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Follicular growth and plasma progesterone patterns in *Bos indicus x Bos taurus* heifers submitted to different PGF2α/progesterone-based synchronization protocols

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Abstract

The effect of different levels of progesterone (P4) concentrations on follicle growth and ovulatory capacity was evaluated in 40 crossbred *Bos indicus* x *Bos taurus* cyclic heifers submitted to distinct PGF2α + progesterone-based protocols. Heifers in CIDR_PGF8 group (n = 10) received 2.0 mg i.m. estradiol benzoate (EB) and a new controlled internal drug release containing 1.9 g of progesterone (CIDR) on day 0 of study. At the time of CIDR withdrawal (day 8), heifers received an i.m. injection of PGF2α and 24 h later a second EB i.m. injection (0.5 mg). The three other groups received EB injections and CIDR insertion/withdrawal as aforementioned, except that an i.m. injection of PGF2α was administered on day 5. In addition, heifers in the CIDR_PGF5 group (n = 10) received a new CIDR, while heifers in the CIDR1x_PGF5 (n = 9) and CIDR2x_PGF5 (n = 11) groups received a previously used CIDR for 8 and 14 days, respectively. Heifers that received a PGF2α injection on day 5 showed lower circulating P4 than heifers treated on day 8 (CIDR_PGF5 = 1.98 ± 0.21 ng/ml; CIDR1x_PGF5 = 1.69 ± 0.17 ng/ml and CIDR2x_PGF5 = 1.33 ± 0.08 ng/ml versus CIDR_PGF8 = 3.31 ± 0.45 ng/ml). The dominant follicle (DF) growth rate was slower in those heifers receiving PGF2α injection on day 8 (CIDR_PGF8 = 0.72 ± 0.13 mm/day) than groups treated on day 5 (CIDR_PGF5 = 0.96 ± 0.12 mm/day; CIDR1x_PGF5 = 1.06 ± 0.15 mm/day and CIDR2x_PGF5 = 1.01 ± 0.06 mm/day). In consequence, preovulatory follicle diameter on day 10 was smaller in those animals injected on day 8 (CIDR_PGF8 = 8.81 ± 6.7 mm) than in those treated on day 5 (CIDR_PGF5 = 10.00 ± 0.58 mm CIDR1x_PGF5 = 10.5 ± 0.69 mm and CIDR2x_PGF5 = 10.5 ± 0.35 mm). For heifers receiving PGF2α injection on day 5, no significant differences on plasma P4 concentrations, follicular growth rate and DF diameters were observed among heifers that received new or previously used CIDR inserts. These results suggest that the presence of corpus luteum during synchronization protocols is the main factor responsible for the increase in the plasma P4 concentrations and inhibition of DF growth.

Keywords: follicle, ovulation, progesterone, prostaglandin.

Introduction

Artificial insemination (AI) and embryo transfer (ET) programs have been employed worldwide in cattle, however, estrous detection remains one of the main limiting factors to implement such technologies (Baruselli *et al*., 2004). Based on the in-depth knowledge of cows' ovarian and hormonal activities, protocols for timed artificial insemination (TAI) and timed embryo transfer (TET) have been developed over the past years to overcome the challenges associated with estrous detection, representing a feasible tool to improve results of these programs (Peters and Pursley, 2003; Baruselli *et al*., 2004; Dias *et al*., 2009).

High circulating P4 concentrations have been associated with decreased growth of the dominant follicle (Stock and Fortune, 1993; Bergfeld *et al*., 1995) as high concentrations of this hormone lead to the decrease of LH pulse frequency and, therefore, suppresses dominant follicle (DF) growth in cattle (Kinder *et al*., 1996). Thus, lower circulating progesterone concentrations during progesterone-based protocols associated with estrogen at the beginning of treatment might increase follicular growth during the synchronization period, with further improvements in both ovulation and conception rates.

In this regard, ovulatory follicles of smaller diameters have been related to ovulation failures and delayed ovulation due to the presence of immature follicles that are unresponsive to the LH surge. Consequently, attempts have been made to improve ovulation and pregnancy rate by increasing the size of the ovulatory follicle in TAI (Vasconcelos *et al*., 2001) and TET programs (Baruselli *et al*., 2001; Mantovani *et al*., 2005). Moreover, increasing preovulatory follicle size is a strategy to increase the size of subsequent corpus luteum (CL) with higher progesterone-secreting capacity (Pfeifer *et al*., 2009) to maintain the early pregnancy (Binelli *et al*., 2001).

