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Estrous Synchronization in Rarámuri Criollo Cattle Using 5- and 7-Day Co-Synch + CIDR Protocols

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ABSTRACT

Objective: To evaluate the reproductive response of Rarámuri Criollo cows to two estrous synchronization protocols: 5- and 7-day Co-Synch + CIDR.

Design/methodology/approach: Twenty multiparous Rarámuri Criollo cows (6 years old; 238±52 kg; body condition score = 5) were randomly assigned to two treatments in a completely randomized design. In Treatment 1 (T1; n=10), the Co-Synch + CIDR protocol was applied for 7 days, while in Treatment 2 (T2; n=10), it was applied for 5 days. Timed artificial insemination (TAI) was performed 48 hours (T1) and 72 hours (T2) after device removal. Body condition score (BCS) was recorded on days 0, 90, and 150. Estrus response, insemination rate, and pregnancy rate were analyzed using logistic regression; time to estrus was assessed using the Kaplan-Meier method; and BCS was analyzed with a mixed model using SAS® at $\alpha=0.05$.

Results: Estrus response was 80% in both treatments. Time to estrus onset was shorter in T1 (41.2±2.36 h) than in T2 (60.0±4.96 h). No significant differences were observed in insemination rates (80% in T1 *vs.* 90% in T2) or pregnancy rates (60% *vs.* 50%, respectively). Body condition score increased progressively throughout the study.

Limitations/implications: The small sample size limits the generalizability of the findings. Future studies with larger cohorts are recommended to confirm the absence of treatment effects.

Findings/conclusions: Both the 5- and 7-day Co-Synch + CIDR protocols were effective for estrous synchronization in Rarámuri Criollo cows.

Keywords: Native cattle, zoo genetic resource, reproduction.

INTRODUCTION

The growth of the livestock sector, along with its contributions to the economy and human livelihoods, underscores the importance of understanding the impacts of climate

change on animal production. Global warming and associated climatic variations affect water and feed resources, as well as livestock health and productivity (Godde *et al.*, 2021), necessitating the implementation of mitigation strategies (Strandén *et al.*, 2022). In this context, Criollo cattle descendants of Iberian livestock introduced to the Americas during colonization possess traits of considerable value for modern cattle production (O'Neill *et al.*, 2010). These native breeds have developed resilience to extreme temperatures, limited water availability, and harsh environments with rugged, difficult terrain (Márquez-Godoy *et al.*, 2024), making them a viable alternative for sustainable meat production (Naranjo *et al.*, 2021). However, Criollo cattle populations have declined significantly over the past century. As a result, reproductive biotechnologies such as estrus synchronization and artificial insemination (AI) have become valuable tools for the conservation and utilization of this zoogenetic resource (Quezada-Casasola *et al.*, 2016). Criollo cattle also exhibit unique reproductive characteristics, including variations in estrous cycle duration, number of follicular waves, dominant follicle size, and hormonal profiles (Quezada-Casasola *et al.*, 2013; 2014), which may lead to variable responses to hormonal protocols originally developed for commercial breeds. Previous studies have reported inconsistent ovulation timing and low pregnancy rates (as low as 9.09%) in Criollo cows inseminated 54 hours after CIDR removal (Zárate-Martínez *et al.*, 2010), likely due to factors such as lactation, postpartum anestrus, or low body condition (Quezada-Casasola *et al.*, 2016). Successful manipulation of the estrous cycle requires the proper development of the ovulatory follicle, with factors such as follicular size, duration of proestrus, and follicular dominance directly influencing oocyte competence (Geary *et al.*, 2013). Estradiol (E_2) and progesterone (P_4) levels are also critical determinants of pregnancy outcomes (Bridges *et al.*, 2008). Extended proestrus duration has been shown to enhance E_2 and P_4 secretion, thereby improving fertility (Geary *et al.*, 2010; Bridges *et al.*, 2014). To this end, reducing the duration of P_4 treatment via 5- and 7-day CIDR protocols, while extending the interval between CIDR removal and Fixed-time artificial insemination (FTAI) has been proposed as a strategy to enhance fertility by mitigating the negative effects of follicular persistence (Geary *et al.*, 2013; Bridges *et al.*, 2014). Therefore, the aim of this study was to evaluate the reproductive response of Rarámuri Criollo cows with a body condition score of 5 to two estrus synchronization and FTAI protocols: 5- and 7-day Co-Synch + CIDR.

