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Importance of estrus on pregnancy per insemination in suckled *Bos indicus* cows submitted to estradiol/progesterone-based timed insemination protocols

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Abstract

The objective was to evaluate the effect of estrus occurrence (based on removal of tail-head marks) on ovarian responses and pregnancy per AI (P/AI; 30 d after AI) in suckled *Bos indicus* beef cows submitted to timed AI (TAI) protocols. Cows received an intravaginal device containing 1.0 g progesterone, and 2.0 mg estradiol benzoate im; 8 d later, the intravaginal device was removed, and they were given $PGF_{2\alpha}$ (0.25 mg of cloprostenol sodium) and 300 IU of eCG, with TAI 48 to 52 h later. In Experiment 1, cows were assigned to receive one of three treatments: 1 mg of estradiol cypionate (ECP) im at progesterone (P4) device removal (N = 178); 10 μg of GnRH im at TAI (N = 190); or both treatments (N = 172). In cows given estradiol (ECP or ECP + GnRH), more displayed estrus (P = 0.002) and became pregnant (P < 0.0001) compared with those receiving only GnRH. In Experiment 2, the effect of the occurrence of estrus on ovarian responses was evaluated in cows (N = 53) synchronized using ECP at device removal. Cows that displayed estrus had a greater diameter of the largest follicle (LF) at device removal (P < 0.0001), a greater diameter at TAI (P < 0.0001), a greater ovulation rate (P = 0.02), a larger CL (P = 0.02), and a greater P4 concentration (P < 0.0001) than cows that did not display estrus. In Experiment 3, the effect of GnRH treatment on P/AI at TAI was evaluated in cows that received ECP at device removal, and either displayed, or did not display, estrus (N = 726). There was no estrus by GnRH interaction (P = 0.22); the P/AI was greater (P < 0.0001) in cows that displayed estrus (61.9%) than cows that did not display estrus (41.4%). However, GnRH did not improve (P = 0.81) P/AI (GnRH = 53.7% vs. no GnRH = 52.6%). In conclusion, exogenous estradiol at device removal increased both the proportion of suckled *Bos indicus* cows that displayed estrus and P/AI. Cows that displayed estrus had better ovarian responses (i.e., larger follicles at TAI, a greater ovulation rate, larger CL, and greater P4 concentrations) following an estradiol/P4-based synchronization protocol. Although occurrence of estrus improved pregnancy outcomes, GnRH at TAI did not improve P/AI in suckled *Bos indicus* cows treated with ECP, regardless of estrus occurrence.

Keywords: Estrus; Estradiol cypionate; Nelore; GnRH; Reproduction

1. Introduction

Estradiol and progesterone-based synchronization protocols have been successfully used to control follicular and luteal dynamics and to synchronize ovulation, enabling AI without estrous detection [1–5]. Currently, these protocols are the main commercial treatment in South America to synchronize follicular wave emergence and ovulation for timed AI (TAI) in suckled beef cows [4,5].

In these estradiol plus progesterone-based synchronization protocols, there is insertion of an intravaginal progesterone (P4) device, or a progestin ear implant,

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plus administration of estradiol on Day 0 (estradiol benzoate [EB] or estradiol valerate to synchronize emergence of a new follicular wave), $PGF_{2\alpha}$ on Days 8 or 9 at device withdrawal (to ensure luteolysis), plus 300 to 400 IU of eCG 9 (as exogenous gonadotrophin support), and subsequent administration of a low dose of estradiol, concurrent with device removal or 24 h later (to induce synchronized ovulation). Giving eCG at device removal has specific importance in sucked *Bos indicus* cows; it increased the growth rate of the dominant follicle from device/implant removal to TAI, diameter of the dominant follicle at TAI, and rates of ovulation and pregnancy in postpartum anestrous beef cows [3–5]. Furthermore, several factors influenced reproductive success following TAI synchronization protocols in suckled beef cows [5–6]. These factors, including the occurrence of estrus between P4 source removal and TAI, have resulted in a greater rate of pregnancy per AI (P/AI) [7]. Cows that displayed estrus were 3.3 times more likely to become pregnant than those without a display of estrus [7].

