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Synchronization Protocol and Its Association with Litter Size in Awassi Ewes

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Abstract | In sheep farming, one of the most crucial economic traits is increasing the number of offspring, particularly female lambs. Therefore, this study aimed to investigate the association between estrus synchronization and litter size in Awassi ewes. A total of 80 multiparous Awassi ewes, aged 3 to 4 years and weighing between 45 and 50 kg, were randomly selected from a flock. Ewes that did not receive any treatment served as the control group. The treatment group received intravaginal sponges saturated with 40 mg of flugestone acetate, inserted for 14 days to induce estrus synchronization. Twenty-four hours after sponge removal, the ewes were administered 500 IU of equine chorionic gonadotropin (eCG) per head to stimulate multiple ovulations. Blood samples were collected to assess reproductive hormone concentrations, and early pregnancy diagnosis was performed on day 40 post-mating using transrectal real-time B-mode ultrasonography. The results showed that the estrus response rate after hormonal treatment (93%) was significantly higher ($P \leq 0.001$) compared to the control group (60%). The onset of estrus was also significantly shorter ($P \leq 0.05$) in the treated group. The average days to lambing were higher in the control group (21 ± 3.10) compared to the treated group (12 ± 1.31). Litter size was significantly affected by hormonal treatment; the treated group had a significantly higher rate of twin births (76%) compared to the control group (3%). Hormonal treatment also had a significant effect ($P \leq 0.05$) on plasma concentrations of estrogen and progesterone. In conclusion, the use of flugestone acetate vaginal sponges, combined with eCG, appears to be a cost-effective and reliable strategy for improving reproductive performance and managing early pregnancy in Awassi ewes. This synchronization protocol is recommended to enhance fertility during both breeding and non-breeding seasons.

Keywords | Estrus, Synchronization, Litter size, Reproductive hormones, Sheep, Twin

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INTRODUCTION

Estrous synchronization plays a crucial role in the functioning of reproductive systems and improves the reproductive capabilities of ewes and does (Farrag, 2019). The use of estrous synchronization enhances reproductive efficiency by inducing ewes to enter estrus during the non-breeding season (Al-Hamedawi *et al.*, 2016; Sun *et al.*, 2025). Synchronizing estrus offers several advantages, including an increase in the number of ewes in estrus, which facilitates insemination and aligns the timing of births (Yu *et al.*, 2022). There are two types of estrous synchronization: Natural (non-hormonal) and pharmacological (hormonal). Various hormones can be utilized in estrous synchronization programs, including prostaglandin F2α (PGF2α), progesterone, estrogen, and gonadotropin-releasing hormone (Saxena *et al.*, 2015; Abiy, 2022; Tsekouras *et al.*, 2025). Estrous synchronization using PGF2α enhances the reproductive and productive characteristics of Abou-Delik and Ile de France ewes (Metodiev, 2015; Farrag, 2019). Despite this, fertility outcomes may vary based on several factors, including

season, dosage, method of hormone administration, body condition, physiological status of ewes, and male factors (Maksimović *et al*., 2020).

