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Research Article

Effects of Combined hCG and eCG Administration on Fertility in Progesterone-Treated Akkaraman Ewes During the Breeding Season

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ABSTRACT

This study aimed to investigate the effect of concurrent hCG injection with eCG in a short-term (7-day) progesterone-based synchronization protocol on fertility parameters and economic profitability during the breeding season of Akkaraman sheep. 110 Akkaraman ewes were used in the study. A sponge impregnated with progesterone was inserted into the vagina of all ewes, and cloprostenol was administered one day before, and on the day the sponge was removed. The sheep were randomly allocated into 3 groups: Group I received 400 IU eCG, Group II received 200 IU hCG, and Group III received 400 IU eCG+200 IU hCG. The rams were incorporated into the flock on day 8 of the study. Pregnancy examinations were performed 30±3 days after mating. Estrus rates were determined as 91.7%, 86.1%, and 94.6% in the groups (Group I, Group II, Group III), respectively. The pregnancy and lambing rates in the group were determined as 80.6% and 96.6%, 75% and 88.9%, and 86.5% and 93.8%, respectively ($P>0.05$). Multiple lambing rates were determined as 42.9%, 20.8%, and 56.7% ($P=0.008$). Litter size was determined as 1.54, 1.21, and 1.77, respectively. In conclusion, eCG+hCG administration numerically improved reproductive performance but was not statistically significant. Only the increase in multiple lambing rate was significant compared to the hCG group.

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Üreme Sezonunda Progesteronla Tedavi Edilen Akkaraman Koyunlarında Kombine hCG ve eCG Uygulamasının Fertilite Parametreleri Üzerine Etkileri

ÖZET

Bu çalışma, üreme sezonunda Akkaraman koyunlarında uygulanan kısa süreli (7 gün) progesteron temelli senkronizasyon protokolünde eCG ile birlikte uygulanan hCG enjeksiyonunun fertilite parametreleri ve ekonomik kârlılık üzerindeki etkilerini değerlendirmek amacıyla yapıldı. Çalışmada toplam 110 Akkaraman koyunu kullanıldı. Tüm koyunlara progesteron emdirilmiş vajinal sünger yerleştirildi ve süngerlerin çıkarılmasından bir gün önce ve çıkarıldığı gün kloprostenol uygulandı. Koyunlar rastgele üç gruba ayrıldı: Grup I'e 400 IU eCG, Grup II'ye 200 IU hCG ve Grup III'e 400 IU eCG + 200 IU hCG uygulandı. Koçlar çalışmanın 8. gününde sürüye katıldı. Gebelik muayeneleri çiftleşmeden 30±3 gün sonra yapıldı. Östrus oranları sırasıyla Grup I, Grup II ve Grup III'te %91,7, %86,1 ve %94,6 olarak belirlenmiştir. Gebelik ve kuzulama oranları ise gruplarda sırasıyla %80,6 ve %96,6; %75,0 ve %88,9; %86,5 ve %93,8 olarak saptanmış olup, gruplar arasındaki fark istatistiksel açıdan anlamlı bulunmamıştır ($P>0,05$). Çoklu doğum oranları sırasıyla %42,9, %20,8 ve %56,7 olarak belirlenmiş ve bu farkın istatistiksel olarak anlamlı olduğu görülmüştür ($P=0,008$). Litter büyüklüğü (kuzu sayısı) ise sırasıyla 1,54, 1,21 ve 1,77 olarak hesaplanmıştır. Sonuç olarak, eCG+hCG uygulamasının üreme performansı üzerinde sayısal bir iyileşme sağladığı gözlenmekle birlikte, bu artış genel olarak istatistiksel açıdan anlamlı değildir. Bununla birlikte, çoklu doğum oranındaki artışın özellikle hCG grubuna kıyasla anlamlı olduğu belirlenmiştir.

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INTRODUCTION

The main objective in sheep breeding is to achieve high productivity by maximizing reproductive performance with minimal costs (Özyurtlu & Bademkıran, 2010). Progesterone or its analogues are commonly used for estrus synchronization. These synthetic hormones mimic the effects of natural progesterone (Abecia et al., 2012). Progestagen treatments can be applied for both short (5-9 days) and long (10-14 days) durations in combination with gonadotropins (Kaya et al., 2013; Kaya et al., 2025; Martinez-Roz et al., 2018). Short-term treatments of 5-7 days have been reported to yield satisfactory results (Ataman et al., 2006; Karaca et al., 2009; Kaya et al., 2023; Kaya et al., 2025; Viñoles et al., 2001).

Supplementation of progesterone-based protocols with eCG enables effective estrus synchronization (Hameed et al., 2021; Kaya et al., 2023). eCG promotes follicular development and ovulation. To increase litter size, a dose of 400–700 IU eCG is recommended 1-2 days before the end of progesterone treatment or immediately after completion of the treatment (Kaya & Koçak, 2022; Wildeus, 2000; Uçar et al., 2002).

Human chorionic gonadotropin (hCG) exhibits luteinizing hormone-like activity (Bruno-Galarraga et al., 2021) and is used to regulate reproductive functions in sheep (Dias et al., 2020; Kaya et al., 2013). Recent studies have shown that hCG administration, either 24 hours after short-term progesterone treatment or at mating, can synchronize estrus and lambing time (Dehkordi et al., 2022) and improve pregnancy rates (Khan et al., 2003). However, contradictory findings have also been reported, with some studies indicating reduced fertility associated with hCG use. These negative effects have been attributed to excessive follicular stimulation, leading to ovulation failure or delayed ovulation (Bruno-Galarraga et al., 2021).