The phenomenon of estrus occurs due to the specific influence of ovarian steroid hormones on behavioral centers in the mammalian brain [8]. Initiation of standing estrus has been associated with peak peripheral estradiol concentrations [9]. Furthermore, estradiol concentration at insemination may influence fertilization (through sperm transport) by altering the uterine environment [10]. After initiation of standing estrus, the uterine environment is modified to enhance sperm motility and longevity [11–13]. Consequently, proestrous estradiol concentrations from either endogenous or exogenous sources may play an important role in sperm transport and the sustained viability of sperm until ovulation and fertilization.

Estradiol has been used to synchronize ovulation in cattle for many years. Most studies that used estradiol as an ovulatory stimulus employed EB [14]. However, estradiol cypionate (ECP), is an interesting alternative to estradiol benzoate, due to its ability to induce the LH surge when administered in a low progesterone environment [15]. Currently, ECP is the main estradiol treatment commercially used to synchronize ovulation in beef cattle in the Brazilian AI industry [5–7]. Its lower cost per dose and convenient time of administration (concurrent with P4 device removal), reduced the number of animal handlings without affecting the overall efficiency of TAI synchronization protocols [5–7].

Therefore, a better understanding of factors influencing the occurrence of estrus following estradiol/P4-based synchronization protocols could improve fertility and therefore, profitability. Thus, the objectives of the current study were to evaluate: (1) effect of estradiol administration, at P4 device removal, on the occurrence of estrus and P/AI following a TAI synchronization protocol (Experiment 1); (2) effect of the occurrence of estrus on follicle diameter, ovulation, and subsequent luteal function (Experiment 2); and (3) exogenous GnRH as an alternative for improving P/AI following the TAI synchronization protocol, especially in cows that did not display estrus (Experiment 3).

2. Materials and methods

2.1. Experiment 1

2.1.1. Cows and management

This experiment was conducted during the 2008/2009 and 2009/2010 spring-summer breeding seasons. A total of 540 suckled cows (370 Nelore [*Bos indicus*] and 170 crossbred [*Bos indicus* × *Bos taurus*; three quarters Nelore) from a commercial beef farm in the state of Mato Grosso, Brazil were enrolled in this study. All cows were maintained on *Brachiaria brizantha* pastures with free access to water and given mineral supplementation. The mineral mix was labeled to contain 11.1% Na; 8.7% Ca; 6% P; 4% S; 0.27% Zn; 0.005% Co; 0.09% Cu; 0.06% F; 0.0048% I; 0.075 % Mn, and 0.0013% Se. Average daily consumption of the mineral mix was estimated at approximately 100–150 g per cow. At the beginning of the TAI protocol, information on the breed and a body condition score (BCS; range, 1 = emaciated to 5 = obese [16]) were collected from each cow.

2.1.2. Reproductive management

After calving, cows were allocated into breeding groups according to calving date; at 30 to 60 d postpartum, they were synchronized using an estradiol/P4-based TAI protocol. Briefly, cows received an intravaginal device containing 1.0 g of P4 (Sincrogest®, Ouro Fino Saude Animal, Sao Paulo, SP, Brazil), plus 2.0 mg of estradiol benzoate im (Sincrodiol®, Ouro Fino Saude Animal). Eight days later, the device was removed, and cows were given 0.25 mg of cloprostenol sodium im (Sincrocio®, Ouro Fino Saude Animal) and 300 IU of equine chorionic gonadotropin im (eCG, Folligon®, Intervet-Shering Plough, Boxmeer, Netherlands). Cows were inseminated 48 to 52 h after the progesterone device was removed. Inseminations were done by two technicians (equally distributed between experimental groups), using frozen-thawed semen from one bull.

