CO-Synch as an economical fixed-time artificial insemination protocol to improve pregnancy rate in cow-calf production systems

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SUMMARY

Fixed-time artificial insemination (FTAI) protocols are a tool to increase the reproductive efficiency in beef herds, however their use in Mexico and worldwide is limited. The aim of this experiment was to compare the pregnancy per artificial insemination (P/AI), overall pregnancy rate (PR), follicular dynamics, and cost among beef cattle treated with three different FTAI protocols. A total of 191 females were assigned to CO-Synch (n=63), CO-Synch+CIDR (n=60) and estradiol/progesterone (n=68) protocols balanced by body condition scores (BCS). Females treated with the estradiol/progesterone protocol had higher (P<0.05) estrus presentation than females in the CO-Synch and the CO-Synch+CIDR protocol. P/AI tend to be higher (P<0.10) in cattle treated with the estradiol/progesterone protocol than females in the CO-Synch follicule, number of dominant follicles (\geq 6 mm) and percentage of females with a dominant follicle at prostaglandin F2- α (PGF2- α) injection were higher (P<0.05) in females of the CO-Synch protocol than females in the estradiol/progesterone protocol. Number of follicles from 2 to 5 mm was higher (P<0.05) in females treated with the estradiol/progesterone protocol than females in the CO-Synch protocol. Cost of treatment in relation to total calves born, was higher in estradiol/progesterone and in the CO-Synch+CIDR protocols than the CO-Synch protocol.

KEY WORDS

Beef cattle; FTAI protocols; economic evaluation.

INTRODUCTION

Fixed-time artificial insemination (FTAI) protocols, represent a significant way to improve reproductive performance in beef herds.^{1,2} Use of these technologies may have a huge impact in regions, like Mexico, where reproductive performance is low.³ Despite of the advantages of these technologies, their use in beef production is limited, not only in México, but worldwide.⁴ In Mexico, less than 15% of the cow-calf operations use synchronization and less than 10% use artificial insemination.³ Similarly, in USA and Brazil, only around of 12% of beef females of reproductive age are inseminated. ^{5,6} Among the reasons that calf producers do not adopt these technologies are time and labor required, low conception rates, lack of facilities, cost, complexity of the FTAI protocols, and requirement for specialized personnel.^{6,7}

Currently, there are two types of FTAI protocols; the gonadotropin release hormone (GnRH)-based protocols and estradiol-based protocols. Estradiol-based protocols are used in combination with progesterone-releasing devices, whereas GnRH based protocols may or may not use progesterone-releasing devices.^{8,9} Even though it has been suggested that use of FTAI protocols result in an increase in pregnancy per artificial insemination (P/AI) of 50% or higher, ⁹ the truth is that P/AI is highly variable. In lactating beef cows treated with the CO-Synch protocol plus a progesterone device, the P/AI reported ranged from 33%¹⁰ to 71%,¹¹ whereas in heifers the lowest P/AI reported is 38%¹² and the highest is 68%.¹³ In lactating beef cows submitted to CO-Synch protocols without progesterone devices,¹⁴ report a P/AI of 31% and¹⁵ a P/AI of 44%. For beef cat-

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tle treated with estradiol-based protocols in heifers the range of P/AI is from 48%¹⁶ to 59%¹⁷ and in lactating beef cows the lowest and highest P/Al reported is 23%¹⁸ and 72%¹⁹ respectively.

Anestrus is one of the most important factors that affect reproductive performance in beef cow-calf operations⁷ and it is well known that FTAI protocols may reduce the period between birth and pregnancy in heifers and between calving and pregnancy in postpartum beef cows.²⁰ Beef heifers and suckled beef cows, submitted to FTAI protocols at the beginning of the breeding season had an increased in the proportion of cows in estrus and in the pregnancy rate at the onset of breading season.^{7,21} The mechanism by which FTAI protocols induce cyclicity in anestrous cattle is associated to the effect of progesterone on LH secretion.^{20,22} Therefore, use of a progesterone device or induction of ovulation of a dominant follicle by GnRH treatment could supply the progesterone necessary to restore cyclicity in anestrus beef cattle.

Among the hormones used for these different FTAI protocols, progesterone-releasing device may represent more than 50% of the costs, thus the use of CO-Synch protocols may represent a good way to reduce the cost of use of FTAI, as long as, the reproductive response is not reduced. Therefore, the aim of this experiment was to compare the P/AI, overall pregnancy rate (PR) and follicular dynamics between beef cattle treated with CO-Synch protocol without progesterone and two protocols that use a progesterone device. Additionally, the influence of FTAI protocol, BCS, and follicular dynamics on the occurrence of P/AI was evaluated.

