# Use of eCG in progestogen based estrus sinchronization programs for hair ewes

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**ABSTRACT**

**Objective**: To determine the importance of eCG use in the reproductive management of hair ewes subjected to progestagen based synchronization protocols.

**Design/methodology/approach**: A total of 120 hair ewes (Blackbelly and Pelibuey) in the peak reproductive season were synchronized using intravaginal sponges containing 20 mg fluorogestone acetate (FGA) and intramuscular equine chorionic gonadotropin (eCG; 200 or 300 IU). Treatments combined eCG dose and timing (200 IU/0 h, 200 IU/−24 h, 300 IU/0 h, and 300 IU/−24 h). Estrus occurrence, interval to estrus, follicular growth, ovulation rate, and number of corpora lutea were analyzed by ANOVA using PROC GLM and PROC MIXED in SAS.

**Results**: The interaction between eCG dose and timing significantly affected ($P<0.05$) estrus occurrence, interval to estrus, and ovulation rate. Regardless of dose and timing, eCG application resulted in an orderly follicular growth pattern similar to that of a natural estrous cycle.

**Limitations/implications**: Crossbred animals were excluded to meet the study's inclusion criteria.

**Findings/conclusions**: Incorporating eCG into progestagen based synchronization protocols enhances estrus expression, concentrates estrus onset within a short time frame, and increases ovulatory rate.

**Keywords**: eCG, estrus, ovulation, follicle.

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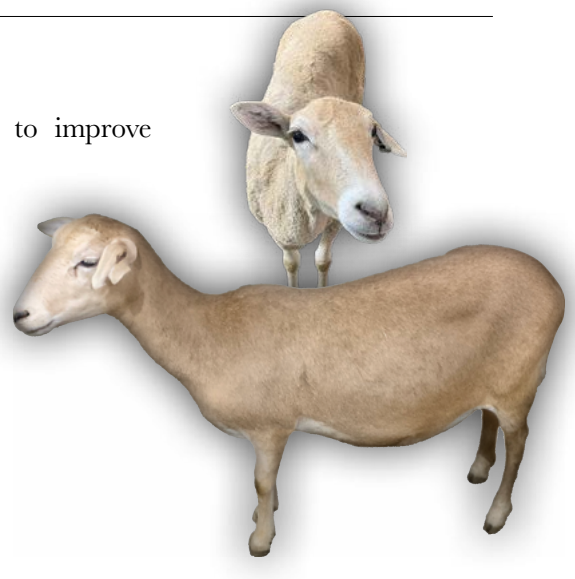
## INTRODUCTION

Estrus synchronization is a strategy used to improve reproductive efficiency in flocks (Balan-May *et al.*, 2021). The use of exogenous hormones to synchronize or induce estrus in sheep is a widely adopted reproductive tool, aimed at optimizing reproductive management in females (López *et al.*, 2023). Furthermore, it plays a crucial role in artificial insemination programs (Deac *et al.*, 2024).



The use of short synchronization protocols based on intravaginal progestagens helps maintain reproductive efficiency in flocks (Balan-May *et al.*, 2021). Various protocols for estrus synchronization and induction have been used in sheep; however, the protocol involving progestagens and equine chorionic gonadotropin (eCG) has shown the best response in terms of estrous and ovulatory activity (Payan *et al.*, 2022; Deac *et al.*, 2024).

Protocols for estrus and ovulation synchronization aimed at fixed-time artificial insemination (FTAI) in sheep primarily rely on the insertion of intravaginal devices containing progesterone [CIDR] or progestagens [sponges impregnated with fluorogestone acetate (FGA)], combined with an intramuscular injection of equine chorionic gonadotropin (eCG). The eCG is administered either at the time of device removal or 24 h prior, with the goal of inducing ovulation and synchronizing its timing across animals within the same group (Martínez *et al.*, 2019), as eCG administration is essential for FTAI.

The administration of eCG induces and concentrates the expression of estrous behavior and ovulation in sheep undergoing a progestagen based synchronization protocol. In eCG treated ewes, the interval to estrus is shorter, with most females exhibiting signs within 24-36 h after progestagen withdrawal (Quintero *et al.*, 2011). When eCG is not included in synchronization protocols, there is a high incidence of ovulatory failure (Martínez *et al.*, 2019). Therefore, synchronization protocols should be complemented with eCG to support follicular growth, steroidogenic activity, and increased ovulation rates, ultimately improving prolificacy (Sánchez *et al.*, 2022).

