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O. G. Sá Filho, E. R. Vilela, T. W. Geary and J. L. M. Vasconcelos

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Strategies to improve fertility in postpartum multiparous *Bos indicus* cows submitted to a fixed-time insemination protocol with gonadotropin-releasing hormone and prostaglandin $F_{2\alpha}$[1]

O. G. Sá Filho,* E. R. Vilela,* T. W. Geary,† and J. L. M. Vasconcelos*[2]

*Departamento de Produção Animal, Faculdade de Medicina Veterinária e Zootecnia-UNESP, P 18618-000, Brazil; and †USDA-ARS, Fort Keogh Livestock and Range Research Laboratory, Miles City, MT 59301

ABSTRACT: In Exp. 1, we evaluated the effects of 2 lengths of progesterone exposure [CIDR (controlled intravaginal drug release); 7 vs. 14 d] before a modified CO-Synch protocol [50.0-μg injection of GnRH 6.5 d before a 25.0-mg injection of $PGF_{2\alpha}$ followed by another injection of GnRH and fixed-time AI (TAI) 2 d after $PGF_{2\alpha}$], with or without temporary weaning (TW) before GnRH treatments, on fertility of suckled multiparous *Bos indicus* cows (n = 283) and on calf performance. Timed AI pregnancy rates for cows receiving 7 d CIDR + TW, 7 d CIDR, 14 d CIDR + TW, and 14 d CIDR were 53, 47, 46, and 41%, respectively ($P > 0.10$). Calves submitted to two 48-h TW 6 d apart had decreased mean BW at 240 d (187.9 ± 2.7 vs. 195.5 ± 2.7 kg; $P < 0.05$), but BW at 420 d was not affected by TW (240.1 ± 5.1 kg). In Exp. 2, we evaluated the effect of no treatment and treatment with or without a CIDR insert between GnRH and $PGF_{2\alpha}$ treatments of a modified CO-Synch protocol on pregnancy rate to TAI, and throughout a 90-d breeding season in suckled multiparous *Bos indicus* cows (n = 453). The inclusion of a CIDR between first GnRH and $PGF_{2\alpha}$ treatments of a modified CO-Synch protocol did not improve pregnancy rate (29 and 33% for cows receiving CO-Synch + CIDR and CO-Synch protocol, respectively), and cycling cows had poorer TAI pregnancy rates than anestrous cows treated with either synchronization protocol (21.7 vs. 40.7%; $P < 0.05$). However, regardless of treatment with CIDR, cows submitted to TAI protocol had greater ($P < 0.05$) pregnancy rates at 30 (54.8 vs. 11.2%), 60 (72.1 vs. 38.8%), and 90 d (82.0 vs. 57.9%) of breeding season than untreated cows.

Key words: anestrous, *Bos indicus*, gonadotropin-releasing hormone, progesterone-releasing intravaginal insert, prostaglandin $F_{2\alpha}$, timed artificial insemination

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INTRODUCTION

Bos indicus cows have long periods of postpartum anestrous, a short length of estrous behavior, and a high incidence of estrus at night (Pinheiro et al., 1998; Meneghetti and Vasconcelos, 2008) that contribute to poor reproductive efficiency. Several treatments have been developed recently allowing induction of ovulation in anestrous and cycling cows and AI without the need for detection of estrus, with positive impacts on reproductive efficiency of beef herds. In a series of studies, Meneghetti et al. (2009) proposed a synchronization of ovulation protocol that used estradiol benzoate + progesterone for synchronization of new follicular wave recruitment and provided satisfactory rates of ovulation (~90%) and pregnancy (~50%) in anestrous and cycling *Bos indicus* cows. Fixed-time AI (**TAI**) protocols that have the ability to consistently achieve pregnancy rates greater than 50% are essential for increasing the use of AI in commercial beef operations.

Timed AI protocols using progesterone + estradiol are less expensive and have been shown to be more efficient in postpartum cows under tropical climates than those based on synchronization of follicular wave emergence with GnRH (Baruselli et al., 2002) because the probability of ovulation to a GnRH treatment is less in anestrous than in cycling cows (Fernandes et al., 2001; Chebel et al., 2006) and in beef than in dairy cows (Vasconcelos et al., 1999; Saldarriaga et al., 2007). A commercial source of estradiol is not available for

[1]Mention of a proprietary product does not constitute a guarantee or warranty of the product by USDA or the authors and does not imply its approval to the exclusion of other products that may also be suitable. USDA, ARS, Northern Plains Area, is an equal opportunity/affirmative action employer. All agency services are available without discrimination.

[2]Corresponding author: vasconcelos@fca.unesp.br

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estrous cycle manipulation in most countries, so other products (e.g., GnRH, LH, or hCG) have been used to induce ovulation of dominant follicles and to synchronize follicular waves. Protocols using GnRH and $PGF_{2\alpha}$ have afforded acceptable results in *Bos taurus* females (pregnancy rate >50%; Lamb et al., 2001; Larson et al., 2006), but reduced pregnancy rates have been reported for *Bos indicus* cows (Saldarriaga et al., 2007; Vasconcelos et al., 2009b). We performed 2 experiments to evaluate strategies to improve fertility of postpartum *Bos indicus* cows submitted to a TAI protocol with GnRH and $PGF_{2\alpha}$. The basis of these strategies were 1) to improve ovulation rate to the first ovulatory stimulus of the protocol by pretreating cows with an intravaginal progesterone insert and temporary weaning (**TW**) and improve ovulation rate to the second ovulatory stimulus of the protocol with TW (Exp. 1); and 2) to improve fertility after the TAI protocol providing progesterone via an intravaginal release insert between first GnRH and $PGF_{2\alpha}$ treatments (Exp. 2).

MATERIALS AND METHODS

All experiments described below were conducted in commercial ranches in Brazil under authority of their managers. 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 (FASS, 1999).

Animals and Treatments

Both experiments described below were started in the first week of November (spring season in Brazil) on commercial ranches. All animals were suckled multiparous Nelore cows maintained on *Brachiaria brizantha* rotated grazing systems with water and mineral ad libitum. Within each experiment, cows were managed in a single group, and treatments were administered simultaneously.