MATERIAL AND METHODS

All animal procedures were performed in accordance with section 2, article 20, 3th title of the Federal law on animal health, (2018) México and approved by the Research Ethics Committee of the División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana, protocol number CEI. 2022.014

Location and animals

The experimental study was performed on three commercial farms in the state of Puebla, Mexico (20°08 48» N, 97°27 42» W) from August 2019 to February 2020. A total of 43 beef heifers (*Bos indicus* × *Bos taurus*) and 148 suckling beef cows (*Bos indicus* × *Bos taurus*; postpartum day = 166 ± 72), were used in this experiment. Animals were allocated in pastureland and fed common Star of Africa grass (*Cynodon plectostachyus*), mixed with native grasses (*Axonopus spp* and *Paspalum spp*) as a basal diet and supplemented with mineral salts (Ganafos 12%; México) containing Ca (12 %), P (12%) and Mn (12,000 ppm) offered ad libitum

Experimental design

Females were randomly assigned to one of three FTAI protocols (Figure 1) balanced by body condition score (BCS) and postpartum days (suckling cows). The BCS using the scale where $1 = \text{emaciated and } 9 = \text{obese}^{23}$ at the beginning of the experiment was 6.6 \pm 1.0 for heifers and 6.5 \pm 1.2 for suckling beef cows, whereas postpartum days in suckling beef cows were 166 \pm 72. Females in the CO-Synch protocol (n=63; 14 heifers and 49 cows) were injected at day zero with 250 µg of GnRH (Sanfer ® Mexico) and seven days later with 25 mg of a PGF2a analog (Lutalyse ® Lutalyse®, Pfizer Animal Health; USA). Animals in the CO-Synch+CIDR protocol, (n=60; 13 heifers and 47 cows) received the same treatment mentioned above but a controlled internal drug release (CIDR) device containing 1.9 g progesterone (CIDR 1900 Cattle Insert; Pfizer Animal Health, Hamilton, New Zealand) was inserted at day zero and removed 7 day later. Between 54 and 60 hours after PGF2α injection females assigned to these protocols were inseminated and a second injection of GnRH was used (250 µg). Females in the estradiol/progesterone protocol (n = 68; 16 heifers and 52 cows) received an injection of 2 mg of estradiol benzoate (Sincrodiol ® Ourofino Animal health, Brazil) and an CIDR was insert on day zero. The CIDR was removed on day 8 and 25 mg of PGF2 α was injected. These animals received a second injection of 1 mg estradiol benzoate 24 hours after CIDR withdrawal and the FTAI

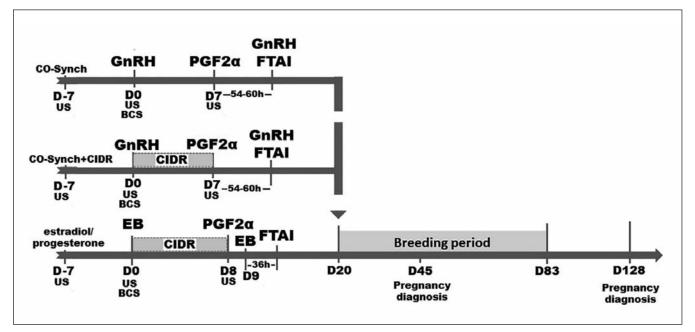


Figure 1 - Experimental design.

was performed at 36 hours later. BCS was assessed at beginning of synchronization (Figure 1). Ten days after FTAI cows and heifers were exposed to bull mating for a breading season of 63-day using a bull:cow ratio of 1:20.

Follicular dynamics

Follicular dynamics were determined by transrectal ultrasonography (Aloka SSD-500, Aloka Hitachi, Tokyo, Japan) using a 7.5 MHz linear transrectal probe. Ultrasounds were performed in the following stages: at animal selection, at beginning of synchronization and at PGF2- α injection (Figure 1). Seven days before the beginning and at the beginning of synchronization, the presence of a corpus luteum was recorded to determine cyclic and anestrus animals. Additionally, at the beginning of the FTAI protocol, the diameter of the largest follicle present in the ovaries was measured. At time of PGF2 α injection, the diameter of the largest follicle presents in the ovaries and the number of follicles larger than 6 mm were recorded. Finally, the number of follicles between 2 and 5 mm was also recorded in this ultrasonography evaluation. The diameter of the dominant follicle (DF) at follicular selection in Bos indicus cattle is around of 6 mm²⁴ and animals in this experiment were Bos indicus × Bos taurus crosses. Therefore, these ultrasonography data could be used to determine which animals had a DF or follicles in the stage of recruitment at the time of proestrus onset, which is supposed to be induced by PGF2 α injection or CIDR withdrawal.

Estrus detection and pregnancy diagnosis.