A commercial preparation containing both eCG and hCG has been developed for simultaneous administration, and its effects have been evaluated in several studies (Habeeb et al., 2019). However, the findings are inconsistent. Some studies reported improved fertility (Morrical et al., 1998), whereas others found no effect (Safranski et al., 1992) or even decreased fertility (Habeeb et al., 2019; Windorski et al., 2008). High-dose applications have also been associated with excessive ovarian stimulation, abnormal follicular development, and reduced embryo survival (Habeeb et al., 2019; Windorski et al., 2008). Unlike previous studies, the concurrent administration of eCG and hCG offers the opportunity to better elucidate their combined effects, particularly in local breeds where such data are limited.

Based on the available literature, we hypothesized that the concurrent use of eCG and hCG in a short-term progesterone-based synchronization protocol would improve reproductive performance and economic outcomes compared to eCG alone. Therefore, this study aimed to evaluate the effects of combined eCG and hCG administration on reproductive performance and economic outcomes in Akkaraman ewes during the breeding season.

MATERIAL AND METHODS

Study Location

The study was conducted in a family-type farm located in the Çıldır district of Ardahan province, in the Eastern Anatolia Region of Turkiye, at an altitude of 1950 m, at 41°7'12''N latitude and 43°8'15''E longitude.

Animal Material

A total of 110 Akkaraman ewes, aged 2-3 years, non-pregnant, clinically healthy, with a body condition score of 2.5-3 and having lambed at least once, were included in the study; none had previously been treated with hCG (Kenyon et al., 2014). Additionally, 10 healthy, fertile Akkaraman-breed rams were used in the study.

Nutrition

Throughout the study, all sheep received a balanced diet consisting of meadows, harvested barley and alfalfa fields, grazed on pastures, and water ad libitum. Blood samples were collected from the sheep during synchronization methods and housed in a closed pen during the evenings. Routine farm care and feeding were provided to the sheep.

Determination of Treatment Groups

Synchronization treatments were performed during the breeding season (September). The ewes were randomly divided into three groups with balanced age, BCS, and parity. Rams were culled from the flock 40 days before synchronization. A progesterone-impregnated sponge (20 mg flugestone acetate, Chronogest®, MSD, France) was inserted into the vagina of all ewes using a special applicator (day 0). 1.5 mL of $PGF_{2\alpha}$ (250 mcg Cloprostenol, Estrumate®, MSD, Germany) was administered intramuscularly the day before (day 6) and on the day of removal (day 7) of the intravaginal sponges, and then the ewes were divided into groups, randomly.

Group I (eCG, n=37): Immediately after the second $PGF_{2\alpha}$ injection, only 400 IU eCG (Chrono-gest/PMSG®, MSD, Germany) was administered intramuscularly to the sheep in this group. One ewe in Group I was excluded due to vaginal sponge loss during treatment.

Group II (hCG, n=36): Immediately after the second $PGF_{2\alpha}$ injection, only 200 IU hCG (Chorulon®, MSD, Germany) was administered intramuscularly to the sheep in this group.

Group III (eCG+hCG, n=37): Unlike the other groups, both 400 IU eCG (Chrono-gest/PMSG®, MSD, Germany) and 200 IU hCG (Chorulon®, Germany) were administered intramuscularly to the sheep in this group immediately after the second $PGF_{2\alpha}$ injection.

Estrus Monitoring

Rams were incorporated on day 8 in all groups. Following the estrus, signs of estrus were monitored at 6-hour intervals for at least half an hour for 5 days. Ewes that accepted mounting were considered to be in estrus, and the day and time of estrus were recorded.

Pregnancy Examination

Pregnancy examination was performed via transrectal ultrasound (4-9 MHz, Draminski iScan®, Draminski, Poland) on the 30±3rd day following breeding.

Blood Sample Collection

Blood samples were collected from all sheep in the study group 10 days before synchronization began (-10th day), on the day synchronization began (0th day), and on the day of estrus into 10 mL tubes via the jugular vein (Figure 1). All blood samples collected from the sheep were centrifuged at 3000 rpm for 10 min (NF800®, Nüve, Turkey) to obtain serum samples. The serum samples were transferred to sterile microcentrifuge tubes and stored at -20°C until the day of analysis.

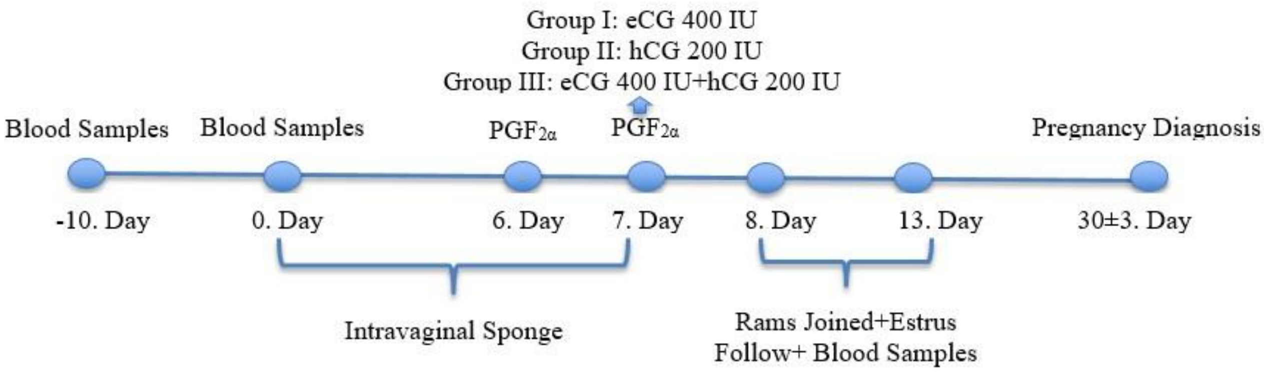

Figure 1. *Synchronization protocol in groups*

Serum Progesterone and Estrogen Analysis

Progesterone and estrogen analyses were performed quantitatively using the chemiluminescence (CLIA) method using the ADVIA Centaur® hormone analysis device (Siemens, Germany) and test kits from the same brand.

