ASFs worldwide, and sustainable food safety is directly related to animal reproduction. However, sustainable reproductive management still needs to be improved. The present study was performed to contribute in this regard. The results of this research showed that the synchronization of nulliparous Assaf ewes using progesterone-based protocols with or without GnRH administered at the end of the season was very effective. However, the administration of GnRH at the time of progesterone-impregnated-sponge insertion did not increase the fertility performance significantly. It was suggested that the nulliparous Assaf ewes could be synchronized using standard short-term progesterone-based protocols without any GnRH injection at the beginning of the protocol.

Conflict of interest

The authors declare no conflicts of interest.

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