After the injection of PGF2 α in the three groups, the tailhead was painted with marker crayons for cattle to detect mounts. Additionally, the presence of estrus behavior was detected by visual evaluation two times a day (06:00 and 18:00) for at least 30 minutes, starting 24 hours after PGF2 α injection and until FTAI. Pregnancy per artificial insemination (P/AI) and pregnancy rate (PR) of breeding season were assessed by ultrasonography using a 7.5 MHz linear transrectal probe 35 days after AI and 45 after the end of breeding period respectively. P/AI was defined as the number of pregnant animals at 35 days after AI on the total number of animals treated in each protocol multiplied by 100. PR of the breeding season was defined as the number of pregnant during the breeding season by bull mating on the total

number of animals treated in each protocol multiplied by 100.

Economic evaluation

For the economic analysis, the cost of each treatment per animal synchronized was obtained and then the total spent (in US dollars) for all the females treated in each protocol was calculated. To evaluate the ratio cost/profit, first we divided the total spend of each treatment by the number of calves born by AI or by the total number of calves born. Secondly, considering a price of sale of a weaned calf in the region of study and in the year that the experiment was carried out of \$ 444.18 US dollars, we calculated the percentage that represents the total spent on each treatment in relation to the value of weaned calves.

Statistical analysis

The effect of treatment, physiologic status (cows and heifers), and their interaction on estrus presentation, P/AI, and PR were evaluated by logistic regression. Differences in BCS and follicular dynamics were evaluated by the standard least square method using treatment, physiological status, and their interaction as fixed variables and location as random effect. For all these variables, homoscedasticity was verified by Levene test. Percentage of cyclic animals at the beginning of the FTAI protocol and presence of DF (≥ 6 mm) at PGF2- α injection were analyzed by logistic regression using treatment, physiological status and their interaction as fixed variables. The odds of P/AI by effect of treatment, cyclicity, presence of DF (≥ 6 mm) at PGF2- α injection and estrus presentation were calculated. Finally, the effect of BCS and follicular dynamics on probability of P/AI were tested by generalized linear model including treatment and physiological status. P values < 0.05 were considered statistically different and P values from 0.05 to <0.10 were considered as tendencies.

RESULTS

Estrus presentation, P/AI, and overall pregnancy rate (PR) was not affected by the interaction treatment by physiological status. Therefore, in table 1, only the main effects are present. Females treated with the estradiol/progesterone protocol had increased frequency of estrus presentation (P<0.05) than females in the CO-Synch and the CO-Synch+CIDR protocol. P/AI tend to be higher in cattle in the estradiol/progesterone protocol com-

 Table 1 - Estrus presentation, pregnancy per artificial insemination (P/AI) and overall pregnancy rate (PR) of breeding season in beef cattle treated with different protocols of estrus synchronization and ovulation.

		Treatment			Physiologic status		P value	
	Estradiol / Progesterone	CO-Synch+ CIDR	CO-Synch	Cows	Heifers	Treat	PS	
Estrus presentation Location 1 Location 2 Location 3	63/68 (92.7) 21/24 (87.5) 30/31 (96.8) 12/13 (92.3)	42/60 (70.0) 11/17 (64.7) 23/30 (76.7) 8/13 (61.5)	39/63 (61.9) 10/21 (47.6) 20/30 (66.7) 9/12 (75.0)	109/148 (73.7) 23/37 (62.16) 57/63 (78.1)	35/43 (81.4) 19/25 (76.0) 16/18 (88.9)	<0.0001 0.0064 0.0058 0.1547	0.297 0.1011 0.4465	
P/AI Location 1 Location 2 Location 3	24/68 (35.3) 5/24 (20.8) 13/31 (41.9) 6/13 (46.2)	17/60 (28.3) 3/17 (17.7) 13/30 (43.3) 1/13 (7.7)	11/63 (17.5) 2/21 (9.5) 8/30 (26.7) 1/12 (8.3)	41/148 (27.7) 6/37 (16.2) 27/73 (37.0)	11/43 (25.6) 4/25 (16.0) 7/18 (38.9)	0.0641 0.5549 0.3263 0.0269	0.7588 0.9166 0.9906	
Overall PR Location 1 Location 2 Location 3	49/68 (72.1) 12/24 (50.0) 25/31 (80.7) 12/13 (92.3)	45/60 (75.0) 11/17 (64.7) 22/30 (73.3) 12/13 (92.3)	39/63 (61.9) 11/21 (52.4) 19/30 (63.3) 9/12 (75.0)	106/148 (71.6) 17/37 (45.9) 53/73 (76.7)	27/43 (62.8) 17/25 (68.0) 10/18 (55.6)	0.2504 0.6232 0.1938 0.3688	0.2695 0.0852 0.0455	