Exp. 1. The goal of this study was to evaluate the effects of 2 lengths of exposure to progesterone (7 vs. 14 d) before a modified CO-Synch protocol with or without TW before GnRH treatments on the rates of ovulation at first GnRH treatment, ovulation at second GnRH treatment, conception, and pregnancy to TAI in suckled *Bos indicus* cows. We expected that a longer treatment with progesterone would result in more cows having a persistent dominant follicle, and consequently, a greater proportion would ovulate to the first GnRH treatment. Cows (n = 283; 35 ± 5 d postpartum; 4 ± 2 parturitions) with BCS at d −8 between 2.75 and 3.25 on a 1 to 5 scale (Houghton et al., 1990) on a ranch at Sao Paulo State were randomly assigned to receive 1 of the following treatments, in a 2 × 2 factorial arrangement of treatment (Figure 1): 1) cows received an intravaginal insert containing 1.9 g of progesterone (controlled intravaginal drug release, **CIDR**; Pfizer Animal Health, São Paulo, Brazil) between d −24 and −10, followed by a 50.0-μg intramuscular injection of GnRH (1.0 mL of Cystorelin, Merial, Paulinia, São Paulo, Brazil) on d −8 (morning), a 25.0-mg intramuscular injection of dinoprost tromethamine ($PGF_{2\alpha}$; 5.0 mL of Lutalyse, Pfizer Animal Health) on d −2 (afternoon), and GnRH on d 0 (afternoon; **CIDR14**; n = 71); 2) cows received a treatment similar to that described for CIDR14 group, with additional 48 h TW between d −10 and −8, and between d −2 and 0 (CIDR14+TW; n = 72); 3) cows received a CIDR insert between d −17 and −10, followed by GnRH on d −8 (morning), $PGF_{2\alpha}$ on d −2 (afternoon), and GnRH on d 0 (afternoon; **CIDR7**; n = 70); or 4) cows received a treatment similar to that described for CIDR7 group, with additional 48 h TW between d −10 and −8, and between d −2 and 0 (CIDR7+TW; n = 70). The $PGF_{2\alpha}$ treatment was performed 6.5 d after GnRH because in a previous study we found that decreasing this interval resulted in better synchronization rate than 7 d (Vasconcelos et al., 2000). All cows were submitted to TAI (d 0) on the same day. The TAI was performed by 3 AI technicians using semen from 3 sires randomly distributed among treatments. In this and the following experiment, during TW, calves were held in pens without physical contact with their dams, with ad libitum access to water. Immediately after TAI, calves were returned to their dams. Ovarian ultrasound examinations were performed in all cows (Aloka SSD-500 with a 7.5 MHz linear-array transrectal transducer, Tokyo, Japan) on d −24, −17, −10, −8, −2, and 2 (d 0 = TAI). Presence or absence of luteal structures on d −24, −17, and −10 was used to determine cyclicity of cows. Cows were considered anestrus when luteal tissue was absent at all examinations. Comparison of sonograms from d −8 and −2 was used to determine ovulation in response to the first GnRH treatment. Ovulation was considered to have occurred in cows having a corpus luteum (**CL**) at d −2 which replaced a >8.0-mm follicle observed on d −8. Comparison of sonograms from d −2 and 2 was used to determine ovulation in response to the second GnRH treatment and the CL regression. Ovulation was considered to have occurred in cows having a >7.5-mm follicle at d −2, and absence of this follicle at d 2 and CL regression was considered in cows that had a visible CL on d −2 but did not have a visible CL on d 2. Furthermore, ovarian follicles were measured on d −10, −8, and −2 by averaging the length and width measurements from sonograms. Pregnancy diagnosis was performed 28 d after TAI by ultrasound examination. Conception rate was calculated by dividing the number of pregnant cows on d 28 by the number of cows that ovulated in response to the second GnRH treatment, and pregnancy rate was calculated by dividing the number of pregnant cows on d 28 by the number of treated cows. Calves were weaned at 240 d of age and were subsequently maintained on *Brachiaria brizantha* pasture with water and mineral available for ad libitum consumption until 420 d of age. Records of BW

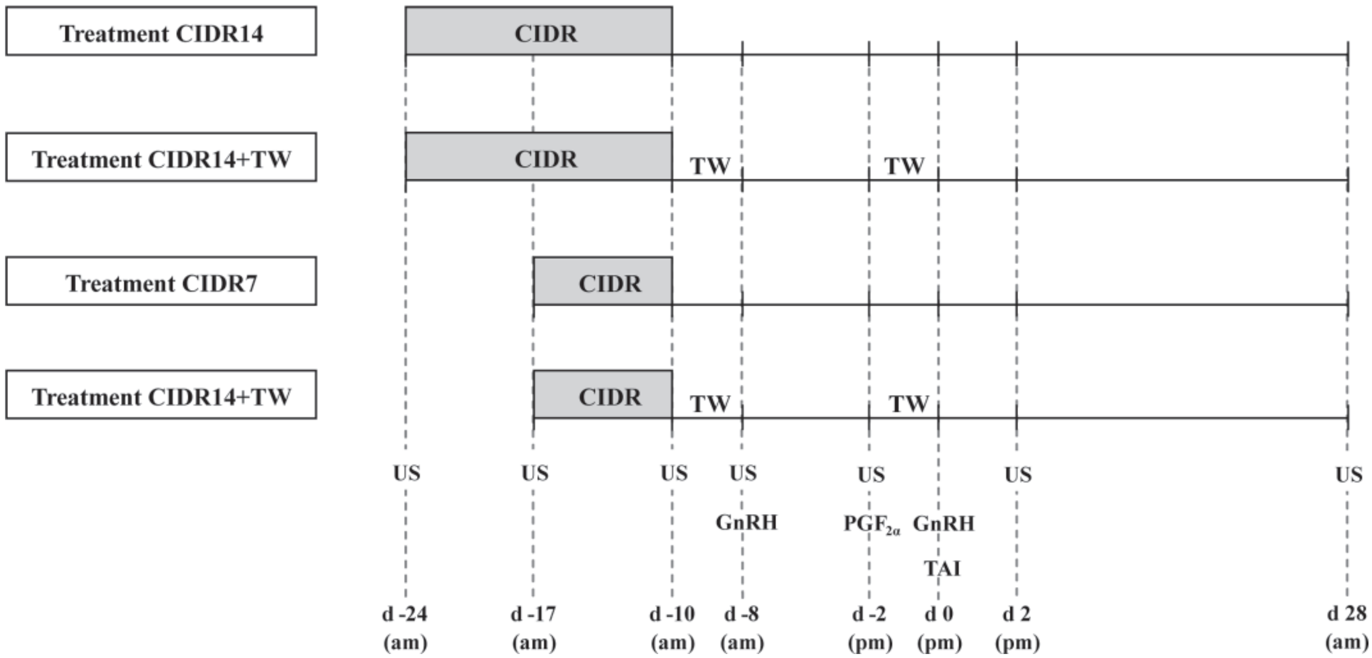

Figure 1. Schematic diagram of the treatments in Exp. 1. CIDR = treatment with an intravaginal insert containing 1.9 g of progesterone, Pfizer Animal Health, São Paulo, Brazil; TW = temporary weaning during 48 h; US = ultrasound examination (ovaries: d −24, d −17, d −10, d −8, d −2, and d 2; pregnancy diagnosis: d 28); GnRH = 50.0-μg intramuscular injection of gonadorelin, Cystorelin, Merial, Paulinia, São Paulo, Brazil; $PGF_{2\alpha}$ = 25.0-mg intramuscular injection of dinoprost tromethamine, Pfizer Animal Health; TAI = fixed-time AI.

at weaning and at 420 d of age were used to evaluate the effect of temporary weaning management on calf performance.