An estrus synchronization protocol utilizing 25 mg of medroxyprogesterone acetate (MAP) co-administered with 300-500 IU of equine chorionic gonadotropin (eCG) improved pregnancy rates in Barki ewes (Mohammed *et al*., 2016). Additionally, estrus in Spanish Merino ewes is successfully synchronized using MAP sponge protocols and eCG on the day of MAP withdrawal, resulting in shortened estrus intervals (Gardón *et al*., 2015). An injection of gonadotropin-releasing hormone administered between two prostaglandin F2α injections enhances estrus synchrony and improves the reproductive performance of synchronized ewes (Duan *et al*., 2025). In contrast, progestagen-impregnated vaginal sponges containing 30 mg of fluorogestone acetate are employed to synchronize estrus in 70 ewes with 500 IU of pregnant mare serum gonadotropin (PMSG), leading to a lower-than-expected lambing rate (Maksimović *et al*., 2020). Another research has indicated that the duration of fluorogestone acetate sponge synchronization and controlled internal drug release (CIDR) protocols ranging from short (5 days) to medium (9 days) and long (13 days) has no significant impact on multiple births and survival rates (Altincekic, 2022). A meta-analysis of studies examining pregnancy rates to identify the most effective synchronization methods revealed that the combination of progesterone (P4) and PMSG resulted in a pregnancy rate of 90.37% during the breeding season. In contrast, the use of P4 combined with PGF2 yielded a pregnancy rate of 69.77%, while P4 alone resulted in a rate of 68.75% during the non-breeding season. The findings indicate that the combination of P4 and PMSG during the breeding season, along with hormone applications during the non-breeding season, leads to the highest pregnancy rates (Arikan *et al*., 2021). Hormonal treatments, such as melatonin or vaginal sponges combined with intramuscular injection of 400 IU of eCG for 35 days, enhance reproductive performance by improving estrus response, lambing rates, and the incidence of twin births (Al-Tai *et al*., 2022). Estrus synchronization among Awassi ewes remains a relatively underexplored area of research. Given the significance of estrus synchronization in improving reproductive traits in livestock, this study investigated the relationship between estrus synchronization techniques and litter size in Awassi ewes.

MATERIALS AND METHODS

Animals and blood collection

The Ethics Committee of Al-Qasim Green University approved the study conducted between July 2024 and March 2025 (Agri, No. 01, 7, 24). This study involved 80 multiparous Awassi ewes, each weighing between 45 and 50 kg before the experiment. All experimental animals were maintained under the same conditions as those at the Babylon station regarding management and nutrition. The diet consisted of 59% barley, 40% bran, and 1% salt, which together constituted 2.5% of their body weight. The animals were also fed three kilograms of hay and one kilogram of straw, and they were allowed to graze in the morning and evening. Fresh water was available throughout the day. Clinical examinations revealed no physical or reproductive abnormalities among animals. The weight of Awassi ewes (in kg) was measured in the morning using a suspended spring balance (Kadhem and Al-Thuwaini, 2022). Blood was centrifuged at 2,000 x g for 15 minutes to separate the serum, which was then stored at -20°C for hormone analysis. Reproductive hormones, including estrogen, progesterone, follicle stimulating hormone (FSH), and luteinizing hormone (LH), were quantified using Enzyme-Linked Immunosorbent Assay (ELISA) kits E0047Sh, E0015Sh, E0105Sh, and E0106Sh from Bioassay Technology Laboratory (Shanghai, China).

Reproductive data collection

Ewes were randomly selected from a herd, before treatment served as control, without treated with vaginal sponges. Then, ewes were injected with vaginal sponges containing 40 mg of flugestone acetate for 14 days to synchronize estrus. This was followed by an injection of 500 IU of equine chorionic gonadotropin (eCG) per head, administered 24 hours after the removal of the sponge, to promote multiple ovulations. After implementing the synchronization protocols, signs of estrus were observed through direct observation (including swollen vulva, mucus discharge, sniffing, excitement, loss of appetite, mounting, and rapid tail movement) as well as by using a fertile ram. Ewes that exhibited signs of estrus were mated to fertile rams. Pregnancy was confirmed via trans-rectal ultrasonography using a 7.5 MHz linear array transrectal probe (Pie Medical LC 100, Netherlands) on days 35 to 40 after mating. Jugular blood samples were collected from each ewe in the morning (8 AM) before the placement of the vaginal sponges and again after their removal on day 14. Reproductive parameters were calculated as follows:

Estrus response (%) = number of ewes showing estrus/total ewes in each group×100

Onset of estrus: Time from sponge removal to first mounting acceptance:

Lambing rate (%) = (number of ewes lambing / number of ewes mated) ×100. Litter size = number of lambs born / number of ewes lambed
Survival rate (%) = lambs born alive - lambs weaned / lambs born alive ×100

Statistical analysis

Statistical Package for Social Sciences 23.0 (SPSS, New York, USA) was used to analyze the association between estrus synchronization and various outcomes with repeated measures ANOVA:

$$Y_{ijk} = \mu + T_i + pk(j) + e_{ij}$$

where μ is the overall mean, T_i is the main effect of treatment (before treatment, after treatment) (fixed w/$\Sigma i\ T_i$=0), $pk(j)$ is the main effect of subjects N (0, σ2 p), and e_{ij} is random error assumed $e_{ij} \sim$ N (0; σ2). The Bonferroni test was used to assess whether the means differed significantly. A chi-square test was used to determine the estrus response, lambing rate, birth type, and survival rate.

