

Response to an estradiol-based fixed-time artificial insemination (FTAI) protocol in postpartum beef cows: interaction between the ovulatory inductor and the genotype

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ABSTRACT

To evaluate if the ovulatory inducer, the genotype, and their interaction affect the reproductive response to a fixed-time artificial (FTAI) protocol and to determine whether this protocol improves the breeding season pregnancy rate (PR), a total of 242 Brahman and 155 Brahman by Brown Swiss cows (F1) were used in this experiment. Cows in the control group (153 Brahman and 115 F1 cows) did not receive hormonal treatment, whereas the estradiol cypionate (EC; 45 Brahman and 19 F1 cows) and the GnRH groups (44 Brahman and 21 F1 cows) were subjected to an estradiol-based FTAI protocol and received EC or gonadotropin-releasing hormone (GnRH) to induce ovulation. Brahman cows treated with EC had a higher pregnancy per artificial insemination (P/AI; $P < 0.05$) than Brahman cows treated with GnRH, but not with F1 cows treated with GnRH or EC. The breeding season PR in synchronized cows was greater ($P < 0.001$) than in control cows, although there was an interaction ($P < 0.05$) between groups and genotypes. In Brahman cows, the use of EC ($P < 0.001$) but not GnRH increased the breeding season PR compared with control cows, but in F1 cows, only the use of GnRH to induce ovulation increased ($P < 0.001$) the breeding season PR compared with the control cows. In conclusion, GnRH is the best option to induce ovulation and improve the P/AI in an estradiol-based FTAI protocol, but the breeding season pregnancy rate in Brahman cows could be compromised with the use of this hormone. Additionally, hormonal treatment improved breeding season PR compared with untreated animals.

Keywords: FTAI protocol, beef cattle, cyclicity, P/AI, GnRH, EC

INTRODUCTION

Use of fixed-time artificial insemination (FTAI) protocols worldwide has increased as a strategy to improve reproductive performance in beef cow-calf systems (Bó and Menchaca, 2023; dos Santos Marques et al., 2022). Use of this biotechnology allows obtaining an acceptable pregnancy per artificial insemination (P/AI) rate (Bó and Menchaca, 2023) and may help to induce cyclicity in anestrus cattle to improve reproductive efficacy during the breeding season (Baruselli et al., 2018; Day, 2004; Lamb and Mercadante, 2016). Ferreira et al. (2018), using

natural service (NS), showed that postpartum primiparous Nelore (*Bos indicus*) beef cows submitted to an estradiol-based protocol plus eCG at the onset of the breeding season had a higher breeding season pregnancy rate (82.1%) than those without treatment (65.0%). Similarly, in suckled Nelore beef cows, females subjected to an estradiol-based protocol and using FTAI plus NS had a higher breeding season (BS) pregnancy rate (92.7%) than those subjected only to NS without hormonal treatment (38.2%; Sá Filho et al., 2013).

In South America, the use of estradiol-based FTAI protocols is preferred because there are no limitations in the use of estradiol (Bó et al., 2016) and because the precise effect of estradiol on the control of ovarian follicular growth and the increase in pregnancy per AI has been shown in anestrus cattle and animals with low body condition (Bó and Menchaca, 2023). Estradiol-based FTAI protocols may differ in the hormone used to induce ovulation and can be used as follows: the application of estradiol benzoate (EB) 24 hour after intravaginal device withdrawal, the application of estradiol cypionate (EC) at intravaginal device withdrawal, or the use of GnRH at the moment of artificial insemination (Hernández-Coronado et al., 2023). The results of P/AI in regard to the ovulatory inducers in estradiol-based protocols are contradictory. In one study, the P/AI was greater in Nelore (*Bos indicus*) multiparous cows treated with EC as the ovulatory inducer (68.2%) than in cows treated with EB (52.4%; Silva et al., 2018a). In contrast, in multiparous suckled beef cows (*Bos taurus* and *Bos indicus*), there is no difference between the use of EC or BE to induce ovulation (Sales et al., 2012; Uslenghi et al., 2014). Similarly, there is no difference in the P/AI between the use of EC or GnRH to induce ovulation in estradiol-based protocols (Silva et al., 2018b; Uslenghi et al., 2016). However, none of these studies evaluated the effect of ovulatory inducers on breeding season pregnancy rates, which could be an important indicator of the effect of these treatments on cyclicity induction in postpartum beef cows.

