AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Effect of breed, breeding season, eCG dose, and eCG application time on the estrous cycle of hair ewe lambs

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ABSTRACT

Objective: To evaluate the effect of breed, breeding season, dose and application time of equine chorionic gonadotropin (eCG) on the estrous cycle and ovarian activity in hair ewe lambs.

Design/methodology/approach: We studied 216 hair ewe lambs (62 Dorper, 69 Katahdin, and 85 Pelibuey) —91 in high breeding season and 125 in low breeding season—, who were synchronized with intravaginal sponges containing 20 mg of fluorogestone acetate (FGA), and intramuscular equine chorionic gonadotropin (eCG; 200 and 300 IU). The treatments are breed, breeding season, eCG dose, and eCG application time. We analyzed the presence of estrus using a logistic regression model, while for the interval to estrus and the ovulation rate we applied an analysis of variance, using a completely randomized design with a 2×2×2×3 factorial arrangement with the PROC LOGISTIC and PROC GLM procedures.

Results: The breed was a factor ($P<0.01$) in the presence of estrus: Dorper ewe lambs presented 9.74 times more possibilities than Pelibuey. The interval to estrus was shorter ($P<0.05$) in Dorper (29.5±0.9 h) and Katahdin (29.1±0.9 h) than in Pelibuey (34.8±0.9 h). The interval to estrus was lower ($P<0.05$) when we applied 200 or 300 IU of eCG 24 h before the end of the protocol, than when we applied 200 IU of eCG at the time of progestogen withdrawal. The ovulation rate was only affected by breed ($P<0.05$): it was higher in Pelibuey (2.4±0.1) than in Dorper (2.0±0.1) and Katahdin (1.9±0.1).

Study limitations/implications: Conducting a second study would be advisable to complement this research. This would include the gestation stage of females and relate it to the ovulation rate, while also measuring the ovarian structures by means of ultrasound.

Findings/conclusions: The main influencing factor on estrus and ovarian activity in hair ewe lambs synchronized with progestogens is breed.

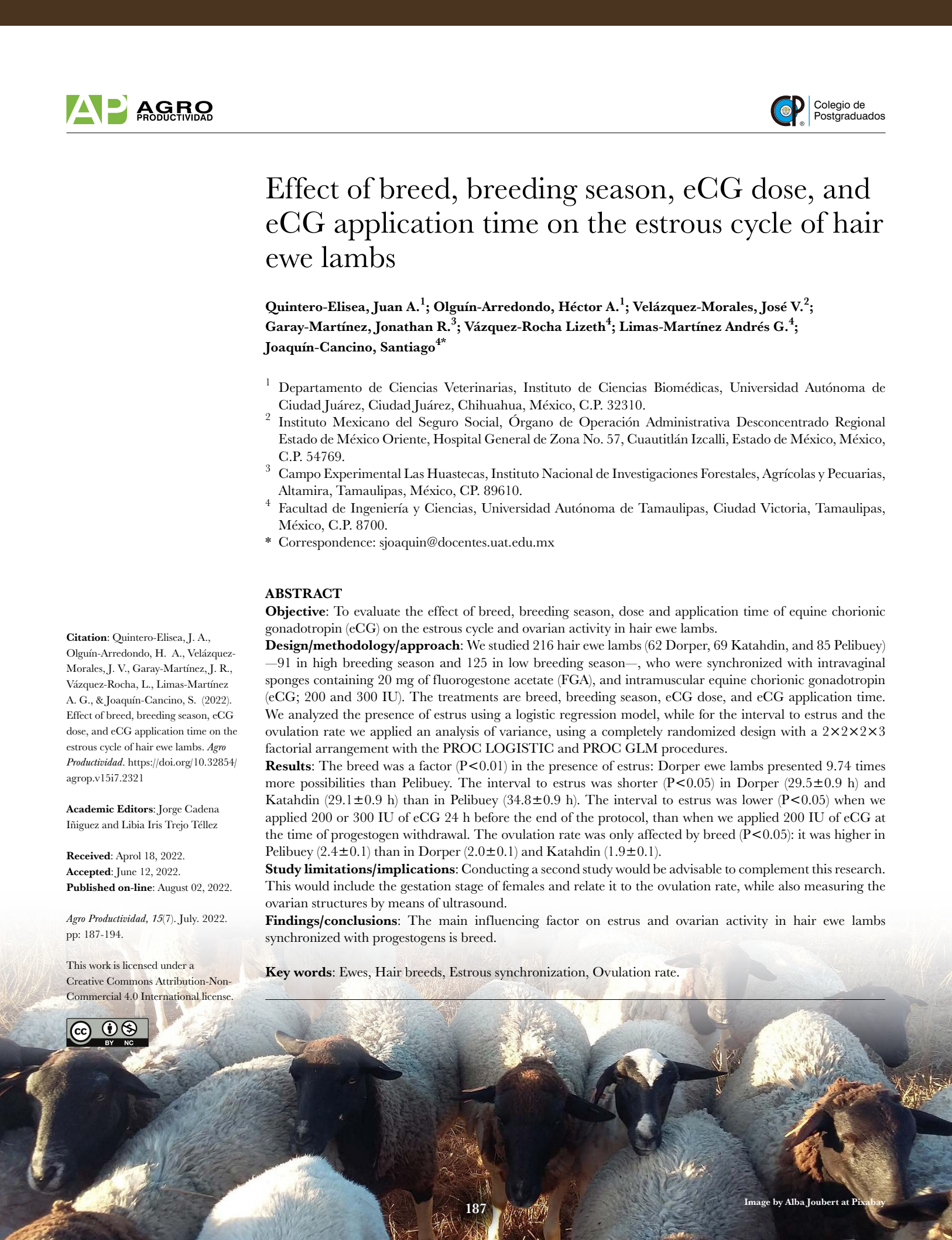
Key words: Ewes, Hair breeds, Estrous synchronization, Ovulation rate.

