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Review article

Alternative programs for synchronizing and resynchronizing ovulation in beef cattle

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A B S T R A C T

Fixed-time artificial insemination (FTAI) has been regarded as the most useful method to increase the number of cows inseminated in a given herd. The main treatments for FTAI in beef cattle are based on the use of progesterone-releasing devices and GnRH or estradiol to synchronize follicle wave emergence, with a mean pregnancy per AI (P/AI) around 50%. However, more recent protocols based on GnRH (named 5-day Co-Synch) or estradiol (named J-Synch) that reduce the period of progesterone device insertion and extend the period from device removal to FTAI have been reported to improve P/AI in beef cattle. Furthermore, treatments to resynchronize ovulation for a second FTAI in nonpregnant cows have provided the opportunity to do sequential inseminations and achieve high P/AI in a breeding season, reducing or even eliminating the need for clean-up bulls. In summary, FTAI protocols have facilitated the widespread application of AI in beef cattle, primarily by eliminating the necessity of estrus detection in beef herds.

1. Introduction

Artificial insemination (AI) is one of the main techniques used worldwide to disseminate desirable genetics among beef and dairy herds. However, the widespread implementation of AI in beef herds is very recent and is mainly due to the use of protocols that allow the AI of large groups of animals at a given time, commonly called fixed-time artificial insemination (FTAI). There are basically two types of FTAI protocols currently used in beef cattle; GnRH-based and estradiol-based protocols, both of which are combined with progesterone-releasing devices. The preference for one of those protocols by practitioners is related to the availability of hormones in a given country; for example, estradiol-based protocols are used in South America and in beef herds in Australia, whereas GnRH-based protocols tend to be used more in North America, Europe, and New Zealand, where use of estradiol is prohibited. In South America, the numbers of cattle involved in FTAI programs during the breeding season has increased dramatically in the past 15 years, from less than 100,000 in the early 2000s to about 3,000,000 in Argentina and about 10,000,000 in Brazil in the last breeding season [1,2]. Although the results are now more consistent than 15 years ago, the mean pregnancy per AI (P/AI) has remained around 50% [2]. However, recently developed protocols that reduce the duration of progesterone-releasing device insertion and extend the proestrus period (defined as the period from device removal to FTAI) have resulted in improved P/AI and have opened new opportunities for increasing P/AI

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following FTAI. Furthermore, treatments to resynchronize ovulation for a second or third round of FTAI have provided the opportunity to do sequential inseminations, without the necessity of estrus detection, in cows not pregnant to the previous AI. The objective of this review is to present and describe the treatments available and discuss their impact on beef cattle fertility.

2. Ovulation synchronization treatments in beef cattle

2.1. Estradiol-based treatments

Estradiol and progestin treatments have been used widely over the past several years in estrus synchronization programs in beef cattle [3] and are the preferred treatment for FTAI of beef cattle in South America [4]. Treatments consist of insertion of a progesterone-releasing device and the administration of 2 mg of estradiol benzoate (EB) on Day 0 (to induce follicle atresia and synchronize follicular wave emergence), $PGF_2\alpha$ at the time of progesterone device removal on Days 7, 8, or 9 (to ensure luteolysis) and the subsequent application of 1 mg EB 24 hours later [5], GnRH or LH 54 hours later [6] or 0.5 or 1 mg of estradiol cypionate (ECP) at the time of progesterone device removal [4,7] to synchronize ovulation.

A recent analysis from 431,000 FTAI performed in Argentina reported a mean P/AI of 50%, ranging from 6% to 100% (L. Cutaia, Syntex S.A., Argentina; unpublished observations). The median P/AI was between 51% and 60% (41% of the herds) followed by 60% to 70% in 24% of the herds and 41% to 50% in 23% of the herds. The mean number of animals inseminated on a given day was 245. In 2015, mean P/AI were higher in *Bos taurus* herds (54.9%; n = 68,878) than in *Bos indicus*–influenced herds (48.7%, n = 95,152). As in previous studies [3,8], body condition scores (BCS) of the animals at the time of progesterone device insertion had a profound effect on P/AI.

Most treatments applied to suckling beef cows in South America involve the application of eCG at the time of removal the progesterone-releasing device [4,5,8]. Probably, the most important effect of eCG is the stimulation of the growth of the dominant follicle and the subsequent increase in ovulation rate [9,10], especially, in cows experiencing postpartum anestrous and/or in low BCS [3]. Furthermore, treatment with eCG increased circulating progesterone concentrations in the subsequent luteal phase, and this was associated with an increased diameter of the CL [5,10] and its progesterone production [10,11].