Fertility Parameters

In the present study, fertility parameters were calculated using the following formulas (Kaya et al., 2023):

Estrus onset time = Time interval between sponge removal and the onset of estrus signs

Estrus rate (%) = (Number of ewes exhibiting estrus/Number of ewes in the group)×100

Pregnancy rate (%) = (Number of pregnant ewes/Number of ewes in the group)×100

Lambing rate (%) = (Number of ewes that lambed/Number of pregnant ewes)×100

Fertility rate (%) = (Number of ewes that lambed/Number of mated ewes)×100

Single lambing rate (%) = (Number of ewes that lambed with one lamb/Number of ewes that lambed)×100

Multiple lambing rate (%) = (Number of ewes that lambed with two or more lambs/Number of ewes that lambed)×100

Fecundity = Number of lambs born/Number of mated ewes

Litter size = Number of lambs born/Number of ewes that lambed

Offspring weight = Determined by weighing lambs within the first 24 hours after birth

Economic Profitability

The costs of the hormones used for synchronization were calculated using market prices during the study period. The treatment cost per ewe included the cost of hormones and consumable materials (injector, cotton, alcohol, etc.) required for synchronization.

The total cost was calculated using the following formula:

Total Cost = Treatment Cost+Transport Cost+Veterinarian Practice Fee

Transport cost included the expenses associated with farm visits during the synchronization period.

The veterinarian practice fee was determined in accordance with the 2024 minimum fee schedule of the Chamber of Veterinarians for the Kars–Ardahan–Iğdır region.

Total revenue was calculated as:

Total Revenue = Number of lambs obtained×Market price per lamb

The market price of lambs was determined by considering both the official prices of the Sheep and Goat Breeders' Association and the prevailing market conditions in Ardahan province for 6-month-old lambs in 2024.

Net return was calculated as:

Net return = Total Revenue-Total Cost

All costs and revenues denominated in Turkish Lira were converted into US dollar equivalents using the exchange rate prevailing on the date of analysis (March 26, 2026).

Statistical Analysis

The normality of continuous variables (lamb birth weight, time to onset of estrus, and serum progesterone [P4] and estrogen [E2] levels) was assessed using the Shapiro–Wilk test. As the data were not normally distributed, comparisons among the three groups (Group I, Group II, and Group III) were performed using the non-parametric Kruskal–Wallis H test. When a significant difference was detected, post-hoc pairwise comparisons were conducted using the Mann–Whitney U test with Bonferroni correction. Continuous variables are presented as mean ± standard error of the mean (SEM) and median (Q1-Q3) values calculated using Tukey's hinges. The effect size for the Kruskal–Wallis test (ε^2) was calculated using the formula $\varepsilon^2 = H/(N-1)$, where H is the test statistic, and N is the total number of observations. Repeated measurements within the same group (changes in P4 levels on day −10, day 0, and the day of estrus) were analyzed using the Friedman test. When significant differences were observed, pairwise comparisons were performed using the Wilcoxon signed-rank test with Bonferroni correction. The effect size for repeated measures was calculated using Kendall's W coefficient ($W=\chi^2/[N (k-1)]$), where χ^2 is the Friedman test statistic, N is the sample size, and k is the number of measurements. Categorical variables (estrus, pregnancy, lambing, multiple birth, and fertility rates) were analyzed using logistic regression. The overall model significance was evaluated using the Omnibus chi-square (χ^2) test. Odds ratios (Exp B) and 95% confidence intervals (CI) were calculated using Group I as the reference category. A value of P<0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA).

RESULTS

In the presented study, a vaginal sponge was observed to have fallen out in one sheep in Group I, and this sheep was excluded from the study. The analyses were conducted on 109 ewes (Group I: n=36, Group II: n=36, Group III: n=37). Of the 109 sheep from which the sponge was removed, 94 developed mild vaginitis. However, these sheep were not excluded from the study. Estrus was observed earliest in Group I (45.74±2.10 hours), and latest in Group II (59.72±2.99 hours). It was determined to be 58.19±3.11 hours in Group III (Table 1). A statistically significant difference was observed among the groups in terms of the time to estrus onset (H=9.997, P=0.007, ε^2=0.109). Pairwise comparisons indicated that Group III had a significantly longer time to estrus onset compared to Group I (P=0.008). Group II showed intermediate values and did not differ significantly from either Group I or Group III (P>0.05).

