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Short-term protocols for the estrus synchronization in hair sheep in Campeche, Mexico

Protocolos cortos para la sincronización del estro en ovejas de pelo en Campeche, México

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ABSTRACT

Estrus synchronization is a strategy to improve the reproductive efficiency of the herd. The objective was to compare different dwells times of an intravaginal progestogen (CIDR®) with a dose of PGF2α in the synchronization to estrus of hair sheeps. Thirty female hair sheeps were used, randomly assigned to one of three treatments. T1: intravaginal progestogen (PI) for 5 days (PI5; *n*=10), T2: PI for 7 days (PI7; *n*=10) and T3: PI for 9 days (PI9; *n*=10). The incidence and onset of estrus, parturition and prolificacy were observed. Data analysis was performed using the life time method, logistic regression models and negative binomial models. The incidence at estrus was not different (p> 0.05) among treatments (T1: 80.0, T2: 100.0 and T3: 100.0%). Onset to estrus was lower (p ≤ 0.0) for T1 (29.8 ± 2.9 h) compared to the rest of the treatments. No differences (p> 0.05) were observed for parturition (T1: 70.0, T2: 90.0 and T3: 90.0%) and prolificacy (T1: 1.4 ± 0.3, T2: 1.4 ± 0.3 and T3: 1.5 ± 0.3). It is feasible to use short synchronization protocols based on intravaginal progestogens (CIDR®) with a dose of PGF2α and maintain the reproductive efficiency of the herd.

Keywords: CIDR, progesterone, devices, fertility, ewes.

RESUMEN

La sincronización de estros es una estrategia para mejorar la eficiencia reproductiva del rebaño. El objetivo fue comparar diferentes tiempos de permanencia de un progestágeno intravaginal (CIDR®) con una dosis de PGF2α en la sincronización al estro de ovejas de pelo. Se utilizaron 30 hembras de pelo, asignadas al azar a uno de tres tratamientos. T1: Progestágeno intravaginal (PI) por 5 días (PI5; *n* = 10), T2: PI por 7 días (PI7; *n* = 10) y T3: PI por 9 días (PI9; *n* = 10). Se observó la incidencia e inicio al estro, parición y prolificidad. El análisis de datos se realizó mediante el método de tiempos de vida, modelos de regresión logística y binomial negativa. La incidencia al estro no fue diferente (p > 0.05) entre tratamientos (T1: 80.0,

T2: 100.0 y T3:100.0 %). El inicio al estro fue menor (p ≤ 0.0) para T1 (29.8 ± 2.9 h) en comparación con el resto de los tratamientos. No se observaron diferencias (p > 0.05) para parición (T1: 70.0, T2: 90.0 y T3:90.0 %) y prolificidad (T1: 1.4 ± 0.3, T2: 1.4 ± 0.3 y T3: 1.5 ± 0.3). Es factible utilizar protocolos cortos de sincronización basados en progestágenos intravaginales (CIDR®) con una dosis de PGF2α y mantener la eficiencia reproductiva del rebaño.
Palabras clave: CIDR, progesterona, dispositivos, fertilidad, ovejas.

INTRODUCTION

In Mexico, most sheep farms are characterized by low reproductive and therefore productive indexes (Hernández-Marín *et al*., 2017), being reproduction a determining factor of profitability and long-term sustainability, which generated, the need to develop different hormonal treatments aimed at regulating reproductive activity, making a reduced use of hormones, contributing to environmental safety and having an acceptable reproductive efficiency.

In this sense, estrus synchronization with progestogens by means of sponges or intravaginal devices are the most used in these protocols, which associated with artificial insemination or controlled mating schemes, turn out to be a tool that improves the reproductive and productive efficiency of herds (Marques *et al*., 2010). However, most of these protocols are traditionally applied for periods of 12 to 14 days in order to imitate the normal luteal phase of a sheep (Viñoles *et al*., 2001; Olivera-Muzante *et al*., 2011; Swelum *et al*., 2015). Long protocols with progesterone and its synthetic analogues have been reported to have several effects on circulating levels of progesterone, resulting in prolonged persistence and aging of ovulatory follicles (Menchaca & Rubianes, 2002), due to a low concentration of progesterone (P_4) towards the treatment end that can negatively affect fertility. Due to the suppressive effect of long-term progestogen treatment on subsequent fertility (Viñoles *et al*., 2001), treatments that decrease progestogen use time and achieve estrus synchronization without decreasing reproductive efficiency are being sought.