Citation: Quintero-Elisea, J. A., Olguín-Arredondo, H. A., Velázquez-Morales, J. V., Garay-Martínez, J. R., Vázquez-Rocha, L., Limas-Martínez A. G., & Joaquín-Cancino, S. (2022). Effect of breed, breeding season, eCG dose, and eCG application time on the estrous cycle of hair ewe lambs. *Agro Productividad*. https://doi.org/10.32854/agrop.v15i7.2321

Academic Editors: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: Aprol 18, 2022.
Accepted: June 12, 2022.
Published on-line: August 02, 2022.

Agro Productividad, 15(7). July. 2022. pp: 187-194.



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INTRODUCTION

The application of exogenous hormones in ewes is a reproductive tool used in artificial insemination programs to synchronize or induce estrus. These hormonal treatments are essential to make lamb production more efficient throughout the year. There are various protocols for the synchronization and/or induction of estrus; however, protocols based on progestogens and eCG result in better estrous activity and fertility (Barrett *et al.*, 2004; Ali, 2007). These protocols consist of administering a progestogen to the ewe for 9-14 days to simulate the luteal phase of the estrous cycle. After this period, the progestogen is withdrawn, and the follicular growth and ovulation rate are stimulated by administering eCG (equine chorionic gonadotropin) (Cline *et al.*, 2001). Although these protocols have been successful in accomplishing estrous synchronization, the response varies according to genetic and environmental factors (Arroyo *et al.*, 2006; Estrada *et al.*, 2006). Moeini *et al.* (2007), for instance, reported different responses to estrus between Iranian Sanjabi and Lori wool ewes treated with FGA and 400 IU of eCG. The observed variation was of 52.2% of Sanjabi ewes in estrus as compared to 91.1% among the Lori breed. Prolificacy and fecundity in Dorper ewes increased up to 20% when the eCG was applied 24 hours before the end of the synchronization protocol, as compared to when it was applied at the end of said protocol (Zeleke *et al.*, 2005). Other factors that affect the response of wool ewes to these hormonal treatments with progestogens are the dose of eCG (Kridli *et al.*, 2006), the time of year (Langford *et al.*, 1983; Rosa and Bryant, 2003), the body condition (Esen, 2001), and the geographic region (Fenton *et al.*, 1997). There are few studies focusing on the factors that influence the reproductive response of ewes subjected to synchronization protocols in Mexico's climatic conditions. Martínez-Tinajero *et al.* (2007) found that the eCG application time affects the presence of estrus in Blackbelly ewes under tropical conditions. Similarly, Macías (2007) reports that the eCG dose, eCG application time, breed, and time of year affect the response to estrus and its onset time under the dry tropical conditions of northeastern Mexico. Therefore, the objective of this research was to evaluate the effect of breed, breeding season, dose, and application time of equine chorionic gonadotropin (eCG) on the estrous cycle and ovarian activity in hair ewe lambs.