Exp. 2. The goal of this study was to evaluate the effect of treatment with a CIDR insert between GnRH and $PGF_{2\alpha}$ treatments of a modified CO-Synch protocol on pregnancy rate to TAI, and the effects of TAI treatments on pregnancy rates throughout a 90-d breeding season in suckled *Bos indicus* cows. Cows (n = 453; 54 ± 10 d postpartum; 5 ± 3 parturitions; BCS between 3.0 and 4.25) in a ranch at Goias State were randomly assigned to receive 1 of the following treatments (Figure 2): 1) cows were submitted to natural service (1 sire per 50 cows) between d 0 and 90 of breeding season (control; n = 152); 2) cows received a 48-h TW between d −10 and −8 of breeding season, followed by GnRH on d −8, $PGF_{2\alpha}$ on d −2, and TW between d −2 and 0; on d 0 (48 h after $PGF_{2\alpha}$ treatment) cows received GnRH, were inseminated, and reunited with their calves immediately after insemination (**GPG+TW**; n = 150); or 3) cows received the same treatment described for GPG+TW group, with addition of a CIDR insert between first GnRH (d −8) and $PGF_{2\alpha}$ (d −2) treatments (**GPG+TW+CIDR**; n = 151). Cows from GPG+TW and GPG+TW+CIDR groups were artificially inseminated the same day by 2 AI technicians randomly distributed among treatments using semen from a single sire and further exposed to natural service between d 14 and 90 of breeding season, with the same sire:cow ratio used in the control group. Blood samples (10 mL) were collected on d −20 and −10 from coccygeal vein of all cows into Vacutainer tubes (Becton Dickinson Co., Franklin Lakes, NJ) to evaluate cyclicity by progesterone concentration analysis. Blood was allowed to clot at 4°C for 24 h and was centrifuged at 1,500 × *g* for 15 min at room temperature. Serum was removed and frozen at −20°C until assays were performed. Concentrations of progesterone were determined in 6 assays using a solid-phase RIA kit containing antibody-coated tubes and ^{125}I-labeled progesterone (Coat-a-count, Diagnostic Products Corporation, Los Angeles, CA) according to the manufacturer's instructions. The assay sensitivity was 0.01 $ng \cdot mL^{-1}$; the intraassay CV were 13.8, 8.4, 4.7, 8.7, 7.4, and 8.3%; and the interassay CV was 4.6%. A cow was considered anestrous if serum progesterone concentrations were $<1.0\ ng \cdot mL^{-1}$ (Sá Filho et al., 2009) in both collections. Rectal palpation examinations were performed at d 44 in GPG+TW and GPG+TW+CIDR groups to evaluate the pregnancy rate of TAI, and at d 74, 104, and 134 in all cows to evaluate the pregnancy rates at 30, 60, and 90 d of breeding season, respectively.

Statistical Analyses

Both experiments were completely randomized designs. Baseline comparisons for parity, BCS, and days postpartum were previously conducted to compare treatment groups using PROC ANOVA (SAS Institute Inc., Cary, NC), and the distributions of cows by parity, BCS, and days postpartum were similar among treatments in both experiments.

Binomially distributed data were analyzed using PROC LOGISTIC of SAS. Explanatory variables such as treatment, cyclicity, sire (semen), and AI technician were used in the model as classes. Explanatory vari-

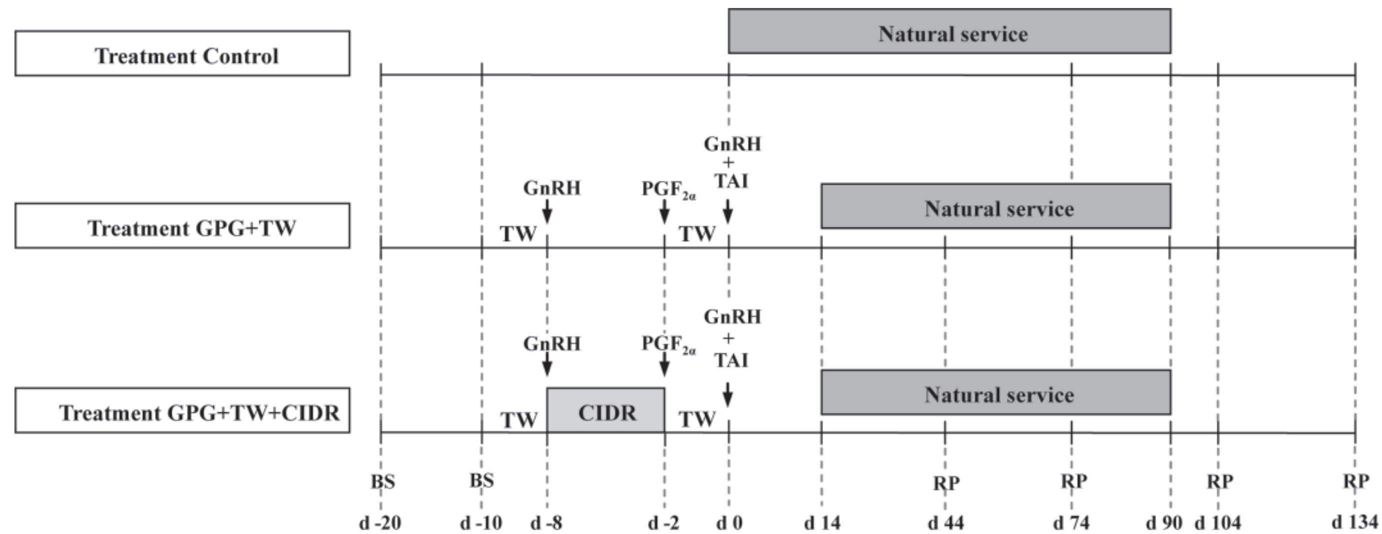

Figure 2. Schematic diagram of the treatments in Exp. 2. TW = temporary weaning during 48 h; GnRH = 50.0-µg intramuscular injection of gonadorelin, Cystorelin, Merial, Paulinia, São Paulo, Brazil; $PGF_{2\alpha}$ = 25.0-mg intramuscular injection of dinoprost tromethamine, Pfizer Animal Health, São Paulo, Brazil; TAI = fixed-time AI; CIDR = treatment with an controlled intravaginal drug release containing 1.9 g of progesterone, Pfizer Animal Health; BS = blood sampling; RP = rectal palpation examination; GPG = cows received an injection of GnRH on d −8 (morning), $PGF_{2\alpha}$ on d −2 (afternoon), and GnRH on d 0 (afternoon).

ables considered covariates, such as days postpartum and BCS, were previously submitted to univariate analysis, and when found to be significant were included in the multivariate models. In the final logistic regression models, variables were removed by a backward elimination (according to the Wald's criterion) when $P > 0.2$. In Exp. 1, for analysis of ovulation rate in response to the first GnRH treatment (**OvGnRH1**), the final model included the effects of length of CIDR treatment (**LCIDR**), TW, cyclicity, and the interactions LCIDR × TW and LCIDR × cyclicity; for analysis of ovulation rate in response to the second GnRH treatment, conception, and pregnancy rate, the final models included the effects of LCIDR, TW, cyclicity, OvGnRH1, and the interactions LCIDR × TW and LCIDR × cyclicity. When interactions were significant, means were compared by orthogonal contrasts (contrast 1: effect of cyclicity in cows treated with CIDR for 7 d; contrast 2: effect of cyclicity in cows treated with CIDR for 14 d; contrast 3: effect of length of CIDR treatment in anestrous cows; contrast 4: effect of length of CIDR treatment in cycling cows). In Exp. 2, a high correlation was found between BCS and cyclicity, which caused multicollinearity, and only cyclicity remained in the model. For analysis of the pregnancy rate of TAI and pregnancy rates at 30, 60, and 90 d of breeding season, the final models included the effects of treatment, cyclicity, and the interaction between treatment and cyclicity. When effects of treatments on a dependent variable were significant, means were compared by orthogonal contrasts [contrast 1: control vs. (GPG+TW) + (GPG+TW+CIDR); contrast 2: GPG+TW vs. GPG+TW+CIDR].