Beef cattle with larger ovulatory follicles had a greater ovulation rate and greater P/AI [3,12,13]. Furthermore, considering only those cows that ovulated following FTAI, P/AI increased as ovarian follicle size increased [12]. Therefore, in addition to the increased ovulation rate, ovulation of larger follicles could be responsible for other events, such as the improvement of endogenous estradiol production, oocyte competence, CL diameter, and concentration of progesterone in the subsequent luteal phase, which may benefit the fertility of beef cows following FTAI. In addition to the effects of eCG on follicle development, it has been shown that treatment with eCG modified specific steroidogenesis-related features (mitochondrial shape and the number of large luteal cells [14]) and increased the expression of steroidogenic enzymes (P450scc, 3b-HSD, and StAR) in the CL [15]. These features are conducive to higher P/AI in cows in postpartum anestrus treated with eCG, especially in those in low BCS.

2.2. GnRH-based treatments

GnRH-based protocols that were developed for lactating dairy cows [16] are widely used in North-American beef cattle [17,18]. The treatment consists of the administration of GnRH to induce LH release and ovulation of the dominant follicle if there is one present, with emergence of a new follicular wave approximately 1.5 to 2 days later. Prostaglandin $F_2\alpha$ is given 7 days later to induce luteal regression and in beef cattle a second GnRH is given at the time of FTAI ($\sim$60 hours later) to synchronize ovulation (named Co-Synch protocol; [17]). Recent studies have shown that the percentage of heifers ovulating to the first GnRH is 26% to 56% [19–22] with reports as low as 15% to 37% in the presence of high progesterone concentrations [23,24]. In beef cows, ovulation rates are around 60% [25], but in both cows and heifers, the emergence of a new follicular wave was synchronized only when treatment caused ovulation [19]. If the first GnRH does not synchronize follicular wave emergence, ovulation following the second GnRH may be poorly synchronized [6], resulting in disappointing P/AI [26]. Therefore, the addition of a progesterone-releasing device to a 7-day GnRH-based protocol has improved P/AI in heifers [6,26] and beef cows and is the most commonly used treatment to synchronize beef cattle in North America [27].

A strategy to increase the number of cows that ovulate after the first GnRH and fertility to the Ovsynch protocol is through presynchronization [28]. However, presynchronization with $PGF_2\alpha$ [29] or by the insertion of a previously used progesterone device (CIDR, Zoetis Animal Health; [25]) before a Co-Synch protocol did not increase P/AI in beef cows or heifers. Furthermore, application of presynchronization treatments in beef cattle is impractical because it is time consuming and requires extra handling of the animals in commercial beef operations.

GnRH protocols have not been used successfully to synchronize beef cows in postpartum anestrus [30], which is a common condition in extensively managed suckled beef herds [8]. Again, the insertion of a progesterone-releasing device significantly improved P/AI in suckled cows [27]. With the Co-Synch protocol, FTAI and the second GnRH administration is carried out at 54 to 66 hours after the removal of the progesterone device [27,31].

Although data on the addition of eCG- to GnRH-based treatment protocols are limited because GnRH-based treatments are not commonly used in beef cattle in countries where estradiol is available and because eCG is not available in countries such as the United States. However, the addition of eCG in a Co-Synch protocol with progesterone devices and FTAI and GnRH at 66 hours after progesterone device removal has resulted in an improvement in P/AI in *B. indicus* cows in postpartum anestrus [32] and in primiparous *B. taurus* cows that had not been presynchronized [25]; however, a lack of improvement in P/AI has been reported in *B. taurus* cows with a high BCS [33].

2.3. Protocols with short progesterone device insertion and prolonged proestrus

2.3.1. The 5-day Co-Synch protocol

The 5-day Co-Synch protocol is a GnRH-based treatment that has gained a lot of attention recently among practitioners and producers in North America, with higher P/AI reported than those obtained with the more traditional 7-day Co-Synch protocol [18]. The physiological basis of this treatment was to reduce the time of insertion of the progesterone device, to avoid the adverse effects of persistent follicles on fertility of the cows not ovulating to the first GnRH and to prolong the proestrus period with high circulating estrogen levels. The importance of a prolonged proestrus derived from a series of studies that evaluated the influence of follicular maturity on fertility in beef cattle [18,34]. Although it was hypothesized previously that follicular diameter was a strong indicator of fertility, the cumulative analysis of several studies has shown that the more consistent predictor of P/AI in cycling *B. taurus* cattle was the duration of proestrus, rather than follicle diameter [18]. To confirm that notion, in one experiment in which cows were induced to ovulate a follicle of similar diameter, P/AI was higher in those receiving GnRH 2.25 days after $PGF_2\alpha$ administration (71%) than in those receiving GnRH 1.25 days after $PGF_2\alpha$ (10%, $P < 0.01$; [35]). The beneficial effect of the prolonged proestrus has been associated with higher circulating estradiol concentrations before ovulation and higher progesterone concentrations in the ensuing luteal phase, especially in those cows not ovulating after the first GnRH [36]. Cows treated with the 7-day Co-Synch that did not ovulate after the first GnRH had a marginal reduction in the diameter of the dominant ovulatory follicle and a substantial reduction in preovulatory estradiol and luteal phase progesterone concentrations than those ovulating after the first GnRH. Conversely, in cows treated with the 5-day Co-Synch protocol, endocrine and follicular characteristics were similar between cows that did or did not ovulate to the first GnRH [36]. In a more recent study, higher estradiol concentrations in the preovulatory period were also related to lower embryonic losses in the period between maternal recognition of pregnancy and placental attachment [37].