	Heifers			Cows			P values				
	Estradiol / Progesterone n=16	CO- Synch+CIDR n=13	CO-Synch n=14	EE	Estradiol / Progesterone n=52	CO-Synch+ CIDR n= 47	CO-Synch n=49	EE	Treat	PS	T*PS
BCS At beginning of synchronization	6.7	6.7	7.0	0.4	6.4	6.5	6.4	0.3	0.8255	0.0656	0.7026
Follicular dynamic Cycling animals at beginning of synchronization %	12 (75)	5 (39)	7 (50)		22 (42)	24 (51)	21 (43)		0.3183	0.2769	0.0895
Diameter of larges follicle at beginning of synchronization	8.6	6.8	10.0	0.9	7.4	7.8	7.5	0.5	0.1181	0.1155	0.0413
Diameter of largest follicle at PGF2- α injection	5.6 1	7.5	7.0	1.0	5.9	7.0	9.3	0.6	0.0075	0.3299	0.2091
Number of DF (\ge 6 mm) at PGF2- α injection	0.6	1.2	1.4	0.2	0.7	1.0	1.2	0.1	0.0008	0.7635	0.5407
Presence of DF (\geq 6 mm) at PGF2- α injection %	8(50)	10 (77)	11 (79)		28 (54)	33 (70)	41(84)		0.0064	0.9056	0.7878
Number of follicles of 2 to 5 mm at PGF2- α	10.7	7.7	4.5	1.4	10.0	7.5	6.3	0.8	<.0001	0.7788	0.4646

Table 2 - Body condition score (BCS) and follicular dynamic in beef heifers and lactating beef cows treated with different protocols of estrus synchronization and ovulation.

pared with females in the CO-Synch protocol. Overall PR was

not different (P>0.05) among protocols.

injection

BCS was similar (P>0.05) by treatment in heifers and suckling cows at the beginning of synchronization, but heifers tend to had higher BCS than suckling cows (Table 2). Percent of cyclic animals at the beginning of the FTAI protocol was similar within physiological status (P>0.05), however the number of cyclic heifers in the estradiol/progesterone protocol tended to be greater than suckling cows in the estradiol/progesterone and CO-Synch protocol. The diameter of the largest follicle at the beginning of the FTAI protocol was higher in heifers of CO-Synch protocol (P<0.05) compared with heifers of CO-Synch+CIDR protocol and with suckling cows in all the protocols (Table 2). Follicular dynamics at PGF2- α injection were not affected by physiological status or the interaction of physiological status by treatment, but there was an effect of treatment (Table 2).

The diameter of the largest follicle, the number of DF (≥ 6 mm) and the presence of DF (≥ 6 mm) at PGF2- α injection were higher (P<0.05) in females in the CO-Synch protocol compared with females in the estradiol/progesterone protocol, but not compared with animals in the CO-Synch-CIDR protocol. In contrast, number of follicles from 2 to 5 mm was greater

(P<0.05) in females in the estradiol/progesterone protocol than in females in the CO-Synch protocol (Table 2).

When the odds of P/AI were calculated (Table 3), it was observed that females treated with the estradiol/progesterone protocol, cyclic females, and females that presented estrus behavior had 2.58, 1.97 and 2.63 (respectively) times more probability of P/AI than females in the CO-Synch protocol, females in anestrus, or without estrus behavior. Additionally, females with a selected follicle at PGF2- α injection tended to have an increased probability of P/AI than females without a selected follicle. Finally, females with higher BCS recorded at the beginning of synchronization, had increased probability (P<0.05) of P/AI (Table 4). Similarly, an increase in the diameter of the largest follicle and in the number of DF (≥ 6 mm) at PGF2- α injection increased (P<0.05) the probability of P/AI (Table 4). Results of economic analysis (Table 5) showed that cost of treatment in relation to number of calves born either by AI or by natural breeding is higher in estradiol/progesterone and in the CO-Synch+CIDR protocols compared with the CO-Synch protocol. Thus, the percentage of synchronization cost in relation to income from sale of weaned calves is only 1% for the CO-Synch protocol compared with 5% for the estradiol/progesterone and CO-Synch+CIDR protocols.

Table 3 - Effect of treatment, ovarian status, presence of dominant follicle (DF) at PGF2- α injection and estrus presentation on the probability of pregnancy per artificial insemination (P/AI) in beef cattle.

Factors	Levels	Odd Ratio	95% CI	Chi value
Treatment	CO-Synch Estradiol/Progesterone CO-Synch+CIDR	1.00 2.58 1.87	1.13 - 5.86 0.79 - 4.41	0.0231 0.1541
Cycling animals at beginning of synchronization	Anestrus Cycling	1.00 1.97	1.03 - 3.78	0.0411
Estrus presentation	Not Yes	1.00 2.63	1.09-6.34	0.031
Presence of DF (\ge 6 mm) at PGF2- α injection	Not Yes	1.00 2.03	0.96-4.30	0.0652

Variable	intercept	estimate	EE	P value
BCS At beginning of synchronization	-3.68	0.40	0.16	0.0092
Follicular dynamic Diameter of larges follicle at beginning of synchronization	-1.43	0.05	0.05	0.3804
Diameter of largest follicle at PGF2- α injection	-1.86	0.11	0.05	0.0173
Number of DF (\geq 6 mm) at PGF2- α injection	-1.53	0.42	0.19	0.0290
Number of follicles of 2 to 5 mm at PGF2- α injection	-0.90	-0.02	0.03	0.5299

Table 4 - Effect of body condition score (BCS) and follicular dynamic on pregnancy per artificial insemination (P/AI) in beef cattle.