Based on the aforementioned facts, this experiment was designed to evaluate the effect of different levels of P4 exposure on follicle growth and ovulatory capacity in *Bos indicus* x *Bos taurus* crossbred heifers submitted to PGF2α/progesterone-based protocols.

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Materials and Methods

Location, animals and feeding

The experiment was conducted on a commercial beef farm located in Southeastern Brazil. Forty crossbred *Bos indicus* x *Bos taurus* (½ Nelore x ½ Simental) cyclic heifers, (previously confirmed by heat detection), 20 to 24 months old and presenting body condition score (BCS) from 2.5 to 3.5 (within a 1 to 5 scale; according to Houghton *et al*., 1990) were selected for the study. Heifers were kept in *Brachiaria decumbens* pasture with free access to mineral supplement and water. Numbered ear tags were used for animal identification. All animal procedures were approved by the Animal Care Committee of the Faculty of Veterinary Medicine and Husbandry, Universidade de São Paulo, São Paulo, SP, Brazil.

Hormonal treatments

All heifers were pre-synchronized with two i.m. injections of PGF2α (25 mg of dinoprost tromethamine - Lutalyse®, Pfizer Animal Health, São Paulo, SP, Brazil) 12 days apart (day - 24 and day - 12). The objective was to synchronize estrus such that heifers would be in diestrus at the beginning of treatment with an intravaginal source of P4.

On day 0, all heifers presented a CL observed by ultrasonoghrafic examination. At this time, heifers were randomly allocated to one of four treatments with the intravaginal sources, as demonstrated in the experimental design (Fig. 1). Heifers in the CIDR_PGF8 group (n = 10) received 2.0 mg i.m. estradiol benzoate (EB - Estrogin®, Farmavet, São Paulo, SP, Brazil) and a new controlled internal drug release containing 1.9 g of progesterone (CIDR, Pfizer Animal Health, Brazil). On day 8, at the time of CIDR withdrawal, heifers received an i.m. injection of PGF2α and, on day 9, an injection of 0.5 mg of EB i.m. Other groups received EB i.m. injections and CIDR insertion/withdrawal as aforementioned, except that an i.m. injection of PGF2α was administered on day 5. Nevertheless, heifers in the CIDR_PGF5 group (n = 10) received a new CIDR, while heifers in the CIDR1 x_PGF5 (n = 9) and CIDR2x_PGF5 (n = 11) groups received a previously used CIDR for 8 and 14 days, respectively.

