Theriogenology 86 (2016) 2238–2243

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Ovulation time in suckled beef cows is anticipated by use of low doses of progesterone and temporary calf removal on fixed timed AI protocol

E.R. Carvalho [a], T. Martins [a], G.C. Lamb [b], J.L.M. Vasconcelos [a,*]

[a] Departamento de Produção Animal, Faculdade de Medicina Veterinária e Zootecnia - UNESP, Botucatu, SP, Brazil
[b] Department of Animal Science, North Florida Research and Education Center, University of Florida, Marianna, Florida, USA

ARTICLE INFO

Article history:
Received 4 December 2015
Received in revised form 12 July 2016
Accepted 14 July 2016

Keywords:
eCG
Nellore cows
Dominant follicle
Estrus behavior

ABSTRACT

This study was designed to evaluate the effect of concentrations of P4 during a fixed-time artificial insemination (TAI) protocol and 48 hours temporary calf removal (CR; i.e., calves were temporally weaned for 48 hours) or treatment with 300 IU of eCG on ovulation time in suckled beef cows. One hundred and fourteen Nellore cows were estrous synchronized by receiving an intravaginal P4 releasing device containing 1.9 g of P4 (CIDR) plus 2.0-mg estradiol benzoate on Day 0, 12.5-mg dinoprost tromethamine on Day 7, and CIDR removal plus 0.5-mg estradiol cypionate on Day 9. Concentrations of P4 (P4 conc.) were manipulated by insertion of a new CIDR containing 1.9 g of P4 (CIDR1) or a CIDR previously used for 9 days (CIDR2), 18 days (CIDR3), or 27 days (CIDR4). On Day 9, within CIDR uses, cows received either CR or eCG. On Day 11 (48 hours after CIDR removal), all cows received a single TAI. Estrus was observed twice, a day between Day 9 and TAI. Ovulation by 60 hours or 72 hours after CIDR removal was defined as the disappearance of the largest follicle (LF) detected on Day 11 and presence of CL on Day 18, determined by transrectal ultrasonography. Cows ovulating after 72 hours were deemed to have a dominant follicle at 60 hours and 72 hours but also had a CL on Day 18. Serum P4 conc. were evaluated on Days 9, 11, and 18. Cows from CIDR4 + CR treatment ovulated at 60 hours (53.3%) tended to be greater ($P = 0.07$) than CIDR4 + eCG (21.4%). For the remaining treatments, this effect was not detected (CIDR1 + eCG: 6.3% vs. CIDR1 + CR: 0.0%; CIDR2 + eCG: 6.7% vs. CIDR2 + CR: 12.5%; CIDR3 + eCG: 0.0% vs. CIDR3 + CR: 25%). As a consequence, the percentage of cows from CIDR4 + CR treatment ovulating by 72 hours (26.7%) was lesser ($P < 0.05$) than that of cows in the CIDR4 + eCG treatment (78.6%), but for the remaining CIDR uses ovulation was not affected. The analysis of ovulating time revealed that earlier ovulation was associated ($P < 0.01$) to: (a) lesser P4 conc. on Day 9 (2.69^b, 3.36^a, and 3.82^a ng/mL, standard error of the mean [SEM]: 0.12); (b) greater LF on Day 11 (12.46^a, 12.09^a, and 11.06^b mm, SEM: 0.34); (c) greater estrus rate ($94.1\%^a$, $80.0\%^a$, and $28.6\%^b$), for ovulation at 60 hours, 72 hours, or >72 hours, respectively. Thereafter, previously used CIDR resulted ($P < 0.01$) in lesser P4 conc. on Day 9 (4.84^a, 3.24^b, 3.00^b, and 2.50^c ng/mL, SEM: 0.12), greater LF (10.50^b, 12.07^a, 11.98^a, and 12.33^a mm, SEM: 0.18) but only CIDR4 increased ($P < 0.05$) estrus rate ($53.1\%^b$, $65.6\%^b$, $57.9\%^b$, and $90.3\%^a$), for CIDR1, CIDR2, CIDR3, and CIDR4, respectively. In conclusion, cows with low concentrations of P4 during a TAI protocol exhibited increased follicle diameter and an increased rate of estrus. This resulted in a greater percentage of cows ovulating by 60 hours when they had experienced calf removal.

1. Introduction

Previously published reports [1,2] have reported that a TAI protocol using the insertion of an intravaginal device

* Corresponding author. Tel.: +55 14 3880 2950; fax: +55 14 3880 2938.
E-mail address: vasconcelos@fmvz.unesp.br (J.L.M. Vasconcelos).