MATERIALS AND METHODS

Study area description

This research was conducted from February 23 to September 27, 2018, at the University Ranch of the Universidad Autónoma de Ciudad Juárez, Ciudad Juárez, Chihuahua. The region is located at 1100 masl; the climate is hot (BWh) and cold desert (BWK); the average annual temperature is 16-18 °C; and the average annual precipitation is 244 mm (INEGI, 2007).

Experimental design and animals

We conducted four estrous synchronization programs during the year, using 216 hair ewe lambs (62 Dorper, 69 Katahdin, and 85 Pelibuey). The ewe lambs were 8 months old, had a live weight of 25-30 kg, and a body condition score (BC) of 2.5-3.5, using the 1-5 scale (Thompson and Meyer, 1994).

The first program was conducted from February 23 to March 14 with 66 ewe lambs (19 Dorper, 20 Katahdin, and 27 Pelibuey); the second from April 30 to May 20 with 59 ewe lambs (17 Dorper, 19 Katahdin, and 23 Pelibuey); the third from July 15 to August 4 with 63 ewe lambs (17 Dorper, 20 Katahdin, and 26 Pelibuey); and the fourth from September 6 to 27 with 28 ewe lambs (9 Dorper, 10 Katahdin, and 9 Pelibuey).

In order to assess the effect of the breed, we defined the following treatments:

T1) Dorper (62 ewe lambs)
T2) Katahdin (69 ewe lambs)
T3) Pelibuey (85 ewe lambs)

Before starting each synchronization program, we randomly distributed the ewe lambs into four groups to apply the following treatments:

T1) 200 IU of eCG 24 h before sponge withdrawal;
T2) 200 IU of eCG at the time of sponge withdrawal;
T3) 300 IU of eCG 24 h before sponge withdrawal, and
T4) 300 IU of eCG at the time of sponge withdrawal.

These treatments were applied in each of the four estrous synchronization programs conducted during the year.

We defined the high breeding season as the time of year when more than 70% of the ewes show estrus and ovulation behavior, and the low breeding season as the time of year when this percentage is less than 50%.

In order to evaluate the breeding seasons, we studied 216 ewe lambs: for the high season, n=91 (28 Dorper, 33 Katahdin, and 30 Pelibuey); and for the low season, n=125 (34 Dorper, 36 Katahdin, and 55 Pelibuey). The treatments were as follows:

T1) High breeding season
T2) Low breeding season

Females were kept in the barn. They were fed twice a day (8 am and 5 pm) with fresh orange pulp (9.6%; CP) on a dry basis; 300 g head^{-1} day^{-1} of supplement (14% CP) on a dry basis, and 2.85 Mcal of ME, made with ground sorghum (70%), wheat bran (7%), soybean meal (12%), chopped buffel grass hay (7%), molasses (3%), and mineral salts (1%). This supplement was offered daily at 8 am, and water was offered *ad libitum*.

Synchronization program and management

The estrous synchronization program consisted of intravaginally applying a sponge impregnated with FGA (20 mg; Chronogest CR®, MSD, Animal Health) to each ewe lamb for 12 d; the eCG treatments (GonActive® eCG, Virbac) were applied before sponge withdrawal. The incidence and distribution of estrus was determined 24 h after sponge withdrawal by introducing marker males provided with an anti-mating apron. The ewe

lambs detected in estrus were registered and separated to facilitate estrus in the remaining ewe lambs. The ovulation rate was determined by direct observation and counting of *corpus luteum* on the surface of the ewe lambs' ovaries through laparoscopy. This was performed 8 d after sponge withdrawal using a rigid laparoscope (Karl Storz Endoscope; Storz).

Study variables

Three variables were determined in each treatment: presence of estrus (ewe lambs that presented estrus after sponge withdrawal); interval to estrus (time interval between sponge withdrawal and presence of estrus), and ovulation rate (number of *corpus luteum* per lamb presenting estrus).

Statistical analysis

The presence of estrus was analyzed under a logistic regression model that included the effects of the eCG dose (200 or 300 IU), eCG application time (−24 h and 0 h), breeding season (high and low), and breed (Dorper, Katahdin, and Pelibuey). To analyze the interval to estrus and the ovulation rate, we applied an analysis of variance using a completely randomized design with a 2×2×2×3 factorial arrangement. The factors included in the analysis of variance were the same as in the logistic regression model, in addition to the possible interactions between factors. Means were compared with the t-student test at $P<0.05$. Trends were established when the analysis indicated $0.05 \geq P \leq 0.10$. All analyses were performed using the PROC LOGISTIC and PROC GLM procedures of the SAS software (SAS, 2004).