Continuous data (Exp. 1) were analyzed by the PROC GLM of SAS. Explanatory variables such as treatment and cyclicity were used in the model as classes. A previous test for normality of residues (Shapiro-Wilk test) and homogeneity of variances (F-max test) indicated that variables fulfilled the assumptions for ANOVA. For the least squares ANOVA models, all 2-way interactions were tested and variables having $P > 0.2$ were removed from the final models. For analysis of follicular diameter on d −10, the final model included the effects of LCIDR, cyclicity, and the interaction between LCIDR and cyclicity; for the analysis of follicular diameter on d −8 and follicular growth between d −10 and −8, the final models included the effects of LCIDR, TW, cyclicity, and the interactions LCIDR × TW and LCIDR × cyclicity; for analysis of follicular diameter on d −2, the final model included the effects of LCIDR, TW, cyclicity, OvGnRH1, and the interactions LCIDR × TW and LCIDR and cyclicity. When interactions were significant, means were compared with the following orthogonal contrasts: contrast 1: effect of cyclicity in cows treated with CIDR for 7 d; contrast 2: effect of cyclicity in cows treated with CIDR for 14 d; contrast 3: effect of length of CIDR treatment in anestrous cows; contrast 4: effect of length of CIDR treatment in cycling cows. For the analysis of calf performance data (BW at 240 d, BW at 420 d, BW gain from d 0 to 240, BW gain from d 0 to 420, BW gain from d 240 to 420, ADG from d 0 to 240, ADG from d 0 to 420, and ADG from d 240 to 420), the final models included the effects of TW, sex of calf, and the interaction TW × sex of calf. For all experiments and dependent variables, differences were considered significant when $P < 0.05$, whereas tendencies were considered when $0.1 < P \leq 0.05$.

RESULTS

Exp. 1

There was an interaction between cyclicity and length of CIDR treatment on follicular diameter on d −10 and

Table 1. Follicular diameters on d −10, −8, and −2, follicular growth between d −10 and −8, ovulation rates to the first and second GnRH treatments, conception, and pregnancy rate according to length of CIDR (controlled intravaginal drug release, Pfizer Animal Health, São Paulo, Brazil) treatment (7 or 14 d) and cyclicity in postpartum multiparous Nelore cows submitted to a modified CO-Synch protocol (Exp. 1)[1]

	CIDR 7 d		CIDR 14 d		*P*-value		
Item	Anestrous	Cycling	Anestrous	Cycling	CIDR	Cyclicity	CIDR × Cyclicity
Follicular diameter (d −10),[2] mm	11.2 ± 0.2	9.3 ± 0.4	13.0 ± 0.2	12.7 ± 0.5	<0.05	<0.05	<0.05*
Follicular diameter (d −8),[3] mm	12.4 ± 0.2	10.9 ± 0.4	13.3 ± 0.2	12.2 ± 0.6	<0.05	<0.05	>0.1
Follicular growth (d −10 to −8),[4] mm	1.2 ± 0.2	1.6 ± 0.3	0.3 ± 0.2	−0.5 ± 0.4	<0.05	<0.05	<0.05**
Ovulation rate (1st GnRH),[5] %	94.7 (107/113)	70.0 (21/30)	93.3 (112/120)	90.0 (18/20)	>0.1	<0.05	>0.1
Follicular diameter (d −2),[6] mm	10.7 ± 0.2	10.7 ± 0.2	10.9 ± 0.2	11.1 ± 0.3	>0.1	>0.1	>0.1
Ovulation rate (2nd GnRH),[7] %	95.6 (108/113)	93.3 (28/30)	95.0 (114/120)	95.0 (19/20)	>0.1	>0.1	>0.1
Conception rate,[8] %	53.7 (58/108)	46.4 (13/28)	45.6 (52/114)	47.4 (9/19)	>0.1	>0.1	>0.1
Pregnancy rate,[9] %	51.3 (58/113)	43.3 (13/30)	43.3 (52/120)	45.0 (9/20)	>0.1	>0.1	>0.1

[1]Cows were diagnosed as anestrous or cyclic before the treatment period by 2 ultrasound examinations 7 d apart. Cows were treated with a CIDR for 7 d (d –17 to −10) or for 14 d (d −24 to −10) in a modified CO-Synch protocol (GnRH on d −8; $PGF_{2\alpha}$ on d −2; GnRH on d 0).

[2]Mean diameter (mm) of the largest follicle on d −10.

[3]Mean diameter (mm) of the largest follicle on d −8.

[4]Average (mm) of diameter of the largest follicle on d −10 subtracted from diameter of the largest follicle on d −8.

[5]Percentage of cows ovulating after 1st GnRH compared with all cows treated.

[6]Mean diameter (mm) of the largest follicle on d −2.

[7]Percentage of cows ovulating after 2nd GnRH compared with all cows treated.

[8]Percentage of cows pregnant to fixed-time AI (TAI) compared with cows that ovulated.

[9]Percentage of cows pregnant to TAI compared with all cows treated.

*Contrast 1 (effect of cyclicity in cows treated with CIDR for 7 d): $P < 0.05$; contrast 2 (effect of cyclicity in cows treated with CIDR for 14 d): $P > 0.1$; contrast 3 (effect of length of CIDR treatment in anestrous cows): $P < 0.05$; contrast 4 (effect of length of CIDR treatment in cycling cows): $P < 0.05$.

**Contrast 1: $P > 0.1$; contrast 2: $P < 0.05$; contrast 3: $P < 0.05$; contrast 4: $P < 0.05$.

on follicular growth between d −10 and −8 ($P < 0.05$; Table 1). Follicle diameter on d −8 was affected by cyclicity (anestrous: 12.87 ± 0.14 mm; cycling: 11.50 ± 0.34 mm; $P < 0.05$; Table 1) and length of CIDR treatment (7 d: 11.70 ± 0.21 mm; 14 d: 12.68 ± 0.24 mm; $P < 0.05$; Tables 1 and 2), but not by TW ($P > 0.1$; Table 2). Follicular diameter on d −2 was not affected (10.89 ± 0.14 mm; $P > 0.1$) by any of the analyzed explanatory variables.