0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2016.07.015

containing 1.9 g of P4 (CIDR) for 9 days, coupled with administration of 2.0 mg intramuscular (i.m) estradiol benzoate, followed by 12.5 mg i.m dinoprost tromethamine 7 days later, and 0.5 mg i.m estradiol cypionate with initiation of 48 hours temporary calf removal (CR) or administration of 300 IU i.m of eCG on day of CIDR removal, resulted in satisfactory pregnancy rates (≈50%) in suckled Nellore cows inseminated 48 hours after CIDR removal. In addition, a CIDR was used and reused successfully in this ovulation synchronization protocol as many as four times for a total duration of 36 days [2].

The opportunities for CIDR reuse in ovulation synchronization protocols permitted the development of strategies to modulate concentrations of P4 [3,4] resulting in enhanced dominant follicle growth due its dependency on LH pulse frequency [5], which is negatively modulated by concentrations of P4 [5,6]. In addition, suckled Nellore cows require a gonadotropic stimulus (CR or eCG) to support final follicle development [2,7]. Previous studies reported the role of temporary CR in increasing the concentrations of LH [8] and estradiol [9] and enhancing onset of the preovulatory LH peak [9]. Similarly, use of a reused CIDR resulted in positive effects on follicle diameter and patterns of LH release [8] and increased the likelihood of ovulation [4,10].

Therefore, the evaluation of the effects of concentrations of P4 during follicle development concurrent with a gonadotropic stimulus (CR or eCG) on the interval to ovulation may provide opportunities to refine TAI systems within different concentrations of P4 and the source of final gonadotropic stimulus.

2. Material and methods

2.1. Animals

This experiment was carried out on a commercial beef operation located in Mato Grosso, Brazil. One hundred fourteen suckled beef cows (Nellore), with an average body condition score of 2.72 (range, 2.00–3.50; 1 = emaciated to 5 = obese, using 0.25 increments), were maintained on pasture (*Brachiaria brizantha*) with *ad libitum* access to water and mineral salt. All animals were cared for in accordance with the practices outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching [11].

2.2. Experimental design

Cows were randomly assigned to receive one of four P4 treatments: (1) a new intravaginal P4 releasing device containing 1.9-g P4 (CIDR; Zoetis, São Paulo, SP, Brazil, first use; CIDR1; n = 32); (2) a CIDR that had been previously used for 9 days in an ovulation synchronization protocol (second use; CIDR2; n = 32); (3) a CIDR that had been previously used for two 9-day ovulation synchronization protocols (third use; CIDR3; n = 19); (4) a CIDR that had been previously used for three 9-day ovulation synchronization protocols (fourth use; CIDR4; n = 31). All cows received a single administration of 2 mg of estradiol benzoate i.m. (2.0-mL Estrogin; Farmavet, São Paulo, SP Brazil) at CIDR insertion (Day 0) and 12.5 mg i.m. of dinoprost tromethamine (PGF; 2.5-mL Lutalyse; Zoetis, São Paulo, SP, Brazil) 7 days later (Day 7).

On Day 9, all CIDR were removed, and cows received 0.5-mg estradiol cypionate i.m. (0.25-mL ECP; Zoetis, São Paulo, SP, Brazil). Cows were randomly assigned within P4 treatment to receive one of two gonadotropic stimulus methods: (temporary 48 hours CR; n = 56) or administration of 300 IU of equine chorionic gonadotropin (eCG; 1.5-mL Novormon; Zoetis, São Paulo, SP, Brazil; n = 58). Therefore, the resulting treatments were arranged in a 4 × 2 factorial arrangement (CIDR1 + eCG, n = 17; CIDR1 + CR, n = 15; CIDR2 + eCG, n = 16; CIDR2 + CR, n = 16; CIDR3 + eCG, n = 10; CIDR3 + CR, n = 9; CIDR4 + eCG, n = 15; and CIDR4 + CR, n = 16). At the time of CIDR removal, the tail head of each cow was painted with marking sticks (Raidex, Walmur, Porto Alegre, RS, Brazil) to aid detection of estrus. Visual detection of estrus in cows occurred twice a day for 1 hour during each period (0730 and 1600), from Day 9 to 11.

On Day 11, cows were randomly assigned within treatments to TAI by experienced technicians (n = 3) using frozen-thawed commercial semen from a single AI sire. At the time of TAI, cows visually observed in estrus between Days 9 and 11, or cows that had evidence of being mounted by the absence of tail paint were all considered to have expressed estrus.

2.3. Ultrasound examinations

Transrectal ultrasound examination of the ovaries were performed by a single technician at CIDR removal (Day 9) and 48, 60, and 72 hours later to assess the diameter of the first and second largest ovarian follicle (defined as the average between measurements of two perpendicular axes of each structure). A map of each ovary was drawn and the positions of follicles were recorded. Ultrasonography was performed with a 7.5-MHz linear-array transrectal transducer (Aloka SSD-500; Aloka, Tokyo, Japan). A fifth ovarian ultrasonography scan was performed on Day 18 (7 days after TAI) to verify the presence of a CL at the approximate topographical location of the dominant follicle detected at the previous ultrasound assessments.

