

Reusing intravaginal progesterone releasing devices for oestrous synchronization in ewes

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Abstract

In this study, the second use of an intravaginal progesterone-releasing device or controlled intravaginal drug release device (CIDR) was evaluated. After a first use of 11 days, the CIDR was again used for either nine or 12 days with 200 or 300 IU equine chorionic gonadotrophin (eCG) being injected on its removal. Sixty-four ewes were randomly distributed to four treatments (n=16/group): CIDR9+eCG200, CIDR9+eCG300, CIDR12+eCG200, and CIDR12+eCG300. The eCG was administered intramuscularly on withdrawal of the device. Thus, the experiment was a completely randomized design with a 2x2 factorial arrangement of treatments. Oestrus presentation did not differ between treatments ($P=0.29$). However, with the dose of 200 IU of eCG, oestrus presentation tended to increase ($P=0.08$). The onset and duration of oestrus, percentage of gestation, and return to oestrus did not differ between treatments ($P>0.05$). Progesterone concentration in serum was greater ($P<0.05$) in ewes treated with CIDR12+eCG300. Prolificacy was greatest (1.44) with the CIDR12+eCG300 treatment and was different ($P=0.001$) from the treatments CIDR9+eCG200 (1.21) and CIDR9+eCG300 (1.20), but not from the CIDR12+eCG200 treated ewes (1.31). The CIDR12+eCG300 treatment produced the highest percentage of twin births (45.8%) ($P=0.001$). Leaving the device in place for 12 days increased ($P=0.001$) the incidence of twin births. Use of the CIDR for a second time synchronized oestrus in ewes successfully with better fertility being obtained when the device was left in place for 12 days, and 300 IU of eCG was injected on its removal.

Key words: gonadotropin, progesterone device, synchronization

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Introduction

Oestrous synchronization has been used for a long time. However, with the increase in knowledge of the dynamics of follicular development (Ginther *et al.*, 1995), experiments have been focused on concentrating the oestrus period to a shorter time if artificial insemination is desired. On the other hand, with the increasing demand to be environmentally friendly and with the costs to the producers in mind, efforts have focused on reusing progesterone-releasing devices without affecting their efficiency, and making their use more affordable.

To improve the profitability of intensive sheep production, it is necessary to increase reproductive efficiency by increasing fertility, prolificacy and the frequency of births. Reproductive seasonality in sheep is a limiting factor to achieving the goal of three parturitions in two years (Rosa *et al.*, 2003). To overcome this limitation various reproductive strategies have been studied. These include treatments with exogenous hormones and their synthetic analogues, melatonin implants either separately or in combination (Abecia *et al.*, 2011). These treatments induce and synchronize ovulation and oestrus in the reproductive period or during the periods of reproductive transition and anoestrus with varying degrees of success (Abecia *et al.*, 2012; Laven, 2019).

Controlled intravaginal release devices with natural progesterone (P_4) stimulate the induction and synchronization of oestrus for a prolonged time (from 12 to 14 days) in ewes (Wheaton *et al.*, 1993; Escobar *et al.*, 2017; Menchaca *et al.*, 2017). This increases the percentage of ewes in oestrus, but with variable effects on fertility (Menchaca & Rubianes, 2004). Controlled intravaginal release devices provide a high serum P_4 concentration immediately after insertion because of their efficient kinetic release, which falls dramatically after the removal of the device, but with no cumulative effect (Mohan, 2017). Prolonged exposure to P_4 in hormonal procedures normally increases the diameter of the ovulatory follicle, but decreases the number of ovulatory follicles (Bartlewski *et al.*, 2017). To reduce the negative effect of long procedures, reproductive effectiveness has been evaluated with medium (8 to 10 days) and short (5 to 7 days) protocols using intravaginal devices to synchronize oestrus in ewes (Martinez-Ros *et al.*, 2019b; Swelum *et al.*, 2016; Swelum *et al.*, 2018a).