Bridges et al. [34] compared a 7-day Co-Synch protocol plus progesterone device with FTAI at 60 hours and a 5-day Co-Synch protocol plus a progesterone device with FTAI at 72 hours in postpartum beef cows. In that study, P/AI was 10.5% higher with the 5-day Co-Synch protocol (70.4%) than in 7-day Co-Synch (59.9%; n = 616 Angus cows; $P < 0.01$). Similar results were reported recently by Whittier et al. [38], i.e., significantly higher P/AI in cows treated with the 5-day Co-Synch than in those treated with the 7-day Co-Synch.

Additional studies have shown that due to a shorter interval between the first GnRH and induction of luteolysis in the 5-day Co-Synch protocol, a single administration of $PGF_2\alpha$ was not effective at inducing luteolysis in beef cows [39]. Thus, two injections of $PGF_2\alpha$ seemed to be necessary to induce complete regression of the GnRH-induced CL in cows (P/AI: one × $PGF_2\alpha$; 53.1%, two × $PGF_2\alpha$; 69.0%; [40]). Therefore, subsequent studies by various laboratories investigated how to modify $PGF_2\alpha$ delivery in the 5-day protocol. Although a reduction in the interval between $PGF_2\alpha$ from 12 to 2 hours seemed to be adequate in one study [41], reduced P/AI was recently reported when the interval between $PGF_2\alpha$ administrations was less than 6 hours [42]. However, in a large field trial with 2465 postpartum beef cows [43], P/AI was greater ($P < 0.05$) in cows receiving two $PGF_2\alpha$ 8 hours apart (55%) than those receiving only one $PGF_2\alpha$ (48%), with those receiving two $PGF_2\alpha$ at the same time being intermediate (51%) and not different from either of the other groups. Hence, double $PGF_2\alpha$ is required in the 5-day protocol; however, P/AI seemed to be acceptable when the two $PGF_2\alpha$ were administered together at the time of progesterone removal.

We have recently completed a study involving 801 suckled beef cows in postpartum anestrus and 183 suckled beef cows that were cycling, to compare the 5-day Co-Synch protocol with the estradiol-based protocol [44]. Cows in the 5-day Co-Synch group were treated as described previously with two $PGF_2\alpha$ given at progesterone device removal and half of the cows also received 400 IU of eCG (Ecegon, Biogenesis-Bagó, Argentina) at the same time. Cows in the estradiol-based treatment group received 2 mg EB and a progesterone device (Cronipres 1 g, Biogenesis-Bagó, Argentina) on Day 0, and one dose of $PGF_2\alpha$, 400 IU of eCG and 1 mg of ECP at progesterone device removal (Day 8). Cows were FTAI 52 to 56 hours after device removal in the estradiol group and 72 hours after device removal in the 5-day Co-Synch groups. Although no differences in P/AI were observed in the cycling cows, in cows in postpartum anestrus P/AI was higher in the two groups receiving eCG (5-day Co-Synch: 120/259, 46.3% and estradiol: 151/277, 54.5%) than in those treated with the 5-day Co-Synch but not receiving eCG (71/265, 26.8%; $P < 0.05$).

The 5-day Co-Synch protocol has also been well studied in heifers [18], where three questions needed to be resolved: (1) the necessity of the first GnRH injection at the time of insertion of the progesterone device, considering that a low percentage of heifers ovulate to that first GnRH [22]; (2) the necessity of one or two injections of $PGF_2\alpha$ at the time of removal of the progesterone device if GnRH is administered; and (3) the optimal time for FTAI. Colazo and Ambrose [22] and Lima et al. [20] found that P/AI did not differ whether dairy heifers received GnRH or not at the time of insertion of a progesterone device. Similar results were obtained by Cruppe et al. [45] in beef heifers.

Although, Rabaglino et al. [21] found no differences in P/AI when one or two doses of $PGF_2\alpha$ were used, Peterson et al. [46] reported a tendency for higher P/AI when two injections of $PGF_2\alpha$ were given 6 hours apart in heifers that received GnRH at the time of progesterone insertion. In addition, greater P/AI in dairy heifers receiving GnRH at device insertion was reported in another study, but only when two $PGF_2\alpha$ were administered 24 hours apart at device removal [47]. In relation to the best timing of FTAI, Kasimanickam et al. [48] reported recently that beef heifers inseminated at 56 hours in a 5-day Co-Synch protocol had, on average, a 10.3% higher P/AI than those inseminated at 72 hours. Moreover, Day [18] suggested that the optimum time of insemination is between 56 and 66 hours after CIDR removal in the 5-day Co-Synch program in heifers.