Table 1. *Time to estrus onset (hours) by groups*

Groups	Mean±SEM	95% CI	Median (Q1–Q3)	Min - Max	H	P	ε^2
Group I	45.74±2.10^{b}	41.48-50.01	50.83 (39.97-53.68)	23.00-73.00			
Group II	59.72±2.99^{ab}	53.61-65.83	50.20 (48.15-69.00)	41.00-98.00	9.997	0.007	0.109
Group III	58.19±3.11^{b}	51.83-64.56	55.08 (50.25-74.12)	26.00-90.00			

Group I: eCG, Group II: hCG, Group III: eCG+hCG. Different superscripts (a, b) indicate statistically significant differences between groups (P<0.05). SEM: Standard Error of Mean, Q1 and Q3 (25th and 75th percentiles) were calculated using Tukey's Hinges algorithm, CI: Confidence Interval. H: Kruskal-Wallis test statistic, ε^2 (Epsilon-squared): Effect size coefficient for the Kruskal-Wallis test (0.01: Small; 0.06: Medium; 0.14: Large effect).

The reproductive performance parameters of the study groups are presented in Table 2. Estrus rates were 91.7%, 86.1%, and 94.6% in Groups I, II, and III, respectively, and showed a significant difference among the groups (χ^2=6.882, df=2, P=0.032). Pregnancy rates were 80.6% (29/36) in Group I, 75.0% (27/36) in Group II, and 86.5% (32/37) in Group III. Logistic regression analysis revealed no significant difference among the groups (χ^2=1.571, df=2, P=0.456). When Group I was used as the reference, the odds of pregnancy were 0.724 in Group II and 1.545 in Group III; however, these differences were not statistically significant (P>0.05). Lambs did not occur in 6 ewes determined to be pregnant. Embryonic death/fetal loss was determined in 1 ewe in Group I, 3 in Group II, and 2 in Group III. In contrast, pregnancy, lambing, and fertility rates were similar across the groups, with no statistically significant differences (P>0.05). Multiple lambing rates were 42.9%, 20.8%, and 56.7% in Groups I, II, and III, respectively, and differed significantly among the groups (χ^2=7.414, df=2, P=0.025). Although some differences were observed in odds ratios, these were not statistically significant in pairwise comparisons (P>0.05). Fertility, fecundity, and litter size were highest in Group III and lowest in Group II. Fecundity was 1.30 in Group I, 0.94 in Group II, and 1.51 in Group III. Similarly, litter size averaged 1.54 in Group I, 1.21 in Group II, and 1.77 in Group III.

Table 2. *Comparison of reproductive performance parameters among groups*

Parameters	Group	n	Rate (%)	Odds Ratio	95% CI	P	χ^2	df	P-value
Estrus rate	Group I*	33 / 36	91.7^{ab}	1.000	-	-	1.603	2	0.449
	Group II	31 / 36	86.1^{b}	0.564	0.124-2.559	0.069			
	Group III	35 / 37	94.6^{a}	1.591	0.250-10.131	0.623			
Pregnancy rate	Group I*	29 / 36	80.6	1.000	-	-	1.571	2	0.456
	Group II	27 / 36	75.0	0.724	0.237-2.215	0.572			
	Group III	32 / 37	86.5	1.545	0.441-5.407	0.496			
Lambing rate	Group I*	28 / 29	96.6	1.000	-	-	1.309	2	0.520
	Group II	24 / 27	88.9	0.286	0.028-2.930	0.292			
	Group III	30 / 32	93.8	0.536	0.046-6.240	0.618			
Multiple lambing rate	Group I*	12 / 28	42.9^{ab}	1.000	-	-	7.414	2	0.025
	Group II	5 / 24	20.8^{b}	0.351	0.102-1.209	0.097			
	Group III	17 / 30	56.7^{a}	1.744	0.616-4.933	0.295			
Fertility	Group I*	28 / 33	84.8	1.000	-	-	0.905	2	0.636
	Group II	24 / 31	77.4	0.612	0.172-2.181	0.449			
	Group III	30 / 35	85.7	1.071	0.280-4.101	0.920			

Group I was used as the reference group in logistic regression analyses. Different superscripts (a, b) within the same parameter indicate statistically significant differences between groups. n: Number of animals per group. χ^2: Chi-square test statistic; df: degrees of freedom. CI: Confidence interval; df: degrees of freedom; p: pairwise comparison versus G1; P-value: overall model significance (Omnibus test). n=109

The hCG group only developed single pregnancies and twin pregnancies. The number of ewes lambing twins was 10 in Group I, 5 in Group II, and 14 in Group III. Triplet and quadruplet lambing occurred only in Groups I and III, with one ewe in each group lambing triplets and another ewe lambing quadruplets. Quintuple pregnancies were only observed in Group III (n=1). The number of lambs born per group was 43, 29, and 53, respectively. Looking at the distribution of lamb genders by group, there were 23 female and 20 male lambs born in Group I, 16 female and 13 male lambs born in Group II, and 30 female and 23 male lambs born in Group III.

Changes in the evaluated parameter over time and differences among groups are presented in Table 3. No statistically significant difference was observed among the groups on Day −10 (H=4.283, P=0.117, ε^2=0.040) or on the day of estrus (H=1.862, P=0.394, ε^2=0.017). On Day 0, the difference among groups approached statistical significance (H=6.035, P=0.051, ε^2=0.056), although no significant pairwise differences were detected. Within-group analyses revealed a significant effect of time in all groups (Group I: χ^2=51.722, P<0.001, W=0.718; Group II: χ^2=55.514, P<0.001, W=0.750; Group III: χ^2=56.595, P<0.001, W=0.765). In all groups, the parameter values decreased markedly on the day of estrus compared to earlier time points.

