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Article

Estrus synchronization in ewes with a six-day protocol using new, second-use, third-use and fourth-use CIDR devices

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Abstract:

Estrus synchronization(is a vital strategy in sheep production and is accomplished most often via controlled internal drug release (CIDR) devices. Reuse of CIDR devices can reduce operating costs and ewe exposure to progesterone (P_4), but remains controversial. A comparison was made of the effects of new and reused natural progesterone-releasing intravaginal (CIDR) devices in six-day protocols on reproductive performance variables and blood serum progesterone concentrations in multiparous ewes. A total of 172 sheep (average body weight = 59 kg) were randomly distributed into four treatments: CIDR1 (control group, new devices), CIDR2 (second-use), CIDR3 (third-use) and CIDR4 (fourth-use). The variables ewes in estrus, pregnancy rate, fertility rate and parturition type did not differ ($P>0.05$) between treatments. Estrus onset was later ($P\leq0.05$) in CIDR1 (40 ± 10 h) than in

CIDR2 (31 ± 9 h). Ewes in estrus ranged from 93 to 100 %, average pregnancy rate was 80.8 %, average fertility rate was 80.2 % and the prolificacy index was 1.26. The single delivery rate was higher ($P \leq 0.05$) than the double delivery rate in all four treatments. Intravaginal CIDR devices can be used in ewes up to four times in six-day estrus synchronization protocols without affecting reproductive variables.

Keywords: CIDR reuse, Sheep, Reproductive variables.

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Introduction

In sheep, estrus synchronization protocols increase reproductive efficiency through hormone administration[1]. The aim is to modify the estrus cycle such that lambs are produced in uniform batches, thus reducing the costs of reproductive management. These protocols are traditionally implemented for 12 to 14 d using controlled internal drug release (CIDR) intravaginal devices (such as inserts and sponges) containing natural progesterone[2]. However, prolonged use of progestogens obstruct sperm transport[3] and modifies follicular dynamics[4].

Progestogens' negative effects can be reduced through short synchronization protocols[2], that is, application of CIDR devices for five to seven days. In addition, prostaglandin F_2 alpha ($PGF_2\alpha$) is administered to control luteal function, as is equine chorionic gonadotropin (eCG) to stimulate follicular development and ovulation by increasing both the number of follicles and the estrogen production rate of the stimulated follicles[4]. This produces a follicle with a greater capacity for fertilization during the reproductive season and seasonal anestrus[5].

The physical characteristics of CIDR inserts allow them to be washed and reused without reducing reproductive variables[6]. When short synchronization protocols of five[6,7] and seven days[8,9,10] are used in sheep, inserts retain a considerable amount of biologically-available progesterone (P_4) after withdrawal[3,11]. Reuse of CIDR inserts remains controversial. Some authors report reduced estrus and pregnancy synchronization rates with reused inserts[6,11], and others[3] have found higher fertility and fecundity rates with new CIDR inserts than with those used a second and third time. It has also been suggested that,

in inserts used for up to six times, reproductive variables begin to decrease beginning with the second reuse[3].

In contrast, there are reports that insert reuse has no effect on reproductive variables. One study comparing second- and third-use inserts to new inserts in six-day treatments found no differences in estrus synchronization rate and ovulatory response, although blood progesterone concentrations and pregnancy rates were lower in ewes treated with reused inserts[11]. Another study found that when used up to three times in six-day protocols, inserts produced similar pregnancy rates with both new and reused devices[12].

Short synchronization protocols theoretically allow for repeated CIDR insert use. This implies a decrease in production costs, a reduction in ewe exposure to progesterone (P_4) concentrations to half that recommended by the manufacturer, and the consequent possibility of using CIDR inserts with lower hormone content and without affecting reproductive performance. When CIDR inserts are used in two 12-d protocols, the main reproductive variables do not differ between the first and second uses because the inserts contain sufficient P_4 to maintain luteotropic function and synchronize estrus in sheep[13]. Based on this result, if inserts are used up to four times in six-day protocols, reproductive variables (ewes in estrus, pregnancy rate, fertility rate) can be expected to remain at the same or similar values to those in new CIDR inserts.