Ovulation in response to the first GnRH treatment was affected by cyclicity [anestrous: 94.0% (219/233); cycling: 78.0% (39/50); $P < 0.05$; Table 1] and TW [with TW: 94.4 (134/142); without TW: 87.9% (124/141); $P < 0.05$; Table 2]. Corpus luteum regression rate of cows that ovulated to the first GnRH was 93.8% (242/258). Ovulation to the first GnRH treatment affected ($P < 0.05$) the ovulation rate to the second GnRH treatment [98.1% (253/258) vs. 64.0% (16/25)] and tended ($P < 0.1$) to affect pregnancy rate [48.1% (124/258) vs. 32.0% (8/25)] for cows that ovulated and did not ovulate to the first GnRH treatment, respectively. Follicular diameter on d −2 and conception rate were not affected by any of the analyzed explanatory variables [10.81 ± 0.18 mm and 49.1% (132/269), respectively; $P > 0.1$]. Pregnancy rate was not affected by treatments or cyclicity ($P > 0.1$; Tables 1 and 2).

Body weights (240 and 420 d), BW gains (0 to 240 d, 0 to 420 d, and 240 to 420 d), and ADG (0 to 240 d, 0 to 420 d, and 240 to 420 d) were affected by sex of the calf ($P < 0.05$; Table 3). Temporary weaning affected BW at 240 d, BW gains (0 to 240 d and 240 to 420 d), and ADG (0 to 240 d and 240 to 420 d; $P < 0.05$; Table 3).

Exp. 2

Pregnancy rate to TAI was affected by cyclicity (Table 3; $P < 0.05$), but no effects of treatment (Table 4; $P > 0.1$) and treatment × cyclicity were detected (GPG+TW/anestrous: 39.5%; GPG+TW/cycling: 19.5%; GPG+TW+CIDR/anestrous: 41.6%; GPG+TW+CIDR/cycling: 24.6%; $P > 0.1$). Pregnancy rates at 30, 60, and 90 d of breeding season were affected by treatment and cyclicity (Table 4; $P < 0.05$).

DISCUSSION

In this series of studies, presynchronization with a CIDR device for 7 or 14 d in postpartum Nelore cows afforded rates of ovulation to the first and second GnRH treatments with synchronization of ovulation greater than previous reports in beef cows. In addition, presynchronization with a CIDR device for 7 or 14 d provided similar synchronized ovulation rates to the second GnRH injection in anestrous (95.3%) and cycling (94.0%) Nelore cows. Also, the inclusion of a CIDR between the first GnRH and $PGF_{2\alpha}$ treatments did not improve pregnancy rates in postpartum Nelore cows submitted to a modified CO-Synch protocol.

Table 2. Follicular diameters on d −10, −8, and −2; follicular growth between d −10 and −8; ovulation rates to the first and second GnRH (Cystorelin, Merial, Paulinia, São Paulo, Brazil) treatments, conception, and pregnancy rate according to length of CIDR (7 or 14 d, controlled intravaginal drug release, Pfizer Animal Health, São Paulo, Brazil); and temporary weaning (TW) treatments in postpartum multiparous Nelore cows submitted to a modified CO-Synch protocol (Exp. 1)[1]

	CIDR 7 d		CIDR 14 d		*P*-value		
Item	With TW	Without TW	With TW	Without TW	CIDR	TW	CIDR × TW
Follicular diameter (d −10),[2] mm	10.84 ± 0.24	10.75 ± 0.24	12.9 ± 0.2	13.0 ± 0.2	<0.05	>0.1	>0.1
Follicular diameter (d −8),[3] mm	12.22 ± 0.27	11.96 ± 0.27	13.2 ± 0.3	13.2 ± 0.3	<0.05	>0.1	>0.1
Follicular growth (d −10 to −8),[4] mm	1.38 ± 0.21	1.22 ± 0.21	0.3 ± 0.2	0.3 ± 0.2	<0.05	>0.1	>0.1
Ovulation rate (1st GnRH),[5] %	94.4 (68/72)	84.5 (60/71)	94.3 (66/70)	91.4 (64/70)	>0.1	<0.05	>0.1
Follicular diameter (d −2),[6] mm	10.8 ± 0.2	10.6 ± 0.2	10.8 ± 0.2	11.3 ± 0.2	>0.1	>0.1	>0.1
Ovulation rate (2nd GnRH),[7] %	95.8 (69/72)	94.4 (67/71)	97.1 (68/70)	92.9 (65/70)	>0.1	>0.1	>0.1
Conception rate,[8] %	55.1 (38/69)	49.2 (33/67)	47.1 (32/68)	44.6 (29/65)	>0.1	>0.1	>0.1
Pregnancy rate,[9] %	52.8 (38/72)	46.5 (33/71)	45.7 (32/70)	41.4 (29/70)	>0.1	>0.1	>0.1

[1]Cows were treated with a CIDR for 7 d (d −17 to −10) ± 2 TW (d −10 to −8, and d −2 to 0), or for 14 d (d −24 to −10) ± 2 TW in a modified CO-Synch protocol (GnRH on d −8; $PGF_{2\alpha}$ on d −2; GnRH on d 0).
[2]Mean diameter (mm) of the largest follicle on d −10.
[3]Mean diameter (mm) of the largest follicle on d −8.
[4]Average (mm) of diameter of the largest follicle on d −10 subtracted from diameter of the largest follicle on d −8.
[5]Percentage of cows ovulating after 1st GnRH compared with all cows treated.
[6]Mean diameter (mm) of the largest follicle on d −2.
[7]Percentage of cows ovulating after 2nd GnRH compared with all cows treated.
[8]Percentage of cows pregnant to fixed-time AI (TAI) compared with cows that ovulated.
[9]Percentage of cows pregnant to TAI compared with all cows treated.

Protocols for TAI involving treatments with GnRH and $PGF_{2\alpha}$ have been extensively studied in *Bos taurus* cows (Thompson et al., 1999; Geary et al., 2001; Lamb et al., 2001; Larson et al., 2006), but few studies have been performed in *Bos indicus* cattle (Williams et al., 2002; Hiers et al., 2003; Saldarriaga et al., 2007; Vasconcelos et al., 2009b). Synchronization of follicular waves is critical for the success of ovulation synchronization programs that utilize TAI (Saldarriaga et al., 2007). In Exp. 1, we used progesterone treatment and TW as strategies to stimulate follicular development before the first GnRH treatment, based on a previous study (Stock and Fortune, 1993) in which cows treated with a CIDR insert had prolonged growth of the dominant follicle. Results from Exp. 1 indicated that CIDR14 created a larger ovulatory follicle than CIDR7 treatment, but no greater ovulatory response at the first GnRH treatment. Furthermore, in cows from CIDR7 treatment, follicular diameter on d −10 was greater in anestrous than in cycling animals, but no effects of cyclicity were observed in cows submitted to treatment with CIDR for 14 d. This interaction between length of CIDR treatment and cyclicity may have been due to an inhibition of LH pulses after the initial rise in circulating progesterone concentration after CIDR insertion, causing follicular turnover in some cycling cows having a dominant follicle at the time of CIDR insertion. Also, the combination between endogenous (CL) and exogenous (CIDR) progesterone during the protocol may have a negative impact on LH secretion, reducing the development of the dominant follicle (Stock and Fortune, 1993; Dias et al., 2009). The lack of a negative

