BURSA ULUDAĞ ÜNİVERSİTESİ ZİRAAT FAKÜLTESİ DERGİSİ
Journal of Agricultural Faculty of Bursa Uludag University
e-ISSN 2651-4044
https://dergipark.org.tr/tr/pub/bursauludagziraat
http://www.uludag.edu.tr/ziraatdergi
Haziran/2026, 40(1), s. 167-183

ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Geliş Tarihi (Received): 31.10.2025
Kabul Tarihi (Accepted): 26.03.2026

Effects and Economic Cost of Pregnant Mare Serum Gonadotropin (PMSG) and Gonadotropin-Releasing Hormone (GnRH) Applications on Reproductive Performance in Ewes[A]

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Abstract: This study aimed to analyze the effects of PMSG and GnRH administration at different times on reproductive and yield traits in 96 head Kıvırcık ewes in the anestrus stage whose estrus was synchronized using a vaginal sponge. All sheep were administered vaginal sponges containing progesterone + 500 IU GKSH. The ewes were divided into four groups: which received 1 ml of GnRH 48 hours after sponge removal (Group I); which received 1 ml of GnRH 48 hours before sponge removal (Group II); sheep that did not receive GnRH (only sponge + PMSG) (Group III), and the group that received 1 ml of GnRH at the time of sponge removal (Group IV). All ewes in the treatment groups became pregnant and gave birth healthly. Although the highest multiple birth rate was observed in Group II (60.9%), the effect of the treatments on birth type was statistically not significant ($p>0.05$). The fecundity and litter size in Groups I, II, III, and IV were comparable: 1.38, 1.65, 1.52, and 1.30, respectively. Net profit was calculated using the application costs per ewe and the total lamb weight at weaning; the differences between the groups were significant ($p<0.01$). In conclusion, considering the lambing rate, multiple birth rate, and hormone costs for estrus synchronization, Group II was the optimal protocol for use on large-scale sheep farms among these four estrus synchronization protocols due to its high profitability.

Keywords: GnRH, hormone costs, PMSG, reproductive parameters, vaginal sponge.

[A] This article has been prepared in accordance with research and publication ethics. It has an ethics committee certificate from the Republic of Turkey Bursa Uludağ University Animal Experiments Local Ethics Committee (HADYEK).

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Atıf/Citation: Öziş Altınçekiç, Ş., Koyuncu, M., Duru, S. 2026. Effects and Economic Cost of Pregnant Mare Serum Gonadotropin (PMSG) and Gonadotropin-Releasing Hormone (GnRH) Applications on Reproductive Performance in Ewes. *Bursa Uludag Üniv. Ziraat Fak. Derg*., 40(1), 167-183. https://doi.org/10.20479/bursauludagziraat. 1814573

Koyunlarda Gebe Kısrak Serum Hormonu (GKSH) ve Gonadotropin Salgılatıcı Hormon (GnRH) Uygulamalarının Üreme Performansı Üzerine Etkileri ve Ekonomik Maliyeti

Öz: Bu çalışmada, östrus senkronizasyonu vajinal sünger kullanılarak sağlanan anöstrus dönemindeki 96 baş Kıvırcık koyunlarında farklı zamanlarda uygulanan GKSH ve GnRH'nin üreme ve verim özellikleri üzerine etkilerinin incelenmesi amaçlanmıştır. Tüm koyunlara progesteron içeren vaginal sünger+500 IU GKSH uygulandı. Koyunlar dört gruba ayrıldı: süngerin çıkarılmasından 48 saat sonra 1 ml GnRH alanlar (Grup I); süngerin çıkarılmasından 48 saat önce 1 ml GnRH alanlar (Grup II); GnRH almayan (sadece sünger + GKSH) koyunlar (Grup III) ve süngerin çıkarılması sırasında 1 ml GnRH alan grup (Grup IV). Uygulama gruplarındaki tüm koyunlar gebe kalmış ve sağlıklı bir şekilde doğum yapmıştır. En yüksek çoğuz doğum oranı Grup II'de (%60.9) gözlenmesine rağmen, uygulamaların doğum tipi üzerindeki etkisi istatistiksel olarak önemli değildir ($P>0.05$). Grup I, II, III ve IV'teki doğurganlık ve yavru sayısı benzerdir: sırasıyla 1.38, 1.65, 1.52 ve 1.30. Net kâr, koyun başına uygulama maliyetleri ve sütten kesimdeki toplam kuzu ağırlığı kullanılarak hesaplanmış; gruplar arasındaki farklar anlamlı bulunmuştur ($P<0.01$). Sonuç olarak, kuzulama oranı, çoğuz doğum oranı ve östrus senkronizasyonu için hormon maliyetleri göz önüne alındığında, bu dört östrus senkronizasyon protokolü arasında, yüksek kârlılığı nedeniyle büyük ölçekli koyun işletmelerinde kullanılabilecek en uygun protokolün Grup II olduğu sonucuna varılmıştır.