RESULTS

Results from the live body weight, estrus response, onset of estrus, days to lambing, lambing rate, birth type, and survival rate of ewes before hormonal treatment and after hormonal treatment are presented in Table 1. The rates of estrus after hormonal treatment (93 %) were significantly higher ($P \leq 0.001$) than before hormonal treatment (60 %). The onset of estrus was significantly ($P \leq 0.05$) shorter after hormonal treatment than before hormonal treatment. The highest days to lambing (21 ± 3.10) was before hormonal treatment when compared with after hormonal treatment (12 ± 1.31). Litter size was significantly different between after hormonal treatment and before hormonal treatment, after hormonal treatment gave more twins (76%) compared with before hormonal treatment (3 %). The mean plasma concentrations of FSH, LH, estrogen, and progesterone are given in Table 2. There were no significant ($P \geq 0.05$) effects of hormonal treatment on FSH and LH concentrations but there were significant ($P \leq 0.05$) effects of hormonal treatment on the concentrations of plasma estrogen and progesterone.

DISCUSSION

A reproductive management program is essential for enhancing the efficiency of sheep production. The implementation of synchronization techniques is a crucial component of effective reproductive management (Reyes-Ramirez *et al*., 2021). The optimal application of these techniques can lead to improved control over estrus and increased ovulation rates, thereby enhancing the reproductive efficiency of agricultural animals (Hameed *et al*., 2021). Due to the seasonal nature of sheep production and the heightened demand during specific periods, specialized systems must be implemented to enhance production and reproduction throughout the year. This can be achieved by synchronizing estrus and increasing the twin rate, particularly outside the insemination season when fertility is at its peak (Khotijah *et al*., 2022). Estrus synchronization in ewes can be accomplished through various methods, including the ram effect or pharmaceutical interventions such as progesterone (Bruno-Galarraga *et al*., 2021). In ewes, progesterone and its structural analogues have been utilized to synchronize estrus by inducing the regression of the corpus luteum (Vilariño *et al*., 2017). The combination of progestogen and progesterone effectively induces synchronized estrus in ewes during the off-breeding

Table 1: Estrus response, onset interval, lambing rate, and birth type following estrus synchronization in Awassi ewes.

Groups	Live weight (kg) (LSM±SE)	Estrus response (%)	Onset of estrus (h)	Days to lambing	Lambing rate (%)	Litter size		Survival rate (%)
						Singleton	Twin	
Before hormonal treatment	44.50 ± 2.24	72.5^{b} (29/40)	71.51 ± 7.21^{a}	21 ± 3.10^{a}	90	72^{a} (97%)	2^{b} (3%)	75 (98.68%)
After hormonal treatment	46.23 ± 3.01	92.5^{a} (37/40)	43.13 ± 4.15^{b}	12 ± 1.31^{b}	93	18^{b} (24%)	56^{a}(76%)	130(100.00%)
P-value	0.17	0.001	0.03	0.04	0.21	0.001	0.001	0.33

Notes: LSM ± SE: Least Square Mean ± Standard Error. a,b: Different superscripts within the same column indicate statistically significant differences ($P < 0.05$). Bolded P-values indicate statistical significance.

Table 2: Reproductive hormone levels following estrus synchronization in Awassi ewes.