The objective of the present study was to test the effect of new and second-, third- and fourth-use CIDR inserts in six-day estrus synchronization protocols on reproductive performance and progesterone concentrations in multiparous ewes.

Material and methods

The study was done during the sheep reproductive season (June – December, 2020) at Montecillo, in the municipality of Texcoco, State of Mexico, Mexico (19°27’18” N, 98°54’26” W; 2220 m asl). Study area climate is subhumid temperate with summer rains[14].

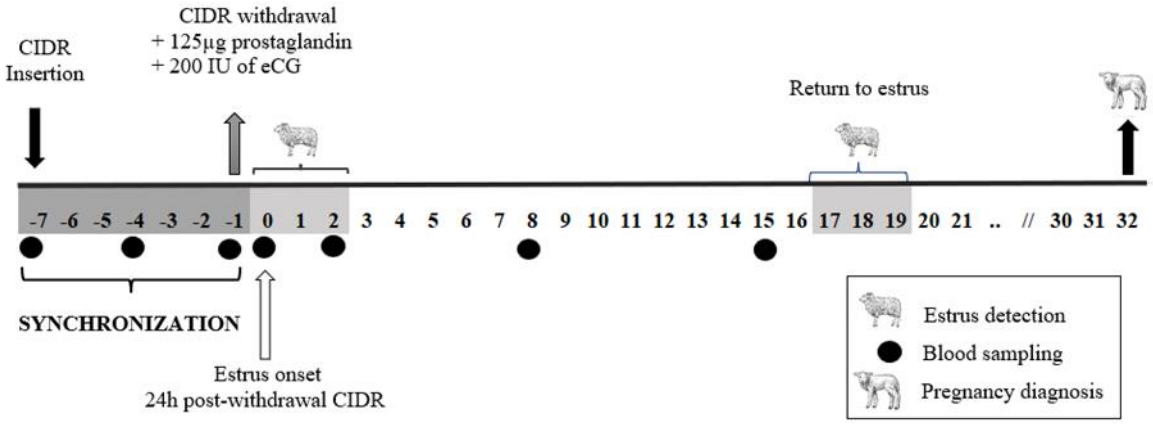
Animals, feeding and treatments

Experimental animals were 172 multiparous female sheep (Katahdin × Dorset) with 59 kg average weight and a body condition of 3 (1-to-5 scale). They were housed in pens, fed a diet of oat hay with grains, alfalfa hay and 300 g commercial breeding sheep feed (PURINA®;

14% CP), and provided free access to water. Prior to beginning the study, the animals were subjected to prophylactic management including a multivitamin (Endovet®; 0.5 ml/ 50 kg L.W., i.m.) and a bacterin-toxoid (Bobact® 8; 2.5 ml subcutaneously), the absence of pregnancy was confirmed by ultrasound. The animals were managed in compliance with federal regulations for the use and care of research animals[15,16].

The animals were randomly distributed into four experimental groups (n=43 sheep/group); each group was administered a progesterone hormone treatment via intravaginal devices (Eazi Breed™ CIDR® 330 sheep & goat insert, Zoetis), 200 IU equine chorionic gonadotropin (eCG) (NOVORMON 5000®, Zoetis; i.m.) and 125 µg prostaglandin (Celosil®, M.S.D). The four treatments were CIDR1 (control group, new devices), CIDR2 (second-use devices), CIDR3 (third-use devices) and CIDR4 (fourth-use devices). All reused CIDR inserts were thoroughly washed with purified water, dried, and stored under refrigeration for up to 24 h prior to reinsertion. In the estrus synchronization protocol, the devices were inserted on day -7 (d-7) and removed on day -1 (d-1); eCG and prostaglandin were applied intramuscularly at removal. Estrus onset was considered to occur on day 0 (d0) (Figure 1).

Figure 1: Estrus synchronization protocol and blood sample timeline



Estrus detection and reproductive variables

Detection of estrus began 24 h after insert removal. Once estrus was confirmed, natural mating was begun using three mounts per ewe at 12-h intervals. Nineteen (19) males of proven fertility were randomly assigned to each female in estrus.

