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Effects of two estradiol esters (benzoate and cypionate) on the induction of synchronized ovulations in *Bos indicus* cows submitted to a timed artificial insemination protocol

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Abstract

The effects of estradiol benzoate (EB) and estradiol cypionate (EC) on induction of ovulation after a synchronized LH surge and on fertility of *Bos indicus* females submitted to timed AI (TAI) were evaluated. In Experiment 1, ovariectomized Nelore heifers were used to evaluate the effect of EB (n = 5) and EC (n = 5) on the circulating LH profile. The LH surge timing (19.6 and 50.5 h; P = 0.001), magnitude (20.5 and 9.4 ng/mL; P = 0.005), duration (8.6 and 16.5 h; P = 0.001), and area under the LH curve (158.6 and 339.4 ng/mL; P = 0.01) differed between the EB and EC treatments, respectively. In Experiment 2 (follicular responses; n = 60) and 3 (pregnancy per AI; P/AI; n = 953) suckled *Bos indicus* beef cows submitted to an estradiol/progesterone-based synchronization protocol were assigned to receive one of two treatments to induce synchronized ovulation: 1 mg of EB im 24 h after progesterone (P4) device removal or 1 mg of EC im at P4 device removal. There was no difference (P > 0.05) between EB and EC treatments on follicular responses (maximum diameter of the ovulatory follicle, 13.1 vs. 13.9 mm; interval from progesterone device removal to ovulation, 70.2 vs. 68.5 h; and ovulation rate, 77.8 vs. 82.8%, respectively). In addition, P/AI was similar (P < 0.22) between the cows treated with EB (57.5%; 277/482) and EC (61.8%; 291/471). In conclusion, despite pharmacologic differences, both esters of estradiol administered either at P4 device removal (EC) or 24 h later (EB) were effective in inducing an LH surge which resulted in synchronized ovulations and similar P/AI in suckled *Bos indicus* beef cows submitted to TAI.

Keywords: Bovine; Ovarian response; Estradiol; Tai; LH; Synchronization

1. Introduction

It is currently possible to efficiently synchronize ovarian follicular growth and induce ovulation of a dominant follicle at a known moment, allowing insemination of beef zebu females on a predetermined day and hour, without the need to detect estrus [1]. Timed

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artificial insemination (TAI) may be employed either in cyclic or anestrous cows and started independent of estrous cycle phase [2].

Recently, many ovulation synchronization protocols have been studied in zebu cows [3–5]. These protocols are based on synchronizing emergence of the wave of follicular growth by simultaneous administration of progesterone and estradiol [6,7]. At the end of a protocol, it is necessary to reduce circulating progesterone concentrations by removal of the progesterone device (exogenous source) and administration of prostaglandin to promote CL regression (endogenous source), so that, ovulation may occur. Lastly, it is necessary to promote the final growth of the follicle and to induce a synchronized ovulation, which allows insemination at a predetermined moment [2].

Adequate results have been achieved by TAI (≈50%) with protocols employing estradiol benzoate (EB) as the ovulation inducing agent in *Bos taurus* [8,9] and in *Bos indicus* [1,4] beef cows. However, it is necessary to handle cows at least four times to perform TAI (Day 0, EB + P4 device insertion; Day 8, PGF + P4 device removal; Day 9, EB; and Day 10, TAI [10]). It is known that estradiol esters are capable of inducing ovulation in beef cows, however, their pharmacokinetics differ. In some studies, EB had a shorter half-life and induced an earlier LH peak compared to estradiol cypionate (EC) [11,12]. Thus, based on the distinct pharmacokinetics of estradiol esters and aiming to reduce TAI-related cow handling, three studies were conducted to evaluate the effect of EB and EC on induction of ovulation of suckled beef cows submitted to TAI. The objective of Experiment 1 was to evaluate the circulating LH-release profile after giving EB and EC administration to ovariectomized *Bos indicus* heifers. Experiment 2 aimed to evaluate the effect of EC (treated at P4 device removal) or EB (treated 24 h post P4 device removal) on ovarian responses of Nelore cows (*Bos indicus*) submitted to a TAI protocol. Experiment 3 aimed to evaluate the effect of EC and EB, as ovulation inducers, on the pregnancy rate of Nelore cows (*Bos indicus*) submitted to TAI. The hypothesis was that EC administration (at device removal) decreases cow handling without affecting fertility of beef cows submitted to TAI programs, compared to EB given 24 h after P4 device removal.

2. Materials and methods

2.1. Experiment 1: circulating LH profiles in ovariectomized Nelore heifers

2.1.1. Animals and handling

Experiment 1 was conducted on the research farm of the University of the State of Sao Paulo (UNESP) in Araçatuba, SP, Brazil. Nelore heifers, 36.0 ± 0.5 mo old, weighing 427.8 ± 9.3 kg had their ovaries removed (via laparotomy) to eliminate endogenous estradiol. Heifers were maintained on *Brachiaria brizantha* pastures and given mineralized-salt and free access to water.

