

Reproductive Response of Hair Sheep to Short or Long Duration Estrus Synchronization Protocols

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Citation: González-Puente, M. A., Buendía-Rodríguez, G., Jiménez-Severiano, H., Villaseñor-González, F., Estrada-Cortés, E., Montiel-Olguín, L. J., & Espinosa-Martínez, M. A. (2024). Reproductive Response of Hair Sheep to Short or Long Duration Estrus Synchronization Protocols. *Agro Productividad*. <https://doi.org/10.32854/agrop.v17i9.3041>

Academic Editor: Jorge Cadena Iñiguez

Guest Editor: Juan Francisco Aguirre Medina

Received: May 03, 2024.

Accepted: August 01, 2024.

Published on-line: September 20, 2024.

Agro Productividad, 17(9) supplement. September. 2024. pp: 193-202.

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ABSTRACT

Objective: Evaluate the reproductive response of hair ewes treated with estrus synchronization protocols that included a progesterone-releasing intravaginal device (CIDR) for a period of 7 or 12 days.

Methodology: During the end of winter, ewes were randomly allotted to one of two estrus synchronization protocols: 1) Short-term protocol (SD), ewes (n=24) received a CIDR on Day 1 and removed on Day 7, and received 400 IU of equine chorionic gonadotropin (eCG) and 0.125 mg of cloprostenol; 2) Long-term protocol (LD), ewes (n=24) received a CIDR on Day 1, retired on Day 12 and received 400 IU eCG. Ewes showing estrous behavior were bred with a mature ram.

Results: There were no differences ($P>0.05$) in the estrus response in both groups of ewes (100 vs. 95.8% for SD and LD, respectively), the pregnancy rate (87.5 vs. 87.5%), prolificacy (1.95 ± 0.1 vs. 2.2 ± 0.1 lambs/ewe), hours between CIDR removing and the beginning of estrus (34.4 ± 8.1 vs. 34.8 ± 9.2 h), and estrus duration (39.7 ± 2.9 vs. 39.9 ± 4.7 h). At CIDR removal, ewes in the LD group showed a greater diameter ($P<0.05$) for the largest follicle in both ovaries (5.42 ± 0.3 and 5.42 ± 0.4 mm for the follicle in the left and right ovaries, respectively) vs. SD ewes (4.25 ± 0.1 and 4.29 ± 0.1 mm).

Limitations of study: Results observed in the follicle diameter could be due to low circulating progesterone levels in the LP group, which must be confirmed in future studies.

Conclusions: The short protocol for estrus synchronization can be used in hair ewes under temperate conditions, without affecting their reproductive response.

Keywords: CIDR, Reproduction, Pregnancy rate, Prolificacy.



INTRODUCTION

Estrus synchronization is a widely used technology in reproductive programs for ruminant species (Habeeb *et al.*, 2021; Hameed *et al.*, 2021). In the literature, various protocols have been published for sheep, such as those considering the use of prostaglandins or their analogs (Alavez-Ramírez *et al.*, 2014; De Carvalho *et al.*, 2018), as well as those using progesterone or progestogens, which can be used alone or combined with other hormones like equine chorionic gonadotropin (eCG) and/or prostaglandins, with a total protocol duration of up to more than 12 days (Oliveira *et al.*, 2016; Rosasco *et al.*, 2019; Santos-Jimenez *et al.*, 2022).

In this context, there is insufficient information on follicular dynamics that supports the use of treatments with different durations, especially in short protocols. This is because after five or six days of inserting intravaginal progesterone-releasing devices (Arroyo-Ledezma *et al.*, 2013; Cox *et al.*, 2012) or progesterone-impregnated sponges (Alavez-Ramírez *et al.*, 2014), the blood concentration of this hormone decreases. It has been suggested not to maintain the devices for more than seven days, which necessarily requires the application of prostaglandins upon removing the source of exogenous progesterone (Arroyo-Ledezma *et al.*, 2013).