MATERIALS AND METHODS

The experiment was conducted at the "La Campana" Experimental Ranch of the National Institute for Forestry, Agriculture, and Livestock Research (INIFAP), located at km 80 of the Chihuahua-Ciudad Juárez highway (29° 26' N, 106° 39''W), in Chihuahua, México. Twenty Rarámuri Criollo cows from the ranch herd were selected in August 2024. These cows had an average body condition score (BCS) of 5 (on a 1-9 scale, where 1=emaciated and 9=obese; Richards *et al.*, 1986), a mean body weight of 238±52 kg, were 6 years old, and were 540 days postpartum. Prior to the experiment, all cows underwent rectal palpation and ultrasonography to rule out reproductive pathologies and confirm cyclicity. A portable ultrasound device with a 7.1 MHz linear transducer (SIUI SCANNER CT-800) was used. All animal procedures followed the guidelines of the Mexican Official Standard NOM-051-ZOO-1995 for the humane handling of animals.

In October 2024, cows were randomly assigned to one of two hormonal treatments for estrus synchronization and Fixed-time artificial insemination (FTAI). In Treatment 1 (T1; n=10), a 7-day Co-Synch + CIDR protocol was used. On day 0, cows received 10 μg of buserelin acetate (Liberactive®, Virbac), a gonadotropin-releasing hormone (GnRH) analog, administered intramuscularly (IM), along with the insertion of a CIDR device (Dibactive® 1200, Virbac). On day 7, cows received 150 mg of D-cloprostenol, a $PGF_{2\alpha}$ analog (Inducelactive®, Virbac) via IM, and the CIDR was removed. FTAI was performed 48 hours after CIDR removal, accompanied by a second IM dose of 10 μg buserelin acetate (Figure 1). In Treatment 2 (T2; n=10), 10 μg of buserelin acetate was administered and a CIDR inserted on day 1. The CIDR was removed on day 6, followed by 150 mg of $PGF_{2\alpha}$ administered IM. FTAI was conducted 72 hours after CIDR removal, along with a second dose of 10 μg buserelin acetate (Figure 1).

Estrus detection was performed by marking the cows' rumps with colored wax crayon. Cows were considered to be in estrus if they allowed mounting for more than 3 seconds, had disrupted crayon marks on the rump, exhibited vulvar edema, and produced clear cervical mucus. Observations were recorded up to the moment of FTAI. At the time of insemination, cows in both treatments were palpated to confirm their response to the hormonal protocol.

The experimental animals grazed in open bunchgrass pasture (*Bouteloua-Aristida*) associated with oak trees and interspersed with invasive Lehmann lovegrass (*Eragrostis lehmanniana*). Upon CIDR removal, animals were kept in a handling corral for estrus detection. During the observation period, they were supplemented with oat hay, a multinutritional block (Mega Pellet, 37% CP, Eragol®), and had *ad libitum* access to water. Body condition score (BCS) was assessed by a single trained observer on days 0, 90, and 150 using the 1-9 scale (Richards *et al.*, 1986). The following variables were evaluated: estrus response rate, time to onset of estrus, percentage of inseminated cows relative to the