Pregnancy rate (pregnant ewes/total ewes × 100) was calculated thirty days after mating by ultrasound (CHISON Eco 6®) and a transrectal transducer (5 to 7 MHz multifrequency) at 7.5 MHz. Fertility rates (ewes that lambed/total ewes × 100), prolificacy index (total lambs born/ewes that lambed) and type of parturition (single or double) were calculated from the lambing record.

Progesterone concentration

Blood samples (seven samples per animal) were taken by jugular puncture from 20 females per group to analyze the progesterone (P_4) secretion profile concentrations in serum (Figure 1). Samples were collected in 5 ml polypropylene tubes and transported to the laboratory. The blood was separated by centrifuging at 1,500 xg for 20 min at 4 °C and stored in 1.5 ml microtubes at -20 °C until analysis. Progesterone (P_4) concentrations were measured by radioimmunoassay (RIA) with a commercial kit (Progest-RIA, Cisbio, Parc Marcel Boiteux-BP 84175-30200 Codolet/France) at 0.05 ng/ml sensitivity. The intra- and interanalysis coefficients of variation were 5.8 and 7.5 %, respectively.

Statistical analysis

The experimental design was completely random. Estrus onset data and prolificacy index results were analyzed using the Shapiro-Wilk normality test and Levene's test of homogeneity of variance. The Kruskal-Wallis test was applied when data did not comply with normality and homogeneity of variance conditions. The χ^2 test was used with the variables ewes in estrus (present/absent), pregnancy rate, fertility rate and parturition type. The P_4 concentration data were analyzed with repeated measures over time using a mixed model with first-order autoregressive error structures (AR1). Least squares means were calculated with the Tukey-Kramer test. Significance was set at α=0.05 for all analyses. The statistical analyses were run with the SAS program[17].

Results

Average ewes in estrus rate was 97 % and did not differ (P>0.05) between treatments. Average estrus onset was 36 ± 11 h. The ewes in CIDR2 began estrus in less time and in a shorter interval than those in CIDR1, while CIDR3 and CIDR4 did not differ (P>0.05) from

CIDR1 and CIDR2. No differences were identified in the variables of pregnancy rate (81 %), fertility rate (80 %), single parturitions (70 %) and double parturitions (30 %) (Table 1).

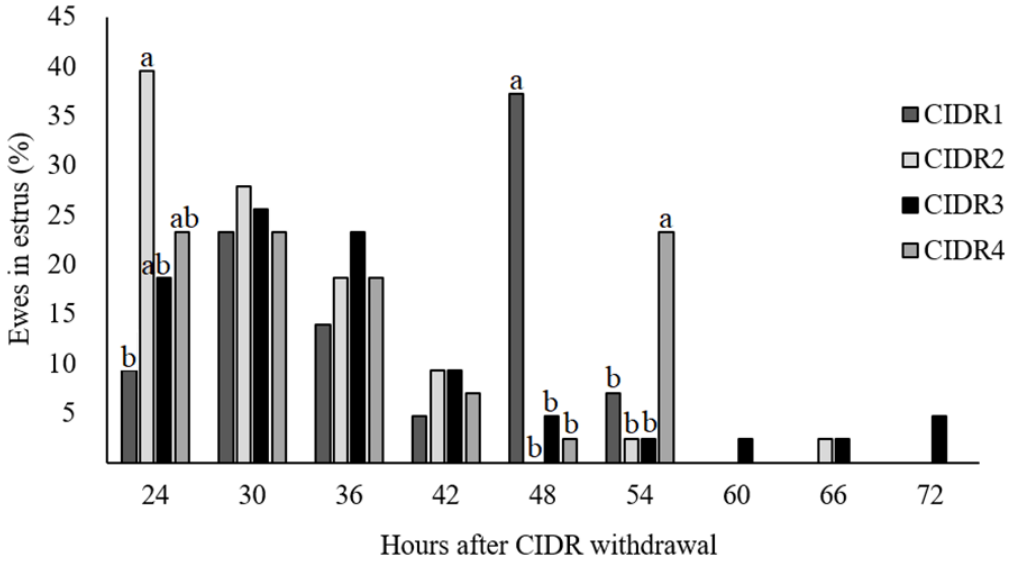
Table 1: Reproductive variables in multiparous ewes after application of CIDR devices (new, second-use, third-use and fourth-use)