Estrus synchronization protocols based on progestogens for only seven days have been previously described (Bruno-Galarraga *et al.*, 2021; Santos-Jimenez *et al.*, 2022), although they are less known among technicians and producers. Their use not only allows for a reduction in cost but also facilitates the development of new protocols involving minimal exposure to exogenous hormones. Although these protocols are effective, the reproductive response to them has not been clearly evaluated in hair sheep outside of warm, tropical, and subtropical regions, where they have their natural habitat (Aguilar-Martínez *et al.*, 2017). Additionally, these sheep exhibit reproductive characteristics different from other breeds, such as a shorter anestrous period (Arroyo, 2011).

The results of estrus synchronization protocols can be influenced by variables such as breed, duration of exposure to progestogens, additional hormones used (prostaglandins, equine chorionic gonadotropin), environmental factors, etc. (Alavez-Ramírez *et al.*, 2014; De *et al.*, 2020; González-Reyna *et al.*, 2014; Teixeira *et al.*, 2016). Therefore, the objective was to evaluate the reproductive response of hair sheep to short and long duration estrus synchronization protocols, based on controlled-release progesterone devices (CIDR), under a temperate climate. The hypothesis was that the use of a short-duration estrus synchronization protocol could elicit at least a similar reproductive response in sheep compared to a long-duration protocol.

MATERIALS AND METHODS

Animals and Geographical Location

Adult hair sheep (1 to 6 years old) of Pelibuey (n=39) and Blackbelly (n=9) breeds were used, with an average body weight of 48.9 ± 1.5 kg. The study was conducted at the end of winter and the beginning of spring, with increasing daylight hours in the state of Querétaro, Mexico (20° 42' N, 100° 01' O). March is routinely considered the breeding season at the production unit. The climate in the study region is temperate, semi-arid,

with an average annual temperature of 17.4 °C. The sheep were fed a diet (forage and concentrate) that met their nutritional requirements according to their physiological stage, as described by the NRC (2007), and always had free access to water.

Treatments

The sheep were randomly assigned to one of two estrus synchronization protocols, which differed in their duration of exposure to exogenous progesterone. In the short-duration protocol (SD; n=24), the sheep received an intravaginal device impregnated with 0.3 g of progesterone (CIDR[®]; Zoetis, NJ, USA) on day 1. On day 7, the CIDR was removed, and the sheep received an intramuscular dose of 400 IU of eCG (Folligon[®]; Intervet, USA) and 0.125 mg of Cloprostenol (Induvel[®]; Virbac, Carros, France) to remove any functional corpus luteum in the sheep (Arroyo-Ledezma *et al.*, 2013). In the long-duration protocol (LD; n=24), the sheep received the CIDR on day 1 and it was removed on day 12, in addition to receiving an intramuscular dose of 400 IU of eCG.

At the beginning of the study, the sheep were weighed, and their body condition was assessed according to Romero (2015), using a scale from 1 to 5, where 1 corresponds to a sheep in a state of emaciation and 5 corresponds to an obese sheep. Additionally, at the beginning and end of the synchronization treatment, both ovaries were scanned using transrectal ultrasonography, with an ultrasound machine (Aloka, model SSD500), equipped with a 5.0 MHz transrectal probe. All follicles equal to or larger than 3 mm in both ovaries were recorded.

Estrus detection was carried out twice a day (08:00 and 17:00 h) between 24 and 96 h after CIDR removal, using a mature ram. Ewes exhibiting signs of estrus (those that remained immobile) were naturally bred with one of three mature rams, each with a history of good fertility during previous breeding seasons, at a ratio not exceeding 1:10. The different durations of the treatments allowed the same rams to be used for both groups of ewes without changing the ratio.

