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Review

Ovarian function and response to estrus synchronization in Creole cattle in Mexico. Review

Elizabeth Pérez-Ruiz [a]

Andrés Quezada- Casasola [b]

José María Carrera-Chávez [b]

Alan Álvarez-Holguín [a]

Jesús Manuel Ochoa-Rivero [a]

Manuel Gustavo Chávez-Ruiz [a]

Sergio Iván Román-Ponce [a*]

[a] INIFAP. Centro de Investigación Regional Norte-Centro. CE La Campana. Km 33.5 Carr. Chihuahua – Ojinaga. 32910. Aldama, Chihuahua. México.

[b] Universidad Autónoma de Ciudad Juárez. Departamento de Ciencias Veterinarias. Ciudad Juárez, Chihuahua, México.

*Corresponding author: roman.sergio@inifap.gob.mx

Abstract:

Nowadays, reproductive biotechnologies have made it possible to conserve and use animal genetic resources. One of these technologies is the estrus synchronization programs, which allow programming the time for mating according to the availability of fodder or the birth of calves for commercial purposes. Another application is the reduction of the calving- first ovulation interval through protocols that facilitate the use of artificial insemination. Creole cattle are a valuable genetic resource due to their hardiness and adaptability to difficult environmental conditions; they are resistant to parasites, take advantage of available forage resources and reproduce in systems with little or no supplementation. In Mexico, the first studies of synchronization of Creole cattle suggest that Creole cows do not respond adequately to hormonal protocols and gestation percentages lower than those obtained in other breeds are obtained. The foregoing gave rise to a series of studies on reproductive physiology and the use of biotechnologies in Creole cattle. The objective of this review is to collect existing information on the use of estrus and ovulation synchronization protocols in Creole cattle from Mexico; in order to be able to identify the lines of research necessary for the development of estrus and ovulation synchronization protocols suitable for Creole cattle.

Key words: Beef cattle, Animal genetic resources, Artificial insemination, Ovulation.

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Introduction

Creole cattle are the descendants of Iberian cattle transported to the Americas during the colonization of these countries by the Spanish and Portuguese[1]. One of the most important traits of Creole cattle is their adaptability to difficult environmental conditions; they reproduce in systems with extreme fluctuations in ambient temperature[2,3], are resistant to parasites[4] and take advantage of a variety of herbaceous plants, in addition to alternating between grazing and browsing[1]. The adaptability of a breed to variable environments, called phenotypic plasticity, is a quality of Creole cattle, which can be used in current selection and reproduction systems[1]. Therefore, these cattle are a genetic resource that could contribute to improving productivity in a challenging environment[1,5]. Regarding animal genetic resources, in March 2018, 7,745 breeds of the 8,803 breeds registered by FAO were classified as local breeds (i.e., reported to be present in only one country) and a total of 594 local breeds became extinct. Among existing local breeds, 26 % were classified as at risk of extinction, 7 % are not at risk and 67 % as unknown[6]. One of the possible causes of the decrease in the population of local breeds is because they are considered unproductive, compared to specialized breeds (European and Zebu). In general, Creole cattle are small animals and can hardly compete with other breeds of cattle specialized in the production of meat or milk, so many farmers have chosen to make crosses with these, or replace Creoles with other cattle breeds[7,8].

Reproductive biotechnologies are useful tools to open the possibility of conserving and exploiting animal genetic resources[9]. With the application of estrus synchronization programs, it is possible to choose the best time for mating (according to the availability of fodder), reduce the interval from calving to first ovulation (CFO), facilitate the use of artificial insemination (AI) and implement genetic improvement programs[10]. The manipulation of the estrous cycle and the induction of ovulation through the different synchronization protocols in conjunction with AI have numerous additional advantages, such as the production of calves in homogeneous batches and the possibility of increasing the sale price, as well as facilitating the nutritional and health management of the herd[11]. However, the use of reproductive biotechnologies, such as estrus and ovulation synchronization, is little used in Creole cattle, and with variable results[12,13]. In Mexico, the first studies related to the synchronization of Creole cattle were carried out in the 1990s, and it was mentioned in them that Creole cows do not respond adequately to hormonal protocols[12]. Therefore, the objective of this review is to collect the existing information on the use of estrus synchronization protocols in Creole cattle from Mexico and in this way, to be able to identify the lines of research necessary for the development of estrus and ovulation synchronization protocols suitable for Creole cattle, which will allow directing research and development efforts.

Fixed-time artificial insemination in cattle

With the use of exogenous hormones and their analogues, it is sought to control follicular development, regression of the corpus luteum (CL) and ovulation, to later perform AI at detected estrus or fixed-time AI (FTAI). With the hormonal protocols for FTAI, the following is achieved: 1) reduce the number and frequency of cattle handling, and 2) eliminate the need for estrus detection to perform AI, which is why they are the most used in beef cattle[14].

Estrus synchronization treatments are mainly based on the use of two types of hormones, progestogens (mainly progesterone, P_4) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) analogues. In ovulation synchronization protocols, estradiol (E_2) analogues and gonadotropin-releasing hormone (GnRH)[15] are additionally used. Preference in the use of these treatments for FTAI may be limited in countries where there are restrictions on the use of E_2[16], such as the United States of America, Australia, the United Kingdom, among others. The percentage of gestation that can be obtained with these FTAI protocols varies in a range of 40-60 %[15]. The establishment of gestation depends to a large extent on the correct function of the CL, the adequate signaling of the embryo for the maternal recognition of gestation and the oviductal and uterine environment that favors the development of the embryo. These factors are modified in part by the preovulatory conditions, that is, there is an effect of the steroidogenic capacity of the follicle, and the consequent competence of the oocyte[17]. Other critical factors for the success of synchronization protocols are the physiological state of the animals (prepubertal heifers, cyclic or anestric cows), body condition (BC)[18], nutrition, semen quality, inseminator dexterity[11] and time of insemination after hormone treatment[19].