Ovulation at 60 or 72 hours after CIDR removal was defined as disappearance of the dominant follicle present on Day 11 and presence of CL on Day 18. Cows identified with a dominant follicle on Day 11 but that had not ovulated by 72 hours and had a CL on Day 18 were considered to have ovulated at an interval exceeding 72 hours.

Seven cows were presumed to have ovulated prematurely (between Days 9 and 11), when the dominant follicle present on Day 9 was not present on Day 11, and a CL was present on Day 18. Data associated with follicle diameter on Day 11 for these seven cows were excluded from statistical analysis (CIDR1 + eCG, n = 2; CIDR1 + CR, n = 1; CIDR2 + eCG, n = 1; CIDR2 + CR, n = 1; CIDR3 + eCG, n = 0; CIDR3 + CR, n = 0, and CIDR4 + eCG, n = 0; CIDR4 + CR, n = 2).

Transrectal ultrasonography was used to determine pregnancy status by evidence of a viable embryo 30 days after TAI. The conception rate was calculated as the proportion of cows ovulating that became pregnant to TAI, and the pregnancy rate included all cows, whether they were deemed to have ovulated.

2.4. Blood sampling for analysis of P4

Blood sampling for determination of concentrations of P4 was performed in the majority of cows on Day 9 (n = 107), Day 11 (n = 109), and Day 18 (n = 104). Blood samples were collected from a coccygeal vessel into silicone coated Vacutainer tubes (Becton Dickison Co., Franklin Lakes, NJ, USA). Blood was allowed to clot at 4 °C for 24 hours and centrifuged at 1500 *g* for 15 minutes at room temperature. Serum was removed and stored frozen at −20 °C until hormonal assays were performed. Concentrations of P4 were measured in all samples using a solid-phase radioimmunoassay kit (Coat-a-count; Diagnostic Products Corporation, Los Angeles, CA, USA) according to manufacturer's instructions. Intraassay and interassay CV were 6.25% and 8.95%, respectively. The assay sensitivity was 0.016 ng/mL. The CV calculations were performed as described by Pereira et al. [12].

2.5. Statistical analyses

Binomial, dependent variables were analyzed by PROC GLIMMIX of the SAS software (version 9.3, SAS Institute Inc., Cary, NC, USA), using binomial distribution. The effects of CIDR uses, gonadotropic stimulus, and their interaction was considered in the models for analyses of ovulation time, expression of estrus, conception, and pregnancy rates. Fisher's exact test and chi-square distribution of SAS were used for comparing the frequency of ovulation at 60 hours, 72 hours, and greater than 72 hours among treatments.

An objective was to evaluate the effects of each individual continuous variable, (follicle diameter and concentrations of P4 on Day 9 and 11) on binomial dependent variables (estrus response). Procedure GLM of SAS was used for this analysis. Based on the effects established by the GLM procedure (linear, quadratic, or cubic), the logistical regression models were designed using intercept and slope values generated by PROC LOGISTIC for the following equation: Probability = $(e^{\text{logistic equation}})/(1 + e^{\text{logistic equation}})$.

Continuous variables from Day 11 and 18 were analyzed using PROC MIXED of SAS, using a similar model that included CIDR uses, gonadotropic stimulus, and their interaction. For analyses of continuous variables for Day 9, only the effect of CIDR uses was considered in the model. Before analyses, the data were assessed for residual normality and variance homogeneity using Shapiro–Wilk and Levene's tests using the UNIVARIATE and GLM procedures of SAS, respectively. For each dependent variable, data that did not follow a normal distribution or variance homogeneity were transformed to natural logarithms. The PROC PEARSON of SAS was used to establish the correlation between continuous variables.

A secondary analysis using PROC GLIMMIX and PROC MIXED was performed including time of ovulation in the statistical models, to assess its effects on the respective variables. Results were reported as least square mean ± standard error of the mean unless otherwise indicated, and comparisons were made using the PDIFF procedure. A probability of $P \leq 0.05$ indicated that a difference was significant, and a probability of $0.05 > P \leq 0.10$ indicated that significance was approached.

3. Results

3.1. Effect of treatments on serum concentrations of P4 expression of estrus

Concentrations of P4 on Day 9 and 11 were influenced by CIDR uses ($P < 0.01$, Table 1). Cows receiving the CIDR4 treatment had lesser concentrations of P4 on Day 9 and 11 compared with those in the CIDR1 treatment. Expression of estrus was negatively affected ($P < 0.01$) by concentrations of P4 on Days 9 and 11 (Figs. 1 and 2). Cows in estrus had lesser concentrations of P4 on Day 9 (3.16 ± 0.14 vs. 3.93 ± 0.21 ng/mL, for cows expressing estrus compared with those not expressing estrus, respectively; $P < 0.01$) and Day 11 (0.32 ± 0.02 vs. 0.42 ± 0.03 ng/mL, for cows expressing estrus compared with those not expressing estrus, respectively; $P < 0.01$). In addition, concentrations of P4 and follicle diameter were inversely correlated ($P < 0.01$) on Days 9 ($r = -0.52$) and 11 ($r = -0.28$). On Day 18, cows ovulating in response to eCG had greater

Table 1
Effect of multiple CIDR treatments during a fixed-time AI protocol on concentrations of P4 and on follicle diameter on Days 9 and 11, and on expression of estrus, in suckled Nellore cows.