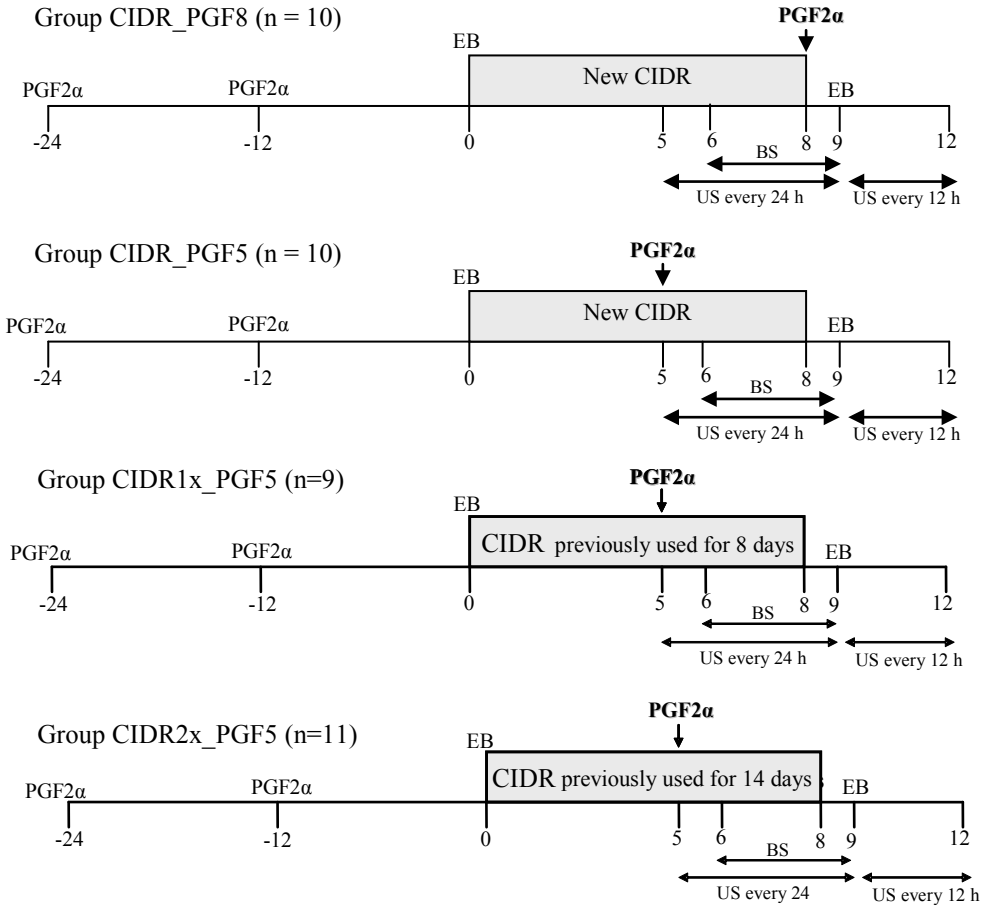

Figure 1. Schematic diagram of experiment. Pre-synchronization with two injections of PGF2α twelve days apart. Ovarian ultrasound evaluations (US) were performed every 24 h from day 5 to day 8, and every 12 h from day 9 to day 12. Blood samples (BS) were collected daily from day 6 to day 9.

Ultrasound examinations

Heifers were submitted to ultrasound examination at day 0 to confirm the presence of CL at beginning of the estrous synchronization protocol. Then, heifers underwent daily transrectal ultrasound (US) examinations from day 5 to day 9 in order to evaluate the DF growth rate and, every 12 h from day 10 to day 12, to establish the time of ovulation. US examinations were done always by the same technician, using a *100 Vet* US equipped with an 8 MHz linear-array transducer (Pie Medical, Netherlands). Follicular growth rate in each group was obtained by the difference in follicle mean diameter daily registered from day 5 to day 10. Ovulation was characterized by the disappearance of DF previously identified and the moment of ovulation was established as 6 h before the last ultrasound examination (Carvalho *et al*., 2008). The ovulation rate was determined by total of animals that had a synchronized ovulation following the ovarian synchronization protocol by the total of synchronized animals.

Blood sampling and progesterone assay

Blood samples from all heifers were collected into heparinized Vacutainer® tubes (Becton Dickinson Co., Franklin Lakes, NJ, USA) once daily from day 6 to day 9 by jugular venipuncture. Afterwards, samples were centrifuged at 1600 x g for 20 min and the plasma stored at -20°C until radioimmunoassay (Coat-A-Count®, Diagnostic Products Corporation, Los Angeles, EUA).

The P4 assay sensitivity was 0.07 ng/ml and the inter- and intra-assay coefficients of variation were 6.7 and 9.0% respectively. Analysis was done at the Laboratório de Análise Hormonal, Universidade de São Paulo, SP, Brazil.

Statistical analyses

Data were statistically analyzed using SAS software (SAS Institute Inc., Cary, NC, USA). Initially, the statistical assumptions of homogeneity of variances and residue normality were verified and, whenever necessary, data were transformed in order to meet the assumptions. Log transformation was used for circulating progesterone to attain normality.

Follicular growth in each group was calculated by the difference in follicle diameter daily registered from day 5 to 10. Punctual (plasma P4, follicular growth, interval from CIDR removal to ovulation and ovulation rate) and temporal (plasma P4 from day 6 to day 9, follicular diameter from day 5 to day 10) variables were evaluated using orthogonal contrast. Orthogonal comparisons were performed to determine the three main effects: day of reatment with PGF2α [CIDR_PGF8 vs. CIDR_PGF5 + CIDR1x_PGF5 + CIDR2x_PGF5], effect of the type (new or previously used) of the intravaginal progesterone device (CIDR_PGF5 vs. CIDR1x_PGF5 + CIDR2x_PGF5) and duration of used CIDR (CIDR1x_PGF5 vs. CIDR2x_PGF5). The orthogonal arrangements were used on the responses variables P4 concentration during CIDR treatment, follicular growth and ovulation rates. The effects of the different contrasts on the P4 concentration and the follicular growth were analyzed using repeated measures (PROC MIXED) and the ovulation rate was analyzed using the logistic regression (PROC Glimmix).