For progesterone treatment to be effective, an adequate amount of gonadotropin is necessary to trigger preovulatory events, so when the CIDR device is removed, eCG is injected to result in similar functions to follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Murphy, 2012; Martinez-Ros *et al.*, 2019a). Equine chorionic gonadotrophin can also be used to accelerate puberty, induce oestrus, improve fertility, induce multiple ovulation for embryo transfer procedures, and induce a greater synchrony of ovulation in sheep (Ramos & Silva, 2018). The application of eCG after the removal of the CIDR ensures the occurrence of fertile ovulations (Martinez-Ros *et al.*, 2019b; Uriol *et al.*, 2019), reduces the interval from oestrus to ovulation, and increases the corpus luteum (CL) diameter (Cox *et al.*, 2012). Generally, recommended doses of eCG vary from 250 to 500 IU (Barret *et al.*, 2004), depending on weight, age (250–300 in young ewes, 350 - 500 IU in adult ewes), season (400 - 500 IU in anoestrus, 300 - 500 IU in cyclic ewes) and breed (lower doses in prolific breeds) (Abecia *et al.*, 2011).

Controlled intravaginal drug release devices are effective in inducing and synchronizing oestrus in sheep. An addition, they can release limited amounts of P_4 for a longer period of time than necessary for synchronization and due to their physical characteristics the devices can be disinfected and sterilized (Ungerfeld *et al.*, 2013; Chacher *et al.*, 2017). However, they are costly compared with sponges, which limits their generalized use in commercial farms, and having to be disposed of after the first use causes environmental contamination owing to the residual hormone they contain (Biehl *et al.*, 2019). For this reason, research has been conducted to determine the efficiency of induction and synchronization of oestrus with reused CIDRs in anoestrus sheep and during the reproductive season (Güngör *et al.*, 2009; Pinna *et al.*, 2012; Swelum *et al.*, 2018b). Likewise, their use has been evaluated in short experiments (Cox *et al.*, 2012; Vilariño *et al.*, 2013; Bazzan *et al.*, 2013) and for multiple times (Da Silva *et al.*, 2014; Swelum *et al.*, 2015; Swelum *et al.*, 2018b). The devices seem to be effective in the second or third use, mainly when combined with eCG. However, there is no consensus in the literature that short procedures of oestrus synchronization improve the pregnancy rate compared with longer ones. For example, Blaschi *et al.* (2014) obtained a high pregnancy rate with long protocols (83.3%) (14 days) compared with short ones (47.8%) (5 days) and with medium duration (60%) (9 days). On the contrary, Viñoles *et al.* (2001) reported greater pregnancy rate with a short protocol (87%) (6 days) compared with a 12-day treatment (67%).

Therefore, the objective of this study was to evaluate the effects on the main reproductive variables and serum progesterone concentration of primal ewes of the reuse of the CIDR device with two periods of duration (9 and 12 days) and two doses of eCG (200 and 300 IU).

Material and Methods

The study was performed in July 2018, during the season of reproductive transition (light/dark hours 13L/11D), at the experimental farm of the Colegio de Postgraduados, Campus Montecillo, México, at 19° 29' N, 98° 53' W and at 2250 metres above sea level. The climate is mild, with an average annual rainfall of 632.5 mm in summer and an annual average temperature of between 12 °C and 18 °C (García, 2004). The handling of the animals was carried out according to the standards of Animal Welfare and Ethics of the Colegio de Postgraduados, México, in compliance with the Official Mexican Standard NOM-062-ZOO-1999 (SAGARPA, 2001).

In the present study, 64 primal ewes (13 months old) were used. These were a Katahdin × Suffolk hybrid, with 54 ± 3.1 kg of initial bodyweight, having been dewormed with Ivomec® (1 mL per 50 kg of bodyweight) subcutaneously and supplemented with Vigantol ADE® (1 ml) intramuscularly, with a body condition score of 3 in a score of 1 to 5. During the experiment, all ewes were fed daily with a diet based on oat hay (*Avena sativa*), alfalfa hay (*Medicago sativa*) and 250 g commercial concentrate with 14% crude protein (CP). Water and mineral salts were offered ad libitum.