Kasimanickam et al. [49] recently performed a study involving a large number of beef (n = 1018) and dairy (n = 1137) heifers synchronized with the 5-day Co-Synch protocol to determine the effects of GnRH injection at the time of insertion of a CIDR device and the number of $PGF_2\alpha$ at CIDR removal on P/AI. The administration of GnRH at the time of insertion of the CIDR device significantly improved P/AI in beef heifers, but not in dairy heifers. In addition, the administration of one or two $PGF_2\alpha$ at CIDR removal did not have a significant effect on P/AI in either beef or dairy heifers.

In summary, although the necessity of giving GnRH at the time of progesterone insertion in heifers is still unclear, increasing the number $PGF_2\alpha$ treatments may not be necessary to achieve high P/AI in heifers. In relation to the timing of insemination, the optimum time for FTAI in heifers may lie between 56 and 66 hours after CIDR removal. The recommended protocol for the 5-day Co-Synch program is illustrated in Figure 1.

2.3.2. *The J-Synch protocol*

We have recently conducted a series of experiments to evaluate an estradiol-based protocol with a prolonged proestrus, that we named J-Synch [50]. Such estradiol-based treatments are commonly used in South America and the idea behind this treatment was that the administration of 2-mg EB at device insertion results in follicle atresia and emergence of a new follicular 2 to 5 days later in 90% of cows and heifers [5,51]. Finally, because estradiol does not induce ovulation and a new CL, only one administration of $PGF_2\alpha$ is required at device removal to induce luteal regression.

In the first study, 28 Angus crossbred beef heifers, that were 16 and 17 months of age were randomly divided into two groups. Heifers in group 1 (J-Synch, n = 12) received 2-mg EB and an intravaginal device with 0.6 g of progesterone (Emefur 0.6 g, Merial Argentina SA) for 6 days, whereas those in group 2 (n = 14) were treated with the 5-day Co-Synch using the same progesterone device. All heifers received 150 μg of D-cloprostenol (Emefur, Merial) at device removal and received GnRH and were FTAI 72 hours later. All heifers were examined by ultrasonography to monitor follicular development and ovulation. Ovulation rate to the first GnRH in heifers in the 5-day Co-Synch was 50% (7/14) and a new follicular wave emerged 1.6 ± 0.2 days later. Heifers that did not ovulate to the first GnRH had a new follicular wave emerging between 1 and 4.5 days after GnRH. However, 91.6% (11/12) of heifers in the J-Synch group had follicle atresia and a new follicular wave 2 to 5 days after EB administration. The mean (±standard deviation) interval from treatment to emergence of a new follicular was earlier ($P < 0.05$) in heifers treated with GnRH (2.1 ± 1.0 days) than in those treated with EB (3.7 ± 0.9 days). Conversely, ovulation rate (91.6% vs. 92.8%), the diameter of the ovulatory follicle (11.7 ± 0.2 mm vs. 12.0 ± 0.5 mm), the interval from $PGF_2\alpha$ to ovulation (97.1 ± 17.4 hours vs. 95.1 ± 12.5 hours), and P/AI (50.0% vs. 57.1%) did not differ between groups. In a follow-up study [52], follicular and luteal dynamics were compared between beef heifers treated with the J-Synch protocol and those treated with the conventional 7-day estradiol-based treatment protocol. Heifers in the conventional group received a progesterone device (0.5-g Dispositivo Intravaginal Bovino (DIB); Syntex SA, Buenos Aires, Argentina) and 2-mg EB (Gonadiol, Syntex SA) on Day 0, 500 μg of cloprostenol ($PGF_2\alpha$; Ciclase DL, Syntex SA) and 0.5 mg of ECP (Cipiosyn, Syntex SA) at the time of DIB removal on Day 7. Heifers in the J-Synch group received the 0.5-g DIB device and 2-mg EB on Day 0, 500 μg of cloprostenol at DIB removal on Day 6 and 100 μg of gonadorelin acetate (GnRH, Gonasyn GDR, Syntex SA) 72 hours later (Day 9). Heifers in the conventional group ovulated 65.0 ± 13.7 hours after DIB removal, whereas those in the J-Synch group ovulated 93.7 ± 12.9 after DIB removal ($P < 0.05$), demonstrating that the proestrus period was on average 28 hours longer in the J-Synch group. Furthermore, plasma progesterone concentrations were higher and the CL was larger ($P < 0.05$) from days 7 to 12 after ovulation in heifers in the J-Synch group than in the conventional group [52].