The differences in the reproductive physiology between *Bos indicus* and *Bos taurus* cattle (Sartori et al., 2010), suggest that they respond differently to FTAI protocols. Recently a study reported a P/AI value of approximately 57% in *Bos taurus* females and a P/AI of approximately 34% in *Bos indicus* cattle subjected to an FTAI protocol (Cooke et al., 2021). On the contrary, Tomazi Filho et al. (2022) did not report the effect of the genotype (*Bos indicus* vs *Bos taurus*) on the P/AI in beef cattle subjected to an estradiol-based FTAI protocol.

In this context, the objective of this study was to evaluate whether the ovulatory inducer, the genotype, and their interaction affect the reproductive response

to an estradiol-based FTAI protocol and to determine whether this protocol improves the BS pregnancy rate compared with that of animals not subjected to hormonal treatment.

MATERIAL AND METHODS

Location and animals

All animal procedures were approved by the Research Ethics Committee of the División de Ciencias Biológicas y de la Salud from the Universidad Autónoma Metropolitana-Xochimilco under protocol number CEI. 2022.014 in accordance with Section 2, Article 20, 3rd Title of the Federal Law on Animal Health (2018), México. The study was performed on one commercial farm located at the parallels 19°59'39" and 20°08'39" of North latitude and the meridians 97°21'39" and 97°27'39" of Western longitude in Ayototxco de Guerrero state of Puebla, Mexico, from August 2021 to May 2022. A total of 242 Brahman cows and 155 F1 (Brahman by Brown Swiss) multiparous postpartum beef cows allocated in pastureland and fed common Star of Africa grass (*Cynodon plectostachyus*), mixed with native grasses (*Axonopus* spp. and *Paspalum* spp.) and supplemented with mineral salts as a basal diet, were used.

Experimental design

From the total number of animals, 89 Brahman (BHM) and 40 postpartum F1 cows were subjected to the following estradiol-based FTAI protocol: The animals received an injection of 2 mg of estradiol benzoate (Sincrodiol® Ourofino Animal Health, Brazil), and an intravaginal progesterone device (IVPD; Sincrogest, Ourofino 20 g, Animal Health, Brazil) was inserted on Day zero. The intravaginal device was removed 8 days later, and 25 mg of PGF2 α (Sincrocio® Ourofino Animal Health, Brazil) was injected. At this moment, within the genotype, the animals were divided into two groups balanced by the body condition score (BCS) and postpartum days to receive GnRH or estradiol cypionate (EC) as ovulatory inducers. BSC was recorded for the same person using a scale from 1 to 9 where 1 is an emaciated cow and 9 is an obese cow (Rosales-Torres et al., 2017; Richards

et al., 1986). The animals of the EC group received 1 mg of EC at device removal and were inseminated 48 to 56 hours later. The animals of the GnRH group were injected with 150 mg of buserelin acetate (SincroForte, Ourofino) at insemination, which was performed also 48 to 56 hours after device removal (Figure 1). The BCS was recorded at the beginning of the FTAI protocol and at the time of intravaginal device removal. At 10 days after FTAI, the cows were exposed to bull mating for a 63-day period. Females of the control group (153 Brahman cows and 115 F1 cows) started a breeding period of natural service for 73 days, starting at the moment of the FTAI of the synchronized cows (Figure 1). For synchronized groups and the control group, a bull: cow ratio of 1:20 was applied.

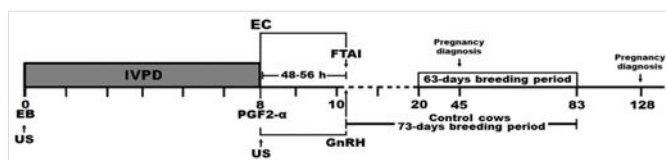


Figure 1. Experimental design

Follicular dynamics

The ovarian structures (antral follicles larger than 2 mm and corpus luteum (CL)) were determined by transrectal ultrasonography (Aloka SSD-500, Aloka Hitachi, Tokyo, Japan), using a 7.5-MHz linear transrectal probe. Ultrasounds were performed at the beginning of the hormonal treatment (Day 0) and when the intravaginal device was removed, together with the injection of PGF2 α (Day 8; Figure 1). On day 0 and day 8, the diameter of the largest follicle (>6 mm) present in the ovaries was measured and it was recorded the population of antral follicles larger than 2 mm. The diameter of the dominant follicle at follicular selection in *Bos indicus* cattle is approximately 6 mm. Because in this experiment, we used *Bos indicus* and *Bos indicus* \times *Bos taurus* animals, the status of the follicular wave was classified as followed: Recruitment phase when both ovaries had follicles \leq 6 mm in diameter, and selection/dominance phase when in one of the ovaries, there was a follicle > 6 mm in diameter.