Subsequently, the time elapsed between CIDR removal and the onset of estrus, as well as the duration of estrus, was recorded. Twenty-eight days after exposure to the rams, pregnancy diagnosis was performed using transrectal ultrasonography with a 5.0 MHz probe. Finally, at lambing, the number of lambs born per ewe (prolificacy) was recorded.

Statistical Analysis

Frequency data (estrus rate and conception rate) were analyzed using Fisher's exact test to determine differences between synchronization protocols. Body weight, body condition, time to exhibit estrus behavior, duration of estrus, diameter of the largest follicle, and number of follicles were analyzed using Student's t-test.

Body condition data, time to estrus presentation, and estrus duration were transformed using the natural logarithm option prior to analysis, while data on the diameter of the largest follicle, number of follicles, and prolificacy were transformed using the square root of (y+0.5). To facilitate understanding, all mean values and standard errors are presented as their original untransformed values. All analyses were conducted using the SAS statistical software package (SAS Institute Inc., Cary NC, USA).

RESULTS

One hundred percent of the devices remained within the ewes’ vaginas during the treatment periods considered. Additionally, no abnormal vaginal discharges were observed after their removal.

Body Weight and Condition. At the beginning of the study, no differences ($P>0.05$) were observed in body weight and body condition score between the synchronization groups (Table 1).

Estrus and Conception. There were no differences between treatments ($P>0.05$) regarding estrus rate, conception rate, and prolificacy (Table 1). For ewes that exhibited estrus exclusively, the conception rate was 87.5% and 91.3% for the SD and LD groups ($P>0.05$). The average time elapsed from CIDR removal to the onset of estrus behavior was less than 35 h ($P>0.05$) for both synchronization protocols, and the duration of estrus lasted nearly 40 h ($P>0.05$).

The timing of estrus occurrence was similar for both treatment groups (Figure 1). Within 24 hours after CIDR removal, only 16.7% of the ewes had shown estrus in both groups;

Table 1. Reproductive Response of Hair Sheep to Short (7 d) or Long Duration (12 d) Estrus Synchronization Protocols.

Variable	Short protocol ¹	Long Protocol ²	P value
n	24	24	-
Body weight, kg	49.4±2.2	48.5±2.0	NS
Body condition score	2.81±0.08	2.68±0.09	NS
Estrus rate, %	100	95.8	NS
Total conception rate, % [†]	87.5	87.5	NS
Onset of estrus ^{††} , h	34.4±1.7	34.8±1.9	NS
Estrus duration, h	39.7±2.9	39.9±4.7	NS
Prolificacy	1.95±0.15	2.2±0.14	NS

¹ Insertion of CIDR on day 1, removal on day 7 + eCG and Cloprostenol; ² Insertion of CIDR on day 1, removal on day 12 + eCG. [†] Pregnant ewes/ewes in the breeding group; ^{††} Time after CIDR removal. NS=Not significant ($P>0.05$). Results are expressed as mean ± standard error.

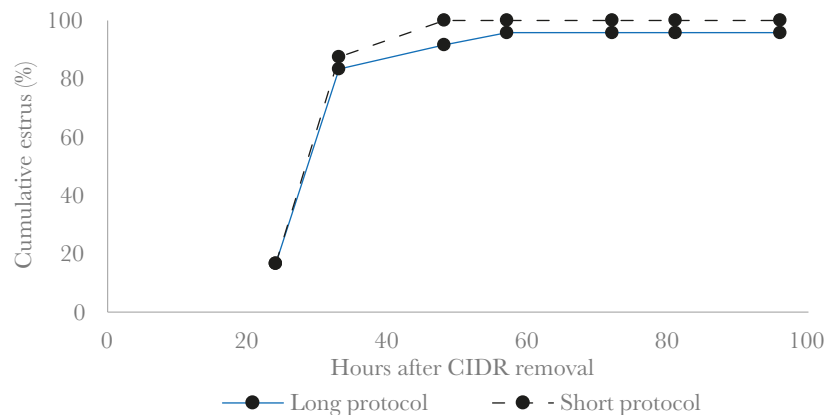


Figure 1. Cumulative Percentage of Estrus in Ewes with Short (CIDR for 7 days) or Long Duration (CIDR for 12 days) Estrus Synchronization Protocol.