Anahtar Kelimeler: GnRH, hormone maliyeti, GKSH, üreme parametreleri, vajinal sünger.

Introduction

Increasing ovulation rates or decreasing embryonic losses in sheep farming are significant traits that must be developed to boost sheep productivity. Hormonal treatments to regulate ovulation and reproduction can help achieve both shorter fertilization and more uniform offspring (Motlomelo et al., 2002; Husein and Kridli, 2003). Methods using progesterone or its analogs rely on its effects in the luteal phase of the cycle and emulate the effects of natural progesterone produced by the corpus luteum after ovulation, which is responsible for regulating Lutein hormone (LH) secretion from the pituitary gland. Progestagen-containing sponges used among ewes are usually placed intravaginally for 12–14 days. Ewes typically display estrus approximately 48 hours after sponge removal (Abecia et al., 2012). Pregnant mare serum gonadotropin (PMSG) is frequently used to stimulate ovarian activity in ewes at the time of vaginal sponge removal. This hormone has simultaneous FSH- and LH-like activities (Emsen, 2004; Köse et al., 2012). In other words, PMSG is used in conjunction with synthetic progestagens to stimulate and synchronize follicular development, aiming for synchronized ovulations and higher fecundation rates (Menchaca and Rubianes, 2007). However, the production of PMSG from pregnant mares raises serious ethical concerns regarding animal welfare. Thus, its production and use may be forbidden in

the future (Vilanova et al., 2019; Santos-Jimenez et al., 2020). Furthermore, repeated use of PMSG, associated with neutralizer antibodies, has been found to lower fertility in ewes (Roy et al., 1999; Maurel et al., 2003; Guner et al., 2022). The use of gonadotropin-releasing hormone (GnRH) instead of PMSG in progesterone-based synchronization protocols in different breeds reportedly resulted in differences in birth rates (Martemucci and D'Alessandro, 2011; Silva et al., 2015; Martinez-Ros and Gonzalez-Bulnes, 2019; Santos-Jimenez et al., 2020).

The basis of gonadotropin-releasing hormone (GnRH) administration stems from the ability of gonadotropins to alter the dynamics of follicular development (Diskin et al., 2002; Ali et al., 2009). Since estrus may occur without ovulation after progestagen use, especially during the non-breeding season, PMSG or GnRH injections are usually recommended to promote follicular development and stimulate ovulation (Wildeus, 2000). GnRH injections are administered simultaneously with or immediately after mating to minimize embryonic and fetal loss or improve reproductive performance in sheep (Ataman et al., 2013; Ahmadi and Mirzaei, 2016; Rostami et al., 2017; Zonturlu et al., 2018). Studies also reported that an endogenous LH release occurs within 1 to 4 hours after GnRH administration. This release is essential for many critical reproductive processes associated with fertility, including dominant follicle development, oocyte maturation, ovulation, and luteinization (Khan et al., 2003; Cam and Kuran, 2004; Cavalcanti et al., 2012).

The effects of GnRH injections on sheep reproductive parameters generally varied depending on the injection time and dosage (Khan et al., 2007; Sirjani et al., 2012; Lashari and Tasawar, 2013; Olfati and Moghaddam, 2013; Hashem et al., 2015). Therefore, this study aimed to identify the effects of PMSG and GnRH injections, administered individually and in combination at different times, on estrus synchronization, their impacts on some fertility traits, and the hormone cost in Kıvırcık ewes whose estrus was synchronized with a progesterone-containing vaginal sponge during the anestrus period.

Material and Method

Location

This study was conducted in the farm of the Department of Animal Science of Bursa Uludağ University. The study was conducted in the Görükle district of Bursa province in the Marmara region during the non-breeding season. This area is located 40° N–29° E and 100 m above the sea level.