Estrus detection and pregnancy diagnosis

When the intravaginal device was removed, the cows' tailhead was painted with marker crayons for cattle to detect mounts. The P/AI and pregnancy rate of the breeding season were assessed by ultrasonography using a 7.5-MHz linear transrectal probe 35 days after AI and 45 days after the end of the breeding period, respectively.

Statistical analysis

The effects of the ovulatory inducer, the genotype, and their interaction on estrus presentation, P/AI, and BS pregnancy rate were evaluated by logistic regression. Differences in BCS and follicular diameter at the beginning of synchronization and at intravaginal device withdrawal were evaluated by the standard least squares method, using treatment, genotype, and their interaction as fixed variables. For all these variables, homoscedasticity was verified by the Levene test. The percentages of animals with CL and the status of follicular wave at the beginning of the treatment and at intravaginal device withdrawal were analyzed by logistic regression, using treatment, genotype, and their interaction as fixed variables. The odds of P/AI by the effect of estrus presentation, animals with CL at the beginning of synchronization, status of follicular wave at the beginning of synchronization and intravaginal device withdrawal, inseminator, and bull were calculated. *P* values < 0.05 were considered statistically different, and *P* values from 0.05 to < 0.10 were considered to indicate tendencies.

RESULTS

The presence of CL and BCS either at the beginning of the FTAI protocol (day 0) or at the time of PGF2 α injection (Day 8) was not different ($P > 0.05$) between ovulatory inducer, genotype, or their interaction (Table 1).

Table 2 shows the reproductive responses to the estradiol-based FTAI protocol. Estrous presentation was not affected by the interaction of ovulatory inducer*genotype, but there was an effect of ovulatory inducer and genotype ($P < 0.05$). Animals treated with EC to induce ovulation ($P < 0.05$) and F1 ($P < 0.001$) cows had

greater estrous presentation than those of the GnRH group and BHM cows, respectively. The P/AI was only affected by the ovulatory inducer, without an effect of genotype and interaction of ovulatory inducer*genotype. Cows in which ovulation was induced with GnRH had a higher ($P<0.05$) P/AI than those in which ECP was used to induce ovulation. The total pregnancy rate was affected by the interaction of ovulatory inducer*genotype ($P<0.01$). Brahman cows treated with GnRH to induce ovulation had a lower total pregnancy rate ($P<0.01$) than those treated with EC and F1 cows treated with GnRH, but not compared to F1 cows treated with EC.

The diameter of the largest follicle at the beginning of the FTAI protocol (Day 0), but not at Day 8, was higher ($P<0.05$) in F1 cows than in Brahman cows (Table 3). The status of the follicular wave on Day 0 was not affected by the ovulatory inducer, genotype, or their interaction. However, on the day of injection with PGF2- α , the proportion of F1 cows with a selected or dominant follicle was greater ($P<0.01$) than in Brahman cows (Table 3).

Table 1. Animals with corpus luteum (CL) and their body condition score (BCS) at the beginning of the FTAI protocol (Day 0) and at the time of PGF2 α injection (Day 8) are presented by genotype (G) and ovulatory inducer (OI)

	BHM			F1			P value		
	EC	GnRH	SE	EC	GnRH	SE	OI	G	OI*G
n	45	44		19	21				
CL Day 0 (%)	32 (71.1)	30 (68.2)		14 (73.7)	16 (76.2)		0.995	0.536	0.753
CL Day 8 (%)	10 (22.2)	17 (38.6)		9 (47.4)	8 (38.1)		0.608	0.16	0.141
BCS Day 0	6	6	0.2	5.9	5.9	0.3	0.93	0.701	0.931
BCS Day 8	5.4	5.4	0.2	5.3	5.5	0.3	0.665	0.874	0.553