Recent experiments were designed to evaluate fertility after the use of the J-Synch treatment compared to the conventional estradiol-based treatment described previously [52]. In this series of experiments, heifers in the J-Synch group were FTAI at the time of GnRH administration (i.e., 72 hours after progesterone device removal), whereas those in the conventional group were FTAI 52 to 56 hours after progesterone device removal. The first experiment was performed during the winter with heifers in a BCS of 4 to 6 (1–9 scale) and losing weight (i.e., on average 1 point of BCS lost from AI to pregnancy detection) due to drought conditions in the area. Results were disappointing, with higher P/AI ($P < 0.01$) in heifers treated with the conventional estradiol-based treatment (49.3%; 138/280) than with the J-Synch treatment (37.8%; 104/275). Therefore, two experiments were performed in the spring to evaluate the fertility in heifers in good BCS (6–7 BCS) and gaining weight after the use of the J-Synch treatment. Experiment 1 was performed in four locations involving 583 Angus, Hereford, and Angus × Hereford crossbred heifers. Results of this study are shown in Table 1. Pregnancy/AI tended to be higher ($P = 0.09$) in the J-Synch–treated heifers than in the conventional estradiol-based treated heifers in the first replicate and was numerically higher in two of the remaining three replicates (Table 1). In a follow-up study (experiment 2; [53]), P/AI was significantly higher in the

Fig. 1. Recommended 5-day CO-Synch protocol in cattle. The recommended interval from progesterone device removal and the second GnRH and FTAI is between 60 and 66 hours in heifers and 72 hours in cows. A second $PGF_2\alpha$ administered at the same time of the device removal or 6 to 12 hours later is recommended in cows and 400 IU of eCG may also be given in cows in postpartum anestrus or poor body condition score. FTAI, fixed-time artificial insemination.

Table 1
Pregnancy rates in beef heifers in good BCS treated with the J-Synch or with the conventional estradiol-based treatment protocols.

Experiment	J-Synch	Conventional	P value
Experiment 1[a]			
Replicate 1	50.7% (33/65)	35.4% (23/65)	0.09
Replicate 2	60.0% (30/50)	66.0% (33/50)	0.6
Replicate 3	58.5% (24/41)	51.2% (22/43)	0.5
Replicate 4	64.2% (88/137)	57.5% (76/132)	0.2
Total experiment 1 (n = 583)	59.7% (175/293)	53.1% (154/290)	0.12
Experiment 2[b]			
Total (n = 208)	67.9% (70/103)	46.6% (49/105)	0.006
Overall (experiments 1 & 2, n = 791)	61.9% (245/396)	51.4% (203/395)	0.0062

Abbreviations: BCS, body condition scores; ECP, estradiol cypionate; FTAI, fixed-time artificial insemination.

[a] In experiment 1, heifers in the conventional treatment received 0.5 mg ECP at progesterone device removal (Day 7) and were FTAI at 52 to 56 h later. In J-Synch treatment, the progesterone device was removed on Day 6, and heifers received GnRH and were FTAI 72 h later.

[b] In experiment 2, all heifers were tail painted at device removal. Heifers with the tail paint rubbed off by 36 h (conventional) or 48 h (J-Synch) after device removal were AI 12 h later. Those without the paint rubbed off at that time were FTAI 52 to 56 h (conventional) or 72 h (J-Synch) after device removal.

Data from the study of de la Mata et al. [52,53].

J-Synch–treated heifers than in those treated with the conventional estradiol-based treatment. In this study, cycling Angus and Hereford heifers (n = 208) were also randomly allocated to receive the estradiol-based conventional or the J-Synch treatment (Table 1). The difference between experiments 1 and 2 was that heifers in experiment 2 were tail painted at the time of DIB removal and observed once for signs of estrus (i.e., tail paint rubbed off). Those in the conventional group with the tail paint rubbed off by 36 hours after DIB removal were inseminated 12 hours later (i.e., 48 hours), whereas those not showing estrus by 36 hours were FTAI at 54 hours. Heifers in the J-Synch group were also tail painted at DIB removal and those with the tail paint rubbed off by 48 hours were inseminated at 60 hours, whereas those not showing estrus received GnRH and were FTAI at 72 hours after DIB removal. Heat-detection rate and P/AI did not differ ($P > 0.1$) between groups (38.8%, 40/103 and 60.3%, 38/63 for heifers in the J-Synch group vs. 28.5%, 30/105 and 45.3%, 34/75 for those in the conventional group). However, P/AI tended ($P < 0.09$) to be higher and the overall P/AI was significantly higher ($P < 0.01$) in heifers observed in estrus and AI early in the J-Synch group (80.0%, 32/40 and 67.9%, 70/103) compared to the conventional group (50%, 15/30 and 46.6%, 49/105). Furthermore, heifers in the J-Synch group that had their tail paint rubbed off by 48 hours after DIB removal and were AI at 60 hours had a higher ($P < 0.05$) P/AI than those in the same group that were FTAI at 72 hours. The cumulative results of the two experiments with heifers in good BCS (Table 1) found a significantly higher ($P < 0.01$) P/AI in those treated with the J-Synch protocol than in those treated with the conventional estradiol-based protocol. It is also important to note that in experiment 1, all heifers were also tail painted and whether the paint was rubbed off was recorded at the time of FTAI. In both treatment groups, heifers with the tail paint rubbed off (i.e., in estrus) at FTAI had higher ($P < 0.01$) P/AI than those that had not shown estrus at FTAI (58% vs. 37% and 66% vs. 45%, respectively, for those heifers in the conventional and J-Synch groups, respectively).