Animals and Management

Ninety-six Kivircik-bred ewes weighing between fifty and fifty-five kilograms, three to four years old, and previously given birth and clinically healthy were used. Before beginning the trial, the ewes got anti-parasitic medications and a vaccination. The animals spent the most of the day grazing, and at night they were housed in a shelter. The local feeds—barley, corn, sunflower meal feed supplement, and pasture herbage—were the basis for the diet. For a full day, they had unlimited access to water, salt lick, and minerals. This study was conducted

with approval from the Ethics Council of Animal Experiments of Bursa Uludağ University University, dated 2024-10-04.

Study Groups

This study involved 96 sheep whose estrus cycles were synchronized by an inserted vaginal sponge with 60 mg medroxyprogesterone acetate (MAP) (Espanjavet®, HIPRA Laboratories Inc., Amer, Spain). The sponges of 24 randomly selected ewes were initially removed on the 12th day following vaginal sponge application, and then 500 IU pregnant mare serum gonadotropin (PMSG; Chronogest PMSG®, Intervet, Turkey) was administered intramuscularly. After 48 hours (day 14), the same ewes were also treated with a 1 ml injection of buserelin acetate (4 µg ml^{-1}) (GnRH, Receptal®, Intervet, Turkey) (Group I). 24 other ewes were randomly selected and treated with 1 ml of GnRH (4 µg ml^{-1}) on the 12th day. After 48 hours (day 14), they also received a 500 IU PMSG injection intramuscularly following the removal of their sponges (Group II). Twenty-four of the 48 ewes were randomly selected and received 500 IU of PMSG intramuscularly on day 14, coinciding with sponge removal (Group III). The remaining 24 ewes, however, received an injection of 1 ml of GnRH (4 µg ml^{-1}) on the 14th day, while their sponges were removed (Group IV) (Figure 1). The concentrations of the synthetic form of GnRH used in this study were selected based on the dosages typically used for ewes in the hormonal protocol (Pereira et al., 2024). Within 24 hours following the last administration, six fertility-proven rams were introduced into the flock, and they were kept there for 34 days (two estrous cycles).

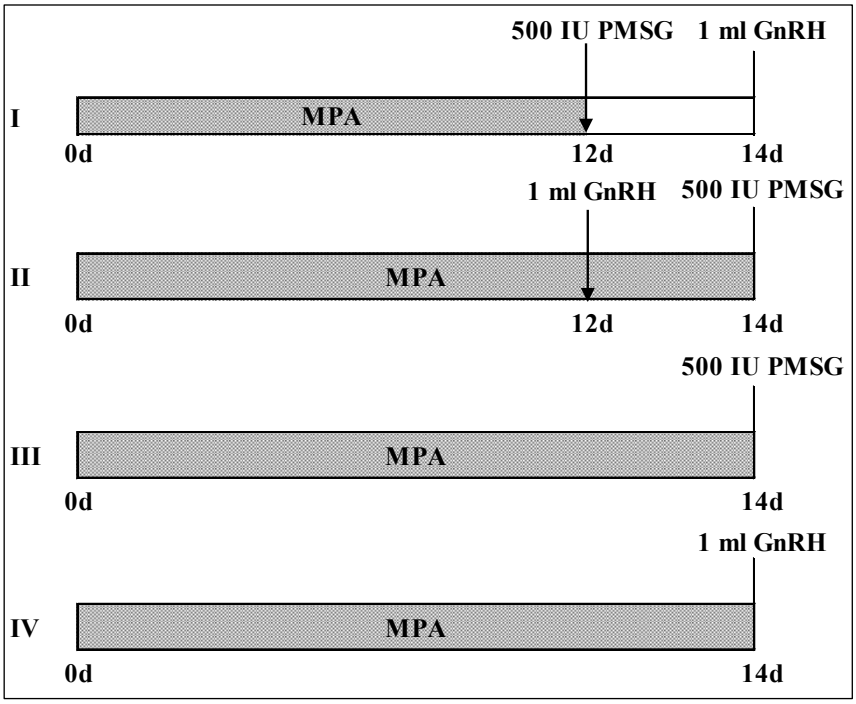


Figure 1. *Treatments protocols*

Determination of Reproductive Parameters

The reproductive parameters determing for each group in this study were the duration of oestrus behaviour (time from removal of sponge to first oestrus behaviour), pregnancy rate (number of pregnant ewes/number of mated/mounted ewes ×100), gestation length (time from mated to birth), lambing rate (number of lambing ewes/number of mated ewes ×100), rate of multiple births (number of multiple lambing ewes / number of pregnant ewes × 100), litter size (number of total lambs/number of lambing ewes in the group ×100), survival rate, %: number lambs weaned / number of lambs born × 100; pregnancy productivity, kg: total weight of lambs live born from each 100 mated ewes; total productivity, kg: total weight of lambs at weaning time from each 100 mated ewes; and daily gain (DG), g: weaning weight (WW), (kg) - birth weight (BW) (kg) / weaning age (WA) (days) (Kaymakci et al., 2024).