BHM = Brahma cows, F1 = Brahman by Brown Swiss cows, EC = estradiol cypionate, GnRH = Gonadotropin-Releasing Hormone

Table 2. Effects of ovulatory inducer (OI), genotype (G), and their interaction (OI*G) on reproductive responses to an estradiol-based FTAI protocol in Brahman (BHM) and F1 (Brahman by Brown Swiss) postpartum beef cows

	Ovulatory Inducer		Genotype		P value		
	EC	GnRH	BHM	FI	OI	G	OI*G
n	64	65	89	40			
Animals in estrous (%)	53 (82.8)	44 (67.7)	59 (66.3)	38 (95.0)	0.038	<0.001	0.251
Pregnancy per AI (%)	17 (26.6)	27 (41.5)	29 (32.6)	15 (37.5)	0.033	0.783	0.195

	BHM		F1		P value	G	OI*G
	EC	GnRH	EC	GnRH			
n	45	44	19	21			
Total pregnancy rate (%)	38 (84.4) ^a	26 (59.1) ^b	14 (73.7) ^{ab}	20 (95.2) ^a	0.597	0.082	0.003

^{a, b} indicates statistical differences ($P<0.05$) among treatments with different superscripts

Table 3. Effects of ovulatory inducer (OI), genotype (G), and their interactions on diameter of the largest follicle (LF) and status of the follicular wave (SFW) at the beginning of the FTAI protocol (day 0) and at the time of PGF2 α injection (day 8) in Brahman (BHM) and F1 (Brahman by Brown Swiss) postpartum beef cows

	Ovulatory Inducer			Genotype			P value		
	EC	GnRH	SE	BHM	F1	SE	OI	G	OI*G
n	64	65		89	40				
LF Day 0	9.8	10.4	0.5	9.3	10.9	0.4	0.318	0.013	0.476
LF Day 8	8.7	9.2	0.5	8.6	9.3	0.4	0.438	0.293	0.581
SFW Day 0									
S/D	56 (87.5)	56 (86.2)		75 (84.3)	37 (92.5)		0.724	0.172	0.368
R	8 (12.5)	9 (13.9)		14 (15.7)	3 (7.5)				
SFW Day 8									
S/D	43 (67.2)	47 (72.31)		56 (63.0)	34 (85.0)		0.309	0.007	0.411
R	21 (32.8)	18 (27.7)		33 (37.0)	6 (15.0)				

S/D = Selection/Dominance phase when in one of the ovaries, there was a follicle > 6 mm in diameter.

R = Recruitment phase when both ovaries had follicles \leq 6 mm in diameter.

The effects of estrus presentation, presence of CL, status of the follicular wave, inseminator, and the bull on P/AI are presented in Table 4. There was no effect of estrus presentation, the presence of CL, the status of the follicular wave at Day 0, the inseminator, or the bull ($P>0.05$) on the P/AI ratio. However, animals with a selected or dominant follicle at PGF2- α injection were three times more likely to become pregnant via AI ($P<0.05$) than those with a follicular wave in the recruitment stage Table 4. Similarly, an increase in the diameter of the largest follicle at Day 8 increased ($P<0.05$) the odds of pregnancy per AI.

The comparison of the total pregnancy rates of synchronized and non-synchronized cows revealed an interaction between treatments and genotypes (Figure 2). In Brahman cows, the use of EC ($P<0.001$) as an ovulatory inducer, but not GnRH ($P>0.05$), increased the total pregnancy rate compared with the control cows. In contrast, in F1 cows, the use of EC to induce ovulation only tended to increase the total pregnancy rate compared with that of the control cows, whereas the

use of GnRH to induce ovulation dramatically increased ($P<0.001$) the total pregnancy rate (Figure 2). Notably, the total pregnancy rate of synchronized cows (76%; 98/129) was greater ($P<0.001$) than that of control cows (50%; 135/268).