DISCUSSION

The results of the present experiment showed that P/AI, but not overall PR, is improved by the use of estradiol/progesterone protocol compared with CO-Synch protocol. Moreover, it was confirmed that P/AI is affected by BCS, follicular dynamics, ovarian status, and estrus presentation. The economic analyses suggested that even though P/AI is low in cows treated with the CO-Synch protocol, this protocol could be more profitable compared with the other two protocols evaluated in this experiment.

P/AI was approximately 100% higher in females in the estradiol/progesterone protocol as compared to those in the CO-Synch protocol. Similarly, in Hereford Shorthorn cross lactating beef cows that were treated with the estradiol/progesterone protocol had higher P/AI than females synchronized with the CO-Synch protocol.²⁵ Success of the CO-Synch protocol depends on ovulation after the first GnRH to ensure synchronization of the follicular wave and proestrus onset after PGF2a injection, ^{26,9} whereas in the estradiol/progesterone protocol, even when treatment with estradiol benzoate does not synchronize the follicular wave, CIDR withdrawal plus PGF2α ensure that females enter proestrus.²² These differences in responses between the estradiol/progesterone and CO-Synch protocols become evident when we observe that estrus presentation was 46% higher in the estradiol/progesterone protocol than in the CO-synch protocol and may explain the differences in P/AI observed. Moreover, at PGF2a injection, females in the CO-synch protocol had a larger dominant follicle (suckling beef cows) and reduced number of recruited follicles (cows and heifers) than females of the estradiol/progesterone protocol. Thus, it is likely that differences in follicular dynamic may also be associated with the reduction in P/AI in the CO-synch protocol as compared with the estradiol/progesterone protocol. 22,26

Use of a CIDR in the CO-synch protocol may improve P/AI.9 Nevertheless, in the present experiment, no differences in P/AI between the CO-synch and CO-synch+CIDR protocol were observed. Similarly, in suckling and non-suckling beef cows, use of a CIDR or melegestrol acetate in the CO-synch did not modify P/AI.²⁷ In contrast, several reports have shown that P/AI is higher in heifers and suckling beef cows synchronized with the CO-synch+CIDR protocol than those synchronized with the CO-synch protocol.^{11,27,14,28,29} Differences between our results and previous reports could be due lack of power in our statistical analysis resulting from the smaller number of animals used herein. Supporting this idea, paired comparisons of P/AI between CO-synch and CO-synch+CIDR protocol reveals a P value of 0.1107. Additionally, P/AI observed in this experiment was low in all protocols, but specially in females in the CO-synch and CO-synch+CIDR protocols, which may also explain the lack of difference in the P/AI.

Although P/AI was lower in the CO-synch protocol compared with the estradiol/progesterone protocol, the overall PR was similar between them and with the CO-synch+CIDR protocol. Prepuberal or postpartum anestrus is one of the main factors that reduces fertility in cow-calf systems^{30,31} and it is well know that FTAI protocols may induce cyclicity. ^{20,5} Thus, the FTAI protocols used in this study could have induced cyclicity similarly in all females, explaining the similar overall PR among them. BCS, follicular development, estrus presentation, and cyclicity are the main factors that affect P/AI in beef cattle submitted to FTAI protocols.^{9,22} In the present experiment, BCS at the beginning had a positive effect on occurrence of P/AI. Similarly, in suckling beef cows and heifers treated with estradiol/progesterone-based protocols or CO-synch+CIDR protocols, BCS at the beginning of the FTAI protocol had positive

 Table 5 - Eff Cost of treatments, spent by calf born, and percentage that represents the spent of all females treated in relation to the sale of weaned calve. To calculate the incoming by sale of weaning calves a price of \$ US 444.18/calve was considered.

	Estradiol / progesterone	CO-Synch +CIDR	CO-Synch
Number of treated animals	68	60	63
Cost of treatment/animal	\$14.92	\$15.31	\$3.20
Total spent	\$1,014.63	\$918.45	\$201.56
Spent by calf born by Al	\$42.28	\$54.03	\$18.32
Spent by all the born calves	\$20.71	\$20.41	\$5.17
Percentage that represents the spent of al females treated in relation to the sale of weaned calves by AI	10%	12%	4%
Percentage that represents the spent of all females treated in relation to the sale of all weaned calves.	5%	5%	1%

effect on P/AI.^{32,33,34,35} BCS is an indirect measurement of fat stores in the body³⁵ and it is well-known that an increase in animal energy reserves promotes GnRH secretion to stimulate reproductive function.^{36,37} This likely explains the positive relationship between BCS and P/AI observed in this experiment and previous reports.