For example, females with low BC (usually ≤ 4 on scale from 1 to 9)[11] tend to be in anestrus and, therefore, have low gestation percentages compared to females with better BC[18]. Additionally, first-calving heifers are more sensitive to weight loss and low BC, compared to multiparous cows[18]. Nutrient intake and energy balance, before and after calving, affect the duration of the postpartum anestrus and the interval from calving to conception, as well as the percentage of pregnancy[11]. When AI is performed, the efficiency of the inseminator is influenced by the quality and handling of the semen, as well as by their technical ability to deposit the semen in the right place[11]. In addition, the correct handling and evaluation of semen straws is essential to ensure the quality of the material used, as it directly influences the fertilization rate and, therefore, the pregnancy rate. Prior to AI, it is recommended to evaluate the semen that will be used, according to the most important semen characteristics[11]. Therefore, it is imperative that inseminator technicians are sufficiently trained to guide the AI gun through the cervix, to deposit the semen completely at the entrance of the uterine body[11].

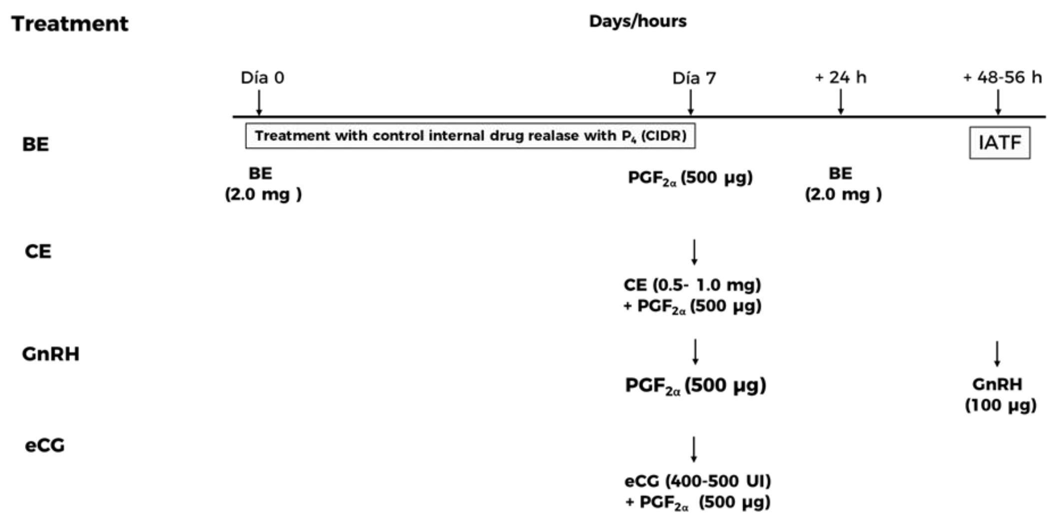
Conventional protocols for FTAI based on estradiol and progesterone in cattle

The protocol based on the use of P_4 and E_2 is known as conventional. P_4 simulates the function of CL, inhibiting the release of GnRH/LH pulses. E_2, applied at the beginning of the treatment with P_4, causes ovulation of the possible existing dominant follicle and atresia of the rest of the subordinate follicles. With the emergence of a new follicular wave, between three and five days later, the presence of a new dominant follicle and a viable oocyte at the end of the treatment with P_4 are sought[20]. The administration of E_2 at the end of the treatment with P_4 induces, in the same way as the first application, a positive feedback on the hypothalamus for the release of GnRH and the consequent increase in the frequency of LH pulses, which synchronizes and reduces the time in which the ovulation occurs, to perform the FTAI[20].

The conventional protocol consists of the insertion of a CIDR and the administration of EB (at a total dose of 2 mg, via IM) on the day of the beginning of treatment with P_4 (day 0)[16]. Estradiol 17 β (E-17β) has a shorter half-life than EB, so the latter has been shown to be more efficient in FTAI protocols[20]. Additionally, $PGF_{2\alpha}$ is administered at the time of removal of the CIDR, to ensure the regression of the CL that has formed after ovulation after the first application of E_2 (d 7, 8 or 9, in case of existence of a CL)[21]. To synchronize ovulation, a total dose of 1 mg of EB can be applied 24 h after the end of the treatment with P_4, and the FTAI can be performed 30 to 34 h after applying the second dose of EB[22]. A modification to this protocol consists of administering a total dose of 0.5 or 1 mg of EC at the time of removing the device with P_4, which simplifies the handling of cattle, with FTAI 48 to 56 h later[15,23]. EC has a longer half-life than EB, so its use allows reducing the number of manipulations carried out on cattle[23]. Another option is to apply a dose of 100 μg of GnRH 54 h after removal of the CIDR (at the time of performing the FTAI), which induces ovulation of the new dominant follicle, in case it has not ovulated spontaneously[14]. One more alternative to this protocol is the administration of equine chorionic gonadotropin (eCG) at the time of finishing the treatment with P_4[24] (Figure 1). The eCG binds to the FSH and LH receptors, causing the increase in the growth rate of the dominant follicle, stimulates the expression of steroidogenic enzymes, mainly follicular E_2, with the consequent occurrence of the preovulatory peak of LH. Additionally, eCG produces an increase in the diameter of the CL and increases the production of P_4 after AI[25]. This effect of eCG is more marked in females with low BC (and that gain weight during the time of mating) and in postpartum (PP) anestrus [24].

Doses of 300-400 IU of eCG are used in beef cattle, these doses are the most used both in conventional protocols[21] and in GnRH-based protocols[26,27]. The eCG can promote the growth of one or more follicles in the same wave, so not only a larger follicular diameter is achieved, but also the increase in the ovulation rate in cows treated with this hormone, when high doses (400-600 IU)[28] are used. The occurrence of multiple births in beef cattle is considered undesirable, so the increase in the ovulation rate due to the effect of eCG is controversial[27]. These eCG effects are most evident during PP anestrus, in cows that are lactating and with low BC[24], because the secretion of GnRH/LH is decreased during this stage[28]. However, in cows with good BC, a beneficial effect is not observed[26].

Figure 1: Schematic representation of synchronization protocols based on progesterone and estradiol



In CE (estradiol cypionate), GnRH (gonadotropin-releasing hormone) and eCG (equine chorionic gonadotropin) treatments, the second dose of BE (estradiol benzoate) is replaced with the respective hormone, at the time of removal of the CIDR (intravaginal progesterone releasing device), to simplify the handling of cattle.