A device that consisted of silicon elastomers (CIDR®, Zoetis, México) impregnated with 0.3 g natural P_4 was inserted intravaginally in each ewe. The devices had previously been used in an 11-day synchronization protocol two months before the present study at the same experimental farm. The devices

were washed with purified water after first use, dried and placed in airtight bags for refrigerated storage. One day before their second use they were exposed to room temperature to avoid moisture in the devices. At the time that the device was inserted, the vulva area was cleaned and bactericidal ointment (Furacine[®], PiSA, México) was applied to the applicator to prevent infection.

The ewes were randomly distributed in four treatments ($n = 16/\text{group}$), namely CIDR9+eCG200, CIDR9+eCG300, CIDR12+eCG200 and CIDR12+eCG300, which combine the days of duration of the device (9 or 12 days) and the dose of eCG (200 or 300 IU). The eCG hormone (Folligon[®] Intervet, México) was injected intramuscularly when the device was removed.

Oestrus detection began at 24 hours after the removal of the CIDR with controlled mating with males of proven fertility. They were subsequently monitored every six hours for 72 hours to determine the duration and end of the oestrous. The ewes received three mounts: the first at the start of oestrus, and two later at 12-hour intervals. The return to oestrus was detected in two periods (morning and afternoon) from 15 to 18 days after mating with fertile males. The ewes that returned to oestrus received three more mounts. Pregnancy was diagnosed 30 and 60 days after the mount with a Sonovet 600[®] ultrasound with a 7.5 MHz linear transrectal transducer to observe embryonic structures (Figure 1). To determine the pregnancy rate, the number of ewes that were diagnosed as pregnant was divided by the number of ewes that had been mounted. The prolificacy index was obtained by dividing the number of lambs born by the number of pregnant ewes and the type of birth by dividing the number of single or twin lambs by the number of pregnant ewes.

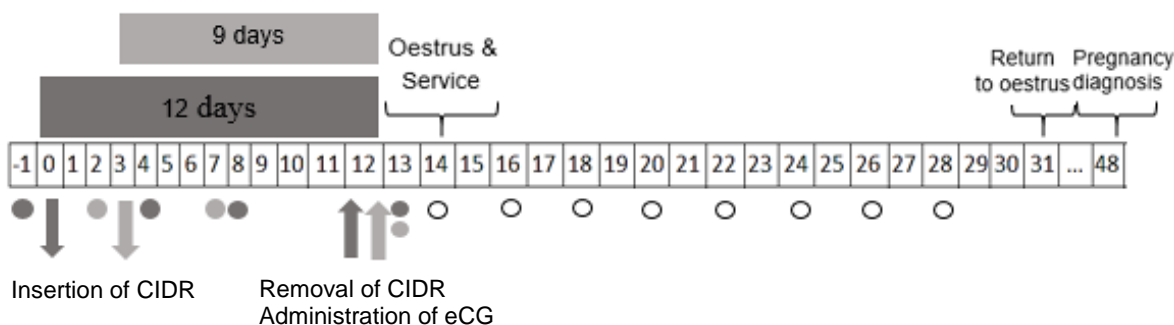


Figure 1 Experimental protocol for oestrous synchronization in ewes with a reused controlled intravaginal release device and equine chorionic gonadotrophin

○ blood sample collection; CIDR: controlled intravaginal release device; eCG: equine chorionic gonadotrophin

Blood samples were collected from 10 ewes per treatment to determine the P_4 levels. Samples were collected in polypropylene tubes (5 mL) by puncturing the jugular vein at 8h00, one day before the insertion of the CIDR, on days 2, 4, 7, and 8, two days after the device was removed, and subsequently every 48 hours. All samples were centrifuged at 1500 g for 20 min at 4 °C to separate the blood serum, which was stored in Eppendorf tubes at -20 °C until analysis. To determine the P_4 concentration, a radioimmunoassay was carried out with the commercial kit PROG-CTRIA[®] (Cisbio International, France) with an intra and inter assay coefficient of variation of 4.1 and 8.7, respectively, and a sensitivity of 0.05 ng mL⁻¹.