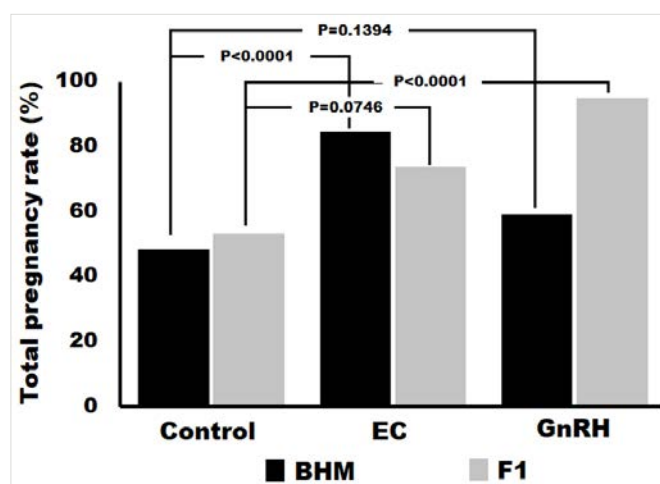


Figure 2. Total pregnancy rates in synchronized and non-synchronized (control) Brahman (BHM) and F1 (Brahman by Brown Swiss) postpartum beef cows. Synchronization was performed using an estradiol-based FTAI protocol with estradiol cypionate (EC) or gonadotropin-releasing hormones (GnRH) to induce ovulation

Table 4. Effects of estrus presentation, presence of CL at the beginning of the FTAI protocol (day 0), status of the follicular wave at day 0 or at the time of PGF2 α injection (day 8), inseminator, and bull on the probability of P/AI in postpartum beef cows

Factor	Levels	Odd ratio	95% CI	Chi value
Estrus presentation	NO	1		
	Yes	0.58	0.25–1.31	0.187
Animals with CL at day 0	NO	1		
	Yes	0.57	0.26–1.26	0.167
Status of follicular wave at day 0	R	1		
	S/D	0.94	0.32–2.74	0.912
Status of follicular wave at day 8	R	1		
	S/D	3.19	1.27–8.00	0.013
Inseminator technician	One	1		
	Two	1.91	0.68–5.32	0.217
	Three	2.15	0.77–5.00	0.143
Bull	Epicenter	1		
	Technique	1.36	0.55–3.36	0.511
	Topper	2.02	0.82–4.97	0.125

cv = cultivarS/D = Selection/Dominance phase when in one of the ovaries, there was a follicle > 6 mm in diameter.

R = Recruitment phase when both ovaries had follicles \leq 6 mm in diameter.

DISCUSSION

Estrous behavior and breeding season pregnancy rate were higher in F1 cows than in Brahman cows. To our knowledge, there are no studies evaluating the effect of genotype on the reproductive response to FTAI protocols. However, several authors have reported important differences in antral follicular dynamics and sexual steroid concentrations between *Bos indicus* and *Bos taurus* cattle (Carvalho et al., 2008; Hernández-Coronado et al., 2023; Sartori et al., 2010, 2016). In general, the consensus is that *Bos indicus* females have smaller preovulatory follicles but need higher serum concentrations of estradiol to present estrus, and the selection of the dominant follicle occurs 0.6 days later than in *Bos taurus* females (Carvalho et al., 2008; Sartori et al., 2016). This might explain the difference in estrous behavior between the genotypes evaluated in this experiment. Hence, it is likely that

Brahman cows at the time of AI (where estrus behavior was recorded) had a young preovulatory follicle because its selection occurred at least 12 hours later (in relation to the induction of the new follicular wave) than in F1 cows. Thus, this preovulatory follicle was unable to produce the estradiol concentration that *Bos indicus* females need to present behavioral signs of estrus.

The breeding season pregnancy rate depends on several physiological processes; however, the most important prerequisite is that the females are undergoing estrous cycles (Crowe, 2008; Diskin and Kenny, 2016). In the present experiment, although at the beginning of the hormonal treatment, the proportion of animals with CL was high, this does not indicate that they were all cyclic, as we only recorded the CL presence once. Additionally, in beef cattle, the main factor that affects postpartum cyclicity is the maternal bond/calf presence

(Crowe, 2008; Orihuela and Galina, 2019). According to a previous study, *Bos indicus* females could be more susceptible to this effect because they form a stronger bond with their offspring than *Bos taurus*-influenced cows (Das et al., 2001). In this regard, *Bos taurus* cows restore their cyclicity 22.5 ± 2.2 days (Hoffman et al., 1996) or 30.7 ± 4.2 (Quintans et al., 2009) days after the onset of suckling restriction, whereas in *Bos indicus* females, the interval from the onset of suckling restriction to first ovulation is 42 ± 9 days (Mukasa-Mugerwa et al., 1991). This makes it likely that the proportion of F1 cows being cyclic during the breeding season is higher compared to that of Brahman cows, which explains the differences in the breeding season pregnancy rates between these two genotypes.