In hair ewes, Arbués *et al.* (2018) found that factors such as eCG dosage and timing of its administration influence the estrus and ovulation response following the completion of a synchronization protocol. Therefore, the objective of this study was to determine the importance of eCG use in the reproductive management of hair ewes subjected to progestagen based synchronization protocols.

## MATERIALS AND METHODS

### Study site

The experiment was conducted at the Research Farm of the Universidad Autónoma de Tamaulipas (23° 56' N, 99° 06''W, 190 m), located in northeastern Mexico. The climate is dry tropical (A)C(w), semi-warm, and sub-humid, with an average annual temperature of 23 °C and an average annual precipitation of 800 mm (INEGI, 2007).

### Animal management

The study was conducted in 2023 and followed an estrus synchronization protocol. A total of 120 hair ewes of the Blackbelly and Pelibuey breeds, aged 2-4 years, with a body condition score (BCS) of 3-4 on a 1 to 5 scale (Thompson and Meyer, 1994), were used. The reproductive program was implemented in November to align with the peak breeding season of hair ewes, as reported by González-Reyna *et al.* (1992). During the study, the hair ewes were housed in an enclosed pen equipped with a feeder, water trough, and shade. They were fed *ad libitum* with hay forage and offered 300 g/sheep/day of a commercial supplement containing 14% crude protein on a dry matter basis. Water was available at all times.

**Treatments**

Prior to initiating the synchronization program, all 120 hair ewes were randomly assigned to four groups to receive the following equine chorionic gonadotropin (eCG; GonActive® eCG, Virbac) treatments: (1) 200 IU administered 24 h before progestagen withdrawal; (2) 200 IU at the time of progestagen withdrawal; (3) 300 IU administered 24 h before progestagen withdrawal; and (4) 300 IU at the time of progestagen withdrawal.

**Synchronization protocol and management**

Fifteen days before the synchronization program, the hair ewes were treated for internal and external parasites with 1.0 mL of ivermectin. Additionally, they received 2 mL of vitamins A, D, and E, and 1 mL of B-complex vitamins. During synchronization, intravaginal sponges containing fluorogestone acetate (a progestagen known as cronolone; 20 mg; Chronogest CR®, MSD Animal Health) were inserted for 12 days. Before sponge removal, the eCG treatments were administered. Estrus detector males were introduced 24 h after sponge removal to confirm estrus incidence. The onset of estrous behavior was recorded, and females exhibiting this behavior were separated to facilitate detection in the remaining hair ewes.

To assess follicular growth (number of follicles), measurements were conducted over a four day period to ensure coverage of a complete follicular phase. Ultrasonographic evaluations were performed once daily between 8:00 and 10:00 a.m. throughout the monitoring period. Follicular dynamics (follicle count) were monitored using transrectal ultrasound (SonoVet 600, SV-600, with a 7.5 MHz transducer), allowing for the evaluation of ovarian dynamics through imaging of ovarian structures. This aimed to investigate the effects of eCG administration or lack thereof on ovarian fluctuations in hair sheep. Additionally, ovulation rate and number of corpora lutea were determined by observing and counting the corpora lutea on the ovarian surface using a rigid laparoscope (Karl Storz Endoscope; Storz) eight days after estrus.

**Study variables**

The study variables included eCG dosage and timing of administration, and their effects on: (1) estrus occurrence (ewes that exhibited estrus after sponge removal), (2) interval to estrus (time between sponge removal and estrus onset), (3) follicular growth (number of follicles per day), (4) ovulation rate (number of corpora lutea per ewe that exhibited estrus), and (5) total number of corpora lutea.

**Statistical analysis**

Estrus occurrence, interval to estrus, follicular growth, ovulation rate, and number of corpora lutea were analyzed using analysis of variance (ANOVA) based on a completely randomized design with a $2\times2$ factorial arrangement. The ANOVA included interactions between eCG dosage and timing of administration (200 IU/0 h, 200 IU/−24 h, 300 IU/0 h, and 300 IU/−24 h). Mean comparisons were performed using the Student's t-test ($P<0.05$). Trends were considered when $0.05\geq P\leq0.10$. Analyses for estrus occurrence, interval to estrus, and ovulation rate were conducted using the PROC GLM procedure, while

follicular growth and number of corpora lutea were analyzed using repeated measures with the PROC MIXED procedure in SAS software (SAS, 2004).