The contradictory results in the experiments with heifers losing weight in the winter and those in good BCS and gaining weight in the spring can probably be explained by smaller follicle diameters and lower estradiol concentrations in the heifers in low BCS and losing weight that were treated with the J-Synch protocol compared to those in good BCS. As indicated previously, high estradiol concentrations in the proestrus period have been associated with a more appropriate uterine environment, higher luteal phase progesterone concentrations and lower incidence of embryo loss [54]. Therefore, it is possible that heifers treated with the conventional protocol may have benefited from the 0.5-mg ECP given at the time of device removal, whereas the heifers in the J-Synch protocol may have had smaller follicles and low estradiol concentration during the proestrus period, since no estradiol was administered at device removal in this protocol. Conversely, the heifers in good BCS and gaining weight in the follow-up studies shown in Table 1 may have had larger estradiol-producing follicles; thus, no additional estradiol was needed to result in high fertility.

To further confirm these results, a large field trial, involving two experiments with 4947 Angus × Hereford crossbred heifers was set up in Uruguay. The experiments were performed in five (experiment 1) and six replicates (experiment 2) during 2013/2014 (reported as preliminary data in [55]) and 2014/2015 (Menchaca unpublished observations). A secondary objective was to determine the effects of the time of FTAI and the addition of eCG at the time of progesterone device removal on P/AI in J-Synch–treated heifers. In experiment 1, all heifers received a 0.5-g DIB device plus 2-mg EB on Day 0. Heifers in the conventional treatment group received $PGF_{2}\alpha$ and ECP and had their DIB removed on Day 7 AM. Heifers were then subdivided to be FTAI on Day 9 AM or PM (i.e., 48 or 56 hours after DIB removal). Heifers in the J-Synch treatment group received $PGF_{2}\alpha$ and DIB removal on Day 6 PM and received GnRH and were FTAI on Day 9 AM or PM (i.e., 60 or 72 hours after DIB removal). All heifers in this experiment were also treated with 300 IU eCG (Novormón 5000, Syntex S.A.) at DIB removal. In experiment 2, all the heifers were treated with the J-Synch protocol as described in experiment 1, but at device removal (Day 6 PM), heifers were divided to receive 300 IU eCG or no eCG treatment at that time, and heifers were further subdivided to receive GnRH and were FTAI on Day 9 AM or PM (i.e., 60 or 72 hours after DIB removal). Results of these two experiments are presented in Table 2. In experiment 1, overall P/AI was higher ($P < 0.05$) in J-Synch–treated heifers, while time of FTAI only affected P/AI after the conventional treatment ($P < 0.05$). However, removal of eCG from the J-Synch protocol in experiment 2 resulted in reduced P/AI ($P < 0.05$) when inseminations were done on Day 9 PM (i.e., 72 hours after DIB removal). In summary, the addition of eCG to the J-Synch protocol provided for a wider window of insemination times facilitating FTAI in large groups of beef heifers. The recommended protocol is shown in Figure 2.

Table 2
Effect of synchronization treatment (J-Synch vs. conventional), time of insemination, and eCG treatment on P/AI in beef heifers.

Experiment 1[a]	J-Synch (+300 IU eCG)	Conventional (+300 IU eCG)	P
FTAI—Day 9 AM	57.1% (335/587)	53.4% (324/607)	0.20
FTAI—Day 9 PM	55.0% (296/538)	48.0% (296/617)	0.02
P	0.49	0.06	
Total (n = 2349)	56.1% (631/1125)	50.7% (620/1224)	0.01
Experiment 2[a]	**J-Synch (+300 IU eCG)**	**J-Synch (No eCG)**	**P**
FTAI Day 9 AM	57.9% (368/636)	56.3% (381/677)	0.56
FTAI Day 9 PM	56.3% (371/659)	49.7% (311/626)	0.02
P	0.57	0.02	
Total (n = 2598)	57.1% (739/1295)	53.1% (692/1303)	0.04

Abbreviations: ECP, estradiol cypionate; FTAI, fixed-time artificial insemination; P/AI, pregnancy per AI.
[a] Heifers in the conventional treatment received 0.5-mg ECP at progesterone device removal (Day 7 AM) and were FTAI at 48 (Day 9 PM) or 56 h (Day 9 PM) later. In J-Synch treatment, the progesterone device was removed on the PM of Day 6 and heifers received GnRH and were FTAI 60 (Day 9 AM) or 72 (Day 9 PM) h later.
Data from the study of Menchaca et al. [55] and Menchaca et al. (unpublished).