Results were expressed as means ± SEM. Differences were considered significant when $P < 0.05$ and tendencies when $0.1 > P \geq 0.05$.

Results

There was a significant effect of earlier administration of the PGF2α treatment on P4 concentrations during CIDR treatment ($P < 0.001$; Table 1) as heifers treated on day 5 showed lower mean circulating P4 from day 6 to day 8 than heifers treated on day 8 (CIDR_PGF5 = 1.98 ± 0.21 ng/ml, CIDR1x_PGF5 = 1.69 ± 0.17 ng/ml, and CIDR2x_PGF5 = 1.33 ± 0.08 ng/ml vs. CIDR_PGF8 = 3.31 ± 0.45 ng/ml; Fig. 2).

Treatment with PGF2α on day 5 also had a positive effect on DF growth rate between days 5 to 10, such that treatment with PGF2α on day 8 reduced ($P < 0.05$) the growth rate of the DF (CIDR_PGF8 = 0.72 ± 0.13 mm/day) when compared to groups treated on day 5 (CIDR_PGF5 = 0.96 ± 0.12 mm/day; CIDR1x_PGF5 = 1.06 ± 0.15 mm/day, CIDR2x_PGF5 = 1.01 ± 0.06 mm/day). As a consequence, heifers receiving a PGF2α injection on day 8 presented smaller DF diameter on day 10 (CIDR_PGF8 = 8.81 ± 6.7 mm) than those that received the same treatment on day 5 (CIDR_PGF5 = 10.00 ± 0.58 mm, CIDR1x_PGF5 = 10.5 ± 0.69 mm, CIDR2x_PGF5 = 10.5 ± 0.35 mm; $P < 0.05$; Fig. 3).

As demonstrated in Table 1, there was a tendency ($P = 0.09$) for a longer interval from CIDR removal to ovulation in heifers that received an injection of PGF2α on day 8 (CIDR_PGF8 = 74 ± 6.2 h) when compared with heifers that received PGF2α on day 5 (CIDR_PGF5 = 69 ± 8.5 h; CIDR1x_PGF5 = 66 ± 0 h and CIDR2x_PGF5 = 72 ± 6.3 h).

In this experiment, ovulation rate among groups were not statistically different according to day of PGF2α treatment, the type of CIDR (new and previously used) or the duration of the previous use (8 or 14 days), as shown in the Table 1.

Table 1. Plasma P4 concentrations from day 6 to day 8, follicular growth rate (mm/day) from day 5 to day 10 and interval from CIDR withdrawal to ovulation in heifers submitted to different PGF2α (day 5 or 8) plus P4 (new or previously used CIDRs) protocols.

Item	Treatments[a]				Contrasts[b]		
	CIDR_PGF8	CIDR_PGF5	CIDR1x_PGF5	CIDR2x_PGF5	C1	C2	C3
Serum P4 concentration (ng/ml)[c]	3.31 ± 0.45	1.98 ± 0.21	1.69 ± 0.17	1.33 ± 0.08	< 0.001	0.16	0.37
Follicular growth rate (mm/day)	0.72 ± 0.13	0.96 ± 0.12	1.06 ± 0.15	1.01 ± 0.06	0.04	0.61	0.78
Interval from CIDR removal to ovulation (h)	74 ± 6.2	69 ± 8.5	66 ± 0.0	72 ± 6.3	0.09	1.0	0.06
Ovulation rate (%)	60.0 (6/10)	80.0 (8/10)	77.8 (7/9)	90.0 (10/11)	0.15	0.27	0.44