Table 3. *Hormonal parameters at different time points*

Groups	Day -10	Day 0	Estrus Day	Within-group change
Group I	3.26±0.30^a Med: 2.91 (2.50-3.80) CI: 2.66-3.86	3.16±0.48^a Med: 2.44 (1.13-4.26) CI: 2.18-4.14	0.19±0.02^b Med: 0.10 (0.10-0.27) CI: 0.15-0.23	χ^2=51.722 P<0.001 W=0.718
Group II	3.14±0.38^a Med: 2.59 (1.18-4.71) CI: 2.38-3.91	4.87±0.60^a Med: 4.10 (2.02-7.05) CI: 3.66-6.08	0.17±0.02^b Med: 0.10 (0.10-0.16) CI: 0.12-0.22	χ^2=55.514 P<0.001 W=0.750
Group III	2.64±0.31^a Med: 1.93 (1.23-3.48) CI: 2.00-3.27	4.95±0.70^a Med: 4.12 (1.73-6.48) CI: 3.52-6.37	0.16±0.02^b Med: 0.10 (0.10-0.21) CI: 0.13-0.19	χ^2=56.595 P<0.001 W=0.765
Between-group statistics	H=4.283 P=0.117 ε^2=0.040	H=6.035 P=0.051 ε^2=0.056	H=1.862 P=0.394 ε^2=0.017	

Different superscripts (a, b) within the same column indicate statistically significant differences between groups ($p < 0.05$). Group I: eCG, Group II: hCG, Group III: eCG+hCG. H: Kruskal–Wallis test statistic, ε^2: Effect size (epsilon squared), χ^2: Friedman test statistic, W: Kendall's coefficient of concordance. Values are presented as mean ± SEM, median (Q1–Q3), and 95% confidence intervals

The serum estrogen levels of the groups on the day of estrus are presented in Table 4. No statistically significant difference was observed among the groups in terms of estradiol levels on the day of estrus (H=4.892, P=0.087, ε^2=0.050).

Table 4. *Estradiol levels on the day of estrus in the groups*

Groups	Mean ± SEM	Median (Q1–Q3)	95% CI	H	p-value	ε²
Group I	87.45±5.98	80.75 (66.00-95.90)	75.29-99.61	4.892	0.087	0.050
Group II	121.34±17.79	84.05 (79.40-112.00)	84.96-157.72			
Group III	113.82±10.67	89.00 (77.20-112.00)	92.13-135.52			

Group I: eCG, Group II: hCG, Group III: eCG+hCG. H: Kruskal–Wallis test statistic, ε^2: Effect size (epsilon squared), Values are presented as mean ± SEM, median (Q1–Q3), and 95% confidence intervals

The effects of treatment groups on lamb birth weights are presented in Table 5. A statistically significant difference was observed among the groups in terms of average female lamb weight (H=10.832, P=0.004, ε^2=0.159). Pairwise comparisons revealed that Group II had the highest values and was significantly higher than Group I (P=0.008) and Group III (P=0.011). No statistically significant difference was found between Groups I and III (P=1.000). Similarly, average male lamb weight differed significantly among the groups (H=12.301, P=0.002, ε^2=0.224). Group II had significantly higher values than Group I (P<0.05), while Group III showed intermediate values and did not differ significantly from either Group I or Group II (P>0.05). For average lamb weight, a significant difference was also detected among the groups (H=14.631, P=0.001,

ε^2=0.118). Group III exhibited significantly higher average lamb weights compared to both Group I and Group II (P<0.05), whereas no significant difference was observed between Groups I and II (P>0.05).

Table 5. *Average weight of lambs born in each group*

Groups	Average Female Lamb Weight (kg)	Average Male Lamb Weight (kg)	Average Lamb Weight (kg)
Group I	3.39±0.19[b] Median: 3.60 (3.15-3.85) Min-Max: 1.2-4.6 95% CI: 3.00–3.77	4.11±0.19[b] Median: 4.20 (3.75-4.80) Min-Max: 1.4-5.1 95% CI: 3.69-4.52	3.76±0.19[b] Median: 3.90 (3.40-4.50) Min-Max: 1.0-6.2 95% CI: 3.37-4.14
Group II	4.18±0.13[a] Median: 4.05 (3.77-4.57) Min-Max: 3.6-5.3 95% CI: 3.91-4.46	5.21±0.16[a] Median: 5.20 (5.00–5.40) Min-Max: 4.1-6.1 95% CI: 4.86-5.56	3.72±0.15[b] Median: 3.80 (3.45–4.35) Min-Max: 1.2-5.1 95% CI: 3.43-4.02
Group III	3.24±0.20[b] Median: 3.75 (2.50-3.90) Min-Max: 1.0-4.6 95% CI: 2.82-3.65	4.43±0.30[ab] Median: 4.60 (4.10-5.40) Min-Max: 0.9-6.2 95% CI: 3.81-5.06	4.64±0.14[a] Median: 4.80 (4.00–5.20) Min-Max: 3.6–6.1 95% CI: 4.36–4.93
P value	0.004	0.002	0.001
H	10.832	12.301	14.631
ε^2	0.159	0.224	0.118

**Different letters in the columns indicate statistical significance. Group I: eCG, Group II: hCG, Group III: eCG+hCG. Differences between group means containing different letters (a, b) in the same column are statistically significant (p < 0.05). SEM: Standard Error of Mean, Q1 and Q3 (25th and 75th percentiles) were calculated using Tukey's Hinges algorithm, CI: Confidence Interval. H: Kruskal-Wallis test statistic, ε² (Epsilon-squared): Effect size coefficient for the Kruskal-Wallis test (0.01: Small; 0.06: Medium; 0.14: Large effect).*

The total cost per ewe and total cost per group were 17.64 USD and 634.94 USD in Group I, 18.68 USD and 672.62 USD in Group II, and 19.33 USD and 715.19 USD in Group III, respectively (Table 6). Total revenue was calculated as 7,660.57 USD, 5,166.43 USD, and 9,442.09 USD for Groups I, II, and III, respectively. Accordingly, the net return was highest in Group III and lowest in Group II.