Protocols for FTAI based on GnRH and $PGF_{2\alpha}$

The initial application of a GnRH analogue causes the release of LH in the form of a preovulatory peak and, therefore, the ovulation of a possible dominant follicle present, with the subsequent appearance of a new follicular wave approximately two days later(29). The administration of $PGF_{2\alpha}$ 7 d after the application of GnRH induces the regression of the possible CL formed after the application of GnRH and a second dose of GnRH will again cause the release of the preovulatory peak of LH, producing the ovulation of a new dominant follicle in a synchronized way(30).

The most commonly used protocol for FTAI in dairy cattle is known as Ovsynch (ovulation synchronization)(29). This protocol requires the handling of cattle three times to apply hormones and a fourth time to perform the FTAI (Figure 2), so it is impractical for its use in beef cattle(31,32). The alternative for this type of cattle is the CO-Synch protocol(33) (the second dose of GnRH is applied at the time of the FTAI), in which the number of times in which cattle are handled is reduced(32). However, with this protocol, 5 to 20 % of females in PP anestrus have estrus before or immediately after the application of $PGF_{2\alpha}$, so a lower pregnancy rate is obtained than with the Ovsynch protocol(34). The insertion of a CIDR, between the first administration of GnRH and the application of $PGF_{2\alpha}$ (Figure 2), increases the pregnancy rate with this protocol[34,35]. The use of the CIDR prevents ovulation before and after the application of $PGF_{2\alpha}$, caused by spontaneous luteolysis of the CL, and as a result, estruses more synchronized with the moment of the FTAI are obtained, therefore, higher pregnancy rates are obtained with the CO-Synch + CIDR protocol[34,35]. The success of this protocol depends largely on the percentage of females that ovulate after the first dose of GnRH[14]. If the induction of the ovulation of the dominant follicle is achieved, the emergence of the next follicular wave and ovulation will be synchronized[22].

In dairy cattle, one more alternative to increase the percentage of pregnant cows in the Ovsynch protocols is the pre-synchronization with one or two doses of $PGF_{2\alpha}$[36], with a difference of 14 d between each dose, and the application of the first dose of GnRH 12 to 14 d after the second dose of $PGF_{2\alpha}$, this protocol is known as Pre-Synch (Figure 3). The objective of this pre-synchronization is that the cows are between d 5 and 12 of the cycle at the time of starting treatment with GnRH[36,37]. In beef cattle, pre-synchronization is impractical, since this protocol involves handling the cattle a greater number of times and does not increase the percentage of pregnant females after FTAI[37]. As a result of the stress produced when introducing beef cows into pens and handling sleeves, animals experience a fight-or-flight response, which activates the adrenal axis and the release of stress hormones (catecholamines and glucocorticoids), which have a negative effect on the reproductive axis[38].

Figure 2: Schematic representation of the Ovsynch, Co-Synch and CO-Synch+CIDR protocols for FTAI (GnRH: gonadotropin-releasing hormone; $PGF_{2\alpha}$: prostaglandin $F_{2\alpha}$; IATF: fixed-time artificial insemination)

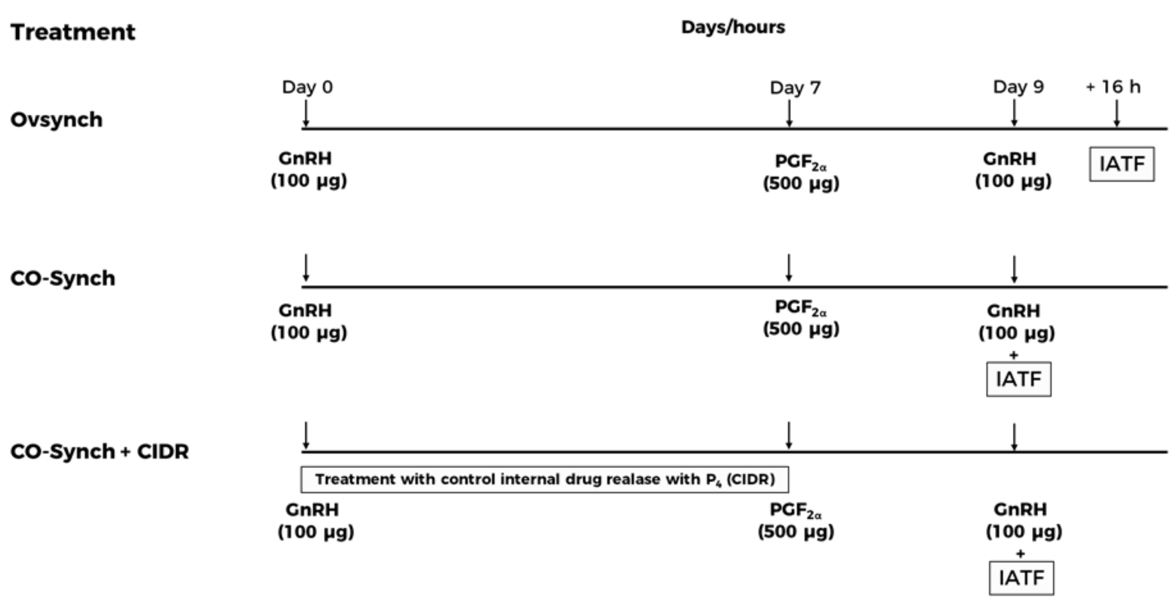
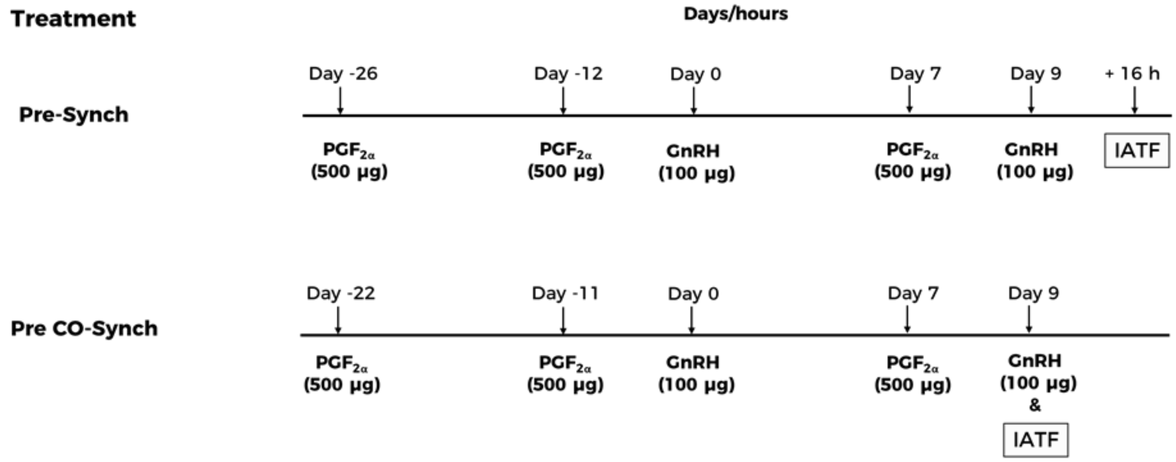