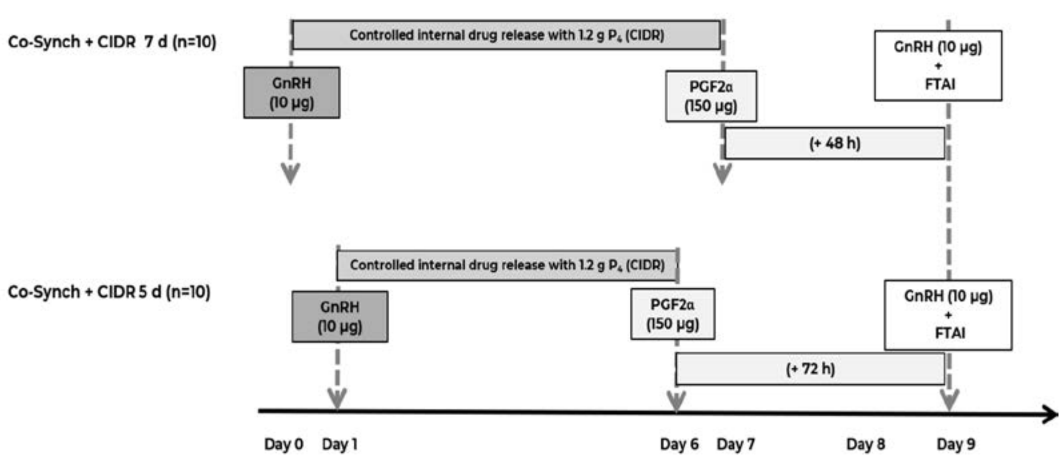

Figure 1. Schematic representation of the 7- and 5-day Co-Synch + CIDR protocols used for estrus synchronization in Rarámuri Criollo cows. GnRH: buserelin acetate (Liberactive®, Virbac); $PGF_{2\alpha}$: D-cloprostenol (Inducelactive®, Virbac); FTAI: Fixed-time artificial insemination.

total in each treatment, percentage of inseminated cows relative to those expressing estrus, and pregnancy rate per treatment relative to the number of inseminated cows.

Time to estrus onset was analyzed using survival analysis with the Kaplan-Meier estimator. Differences between treatments were assessed with the Log-Rank test (Kalbfleisch & Prentice, 2002). Estrus response, insemination rate (relative to total cows and to estrus responders), and pregnancy rate (relative to inseminated cows) were analyzed using logistic regression via the PROC LOGISTIC procedure. Synchronization treatment was included as a fixed effect. Models were fitted using maximum likelihood estimation, and odds ratios (OR) were calculated with 95% confidence intervals using the Wald method. Fisher's exact test was used to assess statistical significance between treatments. A convergence criterion of GCONV=1×10^{-8} was used to ensure model stability. Body condition score was analyzed as repeated measures using a mixed model (Littell *et al.*, 1998) with the PROC MIXED procedure. Treatment means were compared using Tukey's test. All statistical analyses were performed in SAS® (SAS Institute, 2022) at a significance level of $\alpha=0.05$.

RESULTS AND DISCUSSION

During the observation period, 80% of the cows in each treatment group exhibited estrus in response to the synchronization protocol (Table 1), with no statistically significant difference between treatments ($p=1.000$). Thus, both protocols demonstrated equal effectiveness in inducing estrus in the Rarámuri Criollo cows evaluated. The odds ratio was 1.0, with a 95% confidence interval ranging from 0.11 to 8.95, indicating considerable uncertainty due to the small sample size, and suggesting that the study lacked sufficient statistical power to detect potential differences between treatments.

The estrus response observed in this study was lower than the 100% reported for Criollo cows synchronized with a conventional 7-day CIDR + estradiol protocol (Zárate-Martínez *et al.*, 2010), and also below the 90% response reported by Quezada-Casasola *et al.* (2016) using a 7-day CIDR-based protocol. Estrus expression and detection in Criollo cattle can be influenced not only by management conditions but also by social dominance behaviors (Zárate-Martínez *et al.*, 2010). In this study, two cows in each treatment group did not exhibit estrus signs. It is possible that these individuals were either stressed during the observation period or occupied lower positions in the social hierarchy, which may have impaired their response to hormonal treatment. In mammals, the physiological response to stress involves activation of the hypothalamic-pituitary-adrenal (HPA) axis and subsequent glucocorticoid release. These hormones can exert inhibitory effects on

Table 1. Estrus response and time to estrus onset in Rarámuri Criollo cows synchronized with 7- and 5-day Co-Synch + CIDR protocols.

Treatment	Females in estrus	% response to estrus	Time to onset of estrus (h)
T1: Co-Synch + CIDR 7 d (n=10)	8.0	80.0 [a]	41.2±2.36 [a]
T2: Co-Synch + CIDR 5 d (n=10)	8.0	80.0 [a]	60.0±4.96 [b]
Total (n=20)	16.0	80.0	55.5±4.24

[a, b]. Different letter within each column indicates statistical difference ($p\leq0.05$).

GnRH and LH secretion, thereby affecting follicular growth and steroidogenesis (Naranjo *et al.*, 2021). Criollo cattle are traditionally managed under extensive grazing systems, and the confinement of animals in pens for estrus detection during this experiment may have induced stress and negatively impacted hormonal synchronization. An alternative approach for monitoring estrus expression could involve the use of electronic devices, such as pedometers that track activity changes, or sensors that detect shifts in body temperature (Merkelytė *et al.*, 2025). The onset of estrus occurred significantly earlier in cows receiving the T1 protocol (7-day CIDR) compared to T2 (5-day CIDR) ($p \leq 0.05$), as reflected in the survival probability curves (Figure 2). These results indicate that CIDR treatment duration influenced estrus synchronization, reducing the response interval in the 7-day protocol group.