Table 6. *Cost analysis in groups*

Groups	Cost per ewe ($)	Total cost ($)	Total revenue ($)	Net return ($)
Group I	17.64	634.94	7,660.57	7,025.63
Group II	18.68	672.62	5,166.43	4,493.81
Group III	19.33	715.19	9,442.09	8,726.90

Group I: eCG, Group II: hCG, Group III: eCG+hCG

DISCUSSION

In sheep farming, reproductive productivity is essential for sustaining meat, milk, and wool production and plays a key role in both farm profitability and the national economy. Improving pregnancy rates and reducing reproductive losses depend largely on effective reproductive management practices, such as estrus synchronization (Rwaida et al., 2024). In this context, exogenous hormones are widely used to regulate the estrous cycle, synchronize estrus and ovulation, and shorten lambing intervals (Abecia et al., 2012; Dias et al., 2020). When ewes are synchronized and lamb within a short period, producers can reduce labor and related costs during each lambing season (Cross et al., 2019). These hormonal treatments also enable year-round lambing and increase lamb yield. Among the various approaches, progestagen-based protocols combined with eCG provide the most consistent and successful outcomes (Quintero-Elisea et al., 2022).

In vaginal sponge applications, sponge retention directly affects synchronization success. It has been reported that the drop rate of vaginal sponges in sheep is below 10% (Wildeus, 2000). In the present study, only one sheep (0.91%) lost the sponge. When sponges remain in place, synchronization results improve. Proper application technique, the animal's genital anatomy, and environmental conditions influence retention. Infections, excessive stress, and herd behavior can increase the risk of sponge loss.

The time between vaginal sponge removal and the onset of estrus varies depending on the breeding season, breed, feeding conditions, geographic region, climate, and the presence of rams in the herd. In general, estrus begins earlier in eCG applications, while this period has been observed to be longer in eCG+hCG combinations (Bruno Galarraga et al., 2021; Dias et al., 2020). In the presented study, the average estrus onset times in the groups were determined as 45.74±2.10, 59.72±2.99, and 58.19±3.11, respectively. These results are consistent with the above study, showing that estrus started later in the eCG+hCG group than in the eCG group.

Estrus rates are different depending on eCG and/or hCG applications in synchronization protocols. In eCG applications, estrus rates range from 81% to 100% (Bruno Galarraga et al., 2021; Daşkın, 2001; Esen & Bozkurt, 2001; Kulaksız et al., 2011; López-García et al., 2021) between 37.5% and 100% in protocols containing hCG (Afri-Bouzebda et al., 2015, Dias et al., 2018; Dogan et al., 2023), and between 78% and 94.2% in eCG+hCG applications (Afri-Bouzebda et al., 2015; D'Souza et al., 2014; Jabbar et al. 1995). In the presented study, the estrus rates were determined as 91.7%, 86.1%, and 94.6%, in the groups, respectively. Variations in the source of progestagen used, duration of administration, breeding season, breed, and hormone dosage are thought to account for such significant differences between studies.