2.1.2. Experimental design

Ovariectomized heifers (n = 10) were synchronized using an estradiol/progesterone-based TAI protocol 1. Heifers received an intravaginal device containing 1 g of P4 (Sincrogest, Ourofino Agronegocio, Sao Paulo, Brazil) plus 2 mg of estradiol benzoate im (EB; Sincrodiol, Ourofino Agronegocio, Sao Paulo, Brazil). Eight days later, the progesterone device was removed and heifers received 500 μg $PGF_{2\alpha}$ im (sodium cloprostenol; Sincrocio, Ourofino Agronegocio, Sao Paulo, Brazil). Concurrently, EC-Group heifers (n = 5) received 1 mg of estradiol cypionate im (ECP, Pfizer, Sao Paulo, SP, Brazil) and EB-Group heifers (n = 5) received 1 mg EB im (Sincrodiol, Ourofino Agronegocio, Sao Paulo, SP, Brazil).

2.1.3. Blood sampling and LH assay

Jugular veins were cannulated 1 day before onset of treatments with the two estradiol esters. Blood samples were collected in evacuated tubes every 3 h after initiation of treatments (total of 72 h). Within 4 h after collection, samples were refrigerated (4 °C) and then centrifuged (3000*g* for 15 min) and stored at −20 °C until assayed for LH. Plasma concentrations of LH were determined by double antibody radioimmunoassay, as previously described [13]. The intraassay and interassay CVs were 10.3 and 13.1%, respectively. Assay sensitivity was 0.02 ng/mL.

2.2. Experiment 2: ovarian responses in Nelore cows

2.2.1. Animals and handling procedures

The second experiment was conducted at the State Research Farm (APTA - Pindamonhagaba Regional Center), in Pindamonhagaba, Sao Paulo, Brazil. Sixty suckled multiparous Nelore cows scoring 2.8 ± 0.4 on a (1-thin to 5-obese) body condition score (BCS; [14]) scale. All cows were maintained on *Brachiaria brizantha* pastures and given mineralized-salt and free access to water.

2.2.2. Experimental design

All cows were synchronized using an estradiol/progesterone-based TAI protocol 1. Hormonal treatments started between 30 and 60 days postpartum. Cows received an intravaginal device containing 1 g of P4 (Sincrogest, Ourofino Agronegocio, Sao Paulo, Brazil) plus an administration of 2 mg of estradiol benzoate im

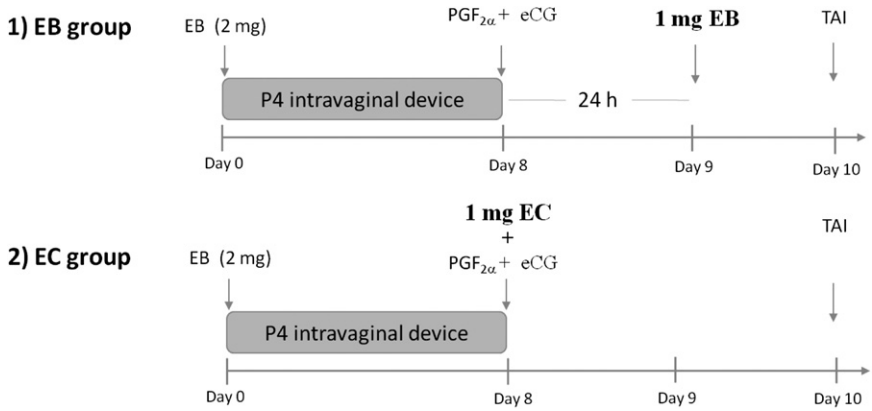

Fig. 1. Experimental design (P4, progesterone; EB, estradiol benzoate; EC, estradiol cypionate; eCG, equine chorionic gonadotropin; TAI, Timed AI).

(EB; Sincrodiol, Ourofino Agronegocio). Eight days later, the device was removed and 500 μg $PGF_{2\alpha}$ (cloprostenol sodium; Sincrocio, Ourofino Agronegocio) and 300 IU of equine chorionic gonadotropin (eCG; Folligon, Intervet-Schering-Plough, Boxmeer, Netherlands) administered im. At P4-device removal, EC-Group cows (n = 30) received 1 mg of estradiol cypionate im (ECP, Pfizer Animal Health, Sao Paulo, SP, Brazil) and EB-Group cows (n = 30) received 1 mg EB im (Sincrodiol, Ourofino Agronegocio) 24 h after P4-device removal (Fig. 1). At the beginning of the estrous synchronization procedures (day 0), ultrasonographic examinations were performed (USA; Chison 600 VET, 5 MHz linear transducer, China) to evaluate ovarian status (presence of CL and ovarian follicles ≥8 or <8 mm [1]). Moreover, ultrasonographic evaluations were performed every 12 h from P4-device removal to detection of ovulation. The moment of ovulation was defined as the time of disappearance of a previously identified dominant follicle (DF) from one ultrasound examination to the next, minus 6 h.