For the T2 treatment (Co-Synch + CIDR for 5 days), Geary *et al.* (2010) recommend a 72-hour interval between CIDR removal and FTAI. This timing is based on the premise that extended development of the dominant follicle in the presence of gonadotropins enhances both nuclear and cytoplasmic maturation of the oocyte, thereby improving its fertilization competence (Bridges *et al.*, 2008). In Criollo cattle, time to estrus onset is variable. Zárate-Martínez *et al.* (2010) reported estrus beginning between 36 and 43 hours after CIDR removal, while Quezada-Casasola *et al.* (2016) observed an average onset of 24.9±2.8 hours when administering an estradiol analog at CIDR removal; however, this interval extended to 31.5±2.8 hours with the use of 500 IU of equine chorionic gonadotropin (eCG). Both values are lower than those recorded in the present study. Since time to estrus onset directly influences pregnancy rates following insemination especially with FTAI precise timing is crucial (Zárate-Martínez *et al.*, 2010). In this experiment, estrus onset in both treatment groups occurred before the scheduled FTAI, allowing insemination to be performed at an optimal time. In T2, the timing met the AM-PM rule (insemination 12 hours after estrus signs), as estrus was detected around 60 hours post-CIDR removal and FTAI was conducted at 72 hours an alignment that

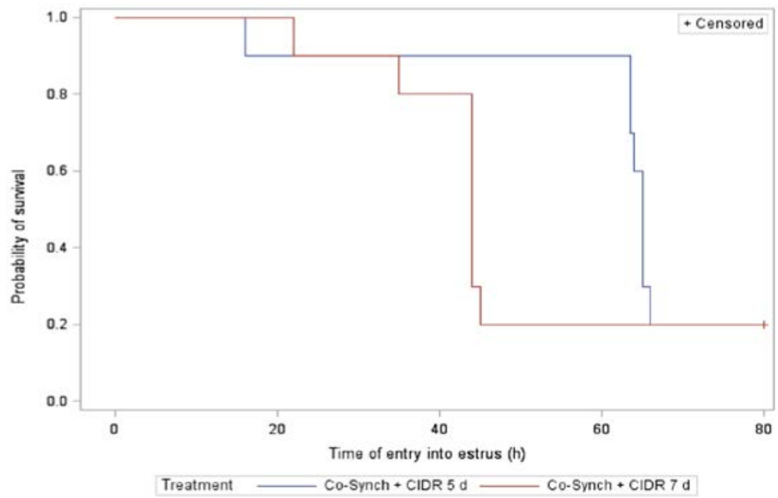

Figure 2. Kaplan Meier survival curves for time to estrus onset in Rarámuri Criollo cows synchronized with 7- and 5-day Co-Synch + CIDR protocols.

likely enhanced pregnancy probability. The proportion of inseminated cows was similar between treatments (p=1.000), both in terms of total cows evaluated and those exhibiting estrus (Table 2). Although T1 showed a lower relative response probability compared to T2, the lack of statistical significance is likely due to the small sample size. In T1, seven of the eight cows that expressed estrus were inseminated; one could not be inseminated due to cervical obstruction. Pre-FTAI palpation revealed that among cows that did not display visible estrus, one in each group exhibited uterine horn turgidity and a palpable follicle, suggesting silent estrus. These cows were inseminated, resulting in insemination rates of 80% for T1 and 90% for T2 (Table 2). Although silent estrus is not commonly reported in Criollo cattle, it may be linked to low estradiol concentrations insufficient to trigger behavioral estrus (Quezada-Casasola *et al.*, 2016). Silent estrus is considered a multifactorial condition potentially influenced by hormonal imbalances, negative energy balance, and stress-related factors (Zduńczyk *et al.*, 2010).

Pregnancy rates were similar between treatments (p = 1.000), with average ranges between 55% and 68%, considering both the total number of cows in each treatment and those that expressed estrus (Table 3). These results suggest that both synchronization protocols 7 and 5-day Co-Synch + CIDR were equally effective in achieving pregnancy in Rarámuri Criollo cows under the conditions of this study.