Our results showed that cattle with a dominant follicle (>6 mm) or diameter of largest dominant follicle at PGF2- α injection as well animals with estrus behavior at AI had increased probability of becoming pregnant at FTAI. Like these results, in beef cattle treated with CO-synch, CO-synch+CIDR, or estradiol/progesterone FTAI protocols, it had been reported that an increase in the preovulatory follicle diameter or estrus presentation, increases probability of P/AI. 11,38,17,39,19,40,41 Large preovulatory follicles are associated with improved oocyte quality and with the formation of a large CL capable of producing a large amount of progesterone^{42,38} which increases the probability of P/AI.¹⁹ These two factors may favor pregnancy success and explain the positive association between follicular diameter and P/A1. Additionally, large preovulatory follicles increase the probability of estrus presentation,³⁸ likely because they produce more estradiol.¹⁷ This may result in animals that are in estrus and ovulate a better-quality oocyte and thus increase the probability of P/AI.

As in previous reports^{43,17} in the present experiment, cyclic animals (measured as animals with CL) had increased probability of P/AI than anestrus animals. Cyclicity is indicative of maturation of reproductive function in heifers or restoration of reproductive function in postpartum cows³⁰ which may explain the positive relationship between cyclicity and P/AI. These results suggest that increasing the number of cyclic females at the beginning of FTAI protocols should be a goal, to improve success of these hormonal treatments.⁴⁴

Average P/AI in present experiment was 27%, which is within the range reported by⁹ and is comparable with reports of Pfeifer et al. ¹⁶ (48%), Williams & Stanko, ¹⁰ (33%), Stevenson et al. ¹⁴ (31%), and Malik et al. ¹⁸ (23.0%). However, this P/AI is lower than the 50% that is expected with the use of the FTAI protocols used in this experiment.^{45,9}

The economic analysis suggested that the CO-Synch protocol, despite the reduced P/IA compared with the estradiol/progesterone protocol, is the most profitable. The cost of the CO-Synch treatment represents only 1 to 4% of the income from the sale of weaned calves. The reasons for this economic outcome are that the estradiol/progesterone and the CO-Synch+CIDR treatments are almost 400% more expensive than the CO-Synch treatment and that the overall pregnancy rate is similar among treatments.

CONCLUSION

The results of the present experiment showed that cattle treated with the estradiol/progesterone protocol had better P/AI than females treated with the CO-synch protocol, likely because differences in follicular dynamic between the two protocols. However, overall PR was similar among protocols. Given that the CO-Synch protocol also had a low cost, this protocol could be used as an economical alternative to improve reproductive performance in beef cattle. Additionally, our results confirm that follicular diameter, body condition, estrus presentation, and cyclicity are main factors that determine P/AI in females subject to the FTAI protocols used herein. Together all these results may be help to take decision when these protocols are implemented in beef cow-calf operations. For instance, if we want to have the better P/AI we should use an estradiol/progesterone protocol but if we want an economical strategy to improve breeding season pregnancy rate, we can use the CO-synch protocol. Moreover, it will be advisable selected animals with better BCS at the beginning of the protocols and use treatments to increase follicular diameter to have good results in the P/AI.

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Author Contributions

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Conflict of interest

The authors declare no conflict of interest.

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References

- Rodgers, J.C., Bird, S.L., Larson, J.E., Dilorenzo, N., Dahlen, C.R., Dicostanzo, A., and Lamb, G.C. 2012. An economic evaluation of estrous synchronization and timed artificial insemination in suckled beef cows. J. Anim. Sci., 90(11): 4055-4062.
- Sá Filho, M.F., Penteado, L., Reis EL, et al. 2013. Timed artificial insemination early in the breeding season improves the reproductive performance of suckled beef cows. Theriogenology., 79(4): 625-632.
- Lassala, A., Hernández-Cerón, J., Pedernera, M., et al. 2020. Cow-calf management practices in México. Reproduction and breeding. Vet. Méx., 7(1): 1-15.
- Knickmeyer, E.R., Thomas, J.M., Locke, J.W.C, et al. 2019. Altering duration of the presynchronization period in a long-term progestin-based estrus synchronization protocol for timed artificial insemination of beef heifers. Theriogenology., 136: 66-71.
- 5. Baruselli, P.S., Ferreira, R.M., Sa, M.F., et al. 2018. Review: Using artificial insemination natural service in beef herds. Animal., 12: S45-S52.
- Marrella, M.A., White, R.R., Dias, N.W., et al. 2021. Comparison of reproductive performance of AI- and natural service-sired beef females under commercial management. Transl. Anim. Sci., 30: 5(3).
- Ferreira, R.M., Conti, T.L., Goncalves, R.L., et al. 2018. Synchronization treatments previous to natural breeding anticipate and improve the pregnancy rate of postpartum primiparous beef cows. Theriogenology., 114: 206-211.
- 8. Colazo, M.G., Mapletoft, R.J. 2014. A review of current timed-AI (TAI) programs for beef and dairy cattle. Can. Vet. J., 55(8): 772-780.
- Bó, G.A., De La Mata, J.J., Baruselli, P.S. et al. 2016. Alternative programs for synchronizing and resynchronizing ovulation in beef cattle. Theriogenology, 86(1): 388-396.
- 10. Williams, G.L., and Stanko, R.L. 2020. Pregnancy rates to fixed-time AI