The experimental design was completely randomized with a 2 × 2 factorial arrangement, in which the main effects were the duration of use for the reused CIDR (9 and 12 days) and the level of eCG (200 and 300 IU). Data analyses were conducted using SAS, version 9.1 (SAS Institute, Inc., Cary, North Carolina, USA). The variables of oestrous onset, return to oestrus, percentage of pregnancy, prolificacy index and type of birth were analysed with an X^2 test using PROC FREQ. Time to the beginning of oestrus and its duration, were analysed with PROC GLM and Tukey's test was used to assess differences between the treatment means. The P_4 concentrations were analysed with PROC MIXED and the Tukey test was performed to determine the differences between treatment means ($P \leq 0.05$).

Results and Discussion

Oestrus response did not differ between treatments ($P = 0.285$). However, with the dose of 200 IU of eCG it tended ($P = 0.078$) to increase (Table 1). The high percentage of ewes that showed oestrus behaviour (95.31%) in response to the reused CIDR suggests that in the second use CIDRs still contain enough P_4 to cause LH blockage and allow the brain to respond to concentrations of circulating oestradiol, so that

oestrous behaviour can be induced in the female (Swelum *et al.*, 2018b). Previous studies have shown that when associated with eCG and a luteolytic agent, use for the third time in short protocols is viable for the synchronization and induction of oestrus and ovulation in ewes without affecting the percentages of oestrus presentation (Pinna *et al.*, 2012; Bazzan *et al.*, 2013; Vilariño *et al.*, 2013; Da Silva *et al.*, 2014) since the eCG favours follicular growth and the consequent production of oestradiol (Murphy, 2012).

Cox *et al.* (2012), using a seven-day protocol with reused CIDR with an injection of 0.125 mg cloprostenol and 350 IU eCG on the removal of the device, obtained 95.7% of ewes that showed oestrus in the reproductive season. These results differ from those reported by Güngör *et al.* (2009), who obtained 66.67% of females in oestrus when evaluating reused CIDR for 12 days plus an injection of 500 IU of eCG in multiparous ewes in anoestrous. They also differ from those of Biehl *et al.* (2019), who found 70% of oestrus of in cyclic multiparous ewes with reused CIDR in protocols of 7 or 11 days plus the application of 300 IU of eCG and 6.70 mg of Dinoprost on withdrawal of the device. Although a luteolytic agent was not used at the end of the treatment in the present study, the high percentage of oestrous response could be attributed to the use of eCG, because a greater percentage of ewes (85.94%) showed P₄ levels greater than 1ng/mL at the beginning of the treatments, suggesting that most ewes were in a regular ovarian cycle.

Table 1 Reproductive variables in primal ewes treated with reused controlled intravaginal release device (9 d or 12 d) and equine chorionic gonadotrophin (200 or 300 IU) at device removal

Response variable	Treatments				P-value		
	CIDR9 + eCG200 (n = 16)	CIDR9 + eCG300 (n = 16)	CIDR12 + eCG200 (n = 16)	CIDR12 + eCG300 (n = 16)	CIDR	eCG	CIDR x eCG
Oestrus response (%)	100	87.50	100	93.75	0.285	0.577	0.078
Oestrus onset (h) ¹	32.62±2.18	30.00±2.35	31.12±2.73	30.80±2.50	0.495	0.792	0.215
Oestrus duration (h) ¹	36.37±3.19	34.71±2.67	37.12±2.94	35.20±3.56	0.610	0.574	0.227
Return of oestrus (%) ²	12.50	18.75	6.25	6.25	0.629	0.269	0.691
Gestation (%) ³	87.50	81.25	87.50	93.75	0.771	0.453	1.000
Lambing rate	1.21 ^{bc}	1.20 ^c	1.31 ^{ab}	1.44 ^a	0.001	0.001	0.823
% Single births	78.57 ^a	66.66 ^{ab}	56.25 ^b	56.25 ^b	0.001	0.001	0.072
% Twin births	21.43 ^b	26.66 ^b	37.50 ^{ab}	43.75 ^a	0.001	0.001	0.044