[a] Treatments Groups:
CIDR_PGF8: heifers were treated with a new CIDR and received a PGF2α treatment concurrent with the CIDR withdrawal on day 8;
CIDR_PGF5: heifers were treated with a new CIDR and a PGF2α treatment was performed on day 5; CIDR was withdrawn on day 8;
CIDR1x_PGF5: heifers received a previously used CIDR for 8 days and the PGF2α treatment was performed on day 5; CIDR was withdrawn on day 8;
CIDR2x_PGF5: heifers received a previously used CIDR for 14 days, PGF2α on day 5 and CIDR was withdrawn on day 8.
[b]C1= Contrast of day of PGF2α treatment (CIDR_PGF8 vs. CIDR_PGF5 + CIDR1x_PGF5 + CIDR2x_PGF5);
C2= Contrast of new vs. used CIDR (CIDR_PGF5 vs. CIDR1x_PGF5 + CIDR2x_PGF5);
C3=Contrast of duration of used CIDR (CIDR1x_PGF5 vs. CIDR2x_PGF5);
[c]Serum P4 concentration: mean of serum progesterone concentration from day 6 to day 8.

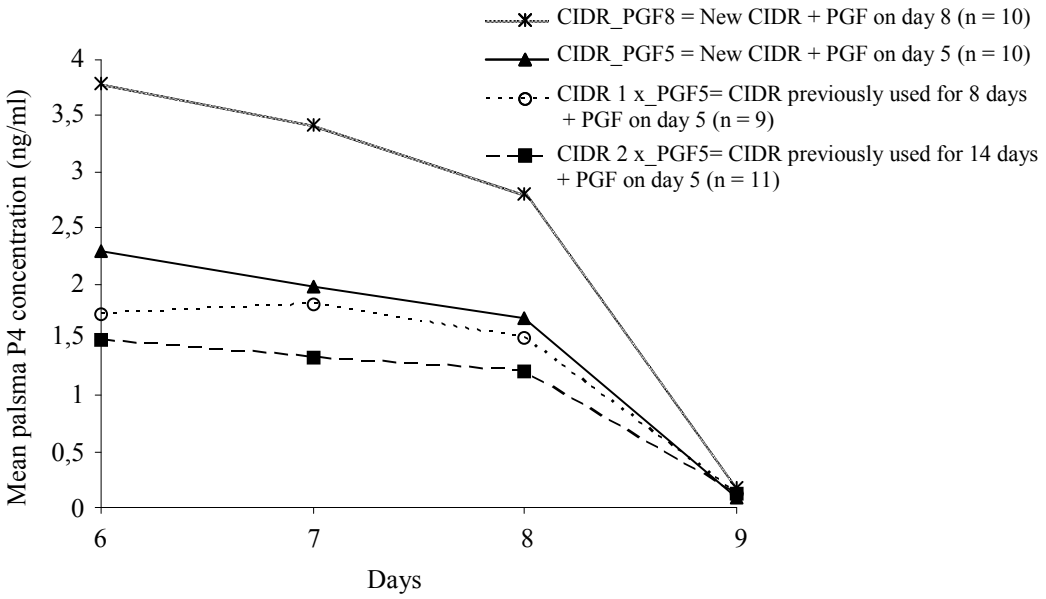

Figure 2. Plasma P4 profile between days 6 and 9 in heifers treated with PGF2α either on day 5 or day 8 and exposed to different levels of exogenous P4. Heifers that received PGF2α treatment on day 5 presented lower P4 concentration (P < 0.001) than heifers treated only on day 8 (C1 = Contrast of day of PGF2α treatment: CIDR_PGF8 vs. CIDR_PGF5 + CIDR1x_PGF5 + CIDR2x_PGF5).