In both treatments, pregnancy rates were higher than those reported by Zárate-Martínez *et al.* (2010), who observed only 9.09% gestation, and by Sánchez-Arciniega *et al.* (2013), who reported 46.7% with an 8-day CIDR protocol, 400 IU of eCG on day eight, and FTAI 56 hours later and only 31.5% when eCG was included. The pregnancy outcomes in the

Table 2. Insemination percentage in Criollo Rarámuri cows synchronized with Co-Synch + CIDR protocols of 7 and 5 days.

Variable	T1 (n=10)	T2 (n=10)	Total (n=20)	OR (CI 95%)
Percentage of females inseminated compared to those exhibiting estrus	87.5 [a]	100.0 [a]	93.7	0.29 (0.01-8.37)
Percentage of females inseminated per treatment	80.0 [a]	90.0 [a]	85.0	0.44 (0.03-5.88)

T1: Co-Synch + CIDR 7 d, T2: Co-Synch + CIDR 5 d. OR: Odds ratios, CI: Confidence interval. *a, b*. Different letter within each row indicates statistical difference ($p \leq 0.05$).

Table 3. Pregnancy rates in Rarámuri Criollo cows synchronized with 7- and 5-day Co-Synch + CIDR protocols.

Variable	T1 (n=10)	T2 (n=10)	Total (n=20)	OR (CI 95 %)
Percentage of pregnant females compared to inseminated females that exhibited estrus	75.0 [a]	62.5 [a]	68.7	1.80 (0.21-15.41)
Percentage of pregnant females per treatment	60.0 [a]	50.0 [a]	55.0	1.50 (0.26-8.65)

T1: Co-Synch + CIDR 7 d, T2: Co-Synch + CIDR 5 d. OR: Odds ratios, CI: Confidence interval. *a, b*. Different letter within each row indicates statistical difference ($p \leq 0.05$).

present study for both synchronization protocols were comparable to the 60-75% reported by Quezada-Casasola *et al.* (2016) using a 7-day CIDR protocol with 500 IU of eCG.

Body condition score (BCS) increased over time. A significant interaction between treatment and time was observed ($p \leq 0.05$), indicating a positive trend in BCS progression throughout the study. However, no significant differences were detected between treatments alone ($p > 0.05$) (Table 4).

Table 4. Body condition score in Rarámuri Criollo cows synchronized with 5- and 7-day Co-Synch + CIDR protocols.

Treatment	Days (Mean ± Standard Error)		
	0	90	150
T1: Co-Synch + CIDR 7 d (n=10)	$4.80 \pm 0.17\ ^{a}{}_{X}$	$5.75 \pm 0.17\ ^{b}{}_{X}$	$6.40 \pm 0.16\ ^{c}{}_{X}$
T2: Co-Synch + CIDR 5 d (n=10)	$5.65 \pm 0.17\ ^{b}{}_{X}$	$6.10 \pm 0.17\ ^{bc}{}_{X}$	$6.45 \pm 0.16\ ^{bc}{}_{X}$

$^{a, b, c}$. Different letters indicate statistical difference ($p \leq 0.05$). $^{X, Y}$. Different letters within each column indicate statistical difference ($p \leq 0.05$).

In this experiment, body condition score (BCS) positively influenced both estrus response and pregnancy rates, in agreement with the findings of Quezada-Casasola *et al.* (2016), who worked with Criollo cows exhibiting a BCS of 4. Furthermore, among Criollo cows with BCS ranging from 3 to 5, those in better condition showed improved responses to hormonal stimulation (Zárate-Martínez *et al.*, 2010).

It has been speculated that one of the inherent traits of Criollo cattle is their low reproductive efficiency (Ruiz-Barrera *et al.*, 2009), which may lead to inconsistent responses to hormonal synchronization protocols and fixed-time artificial insemination (FTAI) typically used in beef breeds (Zárate-Martínez *et al.*, 2010; Sánchez-Arciniega *et al.*, 2013). However, the results of this study, along with those reported by Quezada-Casasola *et al.* (2016), indicate that Criollo cows with a BCS above four can respond satisfactorily to synchronization protocols, achieving pregnancy rates comparable to those observed in other cattle breeds.

CONCLUSIONS

The 7- and 5-day Co-Synch + CIDR protocols were effective in synchronizing estrus in Rarámuri Criollo cows. Therefore, they represent viable tools for reproductive management aimed at conserving and utilizing this unique genetic resource. Nevertheless, due to the limited sample size, it is recommended that these findings be validated through larger-scale studies that consider the effects of social hierarchy and group management to enhance synchronization and reproductive efficiency.

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