

Factors influencing estrus and ovulatory activity in hair ewes

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ABSTRACT

Objective: To evaluate the effects of eCG dose and timing of administration, breed, and reproductive season on estrus and ovarian activity in hair ewes.

Design/methodology/approach: A total of 216 hair ewes (62 Dorper, 69 Katahdin, and 85 Pelibuey), including 91 in the high and 125 in the low reproductive season, were synchronized using intravaginal sponges containing 20 mg of fluorogestone acetate (FGA) and intramuscular equine chorionic gonadotropin (eCG; 200 or 300 IU). Treatments included breed, reproductive season, eCG dose, and timing of eCG administration. The presence of ovulation, ovulation without estrus, and estrus without ovulation was analyzed using a logistic regression model. Statistical analysis was performed with the PROC LOGISTIC procedure in SAS.

Results: None of the studied factors (reproductive season, breed, or eCG dose and timing) significantly influenced the likelihood of ovulation ($p > 0.05$). However, Dorper ewes showed a higher incidence of silent estrus compared to the other breeds (OR=0.56, CI=0.02–0.59; $p = 0.0094$). Estrus without ovulation was also significantly affected by breed; Dorper ewes were 7.41 times more likely ($p = 0.0373$) to exhibit estrus without ovulation compared to Pelibuey ewes.

Limitations/implications: Crossbred animals were excluded to meet inclusion criteria, which limited the sample size.

Findings/conclusions: Estrus and ovarian activity in hair sheep synchronized with progestogens is primarily influenced by breed.

Keywords: follicular dynamics, ewes, silent estrus, follicles.



INTRODUCTION

The administration of exogenous hormones to synchronize or induce estrus in ewes is a widely used reproductive tool in the global sheep industry. Its primary aim is to enhance reproductive management in females and increase lamb production within a given production cycle (López *et al.*, 2023). Additionally, it plays a crucial role in artificial insemination programs (Deac *et al.*, 2024).

Different protocols for estrus synchronization or induction have been employed in ewes. However, the protocol involving progestogens and equine chorionic gonadotropin (eCG) has demonstrated superior responses in terms of estrus activity and fertility (Deac *et al.*, 2015; Payan *et al.*, 2022). Although outcomes using these protocols have generally been satisfactory, several studies conducted on wool breeds and some hair breeds indicate that ovarian response to estrus synchronization and consequently, fertility and fecundity can vary due to multiple factors, including breed (Moeini *et al.*, 2007), eCG dosage (Quintero *et al.*, 2011; Espinoza *et al.*, 2020; López *et al.*, 2021), timing of eCG administration (Payan *et al.*, 2022), season (Deac *et al.*, 2024), and body condition (Legesse *et al.*, 2017).

Under Mexico's climatic conditions, few studies have examined the factors influencing the reproductive response of ewes subjected to synchronization protocols, which affect both estrus and ovulatory responses. Failures in estrus expression and ovulation mechanisms may occur in this context (Al-Suhaimi *et al.*, 2022), potentially resulting in estrus without ovulation (Pool *et al.*, 2020) or ovulation without estrus (Visha and Sejian, 2023). These two events estrus and ovulation are critical for both estrus detected and fixed-time artificial insemination programs, as they determine the number of ewes serviced and the resulting conception rates.

Moreover, factors such as eCG dosage, timing of eCG administration, breed, season, and physiological status have been shown to influence the estrus and ovulatory responses of hair ewes following synchronization protocols (López *et al.*, 2021; Arbués *et al.*, 2018). Therefore, the objective of this study was to evaluate the effects of eCG dosage and its timing of administration, breed, and reproductive season as potential factors affecting estrus behavior and ovarian activity in hair ewes synchronized with progestogens.

MATERIALS AND METHODS

Study area

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

Animals used

The study was conducted in 2022 and involved four estrus synchronization programs. The first took place from February 23 to March 14 with 66 ewes; the second, from April 30 to May 20 with 59 ewes; the third, from July 15 to August 4 with 63 ewes; and the fourth, from September 6 to 27 with 28 ewes. These programs were scheduled to encompass

both the low and high reproductive seasons of hair ewes, as reported by González-Reyna *et al.* (1992). In total, 216 hair ewes were used across the two reproductive seasons (high: $n=91$; low: $n=125$), representing the Dorper, Katahdin, and Pelibuey breeds. The hairs ewes were 2 to 4 years old and had a body condition score (BCS) of 3 to 4 on a 1-to-5 scale (Thompson and Meyer, 1994).

Prior to the start of each synchronization program, the ewes were randomly assigned to four treatment groups to receive the following eCG protocols (GonActive[®] eCG, Virbac): (1) 200 IU administered 24 hours before sponge removal; (2) 200 IU at the time of sponge removal; (3) 300 IU administered 24 hours before sponge removal; and (4) 300 IU at the time of sponge removal.