Variables	CIDR treatment[d]				P value[e]
	CIDR1	CIDR2	CIDR3	CIDR4	
P4 Day 9, ng/mL	$4.84^{a} \pm 0.16$	$3.24^{b} \pm 0.15$	$3.00^{b} \pm 0.19$	$2.50^{c} \pm 0.16$	<0.01
P4 Day 11, ng/mL	$0.42^{a} \pm 0.16$	$0.36^{a,b,Z} \pm 0.15$	$0.34^{a,b} \pm 0.19$	$0.28^{b,W} \pm 0.16$	<0.01
Fol. Day 9, mm	$8.15^{b} \pm 0.26$	$10.76^{a} \pm 0.28$	$10.60^{a} \pm 0.33$	$10.84^{a} \pm 0.26$	<0.01
Fol. Day 11, mm	$10.50^{b} \pm 0.32$	$12.07^{a} \pm 0.31$	$11.98^{a} \pm 0.39$	$12.33^{a} \pm 0.32$	<0.01
Estrus, % (estrus/n)	53.1^{y} (17/32)	65.6^{y} (21/32)	57.9^{y} (11/19)	90.3^{x} (28/31)	<0.05

[a,b,c]Values without a common superscript differed between treatments ($P < 0.01$).
[x,y]Values without a common superscript differed between treatments ($P < 0.05$).
[Z,W]Values without a common superscript tended to differ ($0.05 < P \leq 0.10$).
Abbreviations: CIDR, controlled internal drug release device containing 1.9 g P4; Fol., follicle diameter; P4, concentration of progesterone.
[d] Suckled Nellore cows (n = 114) were assigned randomly to receive a new CIDR (CIDR1) or a CIDR previously used for 9 days (CIDR2), 18 days (CIDR3), or 21 days (CIDR4) plus 2 mg of estradiol benzoate at initiation of synchronization protocol (Day 0) for TAI. Removal of CIDR occurred on Day 9, and ovaries were scanned using transrectal ultrasonography on Days 9 and 11. Cows (n = 7) that ovulated prematurely (Day 11) were not included in follicle diameter data analyses.
[e] Effect of CIDR treatment on the variables.

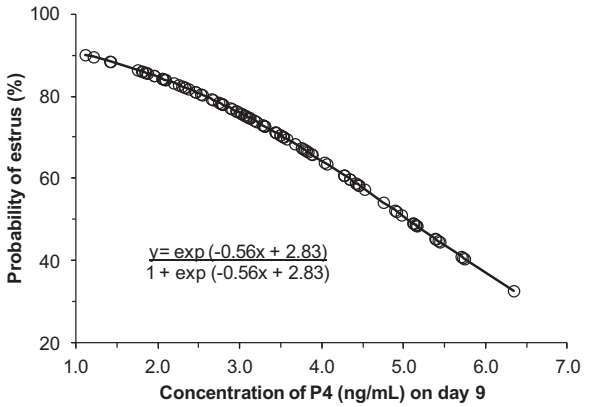

Fig. 1. Relationship between concentrations of P4 (ng/mL) on Day 9 (CIDR removal) and probability of estrus, monitored twice daily for a duration of 1 hour from Day 9 to 11. Cows with lesser concentrations of P4 on Day 9 had greater probabilities of estrus ($P < 0.01$).

($P < 0.05$) concentrations of P4 (6.33 ± 0.26 ng/mL) than cows ovulating in response to the CR treatment (5.50 ± 0.23 ng/mL).

3.2. Effect of treatments on follicle diameter and expression of estrus

Dominant follicle diameters on Day 9 and 11 from cows differed ($P < 0.01$) among the four CIDR treatments, with follicle diameter increasing with the number of previous uses of the CIDR (Table 1). Follicle diameter had a positive effect ($P < 0.01$) on expression of estrus (Fig. 3) and the number of CIDR uses affected ($P < 0.05$) expression of estrus (Table 1), with a greater proportion of cows receiving the CIDR4 treatment expressing estrus. Ovulation rates (92.1% [105/114]), conception rates (48.6% [51/105]), or pregnancy rates (44.7% [51/114]) were not affected ($P > 0.10$) by CIDR use or gonadotropic treatments.