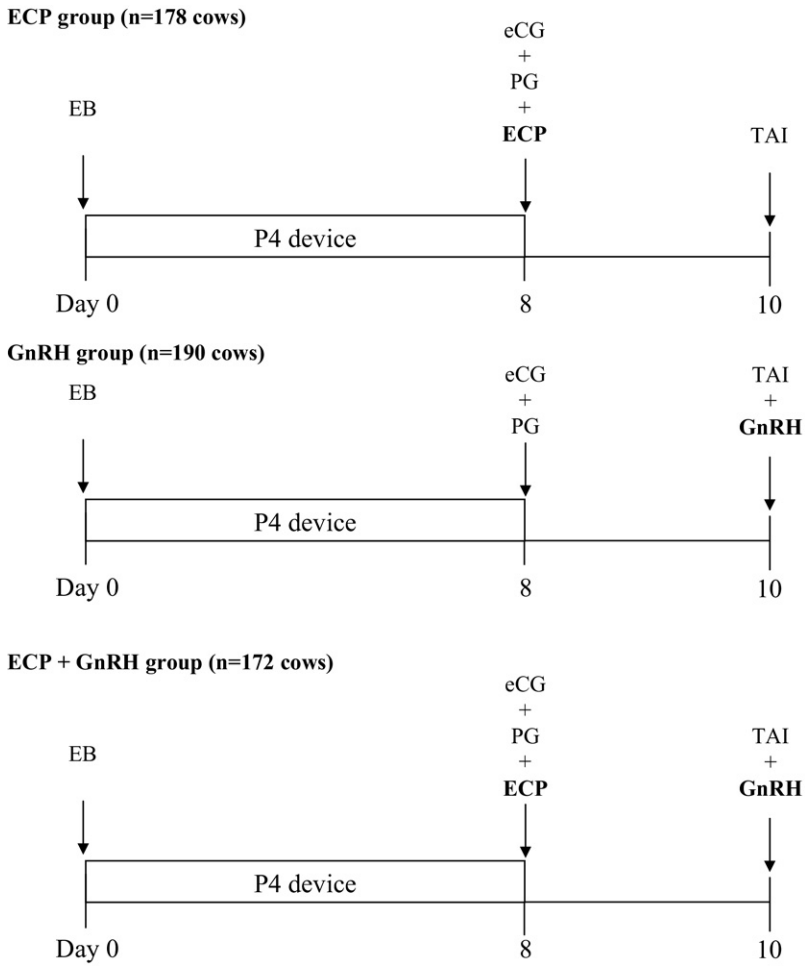

Fig. 1. Schematic diagram of treatments to synchronize ovulation in suckled *Bos indicus* cows. EB = 2 mg of estradiol benzoate; GnRH = 10 μg of Buserelin; ECP = 1 mg of estradiol cypionate; PG = 0.25 mg of cloprostenol sodium; TAI = timed AI, performed 48 to 52 h after progesterone (P4) device removal (Experiment 1). ECP group (N = 178 cows); GnRH group (N = 190 cows); ECP + GnRH group (N = 172 cows).

2.1.3. Treatments

At intravaginal device removal, cows were randomly assigned to receive one of three treatments: 1 mg of estradiol cypionate im (ECP®, Pfizer Animal Health, Sao Paulo, SP, Brazil) at device removal (N = 178); 10 μg of buserelin acetate im (GnRH; Sincroforte®, Ouro Fino Saude Animal) at TAI (N = 190); or both treatments (N = 172). Schematic diagram of synchronization treatments are described in the Figure 1.

2.2. Experiment 2

2.2.1. Animals and management

This experiment was conducted during the 2009/2010 spring-summer breeding season. Fifty-three suckled Nelore (*Bos indicus*) cows from a commercial beef farm in the state of Paraná, Brazil were enrolled in this study. All cows were maintained on *Brachiaria decumbens* pastures, with free access to water and mineralized salt. At the beginning of the TAI protocol, BCS (as described above) and parity (multiparous or primiparous) of each cow were recorded.