It has been reported that short-term protocols with progesterone promote follicular turnover with the appearance of a new follicular wave in 3 or 4 days in most ewes (Vilariño *et al*., 2013), reducing the exposure of the preovulatory follicle to low concentrations of progesterone in serum during prolonged periods, which favors the ovulation of a younger follicle and an oocyte of better quality to be fertilized.

Currently, there are variations in drugs used and the exposure time of progestogens in short treatments, all with the purpose of improving reproductive variables and shortening the management time. Therefore, the aim of this study was to compare different residence times of an intravaginal progestogen (CIDR®) with a dose of prostaglandin F2 alpha (PGF2α) two days before withdrawal, in the synchronization of estrus of hair sheep in Campeche State, Mexico.

MATERIAL AND METHODS

The present study was conducted according to regulations for the use and care of animals destined for research at the Postgraduate College, Mexico (COLPOS, 2016) and in accordance with the Mexican Official Standard NOM-024-ZOO-1995 (SAGARPA, 2015).

Location

The research was conducted during August 2019 to January 2020 months. At the Agricultural Ranch "La Unión" located in Xcampeu, San Francisco de Campeche municipality, Campeche, Mexico. The study site is located at an altitude of 8 meters above sea level, between the geographical coordinates 19º 47' 53" N and 90º 23' 49" W. The predominant climate is warm sub-humid with rainfall in summer, average temperature of 28º C and rainfall of 1200 mm (García, 2004).

Animals and treatments

Thirty Pelibuey x Black Belly, crossbred hair sheeps with an average age of 1 year, weight of 35 (± 2.4) kg and a body condition of 2 (± 0.2) units on the Russel *et al*. (1969) scale were used. Ewes were randomly distributed to one of the following treatments: T1: Intravaginal progestogen for 5 days (PI5; *n* = 10), T2: Intravaginal progestogen for 7 days (PI7; *n* = 10) and T3. Intravaginal progestogen for 9 days (PI9; *n* = 10), where "n" indicates the number of individuals in the treatment.

Lodging and feeding

The ewes were housed in three earth-floored pens, with 2 m^2 space per animal. Feeding was in a semi-stabled system with 4-hour daytime grazing (6:00 to 10:00 h) within paddocks established with invasive wiregrass (*Dichanthium annulatum*). Afterwards, they were supplemented twice a day in the pen with 300 g^{-1} ewe^{-1} of a sorghum-based balanced feed (Campi® borrego; with 15% CP and 3 Mcal of ME kg of DM^{-1}). In addition, maralfafa grass (*Pennisetum purpureum*) silage was offered as a supplement with 2 kg ewe^{-1} of green forage ration. Mineral salts (Campi® Sales) and water were freely available.

Synchronization protocol

The ewes were synchronized to estrus with an intravaginal device (CIDR®, Pfizer) with 0.3 g of progesterone, with permanence according to treatment, starting with ewes with a 9-day protocol and consecutively in descending order. Two days before the withdrawal of the device, one mL of a PGF2α analog (5 mg Dinoprost tromethamine, Lutalyse® Zoetis) was applied intramuscularly. Removal of the progesterone device was on the same day for all ewes, starting the detection of estrus at 4 h intervals for 25 min until 80 h. A ewe was considered to have failed to respond to treatment if she did not show estrus during this time. For the detection of estrus, two Black Belly rams with an average age of 2 years,

provided with an apron, were used. A ewe was considered to be in estrus when she remained immobile and allowed the ram to mount her. Subsequently, 12 h after estrus was detected, ewes were covered by controlled mating, using for this stage two Black Belly rams with an average age of 3 years, which were represented in all treatments and whose seminal quality had been previously verified by macroscopic and microscopic tests.

Variables and statistical analysis

The response variables in the study were: estrus incidence, onset of estrus, lambing and prolificacy. Incidence at estrus was calculated as the number of ewes that showed external signs of estrus divided by the total number of ewes in the treatment. Onset of estrus was calculated as the time elapsed between removal of CIDR® and onset of estrus in ewes. Lambing was calculated as the number of ewes that lambed after the first service out of the total number of ewes in the treatment and prolificacy was measured as the number of lambs born per lambing ewe. The analysis of the variable onset at estrus was carried out using the life time method and differences between treatments were established with the Long-Rank test. The determination of the incidence at estrus and lambing were analyzed with the logistic regression model. Negative binomial regression was used for prolificacy analysis. All analyses were performed using SAS/STAT (SAS, 2002) and with a significance level $\alpha = 0.05$α.