in Bos indicus-influenced beef cows using PGF2a with (Bee Synch I) or without (Bee Synch II) GnRH at the onset of the 5-day CO-Synch+ CIDR protocol. Theriogenology., 142: 229-235.

- Lamb, G.C., Stevenson, J.S., Kesler, D.J., et al. 2001. Inclusion of an intravaginal progesterone Insert plus GnRH and prostaglandin F2α for ovulation control in postpartum suckled beef cows. J. Anim. Sci., 79(9): 2253-2259.
- Oosthuizen, N., Canal, L.B., Fontes, P.L., et al. 2018. Prostaglandin F2α 7 d prior to initiation of the 7-d CO-synch+ CIDR protocol failed to enhance estrus response and pregnancy rates in beef heifers. J. Anim. Sci., 96(4): 1466-1473.
- Martinez, M.F., Kastelic, J.P., Adams, G.P., et al. 2002. The use of progestins in regimens for fixed-time artificial insemination in beef cattle. Theriogenology., 57(3): 1049- 1059.
- Stevenson, J.S., Lamb, G.C., Johnson, S.K., et al. 2003. Supplemental norgestomet, progesterone, or melengestrol acetate increases pregnancy rates in suckled beef cows after timed inseminations. J. Anim. Sci., 81(3): 571-586.
- Small, J.A., Colazo, M.G., Kastelic, J.P., et al. 2009. Effects of progesterone presynchronization and eCG on pregnancy rates to GnRH-based, timed-AI in beef cattle. Theriogenology., 71(4): 698-706.
- Pfeifer, L.F.M., Leonardi, C.E.P., Castro, N.A, et al. 2014. The use of PGF2 as ovulatory stimulus for timed artificial insemination in cattle. Theriogenology., 81(5): 689-695.
- Silva, E.P., Wiltbank, M.C., Machado, A.B., et al. 2018. Optimizing timed AI protocols for Angus beef heifers: Comparison of induction of synchronized ovulation with estradiol cypionate or GnRH. Theriogenology., 121: 7-12.
- Malik, A., Wahid, H., Rosnina, Y., et al. 2010. Effects of timed artificial insemination following estrus synchronization in postpartum beef cattle. Open Vet. J., 2(1): 1-5.
- Rodrigues, A.D., Cooke, R.F., Cipriano, R.S., et al. 2018. Impacts of estrus expression and intensity during a timed-AI protocol on variables associated with fertility and pregnancy success in Bos indicus-influenced beef cows. J. Anim. Sci. 96(1): 236-249.
- 20. Day ML. 2004. Hormonal induction of estrous cycles in anestrous Bos taurus beef cows. Anim. Reprod. Sci. 82: 487-494
- Lucy, M.C., Billings, H.J., Butler, W.R., et al. 2001. Efficacy of an intravaginal progesterone insert and an injection of PGF2alpha for synchronizing estrus and shortening the interval to pregnancy in postpartum beef cows, peripubertal beef heifers, and dairy heifers. J. Anim. Sci., 79(4): 982-995.
- Lamb, G.C., and Mercadante, V.R. 2016. Synchronization and Artificial Insemination Strategies in Beef Cattle. Vet. Clin. North. Am. Food. Anim. Pract., 32(2): 335-347.
- Richards, M. W., Wettemann, R. P., Spicer, L. J., & Morgan, G. L. (1991). Nutritional anestrus in beef cows: effects of body condition and ovariectomy on serum luteinizing hormone and insulin-like growth factor-I. Biology of reproduction, 44(6), 961-966. https://doi.org/10.1095/biolreprod44.6.961
- 24. Sartori, R., and Barros, C.M. 2011. Reproductive cycles in Bos indicus cattle. Anim. Reprod. Sci., 124: 244-250.
- Wichtel, J.J., Charmley, E., Richardson, G.F., et al. 2008. Effects of postpartum energy intake on pregnancy rates in beef cattle subjected to GnRHor CIDR-based timed artificial insemination protocols. Can. J. Anim. Sci., 88(3): 439-447.
- 26. Day, M.L. 2015. State of the art of GnRH-based timed AI in beef cattle. Anim. Reprod., 12(3): 473-478.
- Martínez, M.F., Kastelic, J.P., Adams, G.P., et al. 2000. Estrus synchronization and pregnancy rates in beef Cattle given DIP-B, prostaglandin and estradiol, or GnRH. Can. Vet. J. 41: 786-790.
- 28. Larson, J.E., Lamb, G.C., Stevenson, J.S., et al. 2006. Synchronization of estrus in suckled beef cows for detected estrus and artificial insemination

and timed artificial insemination using gonadotropin-releasing hormone, prostaglandin F2, and progesterone. J. Anim. Sci., 84(2): 332-342.