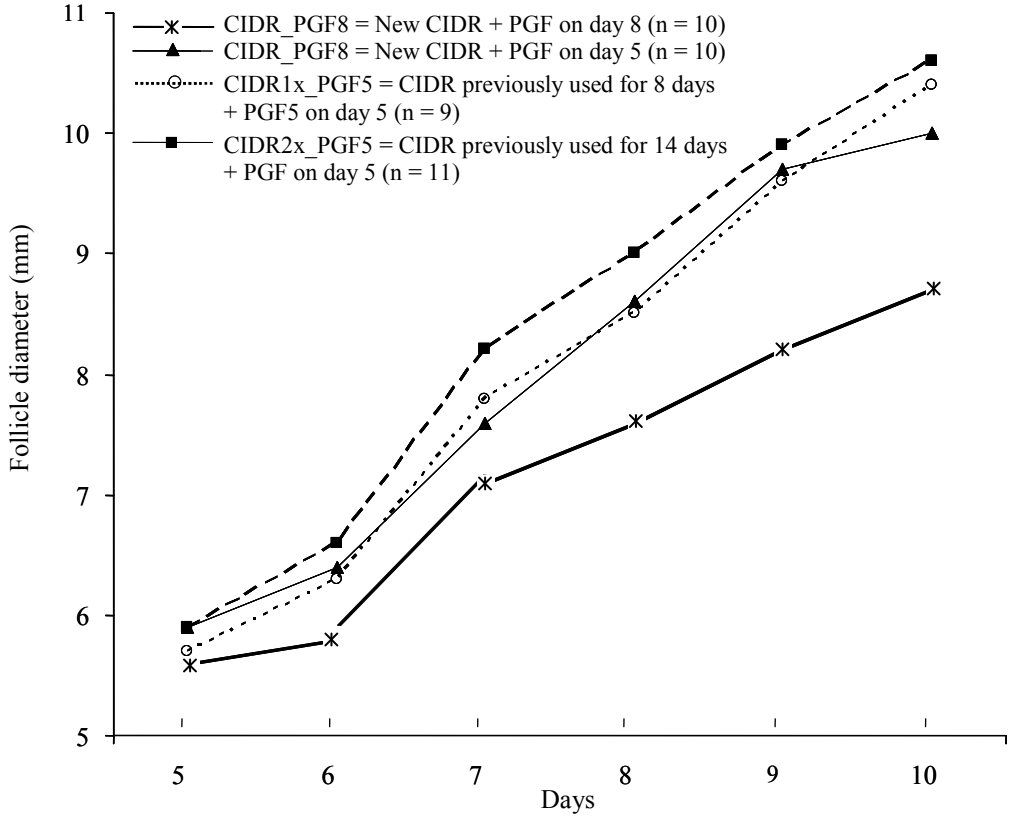

Figure 3. Dominant follicle diameter (mm) in heifers treated with PGF2α either on day 5 or day 8 and exposed to different levels of exogenous P4. Heifers that received PGF2α treatment on day 5 had larger dominant follicle growth (P = 0.04) than heifers treated only on day 8 (C1= Contrast of day of PGF2α treatment (CIDR_PGF8 vs. CIDR_PGF5 + CIDR1x_PGF5 + CIDR2x_PGF5).

Discussion

The current study showed that administration of a PGF2α analog on day 5 was effective in decreasing plasma concentrations of P4 and promoted an increased rate of follicular growth resulting in an ovulatory follicle with larger diameter, which is consistent with findings by others (Carvalho *et al*., 2008).

The negative correlation between plasma P4 concentrations and follicular development found in the current trial was consistent with those found in the study by Cupp *et al*. (1993) which reported that cows with lower circulating P4, approximately 1 to 2 ng/ml, developed follicles with higher diameters than those with higher circulating P4 concentrations (5 to 6 ng/ml).

Additionally, other studies have demonstrated that high plasma P4 concentrations can affect LH pulsatility pattern as LH pulses occur in lower frequency in the presence of higher circulating P4 (Ireland and Roche, 1982; Roberson *et al*., 1989), and the lower LH-pulse frequency is known to reduce follicular growth rate and consequently lower DF diameters (Kinder *et al*., 1996). Moreover, Cutaia *et al*. (2004) found higher ovulatory follicle size in beef cows receiving a PGF2α treatment at the time of P4-device insertion and supposed that lower P4 levels increased LH-pulse frequency, leading to higher DF growth. Similarly, Kinder *et al*. (1996) demonstrated that the use of a CIDR implant in the absence of a corpus luteum (CL) resulted in a sub-luteal plasma P4 concentration (1 to 2 ng/ml), which promoted greater frequency of LH pulses and consequently led to higher follicular diameter.

Although LH pulses were not evaluated in the current study, it is very likely that greater LH frequencies accounted for the greater follicular growth rate found in heifers that had lower plasma P4 concentrations due to PGF2α treatment on day 5.