Statistical Analysis

This study used the chi-square test to measure the effects of treatments on 24-hour estrus rate, multiple birth rate, and sex ratio. It also performed the analysis of variance to identify the factors affecting lambs' birth, weaning weight, and the daily gain from birth to weaning. The study also performed Fisher's LSD test to specify different groups (Minitab, 2019). The statistical differences between the litter size values of the groups were also ascertained using the Kruskal-Wallis test, a nonparametric test that can be utilized as an alternative to the one-way ANOVA approach (Minitab, 2019).

The statistical models used for the birth and weaning weights and daily gains were as follows:

$$y_{ijkl} = \mu + a_i + b_j + c_k + e_{ijkl} \quad (1)$$

$$y_{ijkl} = \mu + a_i + b_j + c_k + b_1 X_{ijkl} + b_2 Z_{ijkl} + e_{ijkl} \quad (2)$$

where:

y_{ijkl} = the birth and weaning weights and daily weight increases

μ = the mean,

a_i = the effect of the *ith* treatment,

b_j = the effect of the *jth* birth type (single vs multiple),

c_k = the effect of the *kth* sex (male vs female),

b_1: regression coefficient of weaning weight and daily weight gain on birth weight

X_{ijkl} = birth weight of *lth* lamb

b_2 = regression coefficient of weaning weight and daily weight gain on weaning period

Z_{ijkl} = weaning period of *lth* lamb

e_{ijkl} = the residual error.

Hormone Cost

The net profit was determined by the study using the total lamb weight at weaning and the administration costs per sheep. Additionally, it determined the cost of live lamb units during the same time frame (National Red Meat Council, 2025). The gross profit for each sheep was calculated by multiplying the unit price by the total weight of the lambs at weaning. Only the cost of hormone administration was considered in calculating the net profit, since labor and other expenses were equal for all ewes (Rosasco et al., 2019). Finally, the study performed an ANOVA test to calculate the difference in net profit between the groups and Fisher's LSD test to identify group differences (Minitab, 2019).

Results

Fertility Parameters

Table 1 provides the findings about estrus rate and fertility parameters among the experimental groups. For estrus, researchers observed the behaviors of ewes during the first 24 hours. Accordingly, they considered it an estrus behavior when ewes remained inactive and allowed the ram to mate. Accordingly, there was no observed estrus behavior in the first 24 hours in ewes in Groups I and IV; however, 69.6% and 86.9% of the ewes in Groups II and III displayed estrus, respectively. All ewes in the treatment groups became pregnant and gave birth healthy. Although Group II had the highest multiple birth rate (60.9%), the effect of the treatments on the type of birth was statistically insignificant. Similarly, there was no statistically significant difference in litter size among groups ($P>0.05$). However, Groups I, II, and III showed numerical differences and were all greater than Group IV when fertility and litter size figures were taken into account. Additionally, Group IV's lamb survival rate (83.3%) was substantially lower than that of the other groups ($P<0.01$). There were no lamb deaths in Groups I and III. The highest pregnancy prolificacy was observed in Group IV, while the highest total prolificacy was in Group II.

Table 1. Fertility parameters in ewes in the treatment groups

Parameters	**Treatments**									
	Group I		**Group II**		**Group III**		**Group IV**			
	n	**mean**	**n**	**mean**	**n**	**mean**	**n**	**mean**	**P-value**	χ^2
Estrus rate (%)	0	0.00	16	69.60	20	86.90	0	0.00	61.48	0.000
Pregnancy rate (%)	24	100.00	23	100.00	23	100.00	23	100.00		
Lambing rate (%)	24	100.00	23	100.00	23	100.00	23	100.00		
Multiple birth rate (%)	9	37.50	14	60.90	11	47.80	6	26.10	0.102	6.195
Fecundity (head)	33	1.38	38	1.65	35	1.52	30	1.30		
Litter size (head)	33	1.38	38	1.65	35	1.52	30	1.30	0.120	
Survival rate (%)	33	100.00	36	94.70	35	100.00	25	83.30	0.000	11.805
Pregnancy prolificacy (kg)		407.00		394.50		425.10		456.70		
Total prolificacy (kg)		2264.80		2684.40		2578.60		2450.00		