RESULTS AND DISCUSSION

No difference ($p > 0.05$) was observed for the estrus incidence variable between treatments (Table 1). Rubianes *et al*., (1998) mention that the insertion of an intravaginal device containing progesterone (CIDR®), causes an increase in serum progesterone concentrations (>5 ng/mL) for three or four days, which is higher than the concentrations observed physiologically during the middle of the luteal phase, which triggers a positive effect on follicular growth, increasing the number of large follicles with greater estradiol secretion and with potential to ovulate. This suggests that in this study, variations in P_4 release by the device were similar between treatments and supports the idea that short-term progestogen-based protocols (CIDR®, P_4) are effective in inducing estrus in ewes. Similar studies report 96 and 100% incidence to estrus using 5 and 9 day protocols respectively with a PGF2α application in ewes (Fraire-Cordero *et al*., 2013; Jackson *et al*., 2014) and 100% with 9 d protocols and an application of equine chorionic gonadotropin (eCG) (Nakafeero *et al*., 2020).

Table 1. Reproductive variables in hair ewes synchronized with short protocols based on an intravaginal progestogen (CIDR®) and prostaglandin

	T1:PI5	T2:PI7	T3:PI9
Total of animals	10	10	10
Incidence of estrus (%)	80.0 a	100.0 a	100.0 a
Onset of estrus (h)	29.8 ± 2.9 a	38.6 ± 3.1 b	39.9 ± 4.4 b
Calving (%)	70.0 a	90.0 a	90.0 a
Prolificacy (Mean ± ES)	1.42 ± 0.3 a	1.44 ± 0.3 a	1.55 ± 0.3 a

[a, b].Different letter within each row indicates difference (p ≤ 0.05). PI5: Intravaginal progestin for 5 days. PI7: Intravaginal progestogen for 7 days. PI9: Intravaginal progestogen for 9 days.

The ewes that did not respond at T1 it is suggested may be related to the fact that some ewes are in the early luteal phase of the estrous cycle at the time of prostaglandin injection, so animals with corpus luteum development at this stage would not respond to prostaglandins (Fierro *et al*., 2013) and would continue to have an estrous cycle of normal length after device removal. Whereas ewes with *corpus luteum* in a more advanced stage would respond to prostaglandin treatment by having a normal follicular phase and, subsequently estrus and ovulation, which possibly occurred for T2 and T3.

The average onset of estrus of ewes was 36.1 ± 3.5 h, T1 ewes initiated estrus in a shorter time (p ≤ 0.05) (Table 1) and better synchronized than the rest of treatments as observed in Figure 1, so it could influence the choice of a desirable protocol for estrus synchronization, when the objective is to use fixed-time insemination. Synchrony could be related to the dynamics of the preovulatory follicle. Authors such as Viñoles *et al*. (2001) and Martínez-Ros *et al*. (2018) mention that follicles that do not have as much contact with progesterone in short-term treatments, it is observed that the newly recruited follicle has larger size, linear growth and higher estradiol concentration thus promoting better estrous behavior.

In studies by Martínez-Ros *et al*. (2018) and López *et al*. (2021) using 6- and 7-day protocols with intravaginal sponge and a dose of PGF2α at withdrawal, they found an onset to estrus of 58.3 ± 10.0 h and 48.0 ± 16.9 h, respectively, being higher than those found in this study. As well as to the 43.6 and 40.3 h reported with similar protocols with 9 d in Pelibuey ewes (Fraire-Cordero *et al*., 2013; Sosa-Pérez *et al*., 2014) and differing to that reported by Nakafeero *et al*. (2020) who report a shorter estrus interval in ewes with CIDR for 14 d (24.9 ± 1.6 h) compared to CIDR at 9 days (30.8 ± 2. 1 h) suggesting that in short protocols the onset of estrus is usually delayed since there is a growing follicle, so it still takes some time to trigger ovulation after progestogen withdrawal, while in long protocols progesterone levels are falling and when the device is removed, it

ovulates and triggers signs of estrus more quickly. However, the conditions were different from those in this study.