2.2.2. Treatments

The same reproductive management described in Experiment 1 was employed. However, only the synchronization protocol using ECP (at device removal) as an ovulatory stimulus was used (no GnRH at TAI). Cows were inseminated 48 to 50 h after the P4 device was removed. Frozen-thawed semen from one bull was used for all inseminations performed by one technician. Cows were retrospectively grouped based on whether or not estrus had occurred.

2.3. Experiment 3

2.3.1. Animals and management

A total of 726 suckled cows (495 Nelore [*Bos indicus*] and 231 crossbred [*Bos indicus* × *Bos taurus*]; three quarters Nelore) from four commercial beef farms (272, 161, 106, and 187 from Farms 1 to 4, respectively) in the state of Paraná, Brazil were enrolled in this study. All cows were maintained on *Brachiaria brizantha* pastures and given mineralized salt and ad libitum access to water. The experiment was conducted during the 2008/2009 spring-summer breeding season. Information on the cow's breed, body condition score (BCS; as described above) at the beginning of TAI protocol, and parity (multiparous or primiparous). Cows were classified as having a low to moderate (BCS between 2.0 and 3.0) or high (BCS $\geq$ 3.25) BCS. This classification was used to determine the relationship between the BCS, the occurrence of estrus, and P/AI. The reproductive management was the same as described for the Experiment 1. Frozen-thawed semen from six bulls equally distributed among the experimental groups was used, with all inseminations done by one technician.

2.3.2. Treatments

All cows were submitted to the synchronization protocol as previously described in Experiment 2. However, cows from each farm location at TAI were assigned to one of four treatment groups according to the occurrence of estrus and GnRH administration (2 × 2 factorial: no estrus-no GnRH, no estrus-GnRH, estrus-no GnRH, and estrus-GnRH). Timed AI was also performed 48 to 52 h after intravaginal device removal.

2.4. Detection of estrus

In all experiments, concurrent with removal of the intravaginal device, the tail-head was marked with chalk (Raidl-Maxi, RAIDEX GmbH, Dettingen/Erms, Germany). Estrus was deemed to have occurred in cattle without a tail-head chalk mark at TAI.

2.5. Ultrasonographic examination

Thirty days after TAI, pregnancy diagnosis (transrectal ultrasonography) was done on all cows from all three experiments. Detection of an embryonic vesicle with viable embryo (presence of heartbeat) was used as an indicator of pregnancy. The P/AI was calculated as the proportion of cows pregnant 30 d after TAI, divided by the total number of cows inseminated.

In Experiment 2, both ovaries were scanned at implant removal and immediately before TAI to measure the diameter of the largest follicle (LF), and 7 d later to determine the presence and the diameter of the resulting CL. A real-time ultrasonic scanner equipped with a 7.5 MHz linear transducer (CTS-3300V, SIUI, Guangdong, China) was used. Ovulation was defined as the appearance of a CL on the same ovary where the LF was detected at TAI.

2.6. Blood sampling and progesterone assay

Blood samples were collected by a puncture of the median coccygeal vein or artery using evacuated tubes, 7 d after TAI. Samples were refrigerated (4 °C) for approximately 4 h, and were then centrifuged (3000 X g for 15 min) and stored at –20 °C. Serum progesterone (P4) concentrations were determined (in unextracted sera) using an antibody-coated-tube RIA kit (Coat-A-Count®, Diagnostic Products Corporation, Los Angeles, CA, USA). The intra-assay coefficient of variation was 2.6%, and assay sensitivity was 0.006 ng/mL.