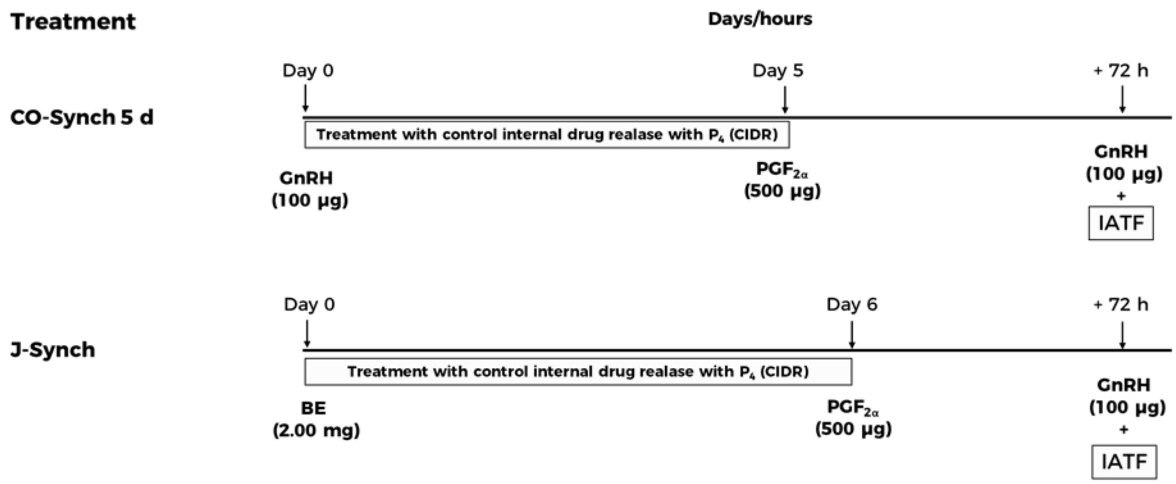
Figure 3: Schematic representation of the Pre-Synch protocols used in dairy cattle and Pre CO-Synch developed for beef cattle (GnRH: gonadotropin-releasing hormone; $PGF_{2\alpha}$: prostaglandin $F_{2\alpha}$; IATF: fixed-time artificial insemination)



5-day Co-Synch protocol

The 5-day Co-Synch protocol with FTAI 72 h later (Figure 4) is based on the idea that increasing the period in which the dominant follicle develops in the presence of gonadotropins can increase the percentage of pregnant females after the hormone treatment(39). The main changes in this protocol are: 1) the reduction of the treatment with P_4 from 7 to 5 d, to avoid adverse effects of persistent follicles on the fertility of cows that do not ovulate with the first dose of the GnRH analogue (total dose of 100 µg of gonadorelin, IM), and 2) prolong the period from removal of P_4 to administration of GnRH, to increase exposure to circulating E_2 concentrations before ovulation(17). With this protocol, a greater diameter of the dominant follicle, an increase in the concentration of E_2, as well as a greater production of P_4 after ovulation, necessary for gestation(40), are observed.

Figure 4: 5-day CO-Synch and J-Synch protocols



In these protocols, proestrus is prolonged (increase of the period between the end of treatment with Progesterone (P_4) and IATF ($PGF_{2\alpha}$= prostaglandin $F_{2\alpha}$; GnRH= gonadotropin-releasing hormone; BE= estradiol benzoate; IATF: fixed-time artificial insemination)

J-Synch protocol

This protocol is based on the use of P_4 and EB, with a longer duration of proestrus than with conventional treatment(41). The protocol begins with the administration of a total dose of 2 mg of EB at the time of insertion of a device with P_4, which is removed 6 d later. At the time of removal of the device with P_4, a single dose of $PGF_{2\alpha}$ is applied. Additionally, 100 µg of GnRH is applied at the time of FTAI, 72 h after (d 9, Figure 4). Similar to the 5-day CO-Synch protocol, with the prolongation of the proestrus (95-97 h), an increase in the concentrations of E_2 (before ovulation) and P_4 (after ovulation) is observed, as well as a higher percentage of gestation (when females are in good BC), compared to the conventional Co-Synch protocol(42). Recent studies indicate that the length of the proestrus is decisive for the establishment of gestation. With a longer proestrus, E_2 production increases before ovulation. Among the functions of E_2 are the modification of cell morphology, secretion and regulation of steroid receptors, which favor the implantation of the conceptus in the uterus(43). Table 1 shows some results obtained for the percentage of pregnancy, with different synchronization protocols.