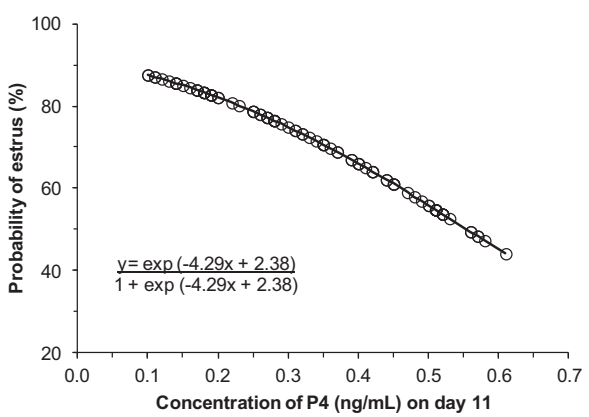

Fig. 2. Relationship between concentrations of P4 (ng/mL) on the day of TAI (Day 11) and probability of estrus, monitored twice daily for a duration of 1 hour from Day 9 to 11. Cows with lesser concentrations of P4 on Day 11 had greater probabilities of estrus ($P < 0.01$).

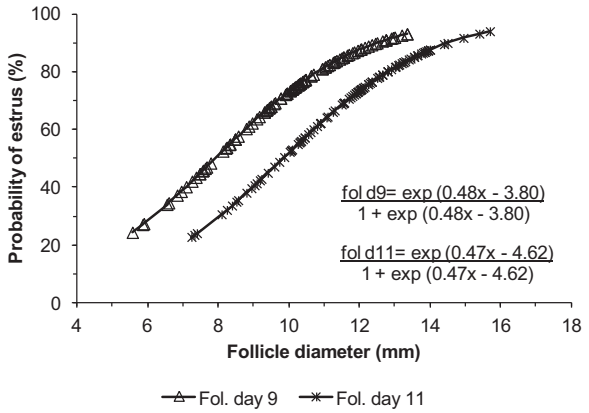

Fig. 3. Relationship between follicle diameter (Day 9 = CIDR removal and Day 11 = TAI) on probability of estrus, monitored twice daily for a duration of 1 hour from Day 9 to 11. Cows with greater follicles on Day 9 or 11 had greater probabilities of estrus ($P < 0.01$).

3.3. Overall effects of treatments on ovulation distribution

At 48 hours, there was no effect ($P > 0.10$) of treatments on proportion of cows that had prematurely ovulated (Table 2).

At 60 hours, an interaction between CIDR treatment and gonadotropic stimulus was detected ($P < 0.05$), ovulation rate from CIDR4 + CR treatment (53.3% [8/15]) tended to differ ($P = 0.07$) from the CIDR4 + eCG treatment (21.4% [3/14]), whereas the CIDR3 + CR treatment (25.0% [2/8]) did not differ from CIDR4 + eCG treatment but differed ($P < 0.05$) from all other treatments (Table 2). For the remaining uses of CIDR, there was no effect of CR or eCG on ovulation rate at 60 hours.

At 72 hours, ovulation rate for the CIDR4 + CR treatment (26.7% [4/15]) was lesser ($P < 0.05$) than CIDR4 + eCG (78.6% [11/14]; Table 2) due to the greater proportion of cows from CIDR4 + CR cows ovulating by 60 hours. For the remaining treatments, this effect was not detected. Also at 72 hours, a significant difference or tendency was detected for the remaining comparisons with CIDR4 + CR (Table 2).

When ovulation occurred at greater than 72 hours, there was an effect of CIDR use on ovulation rate (35.7%^{a} [10/28], 22.6%^{a} [7/31], 17.6%^{a,b,X} [3/17], and 3.4%^{b,Y} [1/29] for CIDR1, CIDR2, CIDR3, and CIDR4, respectively; $^{a,b}P < 0.05$ and $^{X,Y}P = 0.10$).

The analysis considering only time of ovulation (Table 3) demonstrated that cows ovulating earlier had lesser concentrations of P4 and greater follicle diameter than cows ovulating at greater than 72 hours. Estrus and conception rates were also greater for cows ovulating earlier (Table 3).

4. Discussion

The effect of concentrations of P4 and gonadotropic stimulus methods on ovulation time may enhance the ability to improve pregnancy rates in a TAI system, since the interval between TAI and ovulation is important for successful pregnancy outcomes [13]. In the present study, previous use of a CIDR efficiently reduced concentrations of

Table 2
Ovulation rate and distribution in suckled Nellore cows synchronized during 9 days with a CIDR of different duration of uses and treated with eCG or CR on day of CIDR removal.