In conclusion, reducing the time of progesterone device insertion and lengthening the proestrus period, as in heifers treated with the J-Synch protocol, resulted in higher P/AI than those treated with the conventional estradiol-based protocol. Furthermore, the combination of estrus detection and FTAI and the addition of 300 IU of eCG may further improve the pregnancy outcome. The beneficial effect of the J-Synch protocol on fertility has been recently confirmed in a recipient synchronization program involving 945 *in vitro*–produced embryos [56]. In this experiment, Hereford cows were treated with the conventional estradiol-based treatment or the J-Synch treatment, all receiving 400 IU eCG at device removal. Pregnancy rate following embryo transfer 7 or 8 days after GnRH (J-Synch) or 9 or 10 days after ECP (conventional) was higher ($P < 0.01$) in recipients synchronized with the J-Synch protocol (49.3%) than the conventional estradiol-based protocol (40.9%).

3. Resynchronization treatments

Aggressive reproductive management systems comprise three strategies that can be implemented early during the breeding period: (1) inseminate all cows at the beginning of the breeding season, (2) identify nonpregnant cows as early as possible, and (3) rebreed the nonpregnant

Fig. 2. Recommended J-Synch protocol in cattle. The recommended interval from progesterone device removal and the second GnRH and FTAI is between 60 and 72 hours in beef heifers. Administration of eCG (300 IU in heifers and 400 IU in cows) may also be given in those animals in anestrus, with poor body condition score or embryo recipients. EB, estradiol benzoate; FTAI, fixed-time artificial insemination.

cows as soon as possible. The easiest and most commonly used alternative to get nonpregnant cows pregnant soon after the first AI is to use clean-up bulls for the remainder of the breeding season. However, there are other options for breeders that desire a larger percentage of AI-sired calves or wish to limit the use of bulls. One approach is to observe estrus and AI all animals showing estrus between 17 and 24 days after the first FTAI and then confirm pregnancy by ultrasonography 30 to 32 days after the first AI in all cows not returning to estrus. All cows determined to be nonpregnant at that time can then receive GnRH or estradiol and a progesterone device and be FTAI after device removal. Although this alternative is more commonly used in dairy than in beef herds because it requires considerable handling of the animals, all open cows to the first FTAI are reinseminated within 6 weeks [57]. Another alternative is to reinsert progesterone-releasing devices and administer 1-mg EB 12 to 14 days after the first FTAI and carry out estrus detection and AI after device removal 7 or 8 days later [58]. However, this treatment requires accurate estrus detection 3 to 4 days after device removal and the administration of EB 13 days after FTAI in yearling beef heifers has been shown to negatively affect pregnancy to the first FTAI [58]. Furthermore, it has been recently reported that the administration of 1.5-mg EB 13 days after the first FTAI negatively affected the CL (i.e., reduced the vascularization of the CL evaluated by color-flow Doppler) and pregnancy to the first FTAI in dairy cows [59]. Therefore, these data makes the application of resynchronization programs using EB 13 days after the first FTAI questionable, or at least risky, if more than 1-mg EB is administered accidentally. Furthermore, with the banning of estradiol in several countries around the world, reinserting a progesterone-releasing device alone often results in poor return to estrus after the resynchronization [60,61].

Another approach that we have investigated is the use of ultrasonography combined with FTAI of all the nonpregnant cows to the first FTAI, without estrus observation. Suckled beef cows and heifers received a reused progesterone device on Day 16 after FTAI and a dose of GnRH on Day 21. The idea of inserting a device on Day 16 to 21 is to prevent ovulation in cows that would return to estrus before Day 21 and to create a persistent follicle, which will ovulate after the administration of GnRH on Day 21. All cows were then examined by ultrasonography on Day 28 for pregnancy diagnosis and those that were found to be nonpregnant received $PGF_2\alpha$ at that time and 1-mg EB on Day 29 or a second dose of GnRH on Day 30. All cows were FTAI 12 hours after the second GnRH or 30 hours after EB, with a cumulative P/AI after two FTAI around 80% [61]. Pregnancy for the first and second FTAI and the overall P/AI in a field trial involving 6431 beef cows and heifers were 57%, 51%, and 79%, respectively.