Variable	Treatments				
	CIDR1	**CIDR2**	**CIDR3**	**CIDR4**	***P***
Ewes in estrus, %	95 (41/43)	100 (43/43)	93 (40/43)	98 (42/43)	0.3268
Estrus onset, h*	40±10^{b}	31±9^{a}	37±13^{ab}	37±11^{ab}	0.0014
Pregnancy, %	88 (38/43)	86 (37/43)	77 (33/43)	81 (35/43)	0.4849
Fertility, %	88(38/43)	81(35/43)	77 (33/43)	81(35/43)	0.5710
Lambing type:					
Single, %	71(27/38)	72 (25/35)	79 (26/33)	57 (23/35)	0.2648
Twins, %	29(11/38)	28 (10/35)	21 (7/33)	43 (12/35)	
Prolificity	1.3	1.3	1.2	1.4	0.0999

CIDR1= new devices; CIDR2 = second-use; CIDR3 = third-use; CIDR4 = fourth-use. * ± standard deviation.
^{ab} Different superscript letters in the same row indicate significant difference ($P \leq 0.05$).

Most (94 %) of the animals exhibited estrus 54 h after device removal, although in treatment CIDR2 this did not occur until 66 h and in CIDR3 until 72 h (Figure 2).

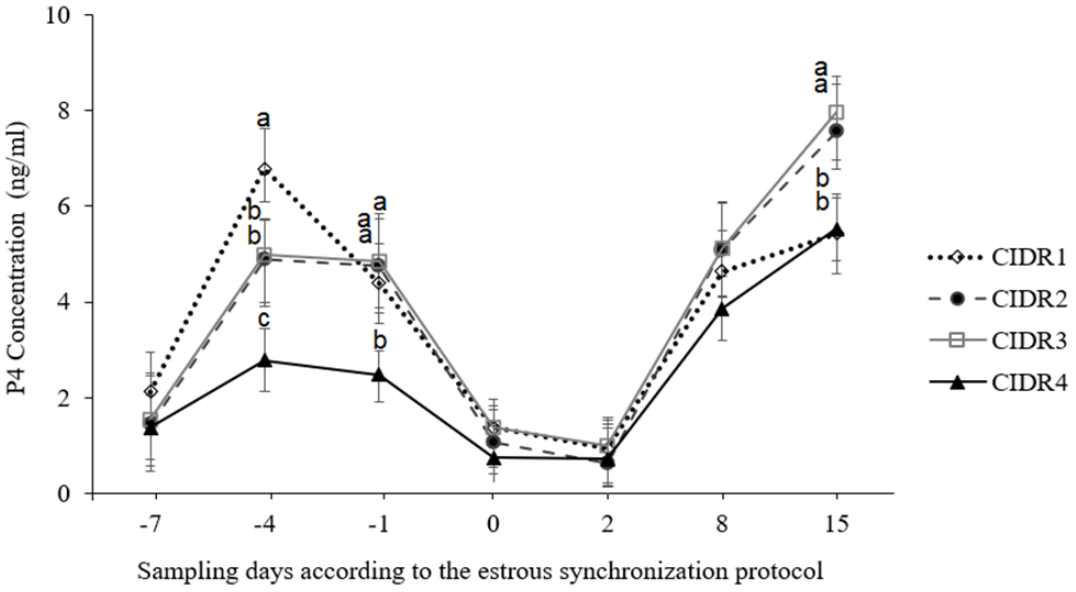
Figure 2: Percentage of ewes in estrus



CIDR1= new devices; CIDR2 = second-use; CIDR3 = third-use; CIDR4 = fourth-use. * ± standard deviation.
^{ab} Different superscript letters in the same set of bars indicate significant difference ($P \leq 0.05$).