however, by 33 hours, this percentage had increased dramatically (86.5%). Ewes that responded to the synchronization protocol did so fully by 57 hours after CIDR removal. The highest percentage of ewes in estrus was observed between 33 and 57 hours for both study groups (Figure 2). Subsequently, this percentage decreased to its lowest level by 96 hours, with 16.7% for ewes in the SD group and 20.8% for ewes in the LD group.

Ovarian Follicular Development

There were no differences ($P>0.05$) between the synchronization groups in the number of ovarian follicles (Table 2) at the time of CIDR insertion and removal. Regarding the diameter of the largest follicle, there were no differences ($P>0.05$) between the groups

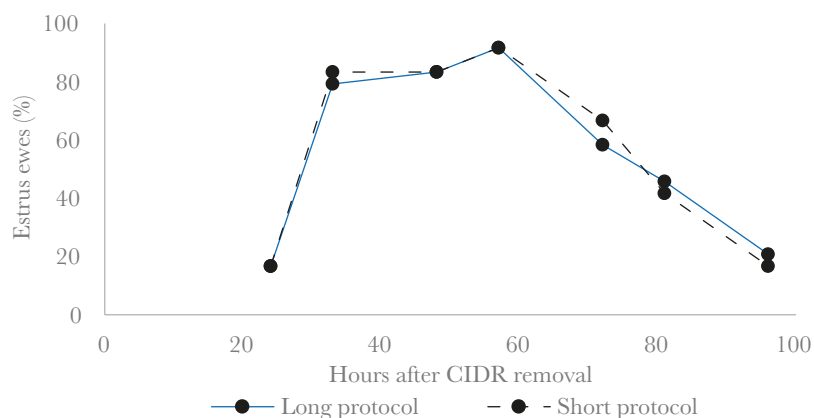


Figure 2. Percentage of Estrus in Ewes with Short (CIDR for 7 days) or Long Duration (CIDR for 12 days) Estrus Synchronization Protocol.

Table 2. Number and Size of Ovarian Follicles at CIDR Insertion and Removal in Hair Sheep with Short (7 days) or Long Duration (12 days) Estrus Synchronization Protocol.

Variable	Short Protocol ¹	Long Protocol ²
Number of ovarian follicles, LO		
CIDR insertion	2.21±0.21	2.62±0.12
CIDR removal	2.50±0.17	2.37±0.13
Diameter (mm) of largest follicle, LO		
CIDR insertion	5.25±0.36	4.46±0.17
CIDR removal	4.25±0.19 ^a	5.42±0.32 ^b
Number of ovarian follicles, RO		
CIDR insertion	2.83±0.27	2.67±0.19
CIDR removal	2.67±0.11	2.50±0.15
Diameter (mm) of largest follicle, RO		
CIDR insertion	5.25±0.37	4.50±0.25
CIDR removal	4.29±0.15 ^a	5.42±0.42 ^b
Total follicles		
CIDR insertion	5.04±0.36	5.29±0.27
CIDR removal	5.17±0.18	4.87±0.21

¹ Insertion of CIDR on day 1, removal on day 7 ± eCG and Cloprostenol; ² Insertion of CIDR on day 1, removal on day 12 ± eCG. LO=Left ovary; RO=Right ovary. ^{a,b} Means with different superscripts in the same row are different ($P<0.05$). Results are expressed as mean ± standard error.

of ewes before CIDR insertion; however, at the end of the treatment, the ewes in the LD group had a larger average diameter than those in the SD group ($P < 0.05$).