Variables	Treatments[c]								P value[d]		
	CIDR1		CIDR2		CIDR3		CIDR4				
	eCG	CR	eCG	CR	eCG	CR	eCG	CR	F1	F2	F1*F2
Ovulation rate, % (n/n)	94.1 (16/17)	80.0 (12/15)	93.4 (15/16)	100.0 (16/16)	90.0 (9/10)	88.9 (8/9)	93.3 (14/15)	93.8 (15/16)	0.93	0.98	0.90
48 h, %	12.5	8.3	6.7	6.3	0.0	0.0	0.0	13.3	0.16	0.64	0.16
60 h, %	6.3^{b}	0.0^{b}	6.7^{b}	12.5^{b}	0.0^{b}	25.0^{a,b}	21.4^{a,Y}	53.3^{a,X}	<0.05	0.05	<0.05
72 h, %	43.7^{a,b,Y}	58.3^{a,b,Z}	66.7^{a}	56.3^{a,b,Z}	77.8^{a,X}	62.5^{a,b,Z}	78.6^{a,X}	26.7^{b,W}	0.17	0.10	<0.05
>72 h, %	37.5	33.3	20.0	25.0	22.2	12.5	0.0	6.7	<0.05	0.92	0.33

^{a,b}Values without a common superscript differed between treatments ($P < 0.05$).
^{X,Y}Values without a common superscript tended to differ ($0.05 < P \leq 0.10$).
^{Z,W}Values without a common superscript tended to differ ($0.05 < P \leq 0.10$).
Abbreviation: CR, calf removal.
[c] Suckled Nellore cows treated with a new CIDR (CIDR1) or a CIDR previously used for 9 (CIDR2), 18 (CIDR3), or 21 days (CIDR4) were assigned within CIDR treatment to receive 300 IU of eCG or temporary 48-h calf removal on day of CIDR removal (Day 9). Cows had the ovaries scanned on Day 9, and 48 h, 60 h, 72 h, and 7 days after CIDR removal. Ovulation was considered to have occurred when dominant follicles were not present on the subsequent ovarian scans, plus a CL was observed on Day 18. When the dominant follicle present on Day 9 was not present on Day 11, and a CL was present on Day 18; the cows were presumed to have ovulated prematurely (48 h). Cows with a dominant follicle at 72 h but with a CL on Day 18, were considered to have ovulated within the interval greater than 72 h.
[d] F1: effect of CIDR treatment; F2: effect of CR or eCG; F1*F2: effect of interaction.

P4, but ovulation time was only hastened in cows synchronized with CIDR4 and submitted to temporary 48 hours CR.

As anticipated, cows in the CIDR1 treatment had smaller follicle diameters on Day 9 and 11. Previous findings from our laboratory [3,4,14] reported smaller follicle diameter and lower estrus response after synchronization of ovulation with CIDR1 in Nellore beef heifers. Our results indicated that the strategy to modulate concentrations of P4 during estrus synchronization by using a previously used CIDR [3,4,14] was efficient in inducing follicles of greater diameter at TAI in suckled Nellore cows. The mechanism responsible has been established and is related to an inverse relationship between LH pulse frequency and concentrations of P4 [5,6], which modulate dominant follicular growth [5].

A positive relationship between dominant follicle diameter and the probability of estrus was detected. This finding is similar to that described by others [14–16], which is likely due to a positive correlation between dominant follicle diameter and concentrations of estradiol [17,18]. A greater concentration of P4 on Days 9 and 11 was inversely correlated to follicle diameter and the probability of estrus. In this study, concentrations of P4 on Day 11 were lesser for cows receiving the CIDR4 treatment. The confounding effect of a CL on these findings can be excluded, since cows received a dose of PGF (i.e., 12.5 mg) 2 days before CIDR removal, which is known to be effective at regressing a CL in *Bos indicus* cows at this time point [1,4]. Further investigation is warranted to evaluate an indirect effect of excitable temperament of *Bos indicus* cattle [19] that may increase circulating concentrations of P4 [20]. In prepubertal Nellore heifers, the excitable temperament is associated with greater concentrations of P4 in heifers that do not contain a CL [21].

Table 3
Association between time of ovulation and follicle diameter and concentrations of P4 on Days 9 and 11, and incidence of estrus and conception in suckled Nellore cows.

Variables	Ovulation time (h)[c]				P value[d]
	48	60	72	>72	
P4 Day 9, ng/mL	3.76^{a,b,Z} ± 0.51	2.69^{b,W} ± 0.27	3.36^{a} ± 0.15	3.82^{a} ± 0.29	<0.01
P4 Day 11, ng/mL	0.40^{a,b,Z} ± 0.04	0.26^{b,W} ± 0.04	0.33^{b} ± 0.02	0.46^{a} ± 0.04	<0.01
Fol. Day 9, mm	9.35^{a,b,y} ± 0.64	11.07^{a,x} ± 0.42	10.33^{a} ± 0.22	9.18^{b} ± 0.42	<0.01
Fol. Day 11, mm[e]	–	12.46^{a} ± 0.38	12.09^{a} ± 0.20	11.06^{b} ± 0.34	<0.01
Estrus, % (n)	57.1^{a,b} (4/7)	94.1^{a} (16/17)	80.0^{a} (48/60)	28.6^{b} (6/21)	<0.01
Conception rate, % (n)	42.9^{x,y} (3/7)	64.7^{x} (11/17)	53.3^{x} (32/60)	23.8^{y} (5/21)	<0.05