Table 3. Body weights (240 and 420 d), BW gains (0 to 240 d, 0 to 420 d, and 240 to 420 d), and ADG (0 to 240 d, 0 to 420 d, and 240 to 420 d) of calves submitted or not to two 48-h temporary weanings (TW) at 39 to 68 d old (Exp. 1)

	Sex of calf			TW[1]		
Dependent variable	Male	Female	*P*-value	Yes	No	*P*-value
BW at 240 d, kg	199.53 ± 1.58	183.27 ± 1.59	<0.05	187.85 ± 2.68	195.46 ± 2.69	<0.05
BW gain 0 to 240 d, kg	169.17 ± 1.58	152.91 ± 1.59	<0.05	157.51 ± 2.68	165.15 ± 2.69	<0.05
ADG 0 to 240 d, $kg \cdot d^{-1}$	0.75 ± 0.01	0.67 ± 0.01	<0.05	0.70 ± 0.01	0.73 ± 0.01	<0.05
BW at 420 d, kg	254.43 ± 3.12	225.31 ± 3.03	<0.05	239.26 ± 5.09	240.98 ± 5.12	>0.1
BW gain 0 to 420 d, kg	224.12 ± 3.12	195.46 ± 3.04	<0.05	208.94 ± 5.09	210.71 ± 5.12	>0.1
ADG 0 to 420 d, $kg \cdot d^{-1}$	0.53 ± 0.02	0.47 ± 0.01	<0.05	0.50 ± 0.09	0.50 ± 0.08	>0.1
BW gain 240 to 420 d, kg	54.90 ± 2.98	42.04 ± 2.71	<0.05	51.41 ± 2.37	45.52 ± 2.32	<0.05
ADG 240 to 420 d, $kg \cdot d^{-1}$	0.31 ± 0.02	0.23 ± 0.01	<0.05	0.29 ± 0.02	0.25 ± 0.01	<0.05

[1]Calves were submitted or not to two 48-h TW 6 d apart. At first TW, calves were 39 to 60 d old, and were 47 to 68 d old at the second TW.

Table 4. Pregnancy rates at fixed-time AI (TAI), 30, 60, and 90 d of breeding season in postpartum multiparous Nelore cows submitted or not to a modified CO-Synch protocol (Exp. 2)[1]

	Treatment				Cyclicity		
Dependent variable	Control	GPG+TW	GPG+TW+CIDR	*P*-value	Anestrous	Cycling	*P*-value
Pregnancy rate to TAI,[2] %	—	32.7 (49/150)	28.5 (43/151)	>0.1	40.7 (57/140)	21.7 (35/161)	<0.05
Pregnancy rate (30 d of BS),[3] %	11.2 (17/152)	50.0 (75/150)	59.6 (90/151)	<0.05*	36.5 (81/222)	43.7 (101/231)	>0.1
Pregnancy rate (60 d of BS),[4] %	38.8 (59/152)	66.7 (100/150)	77.5 (117/151)	<0.05*	54.9 (122/222)	67.5 (156/231)	<0.05
Pregnancy rate (90 d of BS),[5] %	57.9 (88/152)	78.7 (118/150)	85.4 (129/151)	<0.05*	73.0 (162/222)	74.9 (173/231)	>0.1

[1]Cows were diagnosed as anestrous or cyclic before the treatment period by 2 evaluations of serum progesterone concentrations 10 d apart. Cows received 1 of the following treatments: control = natural service between d 0 and 90 of breeding season; GPG+TW = cows received a modified CO-Synch protocol [GnRH (Cystorelin, Merial, Paulinia, São Paulo, Brazil) on d −8; $PGF_{2\alpha}$ on d − 2; GnRH on d 0] associated to 2 TW (d −10 to −8, and d −2 to 0); or GPG+TW+CIDR (controlled intravaginal drug release, Pfizer Animal Health, São Paulo, Brazil) = cows received the same protocol described for GPG+TW, associated to a 6-d CIDR treatment (d −8 to −2).

[2]Percentage of cows pregnant to TAI compared with all cows treated.

[3]Percentage of cows pregnant at 30 d of breeding season (BS) compared with all cows treated.

[4]Percentage of cows pregnant at 60 d of BS compared with all cows treated.

[5]Percentage of cows pregnant at 90 d of BS compared with all cows treated.

*Contrast 1 [control vs. (GPG+TW) + (GPG+TW+CIDR)]: $P < 0.05$; contrast 2 (GPG+TW vs. GPG+TW+CIDR): $P > 0.1$.

effect of cyclicity on follicular diameter on d −10 in cows treated with CIDR for 14 d may have been due to the fact that more cycling cows in the 14-d CIDR treatment group would have experienced spontaneous luteolysis and had older follicles present on their ovary at d −10. Interestingly, the ovulation rate to the first GnRH was found to be reduced (70%) in cycling cows treated with CIDR for 7 d. It is possible that in this category, insertion of the CIDR for presynchronization caused turnover of the dominant follicle (Stock and Fortune, 1993) and decreased subsequent LH pulse frequency in a manner that reduced the rate of growth of the newly recruited follicle after deviation. Alternatively, comparing the ovulation rate of cyclic cows receiving CIDR14 with CIDR7, it is likely that more CIDR14 cows underwent luteolysis earlier on their own and the progesterone from the CIDR itself allowed continued growth of the last dominant follicle but prevented its turnover. When a single treatment with GnRH is performed, a decreased ovulation rate is expected in anestrous than in cycling cows. Our results indicated that presynchronizing with progesterone before the first GnRH injection was an effective strategy to improve ovulation rate because anestrous cows ovulated in a greater proportion than cycling cows (94 vs. 78%).

Several studies have indicated that the addition of temporary weaning to synchronization protocols improved fertility (Geary et al., 2001; Vasconcelos et al., 2009a,b) due to an increase in LH pulse frequency (Mackey et al., 2000). In our knowledge, this is the first study evaluating the effects of temporary weaning in cows presynchronized with progesterone. In Exp. 1, in which cows were presynchronized with a CIDR, TW did not directly affect follicular development before the onset of TAI protocol, but improved the ovulation rate to the first GnRH treatment and thus may have indirectly affected follicular development of the follicle that ovulated to the second GnRH treatment. It is possible that cows submitted to TW had a greater induced-LH peak after GnRH treatment, as observed by Smith et al. (1983). The positive effect of TW on ovulation rate to the first GnRH treatment in CIDR7 cows was probably because their follicles were in the growth phase of development, whereas follicles in CIDR14 cows were more likely to be at the plateau phase. Thus, presynchronization with CIDR for 14 d may be used as an alternative to TW. The lack of effect of TW on ovulation rate to the second GnRH is in contrast with Vasconcelos et al. (2009b), and this may be attributed to the increased rates of ovulation to the first GnRH and, thereby, to follicular wave synchronization. Because there was an increased ovulation rate to the first GnRH treatment in Exp. 1 and the interval between GnRH and $PGF_{2\alpha}$ treatments was 6.5 d, incomplete luteolysis could be a factor that could negatively affect the efficiency of the protocol. However, the rate of CL regression was 93.8%, in agreement with Saldarriaga et al. (2007), who reported a 93% rate of luteal regression in cows treated with $PGF_{2\alpha}$ 7 d after GnRH. These factors together contributed, in Exp. 1, to the achievement of synchronization rates greater than has been reported in beef and dairy cows (Vasconcelos et al., 1999, 2001, 2009b; Moreira et al., 2001). The decision of whether to use long-term treatment with CIDR or use short-term treatment with CIDR plus TW should consider the cost of inserts and the effects of TW on calf performance. In Exp. 1, TW reduced calf performance during the suckling period but did not affect it at 420 d. Producers who maintain ownership of calves to advanced ages could expect compensatory growth in TW calves as observed in Exp. 1.