DISCUSSION

The use of the short-duration estrus synchronization protocol resulted in a reproductive response in ewes similar to that obtained in the group with the long-duration protocol. For many years, protocols have been used in sheep as a tool to help improve reproductive processes. A previous study (Espinosa-Martínez *et al.*, 2020) described the use of short-duration synchronization protocols in hair sheep using progesterone and eCG with good results in estrus presentation (92%). The results of estrus presentation in our study are similar to or better than those reported in the literature (ranging from 70% to 100%), describing protocols with the use of CIDR for varying periods in breeds such as Dorper and Santa Inês (Bruno-Galarraga *et al.*, 2021; Santos-Jiménez *et al.*, 2022; Vinicius *et al.*, 2019).

The onset of estrus in hair sheep in this study showed results similar to (36-41 h; Santos-Jiménez *et al.*, 2022) or different from those observed in other studies, with no consensus as the range varies from 22 to 46 h. This variation could be attributed to the breeds used in the studies and the type of progestogen employed (Alavez-Ramírez *et al.*, 2014; Arroyo-Ledezma *et al.*, 2013; Bruno-Galarraga *et al.*, 2021). The difference in the time periods established for estrus detection likely explains, at least partially, the results obtained; studies with the same frequency of estrus detection as conducted in this work describe similar results (35-42 h; Vilariño *et al.*, 2013). Additionally, another possible explanation could be related to variations in the products used in synchronization protocols; for example, it has been reported that the use of eCG after CIDR removal reduces the onset of estrus (Cox *et al.*, 2012).

On the other hand, the duration of estrus was shorter compared to studies of ewes treated with progesterone (55 h, Alavez-Ramírez *et al.*, 2014; 54 h, Arroyo-Ledezma *et al.*, 2013), although it was longer compared to Dorper ewes (24-29.5 h, Santos-Jiménez *et al.*, 2022). Again, this may be associated with differences in the frequency of estrus detection and even effects related to the breed studied.

The estrus response using a 12-day CIDR was higher than described for hair sheep under tropical conditions using intravaginal sponges (93%, Alavez-Ramírez *et al.*, 2014) and higher than for Dorper sheep with a 7-day CIDR (over 54%, Santos-Jiménez *et al.*, 2022). Additionally, the period of highest estrus response (33-57 h after CIDR removal) was similar to that observed by other authors (Santos-Jiménez *et al.*, 2022). Considering these data, the estrus response obtained with short-duration protocols can be considered appropriate for Pelibuey and Blackbelly genotypes under temperate climate conditions, as observed in their tropical regions of origin.

Perhaps more important than the estrus response is the conception rate achieved with synchronization protocols. This variable also showed good results for both protocols, with rates similar to or greater than those described in previous studies using progestogen protocols (69% in unspecified breed, Bruno-Galarraga *et al.*, 2021; 70% in Ghezel ewes, Hasani *et al.*, 2018; 24-33% in Santa Inês breed, Vinicius *et al.*, 2019). Differences that may

exist with other studies could be associated with genotype (Cox *et al.*, 2012), the number of parturitions in the ewe (Santos-Jiménez *et al.*, 2022), type of service (Vinicius *et al.*, 2019), and climate or time of year (Alavez-Ramírez *et al.*, 2014; De *et al.*, 2020).

Small ruminants are mostly seasonal breeders, showing an anovulatory period during long days (Arroyo, 2011). In this study, the good results obtained in the various reproductive indicators could also be attributed to the fact that the ewes were still in the breeding season when the treatments began. An evaluation of their reproductive status prior to the start of the study was not conducted, but the timing is similar to the period routinely used in the production unit for breeding. However, other studies have shown that hair sheep, such as the Pelibuey, can have a short anestrus period (Arroyo, 2011; Valencia *et al.*, 2001), which could also result in lower pregnancy rates in synchronized ewes, as naturally occurs in seasonal sheep (Habeeb *et al.*, 2021; Morris *et al.*, 2004). For this reason, future studies should consider evaluating the reproductive status prior to the use of estrus synchronization protocols. Prolificacy was not affected by any of the treatments, with results similar to those observed in some hair breeds (2.1 lambs in Pelibuey breed, Avendaño-Reyes *et al.*, 2007; 1.9 lambs in Pelibuey breed, Macías-Cruz *et al.*, 2012).