RESULTS AND DISCUSSION

Presence of estrus

Table 1 shows the results of the logistic regression analysis on factors that influence the presence of estrus. Breeding season, PMSG dose and application time were factors that did not affect ($P>0.05$) the presence of estrus in ewe lambs synchronized with FGA. On the contrary, breed was indeed a factor of influence ($P<0.01$): Dorper ewe lambs presented 23.9% better estrous behavior than Pelibuey ewe lambs; however, only a 6.8% difference ($P>0.05$) was observed between Katahdin and Pelibuey as a result of the synchronization protocol.

Among the factors studied, only breed affected the response to the presence of estrus (OE), which coincides with the results found in wool ewes, as reported by Emsen and Yaprak (2006), and Moeini *et al.* (2007). Both studies reported variations between breeds (Awassi *v.* Red Karaman and Iranian Sanjabi *v.* Lori) in the percentage of ewes presenting estrus after being synchronized with a progestogen and eCG. Meanwhile, after applying a synchronization program similar to the one used in this research, Macías (2007) found a higher percentage of estrus in Pelibuey Canelo ewes than in those of the Pelibuey Blanco, Blackbelly, and Dorper breeds. However, other studies did not find any differences in the presence of estrus between wool breeds (Romano *et al.*, 2000; Boscos *et al.*, 2002). Moeini *et al.* (2007) mention that breeds that have been improved to increase prolificacy are more sensitive and likely to respond to hormonal treatments for estrus synchronization

Table 1. Logistic regression on factors that affect the presence of estrus (OE) in hair ewe lambs treated with FGA.

Treatment/variable	N	PE % (n)	Odd Ratio	Confidence interval 95%	$P>X^2$
Breed					
Dorper	62	96.8 (60)	9.74	1.93-49.19	0.0059
Katahdin	69	79.7 (55)	2.15	0.81-5.69	0.1226
Pelibuey	85	72.9 (62)			
Doses of eCG					
200	104	80.8 (84)	0.73	0.33-1.60	0.4314
300	112	83.0 (93)			
Time application of eCG					
0 h	100	78.0 (78)	0.57	0.26-1.26	0.1648
-24 h	116	85.3 (99)			
Reproductive season					
high	91	74.7 (68)	0.48	0.19-1.26	0.1358
low	125	87.2 (109)			

FGA: Fluorogestone acetate; eCG: Equine chorionic gonadotropin.

and induction. García-Guerra *et al.* (2018) mention that there is occurrence of follicular codominance in ewes. This results in multiple ovulations and therefore increases the response to estrus and ovulation. Hence, multiple ovulation is due to genetic (breed), dietary, and hormonal influence on follicular development and growth, as well as on oocyte-granulosa cell interaction (cumulus oophorus complex).

This might explain the variations found in the presence of estrus among the breeds studied. It is worth mentioning that the Dorper ewe lambs showed a higher increase in the presence of estrus than the Pelibuey. A higher percentage of females of both breeds (and Katahdin) presented estrus between 24 and 36 h after the end of the synchronization protocol. Hashemi *et al.* (2006) and Kridli *et al.* (2006) reported the presence of estrus between 24 and 36 h after progestogen withdrawal in ewes of different breeds synchronized with FGA and eCG (500 and 600 IU). In this sense, Martínez-Tinajero *et al.* (2006) —using the same protocol, with 150 and 300 IU of eCG— found 80% of ewes in estrus between 24 and 48 h after finishing the hormonal treatment. These results suggest that the time it takes for ewes treated with FGA and eCG to present estrus after finishing the synchronization protocol does not depend on the breed, but rather on other factors, such as nutrition and environmental aspects.

Interval to estrus and ovulation rate

Table 2 shows the results of the effect of breed, breeding season, and interaction between eCG dose and application time on the interval to estrus and ovulation rate in ewe lambs synchronized with FGA. The interval to estrus was shorter ($P<0.05$) in Dorper (29.5±0.9 h) and Katahdin (29.1±0.9 h) ewe lambs than in Pelibuey (34.8±0.9 h). The interaction between eCG dose and application time also affected the interval to estrus,

Table 2. Effect of breed, breeding season, and eCG application on interval to estrus and ovulation rate of hair ewes synchronized with FGA.