## RESULTS AND DISCUSSION

### Estrus occurrence

The interaction between eCG dosage and timing of administration on estrus occurrence in hair ewes synchronized with progestagens is shown in Figure 1. The dose × timing interaction significantly affected estrus occurrence ($P<0.05$). Across all four eCG treatment combinations, the highest estrus occurrence was observed within 24-36 h after progestagen withdrawal (71.4% for 200 IU/0 h, 82.3% for 200 IU/−24 h, 75.8% for 300 IU/0 h, and 90.6% for 300 IU/−24 h). The highest proportion of hair ewes expressing estrus occurred with a 300 IU dose administered 24 h before progestagen removal (90.6%; $P>0.05$), compared to the other treatment combinations.

In a study by López *et al.* (2021), a high concentration of hair ewes in estrus (81.25%) was observed within 30 h after progestagen withdrawal when 300 IU of eCG was administered at the time of withdrawal a result similar to that of the present study (75.8%). The same authors reported an increase in estrus occurrence (93.75%) when the eCG dose was raised to 400 IU, showing a trend consistent with the current findings (90.6%). As the eCG dose increases, the number of hairs ewes in estrus also rises; therefore, both dosage and timing of eCG administration influence estrus expression in hair ewes synchronized with progestagens.

One of the key requirements of an estrus synchronization protocol is to achieve the highest possible number of females in estrus (ideally all or most showing signs of receptivity). Thus, obtaining an effective response is critical and depends on the type and dosage of hormones used. The stronger the hormonal effect, the greater the number of females available for breeding.

Currently, sheep breeding is primarily based on artificial insemination. FTAI programs rely on estrus and ovulation synchronization protocols, assuming that all treated females will exhibit estrus and ovulate, allowing insemination of the entire group. In these protocols, eCG plays a crucial role by promoting follicular growth and, consequently, the expression

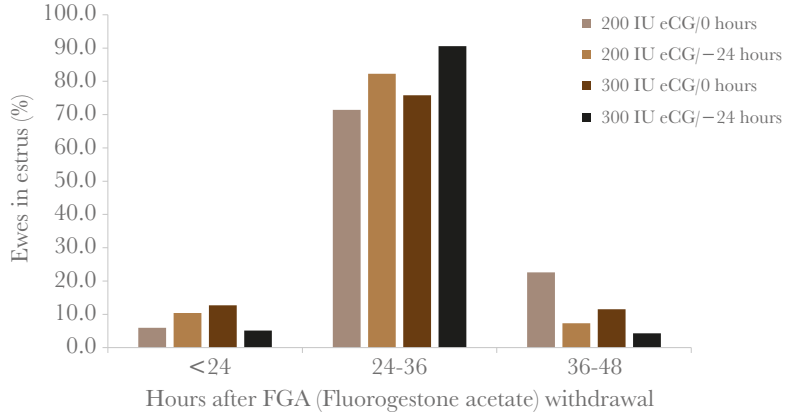



**Figure 1**. Distribution of estrus occurrence as affected by eCG (equine chorionic gonadotropin) administration (dose and timing) in hair ewes synchronized with progestagens.

of estrus and ovulation (Ali, 2007; Dias *et al.*, 2020). Thus, both the dose and timing of eCG administration are critical to ensure the occurrence of these events (Quintero *et al.*, 2011). Most importantly, estrus and ovulation must occur in a synchronized manner to enable insemination within a single 2 to 4 h window.

**Interval to estrus and ovulation rate**

The interaction between eCG dosage and timing of administration on interval to estrus and ovulation rate in hair ewes synchronized with progestagens is presented in Table 1. The dose × timing interaction significantly affected the interval to estrus ($P<0.05$). The longest interval was observed in hair ewes treated with 200 IU of eCG at the time of progestagen withdrawal (34.3±1.0 h), which was significantly longer ($P<0.05$) than in the other treatment groups (mean=29.6±1.0 h). When 200 or 300 IU of eCG were administered 24 h before withdrawal, or 300 IU at the time of withdrawal, the interval to estrus was similar ($P>0.05$).

Ovulation rate was significantly affected ($P<0.05$) by the highest eCG dose administered 24 h before progestagen withdrawal, with a rate of 2.3 observed for the 300 IU/−24 h treatment. As the eCG dose increased, a reduction in the interval to estrus was observed, regardless of administration timing. This resulted in a shorter response period, with females exhibiting estrous behavior more quickly after progestagen withdrawal.

The interaction between eCG dose and timing of administration affected both the interval to estrus and the ovulation rate. This may be due to changes in the follicular development pattern caused by altering the dose and timing of eCG administration (Ali, 2007), which can either favor or hinder the acceleration of follicular growth and the rise in plasma estrogen levels.