In Exp. 2, we hypothesized that postpartum Nelore cows receiving a CIDR between GnRH and $PGF_{2\alpha}$ treatments would have improved pregnancy rates to TAI based on previous studies in *Bos taurus* cows indicating that exogenous treatment with progesterone associated with GnRH-based synchronization protocols improved fertility in anestrous females (Thompson et al., 1999; Lamb et al., 2001; Stevenson et al., 2003; Larson et al., 2006). This positive effect of progesterone in anestrous cows may be attributed to exposure to circulating levels of progesterone that potentially improved follicular development and the likelihood of ovulation to the second GnRH treatment or to prevention of premature luteolysis in cows that did not ovulate to the first GnRH treatment (Anderson et al., 1996; Imwalle et al., 1998; Sá Filho et al., 2009), or both. Although Lamb et al. (2001) reported no beneficial effect of CIDR treatment on pregnancy rates to TAI in cyclic cows with circulating concentrations of progesterone >1.0 ng·mL^{-1} at $PGF_{2\alpha}$, a positive effect of CIDR treatment was realized among cows in which the protocol was initiated at late stages of their estrous cycle and was likely due to prevention of premature ovulation. In Exp. 2, the percentage of cycling cows was 51%, but no effect of CIDR treatment on pregnancy rate at TAI was detected, and anestrous cows had greater pregnancy rate at TAI than cycling cows regardless of CIDR treatment (40.7 vs. 21.7%). In anestrous Nelore cows synchronized with GnRH (d 0), $PGF_{2\alpha}$ (d 7), and estradiol benzoate (d 8), TW beginning 48 h before GnRH treatment increased the follicular diameter and ovulation rate, whereas in cycling cows these effects were not observed (Vasconcelos et al., 2009b). Because in Exp. 2 all cows were exposed to TW, it is likely that the ovulation rate to the first GnRH treatment was improved in anestrous but not in cycling cows in a manner that affected pregnancy rates. Body condition score could not be maintained in the final models because BCS was confounding with cyclicity. However, this high correlation between BCS and cyclicity indicates that BCS can be used as a practical tool to predict the percentage of cycling cows and results to synchronization protocols.

A lack of effect of CIDR in *Bos indicus*-influenced cows submitted to a GnRH-based protocol had been previously observed (Saldarriaga et al., 2007). It is possible that cows treated with CIDR had a reduced ovulation rate to the first GnRH, but this response was not evaluated in the current experiment. Although stores of LH are completely restored to normal concentrations in the anterior pituitary gland by 15 to 20 d postpartum (Lamming et al., 1981), Saldarriaga et al. (2007) observed a greater release of LH after a GnRH treatment in anestrous than in cycling cows and Williams et al. (1982) reported a greater magnitude of LH release after GnRH treatment in suckled than in nonsuckled cows. Also, Stevenson et al. (2000) reported rates of induced ovulation between 38 and 49% when cows were treated only with GnRH, whereas ovulation rates of cows receiving a norgestomet implant at the time of GnRH treatment were between 17 and 28%. Thus, the reduced fertility observed in cycling cows in Exp. 2 might have been from decreased ovulation rates to the first GnRH treatment (negative effect of progesterone from CL + CIDR on first GnRH-induced LH peak, a lack of TW effect, or both), and the ovulation rate to the second GnRH due to negative effect of progesterone (from original CL + CL from ovulation to the first GnRH + CIDR) on follicular development during the protocol (Dias et al., 2009). However, regardless of treatment, TAI protocol reduced the mean interval between calving and conception and improved pregnancy rates throughout the breeding season. Although data from Exp. 1 and 2 cannot be compared, the use of progesterone to presynchronize postpartum *Bos indicus* beef cows appears to result in better results than the use of progesterone between GnRH and $PGF_{2\alpha}$ treatments of the CO-Synch protocol.

In conclusion, in postpartum Nelore cows presynchronized with CIDR, temporary weaning improved ovulation rate to the first GnRH treatment of a modified CO-Synch protocol. Because pregnancy rates at TAI were not affected by length of CIDR treatment (7 vs. 14 d), treatment CIDR7+TW, which is less expensive and shorter in duration, appears to be the best choice to achieve a pregnancy rate benchmark of 50%. Calves submitted to two 48-h TW 6 d apart had decreased average BW at 240 d, but those treatments did not affect BW at 420 d. The inclusion of a CIDR between the first GnRH and $PGF_{2\alpha}$ treatments of a modified CO-Synch protocol with 2 TW did not improve TAI pregnancy rate.

LITERATURE CITED

Anderson, L. H., C. M. McDowell, and M. L. Day. 1996. Progestin induced puberty and secretion of luteinizing hormone in heifers. Biol. Reprod. 54:1025–1031.

Baruselli, P. S., M. O. Marques, N. A. T. Carvalho, E. H. Madureira, and E. P. Campos Filho. 2002. Efeito de diferentes protocolos de inseminação artificial em tempo fixo na eficiência reprodutiva de vacas de corte lactantes. Rev. Bras. Reprod. Anim. 26:218–221.

Chebel, R. C., J. E. P. Santos, R. L. A. Cerri, H. M. Rutigliano, and R. G. S. Bruno. 2006. Reproduction in dairy cows following progesterone insert presynchronization and resynchronization protocols. J. Dairy Sci. 89:4205–4219.

Dias, C. C., F. S. Wechsler, M. L. Day, and J. L. M. Vasconcelos. 2009. Influence of exogenous progesterone treatment, timing of prostaglandin F2α administration and eCG treatment on fertility in Nelore cycling heifers. Theriogenology In press.

FASS. 1999. Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. 1st rev. ed. Fed. Anim. Sci. Soc., Savoy, IL.

Fernandes, P., A. B. Teixeria, A. J. Crocci, and C. M. Barros. 2001. Timed artificial insemination in beef cattle using GnRH agonist, PGF2alpha and estradiol benzoate. Theriogenology 55:1521–1532.

Geary, T. W., J. C. Whittier, D. M. Hallford, and M. D. MacNeil. 2001. Calf removal improves conception rates to the Ovsynch and CO-Synch protocols. J. Anim. Sci. 79:1–4.

Hiers, E. A., C. R. Barthle, M. K. Dahms, G. E. Portillo, G. A. Bridges, D. O. Rae, W. W. Thatcher, and J. V. Yelich. 2003. Synchronization of *Bos indicus* × *Bos taurus* cows for timed artificial insemination using gonadotropin-releasing hormone plus prostaglandin F2α in combination with melengestrol acetate. J. Anim. Sci. 81:830–835.