Indices	FSH (ng/ml)	LH (ng/ml)	Estrogen (pg/ml)	Progesterone (ng/ml)
Before hormonal treatment	3.12 ± 0.39	0.89 ± 0.03	17.78 ± 1.59^{a}	4.56 ± 0.37^{b}
During sponge insertion	3.32 ± 0.46	0.96 ± 0.03	18.82 ± 2.72^{a}	5.52 ± 0.54^{a}
2 days after PMSG injection	3.48 ± 0.54	0.87 ± 0.02	12.58 ± 0.92^{b}	4.91 ± 0.40^{b}
P-value	0.21	0.14	0.001	0.03

Notes: LSM ± SE = Least Square Mean ± Standard Error. a,b: Different superscripts in the same column indicate statistically significant differences ($P < 0.05$). Bolded values indicate statistically significant differences between time points. FSH: Follicle-Stimulating Hormone; LH: Luteinizing Hormone.

season (Texeira *et al.*, 2016). In this study, a vaginal sponge containing P4 was employed. The experiment revealed that ewes exhibited varying responses to estrus and the onset of estrus after the withdrawal of the sponge, in contrast to the control groups. This variation may be attributed to the fact that synchronization protocols enhance the onset of estrus in ewes, while control groups showed only a slight response to estrus (Mohan and Kumar, 2023). A study conducted by Biehl *et al.* (2019) demonstrated that long-term use of a P4 sponge (11 days) resulted in higher pregnancy rates in ewes compared to short-term use (7 days). Conversely, progesterone treatment lasting 18-21 days has been associated with decreased fertility. This decline in fertility is believed to be due to the ovulation of persistent follicles containing low-quality oocytes. Additionally, evidence suggests that reduced fertility levels may arise from the adverse effects of progesterone on the uterine environment, which can negatively influence sperm transport and longevity (Brasil *et al.*, 2022).

The results also indicated a significant difference in litter size before and after hormonal treatment, with the post-treatment group producing a higher percentage of twins (76%) compared to the pre-treatment group. Additionally, there were significant effects ($P \leq 0.05$) of treatment on the concentrations of plasma estrogen and progesterone. Ovarian function is influenced by the expression of hormones and growth factors during estrus. Ovarian follicle development and function depend on sufficient systemic progesterone levels (Turgut and Ağaoğlu, 2023). Intravaginal sponges are utilized to decrease LH secretion, thereby preventing estrus, LH surges, and ovulation until the sponge is removed. Consequently, elevated progesterone levels and decreased LH concentrations lead to increased follicle turnover. As a result, preovulatory follicles should be in the growth phase by the end of treatment and capable of releasing oocytes. Subtropical ewes treated with a progesterone sponge exhibited higher twinning rates, enhanced follicular development, and improved ovulation and conception rates (El-Sherry *et al.*, 2012; Salama *et al.*, 2024). Additionally, the intravaginal sponge impairs GnRH secretion, thereby inhibiting LH production. After the sponge is removed, equine chorionic gonadotropin (eCG) is injected, which stimulates ovulation by promoting FSH and LH release (López-García *et al.*, 2021). According to Ak *et al.* (2011), the administration of eCG or PMSG following a 12-day treatment with progesterone-impregnated intravaginal sponges enhances the synchronization of estrus and ovulation during and outside the breeding season. This factor is largely comparable to other elements that increase granulosa cell proliferation, inhibit FSH receptor expression, and play a crucial role in ovulation rates and litter size (Karam, 2025). This study confirms and corroborates findings of Yadav *et al.* (2021) and Kuru *et al.* (2022), who utilized an intravaginal sponge infused with 350 mg of natural progesterone for 12 days, followed by 200 IU of eCG the day of sponge removal. These results indicate that fecundity rates are higher in the treatment group than in the control group.

CONCLUSION

The synchronization of estrus in Awassi ewes using a progesterone sponge and PMSG was more effective regarding estrus response, onset of estrus, days to lambing, litter size, and reproductive hormone levels. Therefore, it is recommended that this protocol be implemented during breeding and non-breeding seasons to synchronize estrus and enhance reproductive efficiency in sheep.

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NOVELTY STATEMENT

This study investigated the relationship between the using a progesterone sponge with PMSG and number of lambs born in Awassi ewes for the first time. This protocol was more effective regarding twinning rate and enhance reproductive efficiency in sheep.

AUTHORS CONTRIBUTION

TMA-T: Conceptualization, investigation, writing review and editing.
AHK: Investigation, methodology.
All authors checked and approved the final version of the manuscript.

Generative AI and AI-assisted technology statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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