The interaction between eCG dose and timing of administration affected both the interval to estrus and ovulation rate. This effect may be due to changes in the follicular development pattern resulting from variations in eCG dosage and timing (Ali, 2007), which can either promote or hinder follicular development and growth, as well as the increase in plasma estrogen levels.

The time interval between equine chorionic gonadotropin (eCG) administration and the onset of estrus in sheep can vary depending on the eCG dose (Martínez *et al.*, 2019; Nakafeero *et al.*, 2020). For example, when 400 IU of eCG is administered, estrus onset

**Table 1**. Effect of eCG administration (dose and timing) on interval to estrus and ovulation rate in hair ewes synchronized with progestagens.

| Variable | N | Interval to estrus ± EE | Ovulation rate ± EE |
|---|---|---|---|
| eCG Dose × Timing of Administration | | | |
| 200 IU 0 h | 34 | 34.3±1.0a | 1.2±0.2a |
| 200 IU 24 h | 50 | 30.4±1.0b | 1.6±0.2a |
| 300 IU 0 h | 45 | 29.6±1.0b | 1.8±0.2a |
| 300 IU −24 h | 48 | 28.8±1.0b | 2.3±0.2b |

[a,b] Different superscripts within columns indicate significant differences ($P<0.05$); FGA: fluorogestone acetate; eCG: equine chorionic gonadotropin; IU: international unit.

occurs between 24 and 40 h, as observed in the present study, where increasing the eCG dose from 200 to 300 IU resulted in a mean estrus interval of 29.6 h. This effect is attributed to the ability of these eCG doses to shorten the ovulation interval, accelerate estrus onset, and stimulate higher estradiol production in the follicles.

These findings highlight the importance of eCG in enhancing the synchrony of reproductive events and estrus expression in hair ewes treated with progestagens. Based on the results, the timing of estrus onset is similar when 200 or 300 IU of eCG are administered 24 h before progestagen withdrawal compared to administering 300 IU at the time of withdrawal.

One of the primary objectives of administering eCG in progestagen-based synchronization programs is to increase the occurrence of multiple ovulations. This results in a greater number of ova available at insemination, thereby increasing pregnancy rates, multiple births, and the number of lambs per ewe bred and lambed. The occurrence of multiple ovulations is measured by the ovulation rate, which refers to the number of corpora lutea present in the ovaries.

Ewes treated with eCG tend to show higher ovulation rates than untreated ewes. This is because eCG stimulates follicular development in the ovaries, leading to a greater number of follicles with the potential to ovulate.

According to Ali (2007), ovulation rates are higher in ewes treated with eCG compared to those not receiving eCG. By stimulating follicular development, eCG increases the ovulation rate, which in turn leads to a higher number of ova available for fertilization, increasing the number of embryos per female and positively impacting prolificacy. Administering eCG before progestagen withdrawal leads to ovulation associated with the earlier development of large follicles, thereby enhancing the ovulatory response in FTAI programs.

**Number of corpora lutea**

Figure 2 shows the distribution of the proportion of hair ewes that ovulated following treatment with eCG (200 IU/0 h, 200 IU/−24 h, 300 IU/0 h, and 300 IU/−24 h). A high percentage of hair ewes had 1 to 3 corpora lutea post-ovulation, corresponding to the

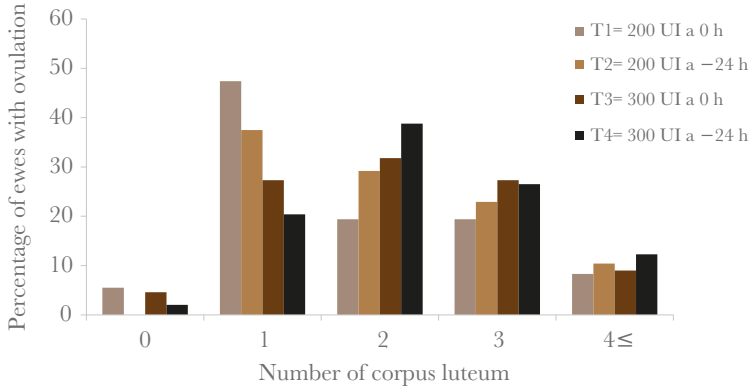



**Figure 2**. Distribution of the percentage of hairs ewes, by eCG administration (dose and timing), presenting 1, 2, 3, or ≥4 ovulations following synchronization with progestagens.

number of ovulations. Among ewes treated with 300 IU of eCG, between 32.8% and 38.8% exhibited 1 to 2 corpora lutea, regardless of timing. Thus, the general trend indicates that most ewes that ovulated presented with 1 to 3 corpora lutea.