Table 1: Percentage of gestation obtained with different protocols for FTAI in cattle

Type of protocol†	Breed type‡	Pregnancy (%)	Reference
Based on P_4 and E_2			
Conventional (EB)	BI, BT	30.0-40.6	(25, 44-46)
Conventional (EC)	BI, BT	34.7-54.0	(16, 42, 44, 47, 48)
J-Synch	BI/BT, BT	47.0-67.9	(16, 41, 42, 47, 49)
Conventional EB + eCG	BI	36.8-57.5	(25, 46, 50, 51)
Conventional EC + eCG	BI, BT	50.3-61.8	(16, 28, 43, 48, 52)
J-Synch + eCG	BI/BT, BT	53.0-60.4	(16, 43, 47, 53)
Based on GnRH and $PGF_{2\alpha}$			
Ovsynch	BT	32.5-57.0	(33, 36, 37, 54, 55)
Pre-Synch	BT	27.0-49.6	(36, 37, 55-57)
CO-Synch	BT	26.7-53.3	(33- 35, 54, 58)
CO-Synch + CIDR	BT	50.0-55.1	(26, 34, 35, 58-60)
5-d CO-Synch	BT	44.4-59.7	(61-63)
5-d CO-Synch + CIDR	BT	48.0-63.9	(59, 60, 63-66)
CO-Synch + CIDR + eCG	BT	43.0	(26)
5-d CO-Synch + CIDR + eCG	BT	42.9	(67)

†CIDR= intravaginal progesterone releasing device; P_4=progesterone; E_2= estradiol; $PGF_{2\alpha}$= prostaglandin $F_{2\alpha}$; GnRH= gonadotropin-releasing hormone; EB= estradiol benzoate; eCG= equine chorionic gonadotropin; EC= estradiol cypionate.

‡ BT: *Bos taurus taurus*; BI: *Bos taurus indicus*.

Creole cattle in Mexico

Creole cattle in Mexico descend from the first specimens brought by the Spanish during the Colonial Period in the sixteenth century(68). These cattle developed qualities of adaptation to the environment in isolated and hard-to-reach areas, which contributed to the formation of breed groups(69,70). In Mexico, of the 53 breeds of cattle recorded in the database of the biodiversity of domestic animals published by FAO(71), the following stand out as local breeds: the Chinampo, from Baja California(72,73); the Coreño, from the Sierra Madre Occidental(74,75); the Creole from the Northern Mountains, also called rodeo Creole or Rarámuri Creole(70); the Mixteco(76); the Creole from the Gulf(77); the Creole from the central region of Chiapas(78) and the Nunkiníen Creole, from the Yucatán Peninsula(79). From 1965, individuals of the tropical dairy Creole and Romosinuano breeds, from the United States and Costa Rica, respectively, were also introduced(80).

The Rarámuri Creole (RC) bovine, also called “Corriente”, is adapted to the northern region of the country, mainly in the Sierra Tarahumara, in the state of Chihuahua[70]. They are animals of small size and large horns; their main zootechnical purpose is for rodeo sports activities, so it is exported in large quantities to the United States[7]. The reproductive physiology of this breed of Creole cattle from Mexico is the most researched so far, this includes the characterization of the estrous cycle, ovarian activity, estrous behavior, hormonal profiles[81-83] and development, and evaluation of synchronization and AI protocols [12,13].

Estrus and ovulation synchronization protocols in Rarámuri Creole cattle

In one of the first studies in RC cattle, a conventional protocol for FTAI was used, with the use of a CIDR (with 1.9 mg of P_4) for 7 d, plus the application of 1 mg of β-estradiol at the beginning of treatment with P_4; administration of 30 mg of $PGF_{2\alpha}$, via IM, when removing the CIDR; and 24 h after removing the CIDR, another dose of 1 mg of β-estradiol was applied. The FTAI was performed 54 h after removal of the CIDR. With this protocol, a percentage of gestation of 9.09 % was observed, despite the fact that 100 % of the cows showed signs of estrus. In this study, administering a dose of 50 mg of P_4, via IM, at the time of removal the CIDR, in conjunction with the application of β-estradiol, did not improve the percentage of gestation (9.09 % was obtained with both treatments). The low percentage of pregnancy obtained with this protocol was attributed to a variation in the time to ovulation[12]. The cows showed estrus between 36 and 43 h after the application of $PGF_{2\alpha}$. The authors mention that letting so much time pass between the beginning of estrus and AI was a determining factor. It should be noted that the cows that had estrus between 36 and 37 h after removal of the CIDR did not become pregnant, while all the cows that showed estrus around 43 h after removal of the CIDR did become pregnant. It is also mentioned that anovulatory estruses occur in RC cows, so this contributes to a low percentage of gestation[12].

In another study with RC cows, the time from the beginning of the $PGF_{2\alpha}$-induced estrus to ovulation was 46.2 ± 8.2 h and 37.6 ± 6.0 h in a natural estrus (all cows had estrus within 24 to 60 h), with a significant proportion of cows that ovulated in the range of 24-35 h in natural estrus (8 cows out of a total of 22). In both types of estrus, the highest percentage of cows ovulated in the range of 36-47 h (12 cows in natural estrus and 11 cows in induced estrus)[82]. This difference in the times from the beginning of estrus to ovulation, in RC females, with respect to *Bos taurus taurus* cattle, should be considered to improve the percentage of gestation with FTAI protocols, or consider the possibility of using the “AM-PM” rule to program AI, as mentioned by Zárate-Martínez *et al*[12]. However, performing this type of handling, which requires estrus detection, is impractical for RC cattle farmers.

The growth pattern of ovarian follicles is in waves; one to four waves may occur in each estrous cycle(84-86). The number of follicular waves in each cycle is variable, with two to three waves being the most common(85,86). In RC females, there is a higher percentage of cows with two follicular waves (77.3 %)(81). Females that have two follicular waves per cycle ovulate on average 6.2 h earlier than cows with three waves(83). In addition, the follicular growth rate in the RC breed (0.6 ± 0.2 mm d^{-1})(82) is lower than in *Bos taurus indicus* cattle (0.9 ± 0.1 mm d^{-1}) and other *Bos taurus taurus* breeds (1.1 ± 0.1 mm d^{-1})(87). In this regard, Quezada *et al*(82) mention that, in order to optimize the response to synchronization protocols in RC cows, it is necessary to modify the time between hormone treatment and AI, so they suggest performing AI ~28 h after the beginning of estrus(82).