Mean progesterone (P_4) concentrations did not differ ($P>0.05$) between treatments on the day (d-7) the devices were inserted (CIDR1 = 2.12 ± 0.72, CIDR2 = 1.46 ± 60, CIDR3 = 1.54 ± 0.49, CIDR4 = 2.78 ± 0.52 ng/ml). While inserted, the highest P_4 concentrations in all treatments were observed three days after insertion (d-4). On this day, the highest ($P<0.05$) concentration was in CIDR1 (6.78 ± 1.23 ng/ml), followed by CIDR2 (4.90 ± 1.36) and CIDR 3 (4.97 ± 1.37 ng/ml), which did not differ, and CIDR4 (2.78 ± 1.56 ng/ml), the lowest ($P<0.05$) (Figure 3).

Figure 3: Serum progesterone (P_4) concentrations in ewes after application of CIDR devices (new, second-use, third-use and fourth-use)



[ab] Different superscript letters in the same day indicate significant difference ($P\leq0.05$).

Immediately after CIDR device removal (d-1), P_4 concentrations in the CIDR1 (4.39 ± 0.98 ng/ml), CIDR2 (4.75 ± 0.79 ng/ml) and CIDR3 (4.86 ±0.75 ng/ml) treatments were higher ($P\leq0.05$) than in CIDR4 (2.48 ± 1.52 ng/ml). However, 24 h later (d0), P_4 concentrations had decreased to levels that did not differ ($P>0.05$) between treatments (CDIR1= 1.38 ± 0.26, CDIR2= 1.07 ± 0.39, CDIR3= 1.38 ± 0.15, CDIR4= 0.74 ± 0.23).

By day d2 (72 h after device withdrawal), 97 % of the animals exhibited estrus and average P_4 was <1 ng/ml in CIDR1 (0.93 ± 0.04 ng/ml), CIDR2 (0.62 ± 0.06 ng/ml) and CIDR4 (0.71 ± 0.6 ng/ml), and only slightly higher in CIDR3 (1.01 ± 0.14 ng/ml). The treatments did not differ ($P>0.05$).

At d8, all four treatments exhibited P_4 concentrations >1 ng/ml with no differences between them ($P>0.05$) (CIDR1= 4.64 ± 1.24, CIDR2= 5.09 ± 1.05, CIDR3= 5.11 ± 1.85 and CIDR4= 3.86 ± 1.11 ng/ml. By d15, which corresponds to endometrium adhesion (12 to 13 days

pregnancy), concentrations were highest ($P<0.05$) in CIDR2 (7.56 ± 0.85 ng/ml) and CIDR3 (7.95 ± 1.0 ng/ml) and lowest ($P<0.05$) in CIDR1 (5.43 ± 0.97 ng/ml) and CIDR4 (5.52 ± 1.04).

Discussion

The 97 % ewes in estrus observed in the present results is similar to that reported in a study using five-day protocols (93 % in second-use devices and 100 % in third-use devices)[6]. The ewes in estrus results observed here suggest that CIDR devices used up to four times in six-day protocols are as effective as new devices in synchronizing estrus and ovulation during the reproductive season[6,11]. Indeed, this ewes in estrus rate is similar to those reported for conventional 12-d treatments[13].

The mean estrus onset results were like those reported for seven-day protocols with new CIDR devices associated with 5 mg dinoprost and 400 IU eCG (33.8 ± 4.0 h)[2]. They were also similar to the 35 ± 0.7 h estrus onset reported at 7 d with devices with 11 d previous use and associated with 300 IU eCG and 6.7 mg dinoprost, and for which ewes in estrus was highest between 36 and 41.9 h[10]. The low ewes in estrus rates after 54 h in CDIR2 (minimum 3 %) and CDIR3 (minimum 9 %) in the present results probably occurred because the ewes were in an early stage of follicular development at the time of removal. This is supported by the fact that low values were not observed in CDIR4. Use of low doses of eCG (200 IU) contributed to a wider interval for ewes in estrus (24 to 54 h). For instance, when applying 400 IU eCG estrus onset occurs from 24 to 40 h[2]. This happens because use of this dose in short- or long-term estrus synchronization protocols reduces the ovulation interval, accelerates ewes in estrus and stimulates greater estradiol production in the follicles[18].