The results of the analysis of variance for lambs' birth, weaning weight, and daily gain are shown in Table 2. Within the first twenty-four hours of their birth, the lambs were weighed to ascertain their birth weights. There was not statistically significant weight difference between male and female lambs, according to the results (P>0.05; Table 2). Nonetheless, there were substantial differences in the average lamb weights between the groups (P<0.05). The lambs with the highest birth weights among treatments were in Groups IV and III. The effect of the treatments on weaning weight and daily gain, on the other hand, was insignificant (P>0.05). While the birth type was significant only for the birth weight of the lambs (P<0.01), its effect on weaning weight and daily gain was not significant. The gender parameter did not affect the birth weight of the lambs; however, its impact on weaning weight and daily live weight gain was significant (P<0.01). Furthermore, there was a substantial (P<0.01) partial regression coefficient of weaning weight on both birth weight (b1) and duration period (b2) (P<0.01).

Table 2. Least squares means and standard errors for some growth characteristics in curly lambs

		BW, kg	**WW, kg**	**DWG, g/day**
Treatments	n	*	ns	ns
Group I	33	4.10±0.12^{b}	25.95±1.01	262.90±12.00
Group II	38	4.13±0.12^{b}	25.08±0.94	252.30±11.20
Group III	35	4.36±0.12^{ab}	23.13±0.99	230.10±11.80
Group IV	30	4.52±0.13^{a}	27.19±1.27	280.50±15.10
Birth type		**	ns	ns
Single	53	4.63±0.09^{a}	25.70±0.60	261.60±7.20
Multiple	83	3.93±0.08^{b}	24.97±0.49	251.40±5.80
Sex		ns	**	**
Female	72	4.30±0.08	24.26±0.5^{b}	243.70±6.00^{b}
Male	64	4.26±0.08	26.42±0.51^{a}	269.20±6.10^{a}
b1			2.034±0.53**	12.20±6.30
b2			0.394±0.10**	1.70±1.20
General	136	4.28±0.06*		

**P<0.01, *P<0.05, ns: non-significant, $^{a, b}$: Differences between means indicated by different letters in the same column are significant.

Economic Analysis of Hormone Treatment

According to the study, the treatment expenses for Groups I, II, III, and IV in August 2024 were 6.5, 6.5, 4.5, and 3.3 dollars per sheep, respectively. During the same time period, live lambs were found to cost 6.67 dollars per kilogram. Group IV's lambs from two single-birth ewes did not live to the conclusion of the weaning period, hence their net profit was calculated to be -3.3 dollars per ewe. The group variations in average net profit were statistically significant (P<0.01). As a result, the study found no statistical difference between Groups II and III, and the highest calculated profit per ewe for these groups was 273.6 $ and 257.0 $, respectively. However, the lowest profitability was in Group IV with 174.2 $.

Table 3. Least squares means and standard errors of net profit per sheep by groups ($)

Groups	**n**	$\overline{X} \pm S_{\overline{X}}$
Group I	24	201.1 ± 19.0 [bc]
Group II	23	273.6 ± 19.4 [a]
Group III	23	257.0 ± 19.4 [ab]
Grup IV	23	174.2 ± 19.4 [c]

[a, b, c]: Differences between means indicated by different letters in the same column are significant.