^{a,b,c} Within a row, values with a common superscript do not differ with ($P=0.05$)

¹ Mean ± SE of onset and duration of oestrus after withdrawal of the device

² Percentage of ewes in oestrus at 17 days after natural service

³ Based on progesterone profiles in serum and ultrasound at 30 days after natural service

CIDR9 + eCG200: controlled intravaginal release device (CIDR) reused 9 d and 200 IU equine chorionic gonadotrophin (eCG), CIDR9 + eCG300: CIDR reused 9 d and 300 IU eCG, CIDR12 + eCG200: CIDR reused 12 d and 200 IU eCG, CIDR12 + eCG300: CIDR reused 12 d and 300 IU eCG

The onset and the duration of oestrus did not differ between treatments ($P>0.05$), showing at 30 to 32 hours and lasting from 34 to 36 hours after the device was removed. These results differ from those recorded by Pinna *et al.* (2012), who obtained an oestrus interval of 46.1 ± 14.1 hours and a duration of oestrus of 52.5 ± 1.2 hours in ewes in anoestrous with reused CIDR for five days plus 5 mg dinoprost and 300 IU of eCG one day before removal of the device. Although there was no significant difference owing to the effect of the eCG dose, ewes that received 300 IU eCG showed an earlier and more compact oestrous synchrony. The addition of eCG to the new or reused CIDR protocols reduces the interval between heat and ovulation and increases the diameter of the CL on day 10 after heat (Cox *et al.*, 2012). However, the variation in ovarian response after treatment with eCG in reproductive programmes in sheep is influenced by differences in doses, breed, nutritional level, seasonal variation, ovarian status and time of application of eCG (Shahneh *et al.*, 2006; Rad & Farzaneh, 2007; Moakhar *et al.*, 2012).

For the duration of the reused CIDR devices (imitation of the luteal phase), the concentration of P₄ was higher ($P<0.05$) with the CIDR12+eCG300 treatment compared with the other groups. In all treatments, P₄ concentration was maintained above 1 ng/mL and then decreased at oestrus (experimental day 14). Subsequently, P₄ concentrations were higher ($P<0.05$) with the CIDR12+eCG300 treatment only during the

first five days after breeding (presence of functional CL). The high P₄ concentration in treatments with a duration of 12 days compared with nine days was probably because of a higher concentration of endogenous P₄ at the beginning of the protocol and a higher rate of absorption of exogenous progestogen, which was reflected in better fertility. In addition, there is evidence of a relationship between P₄ concentrations during treatments, with the ovarian follicular turnover and fertility in sheep (Vilariño *et al.*, 2013).

Elevation of serum P₄ concentration, which is influenced by the release of P₄ from the device, causes persistent follicle regression and inhibits the release of gonadotropins from the anterior pituitary gland, allowing blockage of oestrus behaviour and ovulation until withdrawal of the device (Swelum *et al.*, 2018b). Subsequently, the inhibition disappears on the removal of the device, which causes the stimulation of the gonadotropin-releasing hormone pulse from the hypothalamus, which influences the preovulatory increase of LH and FSH that results in the re-start of ovarian activity, which culminates in ovulation (Laven, 2019; Martínez-Ros *et al.*, 2019a).