Synchronization studies indicate that both the administration and dosage of eCG affect pregnancy rates. Moreover, administering 400 IU of eCG has been reported to reduce the time to estrus onset compared to 300 IU, while also increasing the fertility index and the rate of twin lambing (López-García et al., 2021). However, Zonturlu et al. (2011) determined that a 300 IU dose of eCG increased the pregnancy rate (82.35%) in Ivesi sheep during the transition period more than a 400 IU dose (80.0%). Santos-Jimenez et al. (2021) found that a 300 IU eCG injection in Dorper sheep that had given birth more than once outside the breeding season resulted in a significantly higher pregnancy rate (72.7%) compared to the control group (0%). In the present study, similar to these studies, high pregnancy rates (80.56%) were achieved in the eCG group. Different pregnancy rates have also been obtained in studies using hCG. In a study conducted outside the breeding season, pregnancy was achieved at a rate of 30.7% with hCG (300 IU) injection (43). A pregnancy rate of 33.9% was observed with hCG injection 24 hours after removal of the vaginal sponge (Dias et al., 2018), while the rate was 48% in those injected 36 hours later (Khan et al., 2003). Compared to these studies, pregnancy was achieved at a higher rate (75%) in the presented study. These differences among studies may be attributed to variations in progestagen sources used for synchronization, the duration of progestagen treatment, animal breeds, and the doses of hCG administered. Moreover, animal age appears to affect reproductive outcomes. Preovulatory LH peaks, which induce ovulation at the onset of estrus, are lower in lambs than in ewes (Beck et al., 1993). It is suggested that lamb ovaries are less sensitive to hCG than the ovaries of multiparous ewes (Khan et al., 1998). In the present study, 10 experienced fertile rams were used with 109 ewes to induce the ram effect, which likely contributed positively to the pregnancy rate. The eCG hormone is immunogenic. Therefore, repeated use may reduce the chance of treatment success by causing antibody formation. Since the study material consisted of animals that had not previously undergone synchronization treatment, antibody formation was not observed with repeated use of the hormones, it is thought that a positive response to the hormone doses used. The effect of co-injection of eCG+hCG on reproduction in sheep is controversial. In the study conducted by Morrical et al. (1998), it was determined that when 5 mL of PG-600 (80 IU eCG+40 IU hCG/mL) was injected at the end of 10 days of MGA treatment, a 20% increase in the pregnancy rate was observed, while Safranski et al. (1992) found that when eCG+hCG was used together with 10-day MGA treatment, both the conception rate and the number of lambs born per ewe did not change significantly. Outside the breeding season, PG-600 (3 mL) increased conception and lambing rates, and eCG+hCG treatment may advance lambing (Redden et al., 2023). Habeeb et al. (2019) reported that injection of different doses of eCG+hCG (PG-600; 1.5 mL or 5 mL) to ewes during the breeding season changed the pregnancy rates and that the pregnancy rate (87.5%) was higher in the low-dose eCG+hCG group than in the high-dose group (37.5%). It has been suggested that high-dose eCG+hCG reduces fertility by reducing embryo survival (Windorski et al., 2008). Santos et al. (2010) found that the pregnancy rate (86%) was lower in the group administered 400 IU eCG+200 IU hCG during synchronization of Texel sheep outside the breeding season than in the group administered eCG alone (56%). Dias et al. (2020) compared eCG and eCG (400 IU)+hCG (200 IU) protocols outside the breeding season. The eCG+hCG protocol induced adequate estrus, but pregnancy rates remained below expectations. It has been reported that PG-600 containing eCG+hCG overstimulates the ovaries, resulting in increased serum estradiol-17β concentrations at the time of ovulation (Habeeb et al., 2019; Windorski et al., 2008). Although it was initially thought that increased serum estradiol-17β concentrations might increase endometrial estrogen receptor (ESR1) levels in sheep (Spencer et al., 1995), ESR1 concentrations in the first week following eCG+hCG treatment were found to be similar to those in normally cycling sheep. It has been reported that changes in uterine ESR1 expression and estradiol and progesterone production observed following eCG+hCG administration in sheep did not have a significant effect on the reproductive potential of the animals (Habeeb et al., 2023). eCG+hCG treatments in synchronization protocols can lead to variability in ovulation and may contribute to ovulation failure (Cline et al., 2001; Dias et al., 2020; Santos et al., 2010). In the presented study, although the highest pregnancy rate was observed in Group III (eCG+hCG) (86.5%), no statistically significant difference was found between the groups (P>0.05). The doses of eCG and hCG used in this study were comparable to those in previous research. The absence of fixed-time artificial insemination may have allowed the ewes to tolerate variations during ovulation, and pregnancy rates remained high. Differences in protocols, animal breeds, and breeding season may explain variations in outcomes across studies. Additionally, the favorable body condition of the ewes and the rich pastures in Ardahan likely supported metabolic health, while the cool climate and high altitude may have reduced heat stress, further contributing to the high pregnancy rates. Although all the studies mentioned above used single-dose combination products containing eCG+hCG, the doses in the present study were similar to those in some of these studies.

Akkaraman sheep were administered 500 IU eCG on the day the sponges were removed following a 14-day vaginal sponge application, resulting in a lambing rate of 92.30% (Daşkın, 2001). Santos et al. (2010) found that the lambing rate was 86.66% in the eCG group and 56.66% in the eCG+hCG group in a study conducted on sheep outside the breeding season. Dias et al. (2020) reported a lambing rate of 34.6% in the eCG group and 18.6% in the eCG (400IU)+hCG (200IU) group in their study conducted outside the breeding season. Ayaseh et al. (2021) reported an 87.5% lambing rate in hCG-treated ewes outside the breeding season. In this study, lambing rates in the groups (Group I, Group II, Group III) were determined to be 96.6%, 88.9%, and 93.8%, respectively. These differences in lambing rates are thought to be due to the breed of sheep and the synchronization methods used.