Discussion

Fertility Parameters

The new follicular wave during the period of progesterone administration indicates that the pre-ovulatory follicles in this wave have grown and advanced to ovulation size at the time of sponge removal. Vaginal sponges also contribute to the availability of a sufficient number of oocytes for ovulation by ensuring high serum plasma progesterone concentrations during ovulatory follicle development (Swelum et al., 2015; Menchaca et al., 2018). The current investigation chose varied times before mating for GnRH administration based on the premise that PMSG use causes varying sizes of follicles or that discrete follicular waves arise on the ovarian surface during the estrous cycle. All ewes in Group III that received progesterone-containing vaginal sponges + PMSG outside the mating season demonstrated estrus in the first 24 hours, and the lambing rate in the first cycle rose among these ewes. Hence, it is reasonable to argue that the progesterone+PMSG administration increases the estradiol production capacity of the dominant follicle by stimulating ovarian activity in ewes and shortening the time until estrus onset. Swelum et al. (2015), Biehl et al. (2019), and Yu et al. (2022) reported that ewes typically display estrus behavior within 48 hours after removal of the progesterone-containing sponge. Hajibeman et al. (2022) documented that 85% of ewes whose estrus was synchronized with the progesterone+PMSG during the anestrus season showed estrus on the day the sponges were removed. Duan et al. (2025) noted that PMSG, due to its dual activity of FSH and LH, promotes follicular growth and ovulation, resulting in faster estrus and better ovulation in ewes. The results of the current study also supported these findings. Indeed, ewes in Group II that received GnRH injections 48 hours before sponge removal displayed estrus signs predominantly within the first 24 hours and became pregnant in their first cycle. Hence, the GnRH administration in Group II increased the induced pre-ovulatory LH surge in ewes, potentially promoting oocyte maturation, and PMSG administered after 48 hours stimulated the onset of estrus by supporting follicular growth and ovarian estrogen secretion. This finding is consistent with that of Reyna et al. (2007), who found that GnRH administration 24 hours after estrus synchronization did not increase ovulation or growth atresia of the lower follicles but did improve ovulation time. Different hypotheses can explain the current data. To begin, the formation of antral follicles larger than 2 mm requires FSH, which appears steady but is not acutely responsive to GnRH, despite the fact that GnRH regulates its secretion (Scaramuzzi and Radford, 1983). The second FSH surge, which controls the ensuing follicular wave, is unaffected by GnRH secretion (Bowen et al., 1998). Second, the ewes' genotype may

influence the response; for example, exogenous GnRH release may boost FSH concentrations by 1.6-fold in high-fertility ewes compared to low-fertility ewes (McNatty et al., 1991). Therefore, the anticipated role of GnRH injections around the estrus period is to induce ovulation for ovulating follicles without stimulating the growth or development of subordinate follicles to become ovulating follicles (Scaramuzzi and Radford, 1983). Yu et al. (2022) reported that GnRH may stimulate the anterior pituitary gland to secrete FSH and LH, which can successfully initiate ovulation and shorten the interval between estrus and ovulation. Zhang et al. (2023) emphasized the significance of the timing of GnRH injection in ewes, reporting that when administered during insemination, 48 hours after removal of vaginal sponges, it increased the number of offspring and multiple birth rate but decreased the fecundity rate; therefore, they recommended no GnRH administration during insemination. Cavalcanti et al. (2012) documented that GnRH administered 24 hours after removing the vaginal sponge did not improve the ewe pregnancy rate, indicating no significant change in ovulation rate, fertility, and estrus duration. Dogan et al. (2023) also remarked that GnRH administered 36 hours after vaginal sponge removal had an insignificant effect on estrus behavior, ovarian findings, or pregnancy rate in ewes. Finally, Zhang et al. (2024) reported that GnRH administered 48 hours after sponge removal adversely affected the uterine environment by increasing preimplantation corticosterone and prostaglandin levels, which ultimately reduced pregnancy rates. Given the results of the current study, it is reasonable to argue that low progesterone levels following sponge removal in Group III without GnRH enabled the release of endogenous gonadotropins, which potentially led to estrus and ovulation.

Similar to the results for Group III in the current investigation, Daghash et al. (2017) found that all ewes whose estrus was synchronized with progesterone+PMSG displayed estrus, and the pregnancy and lambing rate was 100%. Differences in results could be caused by a number of things, such as the ewes' breed, their diet, or their physiological states. However, the present study found that GnRH injection given at the time of sponge removal (Group IV) did not have the same effect on inducing estrus alone, and the ewes (in Group IV) became pregnant in the second cycle. Hence, it is reasonable to assert that GnRH without PMSG had no effect in inducing estrus in anestrous ewes. Venkataramanan et al. (2015) documented that GnRH injection at the time of sponge removal in anestrous ewes could not stimulate estrogen release if the initial FSH concentration is insufficient for follicular development. Contrarily, ewes in Group I of the current study, which received GnRH 48 hours after vaginal sponge removal, displayed no estrus during the first 24 hours and became pregnant during the second cycle. Accordingly, GnRH treatment 48 hours after sponge removal in Group I may have inhibited the estrus response by inducing ovulation before the onset of estrus, thereby inducing hormonal alterations in the central nervous system of the ewes that hindered sexual behavior. Indeed, it is widely known that the high levels of LH secreted in estrus are responsible for blocking estrogen secretion by the pre-ovulatory follicle, which then transforms into a progesterone-secreting corpus luteum (Quirke et al., 1979). The acceleration of the LH surge within 1-4 hours after GnRH injection may reportedly lead to premature ovulation of pre-ovulatory follicles, potentially impeding the level of estradiol secretion necessary for estrus behavior (Silva et al., 2015; Martinez-Ros and Gonzalez-Bulnes, 2019). In conclusion, the estrus-inducing effect of GnRH injection in anestrous ewes

varies depending on the time of administration, the physiological state of the ewe, and its interaction with other hormonal treatments.