2.7. Statistical analysis

A binomial distribution was assumed for the categorical response variable. The occurrence of estrus and P/AI were analyzed using the GLIMMIX procedure of SAS (SAS 9.2 Institute Inc., Cary, NC, USA), with cows as a random effect. In Experiment 1, variables initially included in the models were treatments (ECP, GnRH, or ECP + GnRH), breed, and BCS at the first day of the synchronization protocol (categorized as ≤ 3 or > 3), and interactions. For the final logistic regression model, variables were removed through backward elimination, based on the Wald statistics criterion when $P > 0.20$. Variables included in the final model for analysis of occurrence of estrus and P/AI were treatment and breed. For analyses of the effect of the occurrence of estrus on P/AI, the final statistical model also included the occurrence of estrus.

In Experiment 2, the explanatory variable that was included in the statistical model was the occurrence of estrus. Dependent variables (i.e., diameter of the LF at device removal, diameter of the LF at TAI, CL diameter 7 d after TAI, and serum P4 concentrations 7 d after TAI — only for those females that had ovulated after the TAI synchronization protocol) were analyzed by a two-way ANOVA using PROC GLM. Response variables were tested according to their homogeneity and normality of variances using Guide Data Analysis from SAS. The square root transformation of P4 concentrations were subjected to ANOVA. Data are presented as mean ± SD.

In Experiment 3, the occurrence of estrus and P/AI

were analyzed using the GLIMMIX procedure of SAS. Variables initially included in the model were farm (1 to 4), breed (Nelore [*Bos indicus*] or crossbred [*Bos indicus* × *Bos taurus*]), parity (primiparous or multiparous), BCS during the first day of the synchronization protocol (categorized as ≤ 3 or > 3), GnRH treatment, occurrence of estrus, and interactions. Variables included in the final model for analysis of pregnancy rate were: BCS, occurrence of estrus, GnRH treatment, and interaction of occurrence of estrus by GnRH treatment.

An additional retrospective analysis, which regarded the factors that influenced the occurrence of estrus between the intravaginal device removal and TAI, was performed with data from Experiments 2 and 3. Information from individual cows from each experiment was collated into a single data set for statistical analysis. Data were analyzed using a multivariate logistic regression using the LOGISTIC procedure of SAS. A backward stepwise regression model was utilized [17], and explanatory variables were sequentially removed from the model using the Wald statistic criterion if $P > 0.10$. The initial model for analyses of occurrence of estrus included farm (1 to 5), breed (Nelore or crossbred), parity (multiparous or primiparous), and BCS category (≤ 3 or > 3). Final models for the analyses of occurrence of estrus included all of the initial effects. Adjusted odds ratios (AOR) and 95% confidence intervals (CI) were generated during logistic regression. Results were presented as proportions and AOR.

Differences with $P \leq 0.05$ were considered statistically significant, and $0.05 < P \leq 0.10$ were designated as a tendency toward a difference for the explanatory variables evaluated.

3. Results

3.1. Experiment 1

The overall occurrence of estrus was 64.3% (347/540). Cows that displayed estrus after the synchronization protocol had greater P/AI (58.5%; 203/347; $P < 0.0001$) than cows that did not display estrus (32.1%; 62/193). A greater proportion ($P < 0.0001$) of the occurrence of estrus was observed when the cows were treated with ECP at P4 device removal (ECP = 78.7%; 140/178 and ECP + GnRH = 72.7%; 125/172) compared with those treated only with GnRH at TAI (43.2%; 82/190). Cows treated with ECP (56.2%; 100/178) and ECP + GnRH (52.9%; 91/172) also had greater ($P = 0.002$) P/AI than cows treated only with GnRH (39.0%; 74/190).

Table 1
Occurrence of estrus after an estradiol/progesterone-based synchronization protocol on the ovarian follicular response and subsequent luteal phase in suckled *Bos indicus* cows (Experiment 2).