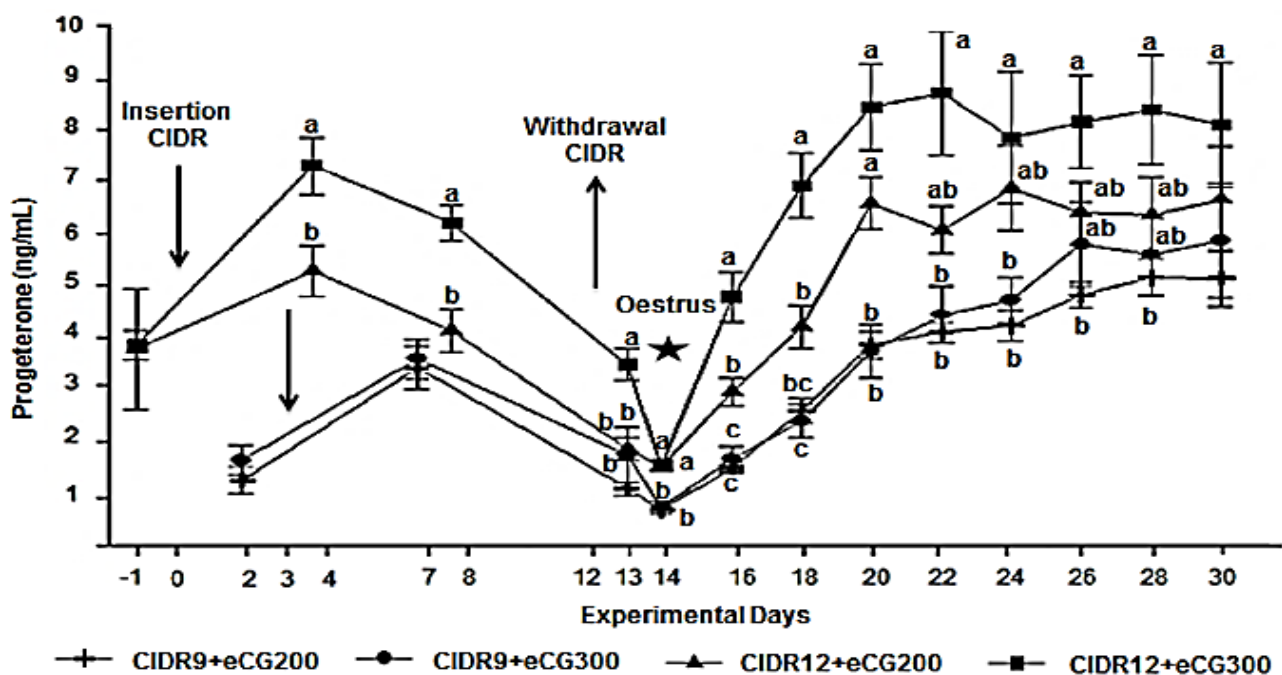


Figure 2 Progesterone concentration in serum from the insertion of the reused controlled intravaginal drug release device to its withdrawal and the posterior oestrus cycle in primal ewes

^{a,b,c} Within a day, means with a common superscript did not differ with $P=0.05$

CIDR: controlled intravaginal drug release, eCG: equine chorionic gonadotropin

CIDR9+eCG200: CIDR reused 9 d and 200 IU eCG, CIDR9+eCG300: CIDR reused 9 d and 300 IU eCG,

CIDR12+eCG200: CIDR reused 12 d and 200 IU eCG, CIDR12+eCG300: CIDR reused 12 d and 300 IU eCG

In previous work it was found that serum P₄ concentrations were lower with reused devices compared with new devices. However, devices on second and third use are as effective as new ones in synchronizing oestrus and ovulation when they are used in short-term protocols in ewes in the reproductive period and in anoestrus (Cox *et al.*, 2012; Pinna *et al.*, 2012; Vilariño *et al.*, 2013). On the other hand, Swelum *et al.* (2015), evaluated devices that were used up to six times in cyclic ewes during a short synchronization protocol, and found no differences in P₄ concentration between treatments, but obtained higher percentages of induced oestrus, pregnancy, fertility and twin births with new devices and those which were only used for a second time.

The percentage of pregnancies and return to oestrus did not differ between treatments ($P>0.05$) in the duration of the reused CIDR or the dose of eCG in primal ewes (Table 1). The ewes that showed oestrus at 17 days after natural service were served and became pregnant (except one in the CIDR9+eCG300

treatment), which suggests that although not all the ewes were pregnant in the first oestrus, they were prepared physiologically to ovulate a healthy follicle in the second oestrus. The average gestation percentage of all reused CIDR treatments was 87.50%, which was higher than those reported by Da Silva *et al.* (2014), who evaluated the application of first-, second-, and third-use CIDR in short protocols plus the injection of 0.263 mg of PGF_{2α} and 250 IU of eCG on removal (73.3%, 72.7%, and 64.7%, respectively) in cyclic ewes, and in those reported by Swelum *et al.* (2018b), who obtained 65% gestation in sheep with natural insemination in the reproductive period with CIDR devices of second use.