Ewes in all treatment groups became pregnant (100%) and gave birth healthily (100%) in the second cycle, despite the fact that the treatment groups showed different rates of estrus based on the administrations they received in the first 24 hours. According to a number of studies, GnRH increases serum progesterone levels, which are essential for maintaining pregnancy (Nikbakht et al., 2022). Furthermore, by causing these follicles to luteinize, GnRH efficiently synchronizes follicular growth and controls progesterone levels in the blood. As a result, it raises embryo survival rate (Cam et al., 2002; Khan et al., 2007; Lashari and Tasawar, 2010). Jordan et al. (2009) reported that estrus response and pregnancy rate were higher in ewes with GnRH injection during anestrus, emphasizing that it was a promising and alternative protocol to progesterone to enhance the ovulation response and reproductive performance of ewes during anestrus. Hashem et al. (2015) documented that GnRH can be used to increase lambing and fertility rates by reducing pregnancy loss. The present study also identified the highest multiple birth rate in ewes administered GnRH 48 hours before sponge removal (Group II). Considering the fecundity and litter size results, the groups were ranked as Group II > Group III > Group I > Group IV, although the intergroup differences were statistically insignificant ($P < 0.05$). Similarly, the pregnancy, lambing, twinning rate, and litter size observed in all GnRH treatment groups of the present study were higher than those reported in Mirzaei et al. (2014) in GnRH-injected ewes 48 hours after sponge removal (69.2%, 66.7%, 31.3%, and 1.3%, respectively). Additionally, these numbers exceeded the pregnancy and lambing rates of 85.71% and 85.71%, respectively, that Farghal and Mostafa (2022) found in GnRH-injected ewes prior to mating. Regarding the impact of GnRH on oocyte maturation (Kleemann et al., 1994) and embryonic development (Khan et al., 2007), there are a number of differing viewpoints. Accordingly, GnRH may have helped improve embryogenesis, suppress luteolysis, and extend the duration of pregnancy (Nephew et al., 1994). Furthermore, it is well acknowledged that GnRH treatments improve placental formation and increase the number of placentomes during pregnancy (Lashari and Tasawar, 2010). However, the number of multiple births and litter size found in the present study were smaller than those reported by Ahmadi and Mirzaei (2016) in sheep following the administration of GnRH and 48 hours of sponge removal (73.3% and 1.73%, respectively). Given its direct effect on follicular development and the subsequent rise in estradiol concentrations with the onset of estrus, which results in an LH surge and ovulation, it is reasonable to justify the induction of ovulation administered by GnRH, even though this study found the difference to be statistically insignificant (Martinez-Ros and Gonzalez-Bulnes, 2019). Accordingly, Quirke et al. (1979) reported a substantial increase in ovulation rate when GnRH analogues were taken 48 hours prior to sponge removal. GnRH injection after progesterone-based estrus synchronization has been shown to improve lambing rate, post-induced estrus pregnancy rate, and estrus induction in anestrus ewes (Husein and Kridli, 2003). Similar findings were reported by Karaca et al. (2009) and Titi et al. (2010), who found that the combination of GnRH and progesterone improved sheep reproductive performance by dramatically increasing the multiple birth rate and litter size. GnRH treatments dramatically raised the multiple birth rate, according to Ahmadi and Mirzaei (2016) and

Lashari and Tasawar (2010). GnRH injections given during mating, however, did not raise the multiple birth rates in ewes, according to Ataman et al. (2013).