	No estrus	Estrus	P
Cows, N	17	36	—
Largest follicle diameter at intravaginal device removal (mm)	8.1 ± 3.1	11.9 ± 1.7	<0.0001
Largest follicle diameter at TAI (mm)	9.5 ± 4.2	13.9 ± 3.3	<0.0001
Ovulation rate (%)	70.6 (12/17)	100.0 (36/36)	0.02
Corpus luteum diameter 7 d after TAI (mm)	20.3 ± 3.9	22.9 ± 3.0	0.02
Progesterone concentration 7 d after TAI (ng/mL)*	3.0 ± 1.2	4.2 ± 1.6	<0.0001

TAI, timed AI.
* Only for cows that had ovulated after the synchronization protocol.

3.2. Experiment 2

The overall occurrence of estrus was 67.9% (36/53). Cows that displayed estrus had a larger LF at device removal ($P < 0.0001$), larger LF at TAI ($P < 0.0001$), greater ovulation rate ($P = 0.02$), larger diameter of the resulting CL ($P = 0.02$), and greater P4 serum concentration 7 d after TAI ($P < 0.0001$) than cows that did not display estrus (Table 1).

3.3. Experiment 3

The overall occurrence of estrus was 57.4% (417/726). There was no interaction between the occurrence of estrus and GnRH treatment ($P = 0.21$) and no effect ($P = 0.19$) of farm location on P/AI. The P/AI was greater ($P < 0.0001$) in cows that displayed estrus (61.9%; 258/417) than those that did not display estrus (41.4%; 128/309). However, P/AI was unaffected by GnRH treatment ($P = 0.55$; Fig. 2).

Estrus was detected in 58.2% (453/779) of cattle in Experiments 2 and 3. Multiparous cows were 2.4 times more likely to display estrus than primiparous cows ($P = 0.0003$; Table 2). Increased BCS (>3.25) on the first day of the synchronization protocol was also associated with an increased occurrence of estrus (AOR = 1.41, 95% CI = 1.00–2.00). Significant differences in estrus rates among farms ($P < 0.0001$) were also found (Farm A = 46.3%, 126/272; Farm B = 54.0%, 87/161; Farm C = 67.9 %, 36/53; Farm D = 68.9%, 73/106; and Farm E = 70.1%, 131/187). Furthermore, a higher percentage of crossbred cows displayed estrus

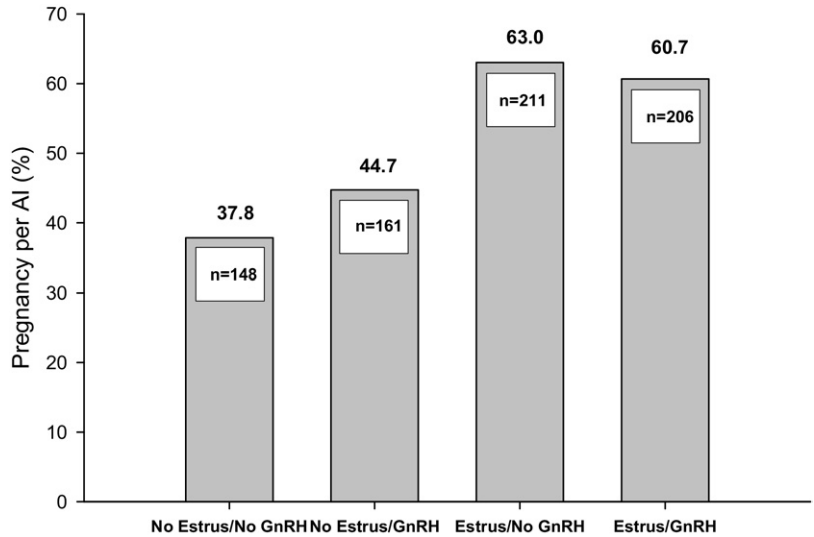

Fig. 2. Pregnancy per fixed-time artificial insemination (TAI) in suckled *Bos indicus* cows according to the GnRH treatment at TAI and the occurrence of estrus between the intravaginal progesterone device removal and TAI (Experiment 3). There was an effect of estrus ($P < 0.0001$), but not of GnRH treatment ($P = 0.55$) nor their interaction ($P = 0.21$).

compared with Nelore cows (56.6% [310/548] vs. 61.9% [143/231]; AOR = 1.61, 95% CI = 1.12–2.31).