Houghton, P. L., R. P. Lemenager, L. A. Horstman, K. S. Hendrix, and G. E. Moss. 1990. Effects of body composition, pre- and postpartum energy level and early weaning on reproductive performance of beef cows and preweaning calf gain. J. Anim. Sci. 68:1438–1446.

Imwalle, D. B., D. J. Patterson, and K. K. Schillo. 1998. Effects of melengestrol acetate on onset of puberty, follicular growth, and patterns of luteinizing hormone secretion in beef heifers. Biol. Reprod. 58:1432–1436.

Lamb, C. G., J. S. Stevenson, D. J. Kesler, H. A. Garverick, D. R. Brown, and B. E. Salfen. 2001. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin $F_{2\alpha}$ for ovulation control in postpartum suckled beef cows. J. Anim. Sci. 79:2253–2259.

Lamming, G. E., D. C. Wathes, and A. R. Peters. 1981. Endocrine patterns of the postpartum cow. J. Reprod. Fertil. 30:155–170.

Larson, J. E., G. C. Lamb, J. S. Stevenson, S. K. Johnson, M. L. Day, T. W. Geary, D. J. Kesler, J. M. DeJarnette, F. N. Schrick, A. DiCostanzo, and J. D. Arseneau. 2006. Synchronization of estrus in suckled beef cows for detected estrus and artificial insemination and timed artificial insemination using gonadotropin-releasing hormone, prostaglandin $F_{2\alpha}$ and progesterone. J. Anim. Sci. 84:332–342.

Mackey, D. R., J. M. Sreenan, J. F. Roche, and M. G. Diskin. 2000. The effect of progesterone alone or in combination with estradiol on follicular dynamics, gonadotropin profiles, and estrus in beef cows following calf isolation and restricted suckling. J. Anim. Sci. 78:1917–1929.

Meneghetti, M., O. G. Sá Filho, R. F. G. Peres, G. C. Lamb, and J. L. M. Vasconcelos. 2009. Fixed-time artificial insemination with estradiol and progesterone for *Bos indicus* cattle: I. Basis for development of protocols. Theriogenology 72:179–189.

Meneghetti, M., and J. L. M. Vasconcelos. 2008. Calving date, body condition score, and response to a timed artificial insemination protocol in first-calving beef cows. Braz. J. Vet. Anim. Sci. 60:786–793.

Moreira, F., C. Orlandi, C. A. Risco, R. Mattos, F. Lopes, and W. W. Thatcher. 2001. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. J. Dairy Sci. 84:1646–1659.

Pinheiro, O. L., C. M. Barros, R. A. Figueiredo, E. R. Valle, R. O. Encarnação, and C. R. Padovani. 1998. Estrus behavior and the estrus to ovulation interval in Nelore cattle (*Bos indicus*) with natural estrus or estrus induced with prostaglandin F2alpha or norgestomet and estradiol valerate. Theriogenology 49:667–681.

Sá Filho, O. G., W. W. Thatcher, and J. L. M. Vasconcelos. 2009. Effect of progesterone and/or estradiol treatments prior to induction of ovulation on subsequent luteal lifespan in anestrous Nelore cows. Anim. Reprod. Sci. 112:95–106.

Saldarriaga, J. P., D. A. Cooper, J. A. Cartmill, J. F. Zuluaga, R. L. Stanko, and G. L. Williams. 2007. Ovarian, hormonal, and reproductive events associated with synchronization of ovulation and timed appointment breeding of *Bos indicus*-influenced cattle using intravaginal progesterone, gonadotropin-releasing hormone, and prostaglandin $F_{2\alpha}$. J. Anim. Sci. 85:151–162.

Smith, M. F., A. W. Lishman, G. S. Lewis, P. G. Harms, M. R. Ellersieck, E. K. Inskeep, J. N. Wiltbank, and M. S. Amoss. 1983. Pituitary and ovarian responses to gonadotropin releasing hormone, calf removal and progestogen in anestrous beef cows. J. Anim. Sci. 57:418–424.

Stevenson, J. S., G. C. Lamb, S. K. Johnson, M. A. Medina-Britos, D. M. Grieger, K. R. Harmoney, J. A. Cartmill, S. Z. El-Zarkouny, C. R. Dahlen, and T. J. Marple. 2003. Supplemental norgestomet, progesterone, or melengestrol acetate increases pregnancy rates in suckled beef cows after timed inseminations. J. Anim. Sci. 81:571–586.

Stevenson, J. S., K. E. Thompson, W. L. Forbes, G. C. Lamb, D. M. Grieger, and L. R. Corah. 2000. Synchronizing estrus and(or) ovulation in beef cows after combinations of GnRH, norgestomet, and prostaglandin F2alpha with or without timed insemination. J. Anim. Sci. 78:1747–1758.

Stock, A. E., and J. E. Fortune. 1993. Ovarian follicular dominance in cattle: Relationship between prolonged growth of the ovulatory follicle and endocrine parameters. Endocrinology 132:1108–1114.

Thompson, K. E., J. S. Stevenson, G. C. Lamb, D. M. Grieger, and C. A. Loest. 1999. Follicular, hormonal, and pregnancy responses of early postpartum suckled beef cows to GnRH, Norgestomet, and Prostaglandin F2α. J. Anim. Sci. 77:1823–1832.

Vasconcelos, J. L. M., T. P. B. Araújo, R. L. A. Cerri, R. L. Valarelli, and F. S. Wechsler. 2000. Ovulation and synchronization rates in Holstein and crossbred lactating dairy cows during two seasons when receiving the PGF2α injection on d 6 or 7 of the Ovsynch protocol. J. Dairy Sci. 83(Suppl. 1):214. (Abstr.)

Vasconcelos, J. L. M., O. G. Sá Filho, G. C. Perez, and A. T. N. Silva. 2009a. Intravaginal progesterone device and/or temporary weaning on reproductive performance of anestrous crossbred Angus × Nelore cows. Anim. Reprod. Sci. 111:302–311.

Vasconcelos, J. L. M., R. Sartori, H. N. Oliveira, J. G. Guenther, and M. C. Wiltbank. 2001. Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. Theriogenology 56:304–314.

Vasconcelos, J. L. M., R. W. Silcox, G. J. M. Rosa, J. R. Pursley, and M. C. Wiltbank. 1999. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology 52:1067–1078.

Vasconcelos, J. L. M., E. R. Vilela, and O. G. Sá Filho. 2009b. Temporary weaning at two different times of the GnRH-PGF2α-EB synchronization of ovulation protocol in post partum Nelore cows. Braz. J. Vet. Anim. Sci. 61:95–103.

Williams, G. L., J. Kotwica, W. D. Slanger, D. K. Olson, J. E. Tilton, and L. J. Johnson. 1982. Effect of suckling on pituitary responsiveness to gonadotropin-releasing hormone throughout the early postpartum period of beef cows. J. Anim. Sci. 54:594–602.

Williams, S. W., R. L. Stanko, M. Amstalden, and G. L. Williams. 2002. Comparison of three approaches for controlling estrus and ovulation for timed artificial insemination in Brahman-influenced cattle. J. Anim. Sci. 80:1173–1178.