In synchronization protocols for multiple pregnancies, the most commonly used hormone is eCG due to its effects on ovarian activity (Boscos ve ark., 2002; Şen, 2020). Multiple lambing rates vary depending on the protocols and the dose of eCG administered. This rate ranges from 18.2% to 57% (Aköz et al., 2006; Daşkın, 2001; Esen & Bozkurt, 2001; Kulaksız et al., 2011; Santos et al., 2010; Takcı et al., 2023). With eCG+hCG injection outside the breeding season, the twin lambing rate was either not achieved at all (Santos et al., 2010) or was determined to be 18.6% (Dias et al., 2020). In the presented study, the multiple lambing rates were 42.9%, 20.8%, and 56.7%, respectively, and the twinning rates were 35.70%, 20.83%, and 46.60%, respectively. Triplet lambing was observed in Groups I (eCG) and II (eCG+hCG), with triplet lambing rates of 3.57% and 3.33%, respectively. Quadruplet lambing was observed in Groups I and III, with quadruplet lambing rates of 3.57% and 3.33%, respectively. Quintuplet lambing was observed only in Group III, with a quintuplet lambing rate of 3.33%. The superovulatory effect of eCG can be influenced by many factors, including breed, live weight, body condition score, age, nutritional level, and season (Quinlivan & Robinson, 1969). The existing differences are thought to be due to these factors. In a study conducted by Köse et al. (2016), eCG 300 IU, eCG 500 IU, and eCG 700 IU were injected to sheep following progestagen administration. As a result of the study, the number of lambs per lambing sheep was reported as 1.40, 1.47, and 1.57 in the groups (eCG 300, eCG 500, and eCG 700), respectively. Gomez-Brunet et al. (2007) administered 500 IU eCG to all sheep on the day the sponges were removed following a 12-day vaginal sponge application. Cervical insemination was performed 55 hours after the injection, and after insemination, the sheep were divided into two groups: the control group received only physiological saline, and the other group received 500 IU hCG. The study reported that the number of lambs per lambing ewe was 1.50 and 1.56, respectively. In animals administered eCG+hCG, the number of lambs per ewe was found to be between 1.68 and 2.33 (Habeeb et al., 2019; Morrical et al., 1998; Safranski et al., 1992). In previous studies on Akkaraman sheep (Akçapınar et al., 1982; Odabaşıoğlu, 1985; Tüfekci, 2023; Yıldız & Denk, 2006), the number of lambs per ewe at birth was reported as 1.30, 1.13, 1.01, and 1.22, respectively. In the present study, the number of lambs per ewe at birth was 1.54, 1.21, and 1.77 in Group I, Group II, and Group III, respectively. Akkaraman sheep, which have a low twinning rate, showed a high twinning rate in groups treated with eCG, indicating that eCG causes the development of multiple follicles and ovulation. In this study, progesterone analysis performed before synchronization initiation revealed that 84.5% of the animals were cyclic. Since eCG had not been used previously in these animals, it is thought that the doses used were successful for superovulation and that ovarian activity at the start of synchronization could positively affect fertility, suggesting that the high lambing rates may be due to these reasons.

Previous studies have shown that 56-88% of sheep had an active corpus luteum on the day the synchronization protocol was initiated (Kaya et al., 2025; Titi et al., 2010). In the present study, progesterone levels >1 ng/mL were found in 84.5% (93/110) of sheep on the day the vaginal sponge was applied (day 0). This indicates the presence of an active corpus luteum in the majority of sheep included in the study and that the animals were in the cyclical phase. Progesterone analysis performed before synchronization can inform the selection of the synchronization method. Although the mean estrogen level on the day of estrus was highest in Group II, the differences in estrogen levels between the Groups were not statistically significant.

Previous studies show that PG-600 injection can cause ovarian overstimulation, leading to abnormally large follicles and increased estradiol-17β levels (Safranski et al., 1992). Similarly, Habeeb et al. (2019) found that serum estrogen levels increase significantly within 24 hours of injection, whereas progesterone levels remain elevated in the subsequent period. In contrast, Celin et al. (2001) observed no change in corpus luteum diameter compared to the control group. Although PG-600 increases the ovulation rate, it appears to reduce the pregnancy rate. Higher estradiol-17β levels may increase estrogen receptor expression in the endometrium and impair cellular function, leading to lower pregnancy rates (Habeeb et al., 2019).

One of the most important considerations for farmers when selecting a synchronization protocol is the cost of hormonal treatments, and farmers generally attempt to reduce costs while maximizing productivity (Yu et al., 2022). The most important factor in profitability is lamb production (Takci et al., 2024). In the present study, although the highest synchronization cost was observed in Group III, the pregnancy rate and multiple lambing rate were also the highest in this group. Group I had the lowest synchronization cost. The net return values were calculated as 7,025.63 USD, 4,493.81 USD, and 8,726.90 USD for Groups I, II, and III, respectively, with the highest net profit per ewe also recorded in Group III. Although lambing rates were similar across the groups, Group III resulted in higher economic returns, indicating that the additional costs of eCG and hCG may be offset by the improved reproductive performance. Overall, the procedure in Group III was more cost-effective than the other treatment options

CONCLUSION

This study demonstrated the effects of eCG and hCG injections on estrus synchronization and reproductive performance in sheep. While hCG alone delayed estrus onset, the combination of eCG and hCG slightly improved estrus and pregnancy rates and significantly increased multiple lambing compared to hCG alone. Fecundity, fertility, and litter size were numerically higher but not statistically significant in the eCG+hCG group; however, the differences were not statistically significant. This may be due to the limited sample size. Unlike previous studies using the commercial product PG-600, this study administered similar doses of eCG and hCG via separate syringes at different sites, resulting in higher pregnancy rates, although it remains unclear whether this improvement was due to the administration method. In conclusion, the eCG+hCG protocol may be preferred when the goal is to maximize multiple lambing and economic return. Despite higher synchronization and feed costs, Group III provided the highest net profit per ewe, indicating superior overall economic efficiency. Considering reproductive outcomes along with synchronization and labor costs, the eCG+hCG protocol proved to be the most profitable. Further studies with larger animal populations are warranted to clarify the effects of simultaneous eCG and hCG administration on reproductive performance.

Compliance With Ethical Standards

This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in MJAVL belongs to the author(s).

Ethıcal Approval

This study was conducted with the approval of the Animal Research Ethics Committee of Kafkas University Ethics Approval No: KAÜ-HADYEK/2023-090.

Conflıct of Interest

The authors declared no conflict of interest.

Author Contribution

SK conceived the idea and planned the manuscript. MY contributed to sample preparation. SK and MY have made significant scientific contributions and also contributed to the interpretation of the results. All authors made significant contributions by providing feedback and helping to shape the manuscript.

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Data Availability

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Statement of Use for Artificial Intelligence and Its Types

Artificial intelligence and its various types were not used in the writing of this article.

Approval for Publication

All authors declare that they have seen and approved the final version of the submitted manuscript

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