Use of eCG in Creole cattle

In *Bos taurus taurus* cattle, the application of eCG in protocol for FTAI helps to increase the follicular growth rate and diameter, as well as the production of P_4, in females with low BC(24). When evaluating the use of a dose of 400 IU of eCG at the time of FTAI (56 h after removal of the CIDR) in RC cows, with the use of a 8-d CO-Synch protocol (application of a dose of 100 µg of GnRH when inserting the CIDR + 25 mg of $PGF_{2α}$ when removing the CIDR, on d 8 of the protocol + 400 IU of eCG, at the time of FTAI), the percentage of gestation (31.5 *vs* 46.6 %) was not improved compared to the same protocol without eCG (in this treatment, 100 µg of GnRH was applied at the time of FTAI). The authors mention that the females were in an acceptable BC (4.5 ± 0.2; on a scale from 1 to 9) and received a good diet during reproductive management, so no positive effect of eCG was observed(88). In this same study, supplementation with concentrate, selenium (0.95 mg Se/50 kg LW) or Ca propionate (100 g), did not modify the percentage of gestation either(88).

In another study in which the use of a dose of 500 IU of eCG in RC cows was evaluated, a percentage of gestation of 60 % of the total number of females in the treatment (and 75 % of gestation compared to those that showed signs of estrus) was obtained(13). The hormonal protocol used in this study consisted of the use of an CIDR for 7 d + 2.76 mg of EB at the start of treatment with P_4 (d 0) + 25 mg $PGF_{2α}$ (d 7) + 1 mg of EC or 500 IU of eCG 24 h after removing the CIDR (d 9); the AI was performed 12 h after the beginning of estrus. The percentage of gestation in the group of cows treated with EC was 27.3 %. It should be noted that, in this study, 100 % of cows treated with EC and 80 % of those treated with eCG showed signs of estrus in response to the AI protocol and all females in both treatments ovulated. The response of the cows to both treatments was similar for the variables: response to estrus, percentage of ovulation and maximum follicular diameter(13). When evaluating these same protocols in RC heifers, the response to estrus was lower in both treatments, 89.5 % and 25.0 % for EC and eCG treatments, respectively. The percentage of heifers that had silent estruses was 10.5 % for heifers in the group treated with EC and 75.0 % for those treated with eCG. The occurrence of silent estruses was attributed to a lower follicular growth than that observed with the use of EC. Because only females that showed signs of estrus were inseminated, the percentage of gestation with the use of eCG was only 10 %, compared to the total in the group (40 % of those that had estrus and were inseminated)[13]. However, 100 % of the heifers treated with eCG ovulated. In this study, in both cows and heifers, estrus occurred in a shorter time with EC (cows 24.9 ± 2.8 h and heifers 25.8 ± 2.9 h) compared to the treatment of eCG (31.5 ± 2.8 and 30.6 ± 2.9). Additionally, the authors mention the possibility of using FTAI successfully, in multiparous cows with the use of eCG, since, with this protocol, the beginning of estrus grouped between 24 and 36 h after removing the CIDR (31.5 ± 2.8 h on average) and the highest percentage of cows were inseminated 36 to 48 h after finishing the treatment with P_4. In addition, the results of response to estrus and percentage of gestation obtained in this study are higher than those obtained by Zárate *et al*[12] and Sánchez-Arciniega *et al*[88] in this same breed of cattle.

Restart of postpartum ovulatory/cyclic activity

Postpartum (PP) anestrus is characterized by the absence of ovulations after calving. In this period, the ovarian follicles begin to grow, but none is able to ovulate, at least during the first weeks[89]. This is partly due to the absence of LH, and often, the first ovulation is not preceded by the manifestation of signs of estrus; the CL may have a reduced mean life, smaller size and limited steroidogenic activity[90]. To reduce the negative effect of suckling, it has been proposed to perform early weaning (EW, a few days after calving); controlled or restricted suckling (RS, it consists of allowing suckling in short periods of the day); or temporary weaning (TW, separating the calf from the mother for a few days)[91]. The RS technique increases the proportion of cows that show signs of estrus during the first 100 d postpartum and reduces the calving-first ovulation interval, without affecting the growth of calves[91].

In RC cattle, with the RS strategy (beginning on day 76 PP), prior to the hormonal protocol for FTAI, 81.4 % of the cows ovulated within an observation period of 22 d after starting the RS, but only 11.1 % of them showed signs of estrus, that is, they had silent ovulations. In this study, the manifestation of estrous behavior was only observed in females with better BC (4-5, regular to good), while cows with poor BC (2-3, on a scale of 1-9) did not ovulate before the synchronization treatment[12].

In another study in RC cattle, when assessing weight loss during lactation, cows that were treated with an EW scheme from 68 ± 3.8 d PP lost less weight than those that remained with the offspring (normal weaning at 180 ± 10.2 days) during the evaluation period. In females with EW, weight loss was 4.8 kg, while females in the group of normal weaning lost 18.9 kg during the evaluation period (68-180 d PP)[92]. As mentioned, as in other specialized bovine breeds[11], BC in RC cattle is a limitation to re-establish reproductive activity after calving, and to establish gestation[92]. RC cattle are rarely supplemented, so there is no control over their body condition[88]. Therefore, performing strategies such as RS or EW in Creole cattle, in conjunction with the implementation of protocols for FTAI, could be useful to improve the percentage of gestation.

Estrus synchronization in other breeds of Creole cattle in Mexico

Information on the reproductive performance in response to protocols of estrus synchronization and AI in other breeds of Creole cattle in Mexico is limited. In Coreño Creole cows from Nayarit, with synchronization with a Norgestomet implant for 9 d + 280 IU of eCG, 80 % response to estrus and a percentage of gestation of 60 %[93] were obtained.

In the Chinampo breed, the use of two doses of $PGF_{2\alpha}$ (11 d apart between each application) to induce estrus and evaluate the estrous behavior in the presence of the bull was evaluated[94]. In the presence of the bull, greater interaction was found between 30 and 60 h after the second dose of $PGF_{2\alpha}$. Cows exposed to the bull started estrus in less time and had a shorter estrus length than those that remained isolated from the bull (10.7 ± 1.1 h *vs* 16.3 ± 2.6 h).