2.3. Experiment 3: pregnancy per AI in Nelore cows (*Bos indicus*)

2.3.1. Animals and management

The experiment was conducted on two commercial farms located in northwestern Brazil, from January to March 2009. A total of 953 multiparous suckled Nelore (*Bos indicus*) cows, 30 to 60 days postpartum and average BCS of 2.79 ± 0.41, were used [14]. All cows were maintained on *Brachiaria brizantha* pastures and given mineralized-salt and free access to water.

2.3.2. Experimental design

Postpartum suckled cows were allocated into treatment groups according to calving date. All cows were synchronized using an estradiol/progesterone-based TAI protocol and treatments similar to those described in Experiment 2. In this experiment, TAI was performed 48 to 52 h after P4 device removal. Frozen semen straws from single ejaculates of each of the three sires were homogeneously distributed between experimental groups. All inseminations were performed by the same technician who had no previous knowledge of cow treatment allocation. Pregnancy examinations were conducted by transrectal ultrasonography (Chison D600VET, Chison Medical Imaging, Co, China) 30 days after AI. The experimental design is shown (Fig. 1).

2.4. Statistical analysis

Statistical analyses were performed using Statistical Analysis System for Windows [15]. In Experiment 1, LH surge was defined as an increase in LH > 2 SD above the overall within-cow mean of LH concentrations. The magnitude of the LH surge (MLH) was defined as the difference between the maximum concentrations observed during the surge and baseline. Timing of LH surge (TLH) was defined as the moment in which the LH surge occurred. Duration of the LH surge (DLH) was determined as the time interval from the onset of the LH surge (baseline concentration before the increase in LH concentration induced by estradiol) to its first return to baseline concentrations. Area under the curve (AUC) of LH release was calculated by the trapezoid method. In Experiment 2, the variables evaluated were diameter of the ovulatory follicle, time from P4-device removal to ovulation, and

ovulation rate. Continuous data were tested for normality of the residues and analyzed by the UNIVARIATE procedure (transformed when necessary) and subjected to Bartlett's test to assess homogeneity of variances. The GLM procedure with Tukey adjustment was used to determine significant differences among groups. All values are expressed as mean ± SEM. In Experiment 3, the variables initially included in the models were treatment (EB and EC), farm and BCS at the first day of the synchronization protocol, and their interactions. Data were analyzed by a multivariate logistic regression using the LOGISTIC procedure of SAS. Variables were removed by backward elimination, based on the Wald statistics criterion when $P > 0.20$. Variables included in the final model for analysis of P/AI were treatment, BCS and farm. Adjusted odds ratio (AOR) and 95% confidence interval (CI) were generated during the logistic regression. Results are presented as proportions and AOR. The P/AI was analyzed using the GLIMMIX procedure of SAS.

3. Results

3.1. Experiment 1: circulating LH profile in ovariectomized Nelore heifers

The LH release profile was different between EC- and EB-Group heifers (Fig. 2). The TLH, MLH, DLH and AUC for the EC and EB treatments are summarized (Table 1). Onset of the LH peak differed ($P = 0.001$) between EC and EB groups, such that in the latter LH peaked 30.9 h earlier than in the former. Additionally, LH peak magnitude was greater ($P = 0.005$) for EB heifers. Moreover, heifers receiving EB as the ovulation inducing agent had shorter ($P = 0.001$) peak duration and smaller ($P = 0.01$) area under the curve for LH concentrations. Finally, LH peak dispersion was smaller ($P = 0.01$) in Group-EB compared to that of EC-Group heifers.

Table 1
Plasma LH profile in ovariectomized Nelore heifers treated with EC or benzoate (EB).

	EB	EC	*P*
No. animals	5	5	
Magnitude of LH surge (ng/mL)	20.5 ± 1.9	9.4 ± 2.2	0.001
Time of LH surge (h)	19.6 ± 1.2	50.5 ± 3.6	0.005
Duration of LH surge (h)	8.6 ± 0.2	16.5 ± 1.0	0.001
Area under LH curve (ng/mL/72 h)	158.6 ± 26.1	339.4 ± 36.4	0.01

Protocol: day 0–1 intravaginal device of P4; 2 mg of EB; Day 8 - progesterone device was removed; 500 μg $PGF_{2\alpha}$; treatments (EC Group —1 mg of estradiol cypionate or EB Group —1 mg estradiol benzoate).