The genotypes evaluated herein responded differently to the ovulatory inducer in regard to the breeding season pregnancy rate compared with untreated cows. In Brahman cows, there was an increase of 75% in the breeding season pregnancy rate when EC was used as an ovulatory inducer; however, such an increase was not observed in GnRH-treated Brahman cows. In contrast, F1 cows treated with GnRH to induce ovulation had 87% higher breeding season pregnancy rate than untreated F1 cows, but when EC was used to induce ovulation, the increase in the pregnancy rate was less pronounced (43%). Because the breeding season pregnancy rate depends on the fact that the cows are cyclic (Crowe, 2008; Diskin and Kenny, 2016), these results suggest Brahman cows treated with EC are kept cyclic at a higher proportion during the breeding season to favor pregnancy via bull-mating, whereas GnRH has this effect on F1 cows. Unfortunately, there is no experimental evidence supporting this hypothesis, and further studies in this field are required.

Regarding the ovulatory inducer, the percentage of animals with estrus behavior was higher for cows treated with EC in contrast to GnRH; however, the P/AI was lower in EC-treated than in GnRH-treated cows. Silva et al. (2018b) showed that animals treated with EC had higher estrus behavior than those treated with

GnRH, but these authors reported no difference in the P/AI. Estrus behavior depends on the estradiol effects on the central nervous system (Woelders et al., 2014). Therefore, it is likely that the EC used in this experiment could have induced estrus behavior even if there were no preovulatory follicles in the ovary, which might explain why animals treated with EC, despite the high estrus presentation, had a lower P/AI.

At Day 8, when PGF2- α was injected, cows with a selected dominant follicle were more likely to become pregnant by AI than those with recruitment follicles, and an increase in the diameter of the dominant follicle at Day 8 increased the odds of P/AI. Similar results have been reported by several authors (Silva et al., 2024; Oosthuizen et al., 2020; Rodrigues et al., 2018; Vázquez-López et al., 2024), and it has been suggested that the presence of a dominant follicle at the start of the proestrus period guarantees the presence of a preovulatory follicle at insemination time to favor the P/AI. Additionally, a large preovulatory follicle may carry a high-quality oocyte and form a large CL capable of producing enough progesterone to maintain the pregnancy (Vázquez-López et al., 2024).

Estrus presentation and animals with CL at day 0 did not affect the P/AI in the present experiment. In contrast, Vázquez-López et al. (2024) and others (Pereira et al., 2016; Nogueira et al., 2019) indicate that estrus presentation increases the odds of P/AI. It is important to note that in this experiment, we used EC and GnRH to induce ovulation, whereas Vázquez-López et al. (2024) used GnRH or estradiol benzoate. As discussed above, EC likely causes estrus signs without ovulation, which would explain the difference between our results and those of previous studies. Regarding the effect of cyclicity on P/AI, several authors have shown that cycling animals, determined by serum P4 concentration or CL presence, had a higher probability of P/AI (Melo et al., 2016; Madureira et al., 2021; Vázquez-López et al., 2024) than anestrus animals. However, in our study, the presence of CL was measured only at the beginning of synchronization, and we could not determine the exact proportion of cycling animals.

CONCLUSION

Based on our results, the use of GnRH to induce ovulation in estradiol-based FTAI protocols improves the P/AI as compared with the use of EC. However, the breeding season pregnancy rate was lower in Brahman cows when ovulation was induced with GnRH compared with Brahman cows treated with EC or with F1 cows treated with GnRH to induce ovulation. Moreover, cows treated with the FTAI protocol used herein showed a higher breeding season pregnancy rate compared with untreated cows, but in Brahman cows, this result was observed only when EC was used to induce ovulation; however, in F1 cows, the best results were observed with the use of GnRH. These findings lead us to confirm that there are important differences in the reproductive physiology between the genotypes evaluated herein, which should be considered when selecting the best treatment to induce ovulation when *Bos indicus* or F1 (*Bos indicus* X *Bos taurus*) cows are submitted to an estradiol-based FTAI protocol.

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