Another option that was recently investigated in *B. indicus* cows and heifers is to administer 1-mg EB or GnRH and insertion of a progesterone device on Day 22 after the first FTAI [62]. Pregnancy diagnosis by ultrasonography was performed at device removal on Day 30. All nonpregnant cattle received $PGF_2\alpha$ and 0.5 mg of ECP at device removal and were FTAI 48 hours later (Fig. 3). One experiment compared the effect of 1-mg EB or GnRH on Day 22 after

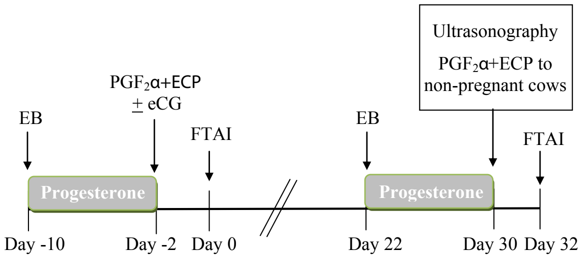

Fig. 3. Resynchronization with unknown pregnancy status using progesterone and estradiol-based fixed-time AI protocols in beef cattle. EB, estradiol benzoate; ECP, estradiol cypionate; FTAI, fixed-time artificial insemination. Adapted from the study of Sá Filho et al. [62].

the first FTAI in *B. indicus* beef heifers. A higher P/AI ($P < 0.05$) was observed in heifers resynchronized with EB (49.2%, $n = 140$) than with GnRH (37.2%, $n = 137$; [62]). In a follow-up experiment [62], pregnancy to the first FTAI and pregnancy loss from 30 to 60 days after FTAI of nonsuckling beef cows and heifers receiving an EB resynchronization protocol ($n = 195$) did not differ from those exposed to natural service with clean-up bulls ($n = 198$) after the first FTAI (61.5% P/AI and 4.1% pregnancy loss in the resynchronized group and 57.1% P/AI and 2.0% in the natural service group). These results demonstrated that 1-mg EB on Day 22 after the first FTAI had no detrimental effect on P/AI. A more recent study compared two different doses of EB (1 vs. 2 mg) administered at the onset of the resynchronization protocol 22 days after first FTAI in suckled beef cows [63]. A total of 1426 cows (768 *B. taurus* and 728 *B. indicus*) were enrolled in this trial. Pregnancy to the first FTAI and pregnancy loss between Days 30 and 62 did not differ between cows receiving 1-mg (44.0% and 3.8%) or 2-mg EB (44.0% and 5.5%) on Day 22. However, pregnancy to the second FTAI (resynchronization) was higher ($P < 0.01$) in cows treated with 2-mg EB (47.3%) than with 1-mg EB (36.1%, $P < 0.01$). Finally, the cumulative P/AI was also higher ($P < 0.01$) in cows treated with 2-mg EB (68.2%) compared to 1-mg EB (62.8%). The difference in P/AI may be due to more effective follicle wave synchronization with 2-mg EB than 1-mg EB in cows [64]. Wave emergence (defined as the appearance of 2 mm follicles) was more variable ($P < 0.05$) in cows treated with 1-mg EB (1.8 ± 1.3 days) than in those treated with 2-mg EB (2.3 ± 0.6 days) 22 days after the first FTAI. Therefore, the dose of 2-mg of EB on Day 22 seems to be more effective to resynchronize cows, but more research needs to be done to determine if 1- or 2-mg EB is more effective in heifers and to confirm that 2-mg EB does not increase pregnancy loses in cows and heifers. Given the positive results, practitioners began to apply these resynchronization protocols in the field and when possible, undertake a third FTAI. Inclusion of a third FTAI in Nelore cattle resulted in an overall P/AI of 83.1% (1531/1843) in the first 64 days of the breeding season (87.4%, 660/755 for multiparous cows, 81.8%, 413/505 for primiparous cows and 78.6%, 458/583 for heifers; [65]). One final approach to resynchronize ovulation for a second FTAI is to use the 5-day Co-Synch protocol on Day 32 after FTAI, with or without the use of GnRH, as it has been reported in dairy cattle [66,67].

4. Summary and conclusions

Protocols that control follicular development and ovulation using GnRH or estradiol and progesterone-releasing devices provide for the opportunity to apply FTAI in beef herds without the need for detecting estrus and P/AI of 50% or higher with a single insemination. The addition of eCG at progesterone device removal to stimulate the growth of the dominant ovulatory follicle has been especially useful in increasing P/AI in cows experiencing postpartum anestrus or in low body condition. Shorter ovulation synchronization treatments that provide for a longer proestrus are an interesting new alternative for FTAI and have resulted in increased P/AI in beef cows and heifers. Finally, similar approaches to those used for the first FTAI can also be combined with early pregnancy diagnosis with ultrasonography for a second or third FTAI without estrus detection resulting in similar or higher P/AI to those obtained with clean-up bulls, maximizing the use of the improved genetics in a herd through AI.

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