The results confirm what was reported by Biehl *et al.* (2019), who concluded that the gestation percentage is not affected by the reuse of the CIDR, in either long or short synchronization treatments when ewes are inseminated during the breeding season. They also support those of Güngör *et al.* (2009), who applying a long procedure with reused CIDR in ewes with natural mount in anoestrus. However, the percentage of pregnancy and fertility might be affected by the number of times that the devices have been used in the past (Swelum *et al.*, 2015; Swelum *et al.*, 2018b) and the type of breeding (natural or artificial insemination (AI)) (Pinna *et al.*, 2012).

In terms of prolificacy, the CIDR12+eCG300 treatment produced the highest (1.44) number of lambs per ewe which was significantly different ($P = 0.001$) from CIDR9+eCG200 (1.21) and CIDR9+eCG300 (1.20) treatments, but not different from the CIDR12+eCG200 group (1.31). However, with the CIDR used for 12 days prolificacy increased ($P = 0.001$) by 13% (1.37) relative to its use for nine days (1.20). These results can be attributed to the correct development of functional CL and the increase in P₄ secretion to provide the endometrium with the appropriate conditions during embryo implantation and for the maintenance of pregnancy. In the CIDR9+eCG200 treatment, the abortions of two lambs in two ewes (one lamb/ewe) were presented in the last third of pregnancy.

According to Vilariño *et al.* (2013), there is a relationship between P₄ concentrations during reused CIDR treatments, ovarian follicular turnover and fertility in ewes. In addition, Cox *et al.* (2012) consider that the increase in the size of the CL 10 days after oestrus in sheep treated with eCG is related to the ovulation of a greater number of healthy follicles. On the other hand, the synthesis of interferon tau (INT_T) by the embryo is positively correlated with P₄ production, so that high amounts of P₄ circulating in the female, induce maternal recognition of pregnancy by increasing INT_T production, which favours embryonic survival and development (Kerbler *et al.*, 1997).

In terms of twinning, the CIDR12+eCG300 treatment obtained the highest percentage ($P = 0.001$) with 43.75%. The lowest percentage was for the CIDR9+eCG200 treatment at 21.43%, with no differences between this treatment and CIDR9+eCG300 and CIDR12+eCG200 (26.66% and 37.50%, respectively). The 12 days of duration of the device influenced ($P = 0.001$) the incidence of twinning births and the 300 IU dose of eCG increased ($P = 0.044$) the presentation of twinning births. This contrasts with that reported by Swelum *et al.* (2018b), who obtained twinning deliveries with new CIDRs (42.9%) and CIDRs used for the second time (63.16%) in combination with 300 IU of eCG without statistical differences compared with devices that were used up to six times, which presented only single deliveries in sheep in the breeding season, which can be attributed to the greater number of mature ovulated follicles during oestrus. In addition, previous studies have shown that the characteristics of the ovulatory follicle and the subsequent luteal phase do not differ among sheep treated with first, second and third use of devices (Pinna *et al.*, 2012; Vilariño *et al.*, 2013). On the other hand, the addition of eCG to ewe synchronization programmes increases the ovulation rate, which triggers multiple births in a greater proportion (Murphy, 2012).

Conclusions

Use of a CIDR device for a second time in primal ewes results in sufficient release of progesterone to synchronize oestrus, which allows the use of CIDR devices to be maximized. The best results in reproductive efficiency in the prolificacy index and twinning deliveries were obtained when the reused CIDR was inserted for 12 days with 300 IU of eCG injected upon its removal.

Authors' Contributions

YIRS was an MSc student in the project. TST, JLCM, JLFV, JCZ, AMA, and MCL were responsible for the design and execution of the project. All co-authors participated in the management and discussion of the results, statistical analysis and writing, and correction of the manuscript. MCL ran the hormonal analysis.

Conflict of Interest Declaration

There are no conflicts of interest.

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