In general, studies do not show information on prolificacy due to the use of progestogens (CIDR) for short or long periods in hair sheep. However, in agreement with this study, dairy ewes treated with CIDR for 6 or 12 days also showed no differences between groups in the number of lambs born (Fleish *et al.*, 2013), which further supports the use of a short synchronization protocol in ewes.

The development of follicles in the ovary is an important aspect to consider in synchronization protocols, as differences in this development could help explain some of the responses to these protocols. In this study, when the devices were removed, the number of follicles was like what has been previously shown in ewes when removing vaginal sponges. The results also show a greater number of follicles compared to studies in ewes (breed not specified by the authors), 24 h after the CIDR was removed (approximately two follicles, Bruno-Galarraga *et al.*, 2021), although without indicating the total at the time of removal. The higher number of follicles observed in the ewes in our study may reflect the prolific condition known for hair breeds like the Pelibuey, predominant (79% in the PC and 83% in the PL) in this study (Aguilar-Martínez *et al.*, 2017).

The diameter of the largest follicle on the day the CIDR was removed was smaller than the follicular diameter described by other authors (greater than 7 mm) in Santa Inês ewes (Oliveira *et al.*, 2016), which could be related to the breed of the ewe or the timing of the ultrasound to determine follicle size. In this study, progesterone concentration in the ewes was not determined; however, it is possible that the larger diameter observed in the ewes treated with long-duration protocols is due to low progesterone concentrations that can occur from 6 or 7 days after CIDR insertion (Arroyo-Ledezma *et al.*, 2013). These low concentrations would allow a higher frequency of luteinizing hormone secretion, which would promote follicular development (Arroyo, 2011). Some studies also describe something similar, ewes treated with intravaginal sponges impregnated with medroxyprogesterone for 6 days had a smaller follicular diameter than those treated for 9 or 12 days (Texeira *et al.*, 2016). In fact, it has been speculated that the decrease in progesterone concentration can

affect follicular turnover and stimulate the presence of persistent follicles (Vilariño *et al.*, 2013). Under the conditions of this study, the larger follicular diameter in ewes with the long-duration protocol did not represent an advantage in the most important reproductive indicators, such as estrus rate and conception rate. Other studies in ewes have described the strong involvement of plasma progesterone concentrations on some indicators of follicular development, such as the shortening of the growth phase of ovulatory follicles and the maximum follicular diameter, but with no consequence on the ovulation rate (Oliveira *et al.*, 2016). In this way, although there are some effects of progesterone concentrations on indicators of follicular development, it does not seem to affect the reproductive response of ewes subjected to the estrus synchronization protocols used in this study, which should be confirmed in future studies.

CONCLUSIONS

In conclusion, short-duration estrus synchronization protocols based on progesterone showed good results in both estrus presentation and pregnancy rate. The different indicators obtained support their use in hair sheep outside tropical regions, under temperate conditions. Additionally, there could be other potential benefits with the use of short-duration protocols, such as reducing production costs, progressing towards minimizing the prolonged use of exogenous hormones for reproductive management, and the possibility of using a CIDR for at least a second application. This is because the devices might maintain a higher concentration of progesterone after removal and using them more than once divides the cost.

ACKNOWLEDGMENTS

The authors thank INIFAP for funding this study through the project with number SIGI 154082642.

The Research Station “Altos de Jalisco”, now known as “Centro Altos de Jalisco” was established in 1974. We acknowledge the institution and its dedicated personnel for their unwavering support and valuable contributions that have benefitted the people of Mexico, marking a significant milestone over decades. This manuscript stands as our tribute, commemorating 50 years of their remarkable achievements.

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