In purebred and crossbred heifers of the tropical dairy Creole breed, an estrus response of 94.1 % and a percentage of gestation of 68.8 % were found; with the addition of a dose of 500 IU of eCG, on day 10, of a protocol with a subcutaneous implant (with 3 mg of norgestomet, for 12 d + 5 mg of estradiol valerate, EV, via IM on d 0). While with a dose of 0.25 mg of GnRH, 24 h after removal of the implant (implant with 3 mg of norgestomet, for 12 d + 5 mg of EV, via IM on d 0), 76.4 % of females showed estrus and 46.2 % of them became pregnant[95]. In this protocol, a dose of 15 mg of $PGF_{2\alpha}$ was applied, via IM, 10 d before placing the subcutaneous implant; to homogenize the estrous cycle. Table 2 summarizes the results obtained in Creole cattle, with the use of various estrous and AI synchronization protocols.

Table 2: Summary of gestation percentages obtained with hormonal protocols in Creole cattle in Mexico

	Breed†	**Protocol‡**	**Gestation (%)**	**Reference**
Cows	RC	CIDR for 7 d + 1 mg E_2 + 50 mg P_4 (d 0) + 30 mg $PGF_{2\alpha}$ (d 7) + 1 mg E_2 (d 8)	9.1	(12)
Cows	RC	CIDR for 7 d^{-1} + 1 mg E_2 (d 0) + 30 mg $PGF_{2\alpha}$ (d 7) + 1 mg E_2 (d 8)	9.1	(12)
Cows	RC	CIDR for 7 d^{-1}, 100 µg GnRH + 25 mg $PGF_{2\alpha}$ (d 8) + 400 IU 56 h after removal of the CIDR	31.5	(88)
Cows	RC	CIDR for 7 d^{-1}, 100 µg GnRH + 25 mg $PGF_{2\alpha}$ (d 8) + 100 µg 56 h after removal of the CIDR	46.6	(88)
Cows	RC	CIDR for 7 d + 2.76 mg EB (d 0) + 25 mg $PGF_{2\alpha}$ (d 7) + 1 mg EC (d 9)	27.3	(13)
Cows	RC	CIDR for 7 d^{-1} + 2.76 mg EB (d 0) + 25 mg $PGF_{2\alpha}$ (d 7) + 500 IU eCG (d 9)	60.0	(13)
Cows	Coreño	Norgestomet implant for 9 days + 280 IU eCG	60.0	(93)
Heifers	RC	CIDR for 7 d + 2.76 mg EB (d 0) + 25 mg $PGF_{2\alpha}$ (d 7) + 1 mg EC (d 9)	27.3	(13)
Heifers	RC	CIDR for 7 d + 2.76 mg EB (d 0) + 25 mg $PGF_{2\alpha}$ (day 7) + 500 IU eCG (d 9)	60.0	(13)
Heifers	TDC	15 mg $PGF_{2\alpha}$ (d-10) + Subcutaneous implant (3 mg norgestomet) for 12 d^{-1} + 5 mg EV, 500 IU eCG (d 10)	68.8	(95)
Heifers	TDC	15 mg $PGF_{2\alpha}$ (d-10) + Subcutaneous implant (3 mg norgestomet) for 12 d^{-1} + 5 mg EV (d 0) + 0.25 mg GnRH (d 10)	46.2	(95)

†RC= Ráramuri Creole. TDC= tropical dairy Creole. ‡CIDR= intravaginal progesterone release device; E_2= estradiol; EV: estradiol valerate; $PGF_{2\alpha}$= prostaglandin $F_{2\alpha}$; GnRH= gonadotropin-releasing hormone; IU= international units; EB= estradiol benzoate; eCG= equine chorionic gonadotropin; EC= estradiol cypionate.

Factors associated with the reproductive response in Creole cattle

There are physiological differences between Creole cattle and European cattle specialized in beef production, as well as with zebu cattle. Among these, those inherent to ovarian functioning stand out: number of follicular waves, follicular growth rate, diameter of the ovulatory follicle and of the CL; and concentration of reproductive hormones and periods between events of the estrous cycle (length of luteal phase *vs* follicular phase; time to ovulation, length of estrus)[82,83,85]. Additionally, external factors such as nutritional status, handling, social and hierarchical relationships[38] influence the response of Creole cattle to hormonal protocols for FTAI.

Reproductive behavior, ovarian function and endocrinology in the Creole female

The estrous cycle in RC cows has an average length of 21.1 ± 1.2 d (range of 19-23 d), with a follicular phase of 6-9 d and a luteal phase of 12-16 d[88]. Follicular growth in cattle occurs in a pattern of follicular waves, and the number of follicular waves in each estrous cycle is variable, but it can range from two to four waves. In RC cows, there is a higher percentage of females with two follicular waves (77.3 %), and a smaller percentage of females with three follicular waves per cycle (22.7 %)[82]. In *Bos taurus taurus* females[84], *Bos taurus in*dicus females[85], Thai Creole females[86], a higher percentage of females with two follicular waves per cycle has also been observed. Contrary to these findings, in Caqueteño Creole cattle from Colombia[9], in Creole heifers with dairy tendency in Ecuador[96], and Creole cattle from the high Andean zone of Peru[86], there is a higher percentage of females with three follicular waves (Table 3). However, so far there is no published information on follicular dynamics in other breeds of Creole cattle from Mexico.

Table 3: Number of follicular waves in the estrous cycle in Creole cattle, *Bos taurus taurus* and *Bos taurus indicus*

Breed	**Number of follicular waves (%)**		
	2	**3**	**4**
Rarámuri from Chihuahua, Mexico [82]	77.3	22.7	
Caqueteño from Colombia [9]	33.3	66.6	
Creole from the high Andean zone of Peru [86]	16.0	78.0	6.0
Creole from the highlands of Ecuador [96]	44.4	55.6	
Native Thai [97]	70.0	30.0	
Nelore [85]	83.3	16.6	
Holstein [84]	81.0	19.0	

The characterization of the estrous cycle and follicular dynamics in different cattle breeds has allowed observing differences and similarities between them (Table 4). In Caqueteño and Nelore Creole cattle, females with three follicular waves have longer estrous cycles than females with two waves[9,85]. In RC cows, the length of the estrous cycle, follicular phase and luteal phase is similar between females with two and three follicular waves, but females with two follicular waves ovulate 6.2 h before than those with three follicular waves[82,83]. The ovulatory follicles of RC females with two waves grow at a lower rate than those with three waves (0.5 ± 0.04 *vs* 0.9 ± 0.08, respectively), while the maximum diameter of the ovulatory follicle is similar between both growth patterns (10.5 ± 0.2 *vs* 10.0 ± 0.4, respectively)[83]. The maximum diameter of the CL in RC cows with two and three follicular waves is similar (13.0 ± 1.0 *vs* 13.2 ± 1.7 mm, respectively)[83]. The size of the CL and the production of P_4 in RC cows are also smaller than those of other cattle breeds, and it is possible that these differences are adaptations to the environmental and nutritional conditions that Creole cattle have developed to survive in difficult environments[83].