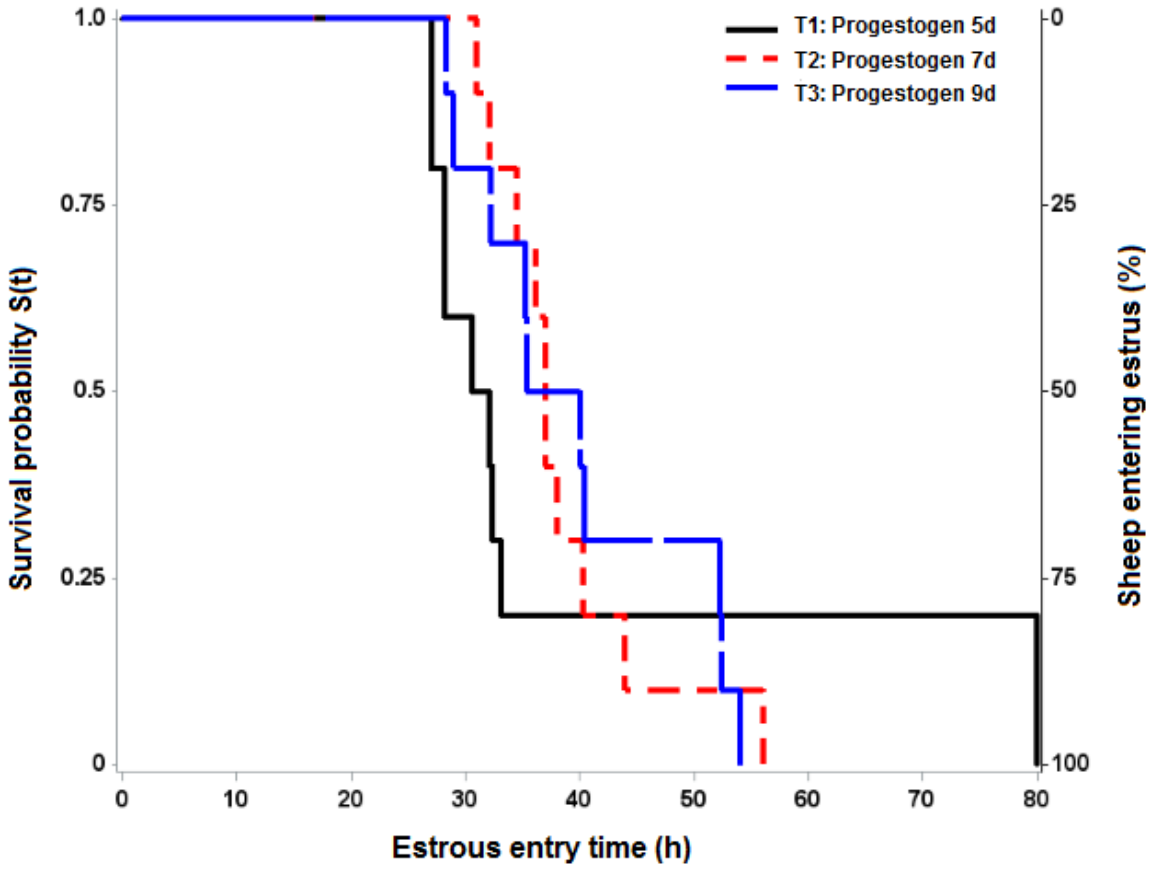

Figure 1. Survival curves of time to estrus onset in hair sheep synchronized with short protocols based on an intravaginal progestogen (CIDR®) and prostaglandin

The average lambing was higher than 83%, being higher than that reported in ewes with protocols where P_4 was used in combination with PGF2a at device removal for five and seven days (83 and 16%) (Martínez-Ros *et al*., 2019) and for nine days with a similar protocol in ewes (50%) (Fraire-Cordero *et al*., 2013). No significant differences ($p > 0.05$) were found between treatments for the percentage of ewes that lambed between treatments (Table 1), apparently, the different estrus synchronization protocols were able to generate corpus luteum with sufficient P_4 production that allowed implantation and embryonic development. Viñoles *et al*. (2001) mention that a higher parturition can be attributed to the ovulation of high quality young follicles, where follicles that have not had much time in contact with progesterone, it is observed that the follicle earlier in the cycle

has a larger size, grows in a more linear way, so it is a follicle that has high levels of estradiol and, at the moment it ovulates and insemination or mating is performed, it will have more possibilities of carrying a gestation to term.

For prolificacy, no differences were found between treatments ($p > 0.05$) as shown in Table 1, results were expected since no hormones were used to stimulate the ovarian response promoting multiple deliveries such as porcine follicle stimulating hormone or eCG. The average of this variable for the flock was 1.47 ± 0.2, higher than the 1.3 ewe lambs-1 reported with a short protocol with intravaginal sponges and eCG (López *et al*., 2021). However, no differences in this variable were detected in this study.

CONCLUSION

Under the conditions of the study, it is feasible to maintain herd reproductive efficiency with the use of short synchronization protocols based on intravaginal progestogens with CIDR® for five, seven or nine days associated with an injection of PGF2α two days prior to withdrawal.

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