4. Discussion

The present study demonstrated that the occurrence of estrus, determined based on removal of tail-head mark, was an important signal of greater ovarian and pregnancy responses in suckled *Bos indicus* cows submitted to estradiol/progesterone-based TAI protocols. Thus, the record of estrus occurrence may be used as a tool to identify females with greater odds of pregnancy for use of expensive and/or sex-sorted semen in TAI synchronization programs. Farm location, parity, breed, and nutritional status estimated by the BCS at synchronization influenced the likelihood of estrus after TAI synchronization protocols in suckled cows. For instance, cows that displayed estrus had a larger follicular

Table 2
Risk factors for occurrence of estrus following an estradiol/progesterone-based synchronization protocol in suckled *Bos indicus* cows (data from Experiments 2 and 3).

Variable	Estrus, % (N/N)	Adjusted OR (95% CI)	P
Farm			
A	46.3 (126/272)*	Referent	
B	54.0 (87/161)†	1.82 (1.16–2.86)	
C	67.9 (36/53)†	2.95 (1.54–5.65)	
D	68.9 (73/106)†	2.88 (1.72–4.82)	<0.0001
E	70.1 (131/187)†	3.10 (2.01–4.77)	
Breed			
Nelore (*Bos indicus*)	56.6 (310/548)	Referent	
Crossbred (*Bos indicus* × *taurus*)	61.9 (143/231)	1.61 (1.12–2.31)	0.01
Parity			
Primiparous	37.1 (45/124)	Referent	
Multiparous	62.1 (407/655)	2.39 (1.49–3.83)	0.0003
BCS category			
Low to moderate (2.0 to 3.0)	54.9 (257/468)	Referent	
High (>3.25)	63.0 (196/311)	1.41 (1.00–2.00)	0.05

BCS, body condition score; CI, confidence interval; OR, odds ratio; Referent, reference group for adjusted risk ratio.
[a,b]Within a column, proportions without a common superscript differed ($P < 0.05$).

diameter at intravaginal device removal, larger follicular diameter at TAI, greater ovulation rate, increased subsequent luteal function, and greater P/AI than those that did not display estrus. This influence of estrus occurrence on ovulation and pregnancy rates has also been described by other studies in *Bos taurus* [18–20] and *Bos indicus* cattle [6,7,21]. Thus, the presence of larger follicles at the intravaginal device removal and at TAI following the synchronization protocol was associated with a greater occurrence of estrus, which was associated with a greater ovulatory capacity and greater pregnancy risk in suckled *Bos indicus* cows.

Overall pregnancy rates following the use of estradiol/P4-based synchronization protocols in *Bos indicus* cattle was 49.6% [5]; these rates were influenced by the farm within location, breed (*Bos indicus*, *taurus*, and crossbred *Bos indicus* × *Bos taurus*), category (nulliparous, suckled primiparous, or suckled multiparous), and body condition score at the synchronization protocol [5]. Additionally, giving eCG at P4 device removal was specifically important in estradiol/P4-based TAI synchronization protocols [4,5,22,23]. In that regard, eCG provided gonadotropin support for the final follicular development [22,23] and increased P4 concentrations during the subsequent diestrus [22]; these positive effects likely benefited anestrus cows [3]. Despite eCG treatment, only 57.8% of the suckled *Bos indicus* cows displayed estrus between removal of the P4 device and TAI [7].