Regarding the number of ovulations observed in ewes treated with eCG, a higher percentage of those administered 300 IU exhibited 2 to 3 ovulations. This finding is consistent with previous studies evaluating high or increasing doses of eCG, which reported that as the dose of PMSG increases, the proportion of hair ewes with two or more ovulations also rises (Boscos *et al.*, 2002; Rekik *et al.*, 2002; Ali, 2007).

**Follicular growth**

Figure 3 presents the results of follicular dynamics in hair ewes treated with eCG. Follicular growth varied across the different eCG dose × timing combinations (200 IU/0 h, 200 IU/−24 h, 300 IU/0 h, and 300 IU/−24 h). A significant increase ($P < 0.05$) in follicle count was observed on the first day of measurement (day 0), which coincided with progestagen withdrawal, particularly when 300 IU of eCG was administered 24 h before removal (6.2 follicles), compared to other treatments (5.3 follicles for 300 IU/0 h, 4.1 for 200 IU/−24 h, and 3.8 for 200 IU/0 h). These results indicate a trend of increasing follicle number with higher eCG doses, especially when administered 24 h prior to progestagen withdrawal.

Additionally, eCG applications regardless of dose or timing produced a consistent and orderly pattern of follicular growth, similar to that observed during a natural estrous cycle. At the start of follicle counting, a high number of follicles were detected, and over the course of three days, these follicles tended to regress through atresia or ovulation.

During the first two days of measurement, a high number of follicles was observed across all four treatment combinations (ranging from 3.8 to 6.2 follicles on day one and 3.3 to 5.8 on day two). Differences in total follicle number during the first three days were attributed to eCG dosage. Higher eCG doses increased the FSH like effect on the ovaries, leading to greater activation of primary (small) follicles. These follicles continued to grow alongside medium and large follicles without undergoing atresia, resulting in a greater number of preovulatory follicles.

In a study on sheep treated with eCG, Uribe-Velázquez *et al.* (2008) found that this hormone increases the recruitment of small follicles, and the maximum diameter of

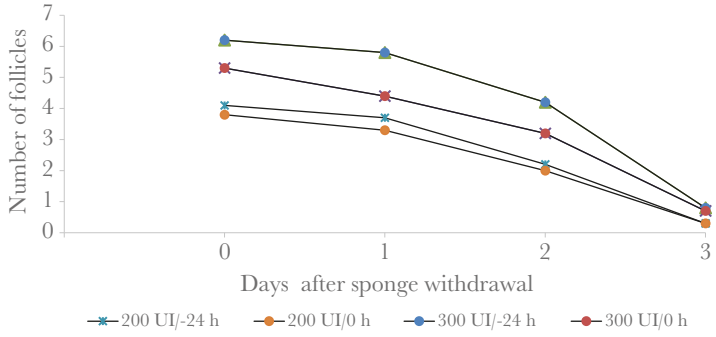



**Figure 3**. Follicular growth pattern as affected by eCG in hairs ewes.

large follicles results consistent with those observed in the present study. eCG positively influences follicular growth by protecting follicles from atresia during selection, leading to an increased number of small and medium follicles. Therefore, the enhanced follicular recruitment and maintenance in eCG treated hair ewes may result from hormonal modulation (López *et al.*, 2021).

This pattern of follicular development induced by eCG in progestagen-based synchronization protocols is critical FTAI programs, as it promotes both estrus and ovulation in treated hair ewes and most importantly, ensures these events occur in a synchronized manner. In FTAI, females are inseminated on a specific day within a narrow time window (4 to 6 h). In contrast, some synchronization protocols that omit eCG result in desynchronized estrus and ovulation, making it difficult to inseminate all females simultaneously (Silveira *et al.*, 2021).

## CONCLUSIONS

Incorporating eCG into progestagen based synchronization protocols promotes estrus expression, concentrates its occurrence within a short time frame, and increases the ovulation rate. For a more predictable and compact estrus response, eCG should be administered 24 h before sponge removal at a dose of 300 IU. This approach shortens the interval to estrus. Regardless of dose or timing, eCG induces an orderly follicular growth pattern similar to that observed in a natural estrous cycle.

### Ethical approval

The experiment was conducted in accordance with the guidelines of the Official Mexican Standard NOM-062-ZOO-1999 (SAGARPA, 1999), which outlines the technical specifications for the production, care, and use of laboratory animals.

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