Synchronization program and management

Throughout the study, ewes were housed in enclosed pens equipped with feeders, waterers, and shade. They were fed *ad libitum* with fresh citrus pulp (9.6% CP DM) and supplemented with 300 g/ewe/day of a commercial feed (14% CP DM). Clean water was available *ad libitum* at all times. Fifteen days prior to each synchronization program, ewes were treated for internal and external parasites with 1.0 mL of ivermectin, and received 2 mL of vitamins A, D, and E, along with 1 mL of B-complex vitamins.

During each synchronization program (Figure 1), intravaginal sponges containing fluorogestone acetate (FGA: 20 mg; Chronogest CR[®], MSD Animal Health) were inserted for 12 days. eCG treatments were administered prior to sponge removal according to the assigned protocol.

Estrus detection rams were introduced 24 hours after sponge removal to assess estrus incidence. The onset of estrus behavior was recorded, and ewes exhibiting such behavior were separated to facilitate detection in the remaining females. Ovulation rate was determined by observing and counting the number of corpora lutea on the ovarian surface using a rigid laparoscope (Karl Storz Endoscope; Storz) eight days after estrus.

Statistical analysis

The presence of ovulation, ovulation without estrus, and estrus without ovulation was analyzed using a logistic regression model that included the effects of eCG dosage (200 or 300 IU), timing of eCG administration (-24 h and 0 h), reproductive season (high and low), and breed (Dorper, Katahdin, and Pelibuey). Ovulation rate was analyzed using ANOVA for a completely randomized design based on a $2 \times 2 \times 2 \times 3$ factorial arrangement to determine treatment differences. The factors included in the ANOVA were the same

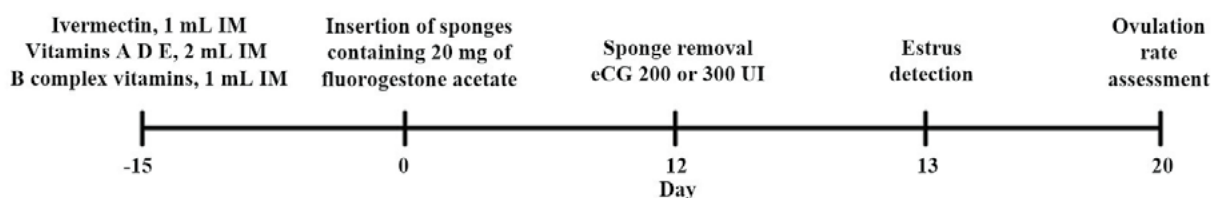


Figure 1. Estrus synchronization protocol.

as those in the logistic regression model, including possible interactions among them. When a significant difference was observed, the Student's t-test was applied at $p < 0.05$ to identify differences between means. All statistical analyses were performed using PROC LOGISTIC in SAS (SAS, 2004).

RESULTS AND DISCUSSION

Ovulation presence

The results of the logistic regression analysis of factors influencing ovulation in ewes synchronized with FGA are presented in Table 1. The percentage of ewes that ovulated across categories within each factor exceeded 94%. This finding contrasts with that of Kusakari *et al.* (1995), who reported seasonal variation in ovulation rates, with May showing the lowest percentage of ovulating ewes. This month corresponds to the anestrus period in wool breeds and the low reproductive season in hair ewes. The reduced ovulation rate during the low reproductive season may be due to failures in the timing of luteinizing hormone (LH) release.

Ovulation without estrus

The results regarding factors influencing the presence of ovulation without estrus signs are presented in Table 2. The variables eCG dosage, timing of eCG administration, and reproductive season did not significantly affect the incidence of ovulation without estrus. However, the variable *breed* did, with Dorper ewes showing a significantly higher incidence of silent ovulation (OR=0.56; CI=0.02–0.59; $p=0.0094$) compared to the other two breeds. Notably, the proportion of Katahdin and Pelibuey ewes that showed no estrus signs was low (4.8% and 3.4%, respectively), in contrast to 12.6% in Dorper ewes. Despite

Table 1. Logistic regression analysis of factors influencing ovulation in hair ewes following treatment with FGA.

Variable	N	Ewes Ovulated, % (n)	Odd Ratio	95% Confidence Interval	$P > \chi^2$
Breeds					
Dorper	62	95.2 (59)	1.06	0.21-5.27	0.9398
Katahdin	69	98.5 (68)	2.86	0.24-34.24	0.4064
Pelibuey	85	98.4 (81)			
eCG dose					
200	104	96.1 (100)	0.90	0.21-3.75	0.8827
300	112	96.4 (108)			
Timing of eCG administration					
0 h	100	94.0 (94)	0.27	0.05-1.37	0.1142
-24 h	116	98.3 (114)			
Reproductive season					
High	91	97.8 (89)	1.36	0.19-9.79	0.7597
Low	125	95.2 (119)			

FGA: Fluorogestone acetate; eCG: Equine chorionic gonadotropin.

Table 2. Logistic regression analysis of factors influencing the presence of ovulation without estrus in hair ewes treated with FGA.