^{a,b}Values without a common superscript differed between treatments ($P < 0.01$).
^{x,y}Values without a common superscript differed between treatments ($P < 0.05$).
^{Z,W}Values without a common superscript tended to differ ($0.05 < P \leq 0.10$).
Abbreviations: Fol., follicle diameter; P4, concentration of progesterone.
[c] Beef cows ovulating (n = 105) that had ovaries scanned on Day 9, and 48 h, 60 h, 72 h, and 7 days after CIDR removal. Ovulation was considered to have occurred when dominant follicles were not present on the subsequent ovarian scans, plus a CL was observed on Day 18. When the dominant follicle present on Day 9 was not present on Day 11, and a CL was present on Day 18; the cows were presumed to have ovulated prematurely (48 h). Cows with a dominant follicle at 72 h but with a CL on Day 18 were considered to have ovulated within the interval greater than 72 h.
[d] Effects of the association.
[e] Cows ovulated prematurely (Day 11) did not present the follicle diameter data.

Cows ovulating at 60 hours exhibited greater follicle diameter at TAI, had increased expression of estrus and reduced concentrations of P4 on Day 9. The CIDR4 treatment with CR was the treatment most capable of producing a response to alter the distribution of ovulation, indicating that the beneficial effect of CR was dependent on increased dominant follicle diameter. This is in agreement with a report that indicated a positive influence of follicle size on pattern of LH release after cows were exposed to CR [8]. In addition, this approach may provide beneficial opportunities for TAI with sex-sorted semen, since improvements in pregnancy rates were noted when TAI occurred closer to ovulation [22]. Few cows ovulated by 48 hours and were not affected by treatments.

The importance of dominant follicle diameter [14,18], estrus behavior [14,16], and low concentrations of progesterone [12] at the time of AI on fertility have been discussed previously. However, results from this study support the contribution of these factors to hastening ovulation and potentially enhancing conception. In contrast, data from this study contradict results that indicated a broad optimum insemination window in beef heifers from 4 to 24 hours after estrus [23] and agree with studies in dairy cows [13,24] that recommend a more restricted window from 4 to 12 hours to AI. Therefore, timing of the TAI among multiple TAI protocols warrants further investigation, especially when evaluating potential improvements in pregnancy rates.

In conclusion, synchronization of ovulation with a previously used CIDR was effective in reducing concentrations of progesterone and inducing greater follicle diameter in suckled beef cows. The combination of greater follicle diameter and low concentrations of P4 at TAI had positive effects on expression of estrus and hastened the interval to ovulation. In addition, CR reduced the interval to ovulation in cows synchronized with a CIDR that had been used previously for 3 uses, indicating a response dependent on dominant follicle diameter.

Acknowledgments

The authors acknowledge the Coordination for the Improvement of Higher Education (CAPES) for financial support, Zoetis (São Paulo, SP, Brazil) for donation of Lutalyse, CIDR, E.C.P and Novormon, and the São Marcelo farm for providing cows and labor used in this study.