Considering a 3.1-day interval between EB treatment and the emergence of a new follicular wave (Bó *et al*., 1995; Burke *et al*., 2001) followed by follicular deviation approximately 2.6 days later (Gimenes *et al*., 2008), the PGF2α treatment on day 5 ensured a lower circulating P4 by the time follicles' gonadotropin dependency changed from FSH to LH.

According to Baruselli *et al*. (2001), lower pregnancy responses following TAI programs applied to *Bos indicus* heifers and post partum cows can be, at least partially, attributed to ovulation failures due to small dominant follicle diameter at the time of expected

ovulation. In this regard, the PGF2α administration at the beginning of progesterone-based protocols could be a strategy to overcome this problem in cycling animals. In fact, Meneghetti *et al*. (2009) verified a positive effect on pregnancy rate to TAI when a PGF2α treatment was administered 48 h before CIDR withdrawal compared to the same treatment immediately after CIDR withdraw.

Independently of the number of days the CIDR (new or previous used for 8 or 14 days was used), the groups that received PGF2α on day 5 presented lower plasma P4 concentration and higher follicular growth and DF diameter. These findings show that treatment with a P4-releasing device (both new or previously used) in the absence of a CL results in lower circulating P4 concentration of those found during the luteal phase that leads to higher follicular growth.

Despite the greater diameter of DF in heifers with lower plasma P4, there was no statistical difference among groups' ovulation rates in the current study. These findings could be partially attributed to the relatively small number of animals used in this trial, which is likely low for this type of analysis. Moreover, these results were not consistent with previous findings from Cutaia *et al*. (2004) and Carvalho *et al*. (2008) which observed that the treatment with PGF2α at the beginning of P4-based protocols increased ovulation rates.

The tendency for a longer interval from CIDR removal to ovulation in heifers that received a PGF2α treatment on day 8 than in heifers that received on day 5 might be attributed to a greater immature and unresponsive follicle as a result of higher plasma P4 concentrations and lower follicular growth rate. Therefore, LH pulsatility was not only responsible for follicle growth, but potentially caused increased LH receptors in the DF (Lopez *et al*., 2005). Thus, greater circulating P4 might have decreased the frequency of LH pulses, leading to delayed expression of LH receptors in the DF and consequently time to ovulation.

Results of the present study led to the conclusion that a PGF2α injection at early stages of P4-based protocols (i.e day 5) may be a feasible alternative to increase DF growth rate and the diameter of the ovulatory follicle. This treatment could be an alternative to improve results of synchronization protocols both to IATF and ET programs. Moreover, results showed that CIDR can be satisfactory used for up to 3 times, without any impairment on follicular growth and on synchronization of ovulation.

References

Baruselli PS, Reis EL, Marques MO, Nasser LF, Bo GA. 2004 The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates. *Anim Reprod Sci*, 82/83:479-486.

Baruselli PS, Marques MO, Madureira EH, Costa Neto WP, Grandinetti RR, Bo GA. 2001. Increased pregnancy rates in embryo recipients treated with CIDR-B devices and eCG. *Theriogenology*, 55:157. (abstract).

Bergfeld EGM, Kojima FN, Wehrman ME, Cupp AS, Peters KE, Mariscal V, Sanchez T, Kittok R, Garcia-Winder M, Kinder JE. 1995. Frequency of luteinizing hormone pulses and circulating 17β-estradiol concentration in cows is related to concentration of progesterone in circulation when the progesterone comes from either an endogenous or exogenous source. *Anim Reprod Sci*, 37:257-265.

Binelli M, Thatcher WW, Mattos R, Baruselli PS. 2001. Antiluteolytic strategies to improve fertility in cattle. *Theriogenology*, 56:1451-1463.

Bó GA, Adams GP, Caccia M, Martinez M, Pierson RA, Mapletoft RJ. 1995. Ovarian follicular wave emergence after treatment with progestogen and oestradiol in cattle. *Anim Reprod Sci*, 39:193-204.

Burke CR, Mussard ML, Grum DE, Day ML. 2001. Effects of maturity of the potential ovulatory follicle on induction of oestrous and ovulation in cattle with oestradiol benzoate. *Anim Reprod Sci*, 66:161-174.