Variable	n	Interval to estro ±SE	Ovulation rate ±SE
Breed			
Dorper	60	29.5 ± 0.9a	2.0 ± 0.1b
Katahdin	55	29.1 ± 0.9a	1.9 ± 0.1b
Pelibuey	62	34.8 ± 0.9b	2.4 ± 0.1a
Reproductive season			
high	68	29.0 ± 0.8a	2.0 ± 0.1a
low	109	32.6 ± 0.8a	2.2 ± 0.1a
Dose × Time of application of eCG			
200 UI 0 h	34	34.6 ± 1.0a	1.8 ± 0.2a
200 UI −24 h	50	30.4 ± 1.0b	2.1 ± 0.2a
300 UI 0 h	45	31.5 ± 1.0b	2.1 ± 0.2a
300 UI −24 h	48	30.8 ± 1.0b	2.2 ± 0.2a

[a, b] Different superscripts within the same column indicate a significant difference ($P<0.05$); FGA: Fluorogestone acetate; eCG: Equine chorionic gonadotropin.

which was longer ($P<0.05$) in ewe lambs treated with 200 IU at progestogen withdrawal (34.6±1.0 h) than in ewe lambs with other treatments (mean=30.9±1.0 h). When 200 and 300 IU of eCG were applied at −24 h, and 300 IU at 0 h, the interval to estrus was similar ($P>0.05$). The variation observed in the ovulation rate was due to the effect of breed ($P<0.05$). Pelibuey had a higher ($P<0.03$) ovulation rate (2.4±0.1 *corpus luteum*) than Dorper (2.0±0.1 *corpus luteum*) and Katahdin (1.9±0.1 *corpus luteum*).

In this research, we observed that Pelibuey ewe lambs took longer (5.5 h) to present estrus after progestogen withdrawal; however, they had a better ovulation rate than other breeds (2.4±0.1 *v.* 1.95±0.1 *corpus luteum* on average). In this regard, the increase in the ovulation rate in Pelibuey ewe lambs may be due to the better prolificacy that this breed presents naturally in relation to the Dorper and Katahdin breeds (Bartlewski *et al.*, 1999).

The FSH affinity of the follicles depends exclusively on the receptors presented by the follicles during their growth and is related to the number of receptors. Hence, when there are more receptors, the dominant follicle or follicles tend to manifest in growth and, therefore, to be dominant and/or ovulatory. The ovulation rate increases for this reason. However, this ovulation rate should go hand in hand with an increase in estrogen levels, since the larger amount of dominant follicles would be expected to produce a greater amount of estrogens. This would result in an interval to estrus similar or shorter than the one found in the other two breeds. In this regard, Rekik *et al.* (2002) mention that the response of ewe lambs to hormonal treatments that seek to induce or synchronize estrus varies due to immaturity of the reproductive system, environmental factors such as nutrition, live weight, season of birth, among others (Martínez *et al.*, 2001; Madani *et al.*, 2009). In an estrous synchronization program with FGA and eCG in Pelibuey Blanco, Pelibuey Canelo, Dorper, and Katahdin ewes, Macías (2007) found that the interval to estrus did not vary

between breeds (average of 28.8 h). This value was similar to that observed in this research for Dorper and Katahdin ewe lambs. Romano *et al.* (2000) compared the interval to estrus between Suffolk, Hampshire, and White Face breeds of wool ewes, and did not find variations derived from breed. These results are different from those found in the present research, probably due to the type of ewes used (adults).

The interaction between eCG dose and application time affected the interval to estrus ($P<0.05$), but not the ovulation rate. This is probably due to the fact that modifying the eCG dose and administration time generates changes in the pattern of follicular development (Ali, 2007), which may be favorable or unfavorable for the acceleration of follicular development and growth, and for the elevation of plasma estrogen levels (Ustuner *et al.*, 2007). These results demonstrate the importance of the eCG hormone to increase the degree of synchrony between reproductive events and the presence of estrus when ewes are treated with progestogens. Based on our findings, the onset time of estrus is the same when applying doses of 200 or 300 IU of eCG 24 h before withdrawing the intravaginal device and when applying one dose of 300 IU of eCG at the time of withdrawal. In general, these results coincide with those reported in other studies (Ali, 2007; Macías, 2007; Ustuner *et al.*, 2007).

CONCLUSIONS

The main influencing factor on estrous cycle and ovarian activity in hair ewe lambs synchronized with progestogen is breed. When a more predictable and compact estrus is preferred, the eCG should be administered prior to sponge withdrawal (preferably 24 hours).

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