- 29. Echternkamp, S.E., and Thallman, R.M. 2011. Factors affecting pregnancy rate to estrus synchronization and fixed-time artificial insemination in beef Cattle. J. Anim. Sci. 89(10): 3060-3068.
- Short, R.E., Bellows, R.A., Staigmiller, R.B., et al. 1990 Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle. J. Anim. Sci., 68(3): 799-816.
- Crowe, M.A. 2008. Resumption of ovarian cyclicity in post-partum beef and dairy cows. Reprod. Domest. Anim., 43(5): 20-28.
- Esterman, R.D., Alava, E.N., Austin, B.R, et al. 2016. Select Synchand CO-Synch protocols using a DIP yield similar pregnancy rate after a fixedtime insemination in suckled Bos indicus x Bos taurus cows. Theriogenology., 85(5): 870-876.
- 33. White, S.S., Kasimanickam, R.K., and Kasimanickam, V.R. 2016. Fertility after two doses of PGF2 concurrently or at 6-hour interval on the day of CIDR removal in 5-day CO-Synch progesterone-based synchronization protocols in beef heifers. Theriogenology., 86(3): 785-790.
- 34. Oliveira, Filho. R.V., Cooke, R.F., de Mello, G.A., et al. 2020. The effect of clitoral stimulation post artificial insemination on pregnancy rates of multiparous Bos indicus beef cows submitted to estradiol/progesteronebased estrus synchronization protocol. J. Anim. Sci., 98(7): skaa195.
- Randi, F., Kelly, A.K., Parr, M.H., et al. 2021. Effect of ovulation synchronization program and season on pregnancy to timed artificial insemination in suckled beef cows. Theriogenology., 172: 223-229.
- Guzmán, A., Rosales-Torres, A.M., and Gutiérrez, C.G. 2012. Neuroendocrine effects of insulin, IGF-I and leptin on the secretion of the gonadotropin-releasing hormone (GnRH). Trop. Subtrop. Agroecosyst., 15(1): S79-S90.
- Guzmán, A., Hernández-Coronado, C.G., Rosales-Torres, A.M., et al. 2019. Leptin regulates neuropeptides associated with food intake and GnRH secretion. Ann. Endocrinol., 80(1): 38-46.
- Pessoa, G.A., Martini, A.P., Carloto, G.W., et al. 2016. Different doses of equine chorionic gonadotropin on ovarian follicular growth and pregnancy rate of suckled Bos taurus beef cows subjected to timed artificial insemination protocol. Theriogenology., 85(5): 792-799.
- Bishop, B.E., Thomas, J.M., Abel, J.M., et al. 2017. Split-time artificial insemination in beef cattle: III. Comparing fixed-time artificial insemination to Split-time artificial insemination with delayed administration of GnRH in postpartum cows. Theriogenology., 99: 48-52.
- Santos, M.H., Junior, M.V.C.F., Polizel, D.M., et al. 2018. Decreasing from 9 to 7 days the permanence of progesterone inserts make possible their use up to 5 folds in suckled Nellore cows. Theriogenology., 111: 56-61.
- Oosthuizen, N., Cooke, R.F., Schubach, K.M., et al. 2020. Effects of estrous expression and intensity of behavioral estrous symptoms on variables associated with fertility in beef cows treated for fixed-time artificial insemination. Anim. Reprod. Sci., 214: 106308.
- Atkins, J.A., Smith, M.F., Wells, K.J., et al. 2010. Factors affecting preovulatory follicle diameter and ovulation rate after gonadotropin-releasing hormone in postpartum beef cows. Part I: Cycling cows. J. Anim. Sci., 88(7): 2300-2310.
- 43. Marquezini, G.H.L., Dahlen, C.R., Bird, S.L., et al. 2011. Administration of human chorionic gonadotropin to suckled beef cows before ovulation synchronization and fixed-time insemination: replacement of gonadotropin-releasing hormone with human chorionic gonadotropin. J. Anim. Sci., 89(10): 3030-3039.
- 44. Helguera, I.L., Whittaker, P., Behrouzi, A., et al. 2018. Effect of initial GnRH and time of insemination on reproductive performance in cyclic and acyclic beef heifers subjected to a 5-d CO-synch plus progesterone protocol. Theriogenology., 106(15): 39-45.
- 45. Bó, G.A., and Baruselli, P.S. 2014. Synchronization of ovulation and fixedtime artificial insemination in beef cattle. Animal., 8(1): 144-150.