References

[1] Meneghetti M, Filho OG, Peres RF, Lamb GC, Vasconcelos JL. Fixed-time artificial insemination with estradiol and progesterone for *Bos indicus* cows I: basis for development of protocols. Theriogenology 2009;72:179–89.
[2] Filho OG, Meneghetti M, Peres RF, Lamb GC, Vasconcelos JL. Fixed-time artificial insemination with estradiol and progesterone for *Bos indicus* cows II: strategies and factors affecting fertility. Theriogenology 2009;72:210–8.
[3] Dias CC, Wechsler FS, Day ML, Vasconcelos JL. Progesterone concentrations, exogenous equine chorionic gonadotropin, and timing of prostaglandin F2α treatment affect fertility in postpuberal Nelore heifers. Theriogenology 2009;72:378–85.
[4] Peres RF, Júnior IC, Filho OG, Nogueira GP, Vasconcelos JL. Strategies to improve fertility in *Bos indicus* postpubertal heifers and nonlactating cows submitted to fixed-time artificial insemination. Theriogenology 2009;72:681–9.
[5] Kojima FN, Bergfeld EG, Wehrman ME, Cupp AS, Fike KE, Mariscal-Aguayo DV, et al. Frequency of luteinizing hormone pulses in cattle influences duration of persistence of dominant ovarian follicles, follicular fluid concentrations of steroids, and activity of insulin-like growth factor binding proteins. Anim Reprod Sci 2003;77:187–211.
[6] Kinder JE, Kojima FN, Bergfeld EG, Wehrman ME, Fike KE. Progestin and estrogen regulation of pulsatile LH release and development of persistent ovarian follicles in cattle. J Anim Sci 1996;74:1424–40.
[7] Sá Filho MF, Ayres H, Ferreira RM, Marques MO, Reis EL, Silva RC, et al. Equine chorionic gonadotropin and gonadotropin-releasing hormone enhance fertility in a norgestomet-based, timed artificial insemination protocol in suckled Nelore (*Bos indicus*) cows. Theriogenology 2010;73:651–8.
[8] Smith MF, Lishman AW, Lewis GS, Harms PG, Ellersieck MR, Inskeep EK, et al. Pituitary and ovarian responses to gonadotropin releasing hormone, calf removal and progestogen in anestrous beef cows. J Anim Sci 1983;57:418–24.
[9] Marquezini GH, Mercadante VR, Olson KC, Jaeger JR, Perry GA, Stevenson JS, et al. Effects of equine chorionic gonadotropin on follicle development and pregnancy rates in suckled beef cows with or without calf removal. J Anim Sci 2013;91:1216–24.
[10] Gimenes LU, Sá Filho MF, Carvalho NA, Torres-Júnior JR, Souza AH, Madureira EH, et al. Follicle deviation and ovulatory capacity in *Bos indicus* heifers. Theriogenology 2008;69:852–8.
[11] FASS. Guide for the care and use of agricultural animals in agricultural research and teaching. Savoy, IL: Federation of Animal Science Societies; 1999.
[12] Pereira MH, Sanches CP, Guida TG, Rodrigues AD, Aragon FL, Veras MB, et al. Timing of prostaglandin F2α treatment in an estrogen-based protocol for timed artificial insemination or timed embryo transfer in lactating dairy cows. J Dairy Sci 2013;96:2837–46.
[13] Dalton JC, Nadir S, Bame JH, Noftsinger M, Nebel RL, Saacke RG. Effect of time of insemination on number of accessory sperm, fertilization rate, and embryo quality in nonlactating dairy cattle. J Dairy Sci 2001;84:2413–8.
[14] Martins T, Peres RF, Rodrigues AD, Pohler KG, Pereira MH, Day ML, et al. Effect of progesterone concentrations, follicle diameter, timing of artificial insemination, and ovulatory stimulus on pregnancy rate to synchronized artificial insemination in postpubertal Nellore heifers. Theriogenology 2014;81:446–53.
[15] Sá Filho MF, Crespilho AM, Santos JE, Perry GA, Baruselli PS. Ovarian follicle diameter at timed insemination and estrous response influence likelihood of ovulation and pregnancy after estrous synchronization with progesterone or progestin-based protocols in suckled *Bos indicus* cows. Anim Reprod Sci 2010;120:23–30.
[16] Sá Filho MF, Santos JE, Ferreira RM, Sales JN, Baruselli PS. Importance of estrus on pregnancy per insemination in suckled *Bos indicus* cows submitted to estradiol/progesterone-based timed insemination protocols. Theriogenology 2011;76:455–63.
[17] Jinks EM, Smith MF, Atkins JA, Pohler KG, Perry GA, MacNeil MD, et al. Preovulatory estradiol and the establishment and maintenance of pregnancy in suckled beef cows. J Anim Sci 2013;91:1176–85.
[18] Perry GA, Smith MF, Roberts AJ, MacNeil MD, Geary TW. Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. J Anim Sci 2007;85:684–9.
[19] Cooke RF, Bohnert DW, Meneghetti M, Losi TC, Vasconcelos JL. Effects of temperament on pregnancy rates to fixed-timed AI in *Bos indicus* beef cows. Livest Sci 2011;142:108–13.
[20] Curley Jr KO, Neuendorff DA, Lewis AW, Cleere JJ, Welsh Jr TH, Randel RD. Functional characteristics of the bovine hypothalamic–pituitary–adrenal axis vary with temperament. Horm Behav 2008;53:20–7.
[21] Júnior IC, Filho OG, Peres RF, Aono FH, Day ML, Vasconcelos JLM. Reproductive performance of prepubertal *Bos indicus* heifers after progesterone-based treatments. Theriogenology 2010;74:903–11.
[22] Sales JN, Neves KA, Souza AH, Crepaldi GA, Sala RV, Fosado M, et al. Timing of insemination and fertility in dairy and beef cattle receiving timed artificial insemination using sex-sorted sperm. Theriogenology 2011;76:427–35.
[23] Dorsey BR, Kasimanickam R, Whittier WD, Nebel RL, Wahlberg ML, Hall JB. Effect of time from estrus to AI on pregnancy rates in estrous synchronized beef heifers. Anim Reprod Sci 2011;127:1–6.
[24] Dransfield MB, Nebel RL, Pearson RE, Warnick LD. Timing of insemination for dairy cows identified in estrus by a radiotelemetric estrus detection system. J Dairy Sci 1998;81:1874–82.