Carvalho JPB, Carvalho NAT, Reis EL, Nichi M, Souza AH, Baruselli PS. 2008. Effect of early luteolysis in P4-based timed AI protocols in *Bos indicus, Bos indicus* x *Bos taurus* and *Bos taurus* heifers. *Theriogenology*, 69:167-175.

Cupp A, Garcia-Winder M, Zamudio A, Mariscal V, Wehrman M, Kojima N, Peters K, Bergfeld E, Hernandez P, Sanchez T, Kittok R, Kinder J. 1993. Concentration of progesterone (P4) in circulation has differential effect on biochemical characteristics of dominant follicles in cows. *J Anim Sci*, 71(suppl. 1):211. abstract.

Cutaia L, Chesta P, Morebo D, Aviles M, Bertero F, Bó GA. 2004. Effect of time of prostaglandin F2 and estradiol benzoate administration on follicular wave emergence and ovulation in beef cattle treated with progesterone vaginal devices. *In*: Abstracts of the 15th International Congress of Animal Reproduction, 2004, Porto Seguro, Brazil. Porto Seguro: ICAR. pp. 111.

Dias CC, Wechsler FS, Day ML, Vasconcelos JLM. 2009. Progesterone concentration, exogenous equine chorionic gonadotropin, and timing of prostaglandin F2α treatment affect fertility in postpuberal Nelore heifers. *Theriogenology*, 72:378-385.

Gimenes LU, Sá Filho MF, Carvalho, NAT, Torres-Junior JRS, Barros CM, Carvalho JBP, Mapletoft RJ, Baruselli PS. 2008. Follicle deviation and ovulatory capacity in Bos indicus heifers. *Theriogenology*, 69:852-858.

Houghton PL, Lemenager RP, Hendrix KS, Moss GE, Stewart TS. 1990. Effects of body condition pre- and postpartum energy intake and stage of production of energy utilization by beef cows. *J Anim Sci*, 68:1447-1456.

Ireland JJ, Roche JF. 1982. Effects of progesterone on

basal LH and FSH secretion in heifers. *J Reprod Fertil*, 64:295-302.

Kinder JE, Kojima FN, Bergfeld EG, Wehrman ME, Fike KE. 1996. Progestin and estrogen regulation of pulsatile LH release and development of persistent ovarian follicles in cattle. *J Anim Sci*, 74:1424-1440.

Lopez H, Sartori R, Wiltbank MC. 2005. Reproductive hormones and follicular growth during development of one or multiple dominant follicles in cattle. *Biol Reprod*, 72:788-795.

Mantovani AP, Reis EL, Gacek F, Bó GA, Binelli M, Baruselli PS. 2005. Prolonged use of a progesterone-releasing intravaginal device (CIDR) for induction of persistent follicles in bovine embryo recipients. *Anim Reprod*, 2:272-277.

Meneghetti M, Sá Filho OG, Peres RFG, Lamb GC, Vasconcelos JLM. 2009. Fixed-time artificial insemination with estradiol and progesterone for Bos indicus cows I: Basis for development of protocols. *Theriogenology*, 72:179-189.

Peters MW, Pursley JR. 2003. Timing of final GnRH of the Ovsynch protocol affects ovulatory follicle size, subsequent luteal function, and fertility in dairy cows. *Theriogenology*, 60:1197-1204.

Pfeifer LFM, Mapletoft RJ, Kastelic JP, Small JA, Adams GP, Dionello NJ, Singh J. 2009. Effects of low versus physiologic plasma progesterone concentration on ovarian follicular development and fertility in beef cattle. *Theriogenology*, 72:1237-1250.

Roberson MS, Wolfe MW, Stumpf TT, Kittok RJ, Kinder JE. 1989. Luteinizing hormone secretion and corpus luteum function in cows receiving two levels of progesterone. *Biol Reprod*, 41:997-1003.

Stock AE, Fortune JE. 1993. Ovarian follicular dominance in cattle: relationship between prolonged growth of the ovulatory follicle and endocrine parameters. *Endocrinology*, 132:1108-1114.

Vasconcelos JLM, Sartori R, Oliveira HN, Guenther JG, Wiltbank MC. 2001. Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. *Theriogenology*, 56:307-314.