Table 4: Follicular and ovulatory dynamics in cattle with two and three follicular waves

	Breed									
	Rarámuri from Mexico (83)		**Creole from Ecuador** (96)		**Caqueteño from Colombia** (9)		**Native Thai** (97)		**Holstein** (84)	
Number of waves	**2**	**3**	**2**	**3**	**2**	**3**	**2**	**3**	**1**	**2**
Estrous cycle length, days	21.1±0.3	21.4±0.6	20.3±0.0	23.6±0.0	20±0.6	22±0.5	18.60±0.1	20.38±0.1		
First wave (non-ovulatory)										
Emergence, days †	−0.5±0.2	−0.4±0.2	1	1	3	4	1.53±0.1	1.54±0.1	-2.0+0-1	-0.5±0.3
Maximum DF diameter‡, mm	8.0±0.1	7.6±0.3	13.2±2.2	12.2±1.7	8.4±1.3	11.7±3.3	7.63±0.1	7.67±0.1	17.1±0.5	16.0±0.4
Growth rate, mm day^{-1}	0.5±0.03	0.7±0.0	1.0±0.1	1.2±0.0	-	-	0.65±0.0	0.75±0.1	-	-
Regression, days	11.0±0.8	7.8±0.4	-	-	11	10.0±0.5	8.34±0.1	8.79±0.1	13.0+0.4	12.2±0.5
Second wave (non-ovulatory)										
Emergence, days	-	9.6±0.5	-	6.6±0.0	-	10.0	-	8.38±0.1		9.0
Maximum DF diameter, mm	-	7.2±0.2	-	10.2±0.0	-	12.2±4.4	-	6.79±0.2		12.9±0.7
Growth rate, mm day^{-1}	-	0.4±0.08	-	1.1±0.1	-	-	-	0.75±0.1	-	-
Regression, days	-	14.8±0.8	-	-		17.0±0.5	-	12.58±0.1		19
Ovulatory wave										
Emergence, days	11.2±0.8	14.8±0.8	7.8±1.6	13,2±1.3	11.0	17.0	11.02±0.1	11.33±0.1	9.6±0.2	16.0±1.1
Maximum DF diameter, mm	10.5±0.2	10.0±0.4	15.3±0.0	13.8±1.4	7.5±1.1	13.8±3.6	8.81±0.2	8.14±0.2	16.5±0.4	13.9±0.4
Growth rate, mm day^{-1}	0.5±0.04	0.9±0.08	0.9±0.1	1.1±0.2	-	-	1.07±0.0	1.48±0.1	-	-
Ovulation, days	22.0	22.5	20.0	23.0	20.0±0.6	22.0±0.5	19.44±0.1	21.13±0.2	20.4+0.3	22.8+0.6
Corpus luteum (CL)										
Maximum CL diameter, mm	13.0±1.0	13.2±1.7	21.7±1.4	23.5±0.6	11.3±4.3	11.2±3.2	13.55±0.1	15.14±0.1	-	-
Maximum P_4 concentration, ng/ml	6.5±0.1	6.5±0.2	20.6±5.4	20.6±3.1	-	-	4.13±0.1	4.25±0.1	-	-
Regression, days	16.3±1.6	16.8±1.1	18.0	20.0	17.0±1	19±0.96	17.11±0.1	19.29±0.1	16.5±0.4	19.2±0.5

† The moment of ovulation (day 0) was used as a reference to determine the beginning of the cycle, so it is possible to observe the emergence of the next follicular wave before ovulation. In the Rarámuri, the evaluation of both ovaries was performed every 8 h after the start of estrus (day 0 = day of ovulation).

‡DF: Dominant follicle.

Social and hierarchical interaction of the RC

In RC cattle, the existence of dominance relationships between females of higher rank over those of lower hierarchical rank has been observed(12). The social dominance in this type of cattle is not only determined by age, but also by the type of horns (as mentioned, this type of cattle has large horns in relation to the size of the body, which is why they are used for rodeo). Females with horn defects have difficulty defending themselves and their offspring during suckling, so they end up escaping or giving up space(12).

Another factor that can influence the percentage of gestation is the temperament of animals; animals with aggressive temperament tend to have a lower reproductive performance than animals with a docile temperament(98). Aggressive temperament disrupts physiological events necessary for reproduction, is associated with increased synthesis and circulating concentrations of adrenocorticotropin (ACTH) and cortisol, which can alter the key physiological events necessary to achieve puberty and the release of the preovulatory wave of GnRH/LH(99). On the contrary, a calmer temperament leads to higher rates of estrus occurrence and gestation, as well as fewer embryonic losses(100). Additionally, the social system of cattle is very hierarchical. Hierarchy influences the intake of feed and social behavior(101). In addition, low-ranking cows adopt a passive attitude as a behavioral strategy to reduce stress. Hierarchy also influences reproduction: stressed and low-ranking mothers produce less LH, which interferes with ovulation and estrous behavior(38).

Conclusions

The use of reproductive biotechnologies such as estrus and AI synchronization is limited in Creole cattle and the results obtained are variable. Among the factors that modify the response to synchronization are the reproductive physiology of the Creole cattle, body condition and hierarchical relationships. The review of the existing information on the response in Creole cattle to the use of synchronization protocols allows proposing the lines of research to be developed, and they should be focused on the comparative study of reproductive biology, adaptation of hormonal protocols considering the particularities of Creole cattle and the nutrition, handling and reproduction interaction. Finally, reproductive biotechnologies adapted to Creole cattle may be incorporated into programs of conservation and rational use of this animal genetic resource.

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