Variable	N	Ewes Ovulated, % (n)	Odds Ratio	95% Confidence Interval	P> χ^2
Breeds					
Dorper	59	12.6 (10)	0.56	0.02-0.59	0.0094
Katahdin	69	4.8 (3)	0.12	0.21-1.52	0.2550
Pelibuey	81	3.4 (2)			
eCG dose					
200	100	18.0 (18)	1.32	0.59-2.95	0.4957
300	109	16.5 (18)			
Timing of eCG administration					
0 h	95	21.0 (20)	1.71	0.77-3.82	0.1885
-24 h	114	14.0 (16)			
Reproductive season					
High	89	23.6 (21)	1.78	0.67-4.77	0.2486
Low	120	12.5 (15)			

FGA: Fluorogestone acetate; eCG: Equine chorionic gonadotropin.

the absence of behavioral estrus, at least one *corpus luteum* was detected in all ewes during ovulation rate assessment, indicating that ovulation had occurred. This outcome confirms the presence of silent estrus. It is well established that the Pelibuey breed is less sensitive to factors directly affecting reproductive behavior (Trujillo-Quiroga *et al.*, 2007), which explains the generally low expression of estrus signs.

Estrus without ovulation

The results of the logistic regression analysis of factors influencing the presence of estrus without ovulation are shown in Table 3. Breed had a significant effect ($p < 0.04$) on the occurrence of estrus without ovulation. Specifically, Dorper ewes were 7.41 times more likely ($p = 0.0373$) to exhibit estrus without ovulation compared to Pelibuey ewes. The proportion of ewes that ovulated showed no significant differences across the evaluated factors, with values consistently above 94%. This suggests that ovulation is not limited by these factors. However, Mufti *et al.* (1997) reported an ovulation rate of 92% in ewes treated with 200 IU of eCG, compared to untreated ewes results that align with this study. This response is likely due to the exogenous stimulation of FSH and LH release from the anterior pituitary by gonadotropins such as eCG, leading to a higher ovulation rate.

Estrus and ovulation are critical for both estrus detected and fixed-time artificial insemination (AI) programs (Hinojosa *et al.*, 2019), as they directly impact the number of ewes serviced and conception rates. Therefore, it is essential to document the incidence of ewes without estrus, as well as cases of estrus without ovulation. The timing of AI is fundamental; insemination even 24 hours after the optimal time can significantly reduce

Table 3. Logistic regression analysis of factors influencing the presence of estrus without ovulation in hair ewes treated with FGA.

Variable	N	Ewes Ovulated, % (n)	Odds Ratio	95% Confidence Interval	P> χ^2
Breeds					
Dorper	60	5.0 (3)	7.41	0.55-10.75	0.0373
Katahdin	55	1.8 (1)	2.44	1.13-48.77	0.2387
Pelibuey	62	1.6 (1)			
eCG dose					
200	84	2.4 (2)	0.71	0.21-2.36	0.5732
300	93	3.2 (3)			
Timing of eCG administration					
0 h	78	5.1 (4)	5.32	1.27-22.37	0.2225
-24 h	99	4.0 (4)			
Reproductive season					
High	68	0.0 (0)	<0.001	<0.001->999.99	0.8963
Low	109	4.6 (5)			

FGA: Fluorogestone acetate; eCG: Equine chorionic gonadotropin.

fertility. AI failure is costly, as some females may be incorrectly diagnosed as infertile due to the absence of visible estrus behavior (Spanner *et al.*, 2024).

A portion of undetected (missed) estrus cases can be attributed to the absence of receptive behavior, such as increased locomotor activity and tail movement, a condition referred to as silent estrus (Rodríguez *et al.*, 2007). Females not showing estrus signs may still cycle normally, exhibiting estrus periods approximately every 17 days.

In synchronized hair ewes, silent estrus poses a major reproductive challenge, likely associated with insufficient estrogen levels, which are crucial for the expression of receptive behavior (Visha and Sejian, 2023). Although hormone levels were not directly measured in this study, the absence of estrus signs in these ewes suggests a possible estrogen deficiency as a key physiological trigger. Estrogens, mainly produced by dominant follicles, regulate critical processes such as follicular maturation, LH receptor upregulation, and endometrial sensitization, all of which are necessary for proper ovulation and fertilization (Al-Suhaimi *et al.*, 2022).

Breed significantly influenced the incidence of silent estrus; Dorper ewes exhibited a higher prevalence compared to Katahdin and Pelibuey breeds (OR=0.56; p<0.01). This variability suggests that the ability to express estrus signs may be modulated by genetic and physiological differences affecting estrogen production and action. Silent estrus represents a major obstacle in AI programs, as undetected estrus can reduce reproductive efficiency. This underscores the need for more precise hormonal strategies to optimize estrus activity (Saint-Dizier and Chastant-Maillard, 2018; Pool *et al.*, 2020). Implementing tailored protocols that consider factors such as breed and reproductive season is essential to mitigate this issue (Año-Perello *et al.*, 2020).

CONCLUSIONS

Estrus and ovarian activity in hair ewes is influenced by multiple factors, with breed being the most significant. This affects the occurrence of estrus without ovulation and ovulation without visible estrus, both of which may compromise the effectiveness of artificial insemination programs.

Ethical approval

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

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