In the current study, suckled *Bos indicus* cows that displayed estrus had a larger CL and greater serum P4 concentrations in the subsequent estrous cycles, as described for high-producing dairy cows [19]. Cows that did not display estrus due to a reduced diameter of the dominant follicle during the synchronized proestrus may also have suboptimal concentrations of estradiol [18,19], which might result in a suboptimal LH surge. Consequently, the rise of P4 concentrations in the subsequent diestrus may be reduced [23]. Furthermore, suboptimal concentrations of estradiol might result in premature luteolysis in the subsequent estrous cycle [24], which would decrease embryo survival rates [25]. When crossbred embryo recipients were submitted to fixed-time embryo transfer, heifers displaying estrus after induction of luteolysis had larger CL diameters after embryo transfer, a greater concentration of P4 during the subsequent diestrus, and a greater pregnancy per embryo transfer [26]. Therefore, the lack of estrus before TAI, which has been associated with the concentration of estradiol during the proestrus, might have influenced the fertility of suckled beef cows by reducing subsequent luteal function, altered the frequency of cows with premature luteolysis, or decreased embryo survival after TAI.

In the current study, ECP increased the proportion of suckled *Bos indicus* cows that displayed estrus after removal of the intravaginal device, compared with cows that received only GnRH as an ovulatory stimulus. Estrus behavior is driven by estradiol action in the hypothalamus [27]; administration of 1 mg of ECP resulted in serum estradiol concentrations similarly to those in cows with physiological estrus [28,29]. Furthermore, exogenous estradiol has induced estrus behavior in ovariectomized cows [30,31].

Pregnancy outcomes were improved when suckled *Bos indicus* beef cows were treated with ECP at P4 device removal compared with cows that received only GnRH as an ovulatory stimulus. Inducing ovulation with 1 mg of ECP increased P/AI in Holstein dairy cows compared with GnRH as an ovulatory stimulus [32]. This improvement in P/AI was suggested to be caused by increased exposure to estradiol during proestrus, which may have improved fertilization, or better primed the uterus for the subsequent luteal phase. This priming may be important for the induction of endometrial progesterone receptors [33] and to avoid premature luteolysis and short cycles [34]. However, in spite of positive effects on fertility by supplementing estrogen in the proestrus period found in the current study, other studies [35] have not reported an overall positive effect of ECP supplementation on conception rates. Therefore, beneficial effects of ECP supplementation on pregnancy rates were more likely in low BCS or anestrus cows and requires further investigation.

As described below, several factors affected the likelihood of ovulation and pregnancy success in suckled beef cows [5–7,18]. The present data provided evidence for some factors that influenced the likelihood of occurrence of estrus in suckled *Bos indicus* cows submitted to estradiol/progesterone-based TAI synchronization protocols. Farm location, breed, parity, and BCS had a significant influence on the proportion of cows that displayed estrus between P4 device removal and TAI. Because the occurrence of estrus is indicative of a successful synchronization response, differences between farms in occurrence of estrus may be an important aspect that can predict pregnancy outcomes in TAI programs in *Bos indicus* cattle. Differences within herd composition, such as the proportion of primiparous and multiparous cows, presence of crossbred cows, and proportion of cows with low BCS at the first day of the synchronization protocol, may be related to this disparity.

Similar to the current results, it was reported that increasing BCS during the synchronization protocol enhanced the proportion of high-producing dairy cows that displayed estrus after the estrus synchronization protocol [20]. In the current study, primiparous *Bos indicus* beef cows had lesser odds of displaying estrus after the TAI synchronization protocol than multiparous cows. Factors associated with precalving body growth rate, BCS at parturition, BCS loss, and metabolic status dynamics during the postpartum period may affect primiparous *Bos indicus* beef cows more profoundly than multiparous cows, and may be associated with the lesser estrus responses in this category.

Administration of estradiol increased the occurrence of estrus and P/AI in suckled *Bos indicus* beef cows submitted to an estradiol/progesterone-based synchronization protocol with TAI. The occurrence of estrus, based upon the removal of the tail-head mark, was an important factor associated with larger follicle diameters at TAI, greater serum concentrations of P4 during the subsequent luteal phase, and greater P/AI. Although the occurrence of estrus improved pregnancy outcomes, GnRH at TAI did not improve pregnancy, regardless of whether estrus occurred following TAI. Fig 2.

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