The pregnancy rates in the present results (77 to 88 %) were higher than reported in a study of CIDR devices used up to six times in 6-d protocols[3]. In this case, the difference may be due to the use of Awassi breed ewes, in which pregnancy and fertility rates decrease after the third use of a CIDR device. This is attributed to the low ewes in estrus rates in this study, and the use of fixed-time artificial insemination (FTAI)[3]. The present rates were also higher than the 70.1 % reported in a study of CIDR devices (new, second-use and third-use; no difference between treatments) in six-day protocols associated with 250 IU eCG and 0.133 mg cloprostenol sodium administered at removal[12]. This discrepancy may be due partially to the use of natural mounting and mating for five days in an extensive system. In the present study, the ewes remained penned and were mated three times at a 12-h interval, which increased the probability of oocyte fertilization. The average prolificacy index in the present

results was 1.26, which coincides with reported prolificacy values in Dorset and Katahdin ewes[19].

Blood P_4 concentrations prior to device insertion indicate the ewes had begun their reproductive season. After insertion, concentrations increased in response to the supply of exogenous P_4 and remained above 1 ng/ml until device removal, which stimulated release of GnRH from the hypothalamus. Preovulatory increases in LH and FSH caused follicular growth and subsequent ovulation[20]. This is deduced from the decrease of P_4 to 0.7 ng/ml at 72 h post device removal. Apparently, concentrations near 1 ng/ml did not negatively affect estrus synchronization since only 5% of the ewes did not exhibit estrus within 72 h post device removal.

Progesterone (P_4) is fundamental to maintaining early pregnancy and establishing implantation[20]. Monitoring P_4 concentrations can therefore be useful in confirming pregnancy in ewes. The corpus luteum (CL) increases P_4 production on day three of pregnancy, increasing concentrations in maternal plasma to approximately 4 ng/ml by day seven[21]; this corresponds to the concentrations measured beginning on d8 in all four treatments of the present study. Increases in P_4 during this period are important because in sheep the CL is sensitive to the luteolytic action of $PGF_2\alpha$ between days four and fourteen of the estrous cycle[22], and levels <1 ng/ml at this stage would not maintain a pregnancy. Remarkably, in the present results is that at the final sampling (d15) average P_4 concentration was lowest in CIDR1 (5.43 ng/ml), which did not differ from CIDR4. Higher levels can be linked to the higher CL number and total weight in sheep administered hormonal treatments[23]; however, the differences in P_4 concentrations at the final sampling were not related to pregnancy rate or parturition type.

The use of CIDR devices in up to four six-day protocols with natural mating did not cause variation in reproductive variables between the four treatments. Progesterone (P_4) concentrations did not differ during the first three uses but did decline somewhat in the fourth. This highlights the potential of this estrus synchronization program since it can generate synchronization in a relatively short period. An added advantage of using CIDR devices in short periods (six days) is that it reduces ewe hormone exposure without negatively affecting reproductive variables (ewes in estrus, pregnancy and fertility rates, and prolificacy index). Clearly, CIDR intravaginal devices can be reused, although, because the devices are designed to release P_4 constantly, their effectiveness in estrus synchronization will depend on number of uses, sheep body weight and season (anestrus or reproductive). A final note is that reuse must occur within the same flock to avoid transmission of sexually-transmitted diseases between breeding animals.

Conclusions and implications

Use of six-day protocols allowed CIDR device reuse up to four times (i.e. new + three reuses) since they continue to release sufficient progesterone to block ovulation and synchronize estrus as effectively as new devices. The ewes in estrus, pregnancy and fertility rates did not decrease, while estrus onset interval, double births and the prolificity index were unaffected.

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Conflicts of interest

The authors declare no conflicts of interest.

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