Group IV ewes had the highest pregnant prolificacy in the current study, while Group II ewes had the highest overall prolificacy. Therefore, the GnRH injection given to Group IV sheep on the day of mating may have improved the ewes' reproductive success, possibly by improving luteal function and improving embryo survival. Nevertheless, Farghal and Mostafa (2022) found that ewes receiving the pre-mating GnRH injection had no increase in prolificacy rate. Except for the mortality of two lambs in Group II and five lambs in Group IV, all lambs successfully reached weaning age healthily. The higher survival rate in lambs may be attributed to the fact that ewes in all groups raised their lambs with care, produced more milk, and thus displayed better maternity abilities. The GnRH injection administered to ewes also significantly affected lamb birth weight ($P<0.01$); however, it had no effect on weaning weight or daily live weight gain ($P>0.05$). In fact, a number of studies suggested that GnRH might influence the birth weight of lambs by either promoting ovulation, which prolongs the time for embryo growth and development, or directly influencing mature oocytes carried toward embryogenesis and implantation (Kleemann et al., 1994; Lashari and Tasawar, 2007). Additionally, Group IV and Group III ewes had the highest average birth weights (4.52 kg and 4.36 kg, respectively) according to this study. Singular and numerous lambs weighed an average of 4.63 kg and 3.93 kg, respectively ($P<0.05$). However, studies in the literature have generally indicated no statistically significant difference in lamb birth weight when ewes were injected with GnRH. For instance, Farghal and Mostafa (2022), performing a study on the Ossimi ewe breed, reported that the birth weight of lambs in the GnRH-treated group was slightly higher than that of the control and other groups; however, this difference was statistically insignificant. Similarly, Cam et al. (2002), in Karayaka and Karayaka x Chios crossbred ewes, and Kaya et al. (2025), in Morkaraman ewes, observed that GnRH injection did not affect the birth weights of lambs. This difference may originate from factors such as the ewe's breed, time of administration, dosage, and nutrition.

Economic Analysis of Hormone Treatment

In practise, hormone prices are a major factor for breeders, who always want to increase revenues while reducing expenses. At $3.3 per sheep, the current study had the lowest hormone cost in the Group IV protocol. Because of its incredibly low multiple birth rate and litter size value, Group IV was the least profitable when taking into account the balance of income and expenses, even if it had the highest weaning weight and pregnancy prolificacy. Furthermore, every sheep in the groups had the highest pregnancy rate—100%. Group II exhibited a greater multiple birth rate and litter size despite having similar hormone costs to Group I. Interestingly, despite the absence of GnRH and PMSG costs in Groups III and IV, respectively, the high multiple birth rate, litter size, and weaning weight in Group II resulted in higher net profit margins compared to the other groups in terms of the balance of income and expenses. Group II also yielded the highest income per ewe, which is 57% and 36% higher than Group IV (the lowest-income) and Group I, respectively (Table 3). Similarly, Group III had 47% more income than Group IV. Hasani et al. (2018) reported that the most expensive estrus synchronization protocol did not frequently induce estrus and reproductive performance, whereas ewes using the medium-cost

protocol had higher birth and lambing rates. Indeed, the present study found no difference between the highest groups and Group IV in terms of pregnancy and lambing rates, despite the lowest hormone costs in Group IV. Yet, Group I had the highest hormone costs; however, it did not yield the maximum profit in terms of fertility parameters. Although the present study found intergroup differences to be statistically insignificant for the litter size parameter, these variations were still non-negligible when considering per-ewe profit ($P<0.01$). These results explicitly demonstrate the economic disparities between the treatments. Consequently, this treatment improved the progesterone-based protocol's efficiency, even reaching an efficiency rate comparable to the conventional progesterone-based method, even though adding GnRH administration to the ewe-raising program increased some costs in comparison to the traditional progesterone protocol. In summary, the application of the GnRH protocol in anestrous ewes seems to be both economically and technically successful, enhancing sheep pregnancies and raising farm profitability.

Conclusion

Ethical concerns about PMSG production in a progesterone-based synchronization protocol in ewes and the welfare of pregnant mares require the use of alternative gonadotropins instead of PMSG. GnRH can be effective in synchronizing estrus and improving ewes' fertility if combined with various protocols. Using progestagen sponges during GnRH administration in the present study appears to have advanced reproductive parameters, most likely through enhancing follicular development. Such a combination may generate better responses than the use of traditional progestagen sponges and PMSGs. Hence, further research is necessary to analyze the effects of such treatments using a larger ewe population and different breeds in varying seasons (breeding and non-breeding seasons). In conclusion, using a sponge combined with GnRH administration may have an enhancing effect on fertility in Kıvırcık ewes during anestrus.

Acknowledgment

This article has been prepared in accordance with research and publication ethics. The authors who prepared this article have contributed jointly to the work, and there is no conflict of interest among the authors.

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