3.2. Experiment 2: ovarian responses in Nelore cows

Of the 60 cows initially used in this experiment, four (6.7%) did not respond (absence of a DF ≥6 mm at P4-device removal) to the TAI protocol and were therefore removed from the study. Follicular response data are shown (Table 2). Maximum diameters of the DF ($P = 0.12$), and of the ovulatory follicle ($P = 0.11$) and interval from P4-device removal to ovulation did not differ between cows that received EB 24 h after P4-device removal and those receiving EC as described. Ovulation rate after the TAI protocol was similar between the EB (77.8%; 21/27) and the EC groups

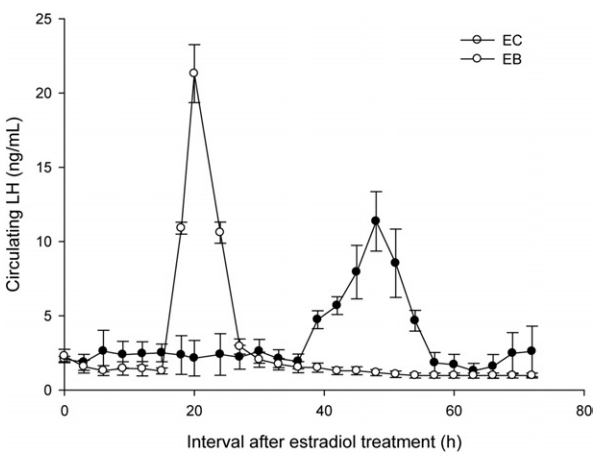

Fig. 2. Mean ± SEM effect of treatment with EC or EB on circulating LH concentrations in Nelore cows.

Table 2
Effects of EB - estradiol benzoate or EC - estradiol cypionate, as ovulation inducers in suckled Nelore cows submitted to progesterone-based TAI protocols, on ovarian responses.

	EB	EC	*P*
No. animals	27	29	
Diameter of DF on Day 8 (mm)	10.2 ± 0.3	9.9 ± 0.3	0.76
Maximum diameter of the DF (mm)	13.4 ± 0.4	14.2 ± 0.3	0.12
Maximum diameter of the OF (mm)	13.1 ± 0.4	13.9 ± 0.4	0.11
Interval P4 device removal to ovulation (h)	70.2 ± 1.8	68.5 ± 1.9	0.53
Ovulation rate (%)	77.8 (21/27)	82.8 (24/29)	0.82
Double ovulation	4.8 (1/21)	4.2 (1/24)	0.89

DF, dominant follicle; OF, ovulatory follicle.

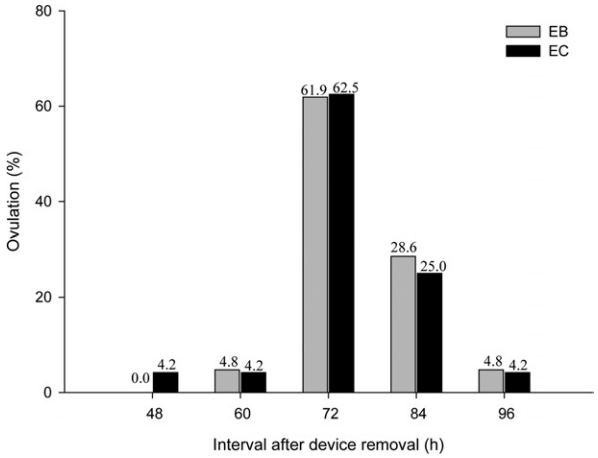

Fig. 3. Distribution of the time of ovulation (h) after P4 device removal in Nelore cows (*Bos indicus*) submitted to TAI according to EC treatment at P4 device removal (handled three times; n = 29) or EB 24 h after (handled four times; n = 27).

(82.8%, 24/29). Similarly, there was no difference in double ovulation rates (EB = 4.8%; 1/21 and EC = 4.2%; 1/24). Moreover, the variability of ovulation was also similar (P = 0.59) between treatments (Fig. 3).

3.3. Experiment 3: pregnancy per AI in Nelore cows (*Bos indicus*)

There was no interaction between ovulation inducer and explanatory variables, such as BCS (P = 0.96) and farm (P = 0.21) on P/AI. There was no significant effect of ovulation inducer (EB and EC; P = 0.22) and farm (P = 0.15) on P/AI (Table 3). However, increased BCS on the first day of the synchronization protocol was associated with increases in P/AI (Table 3).

4. Discussion

To our knowledge, this is the first report studying the effects of distinct ovulation inducers in estradiol and progesterone based protocols on LH profiles, follicular responses and fertility of *Bos indicus* females submitted to TAI. In the present study, despite differences between the LH releases characteristics of the two ovulation inducers, ovarian response and fertility of *Bos indicus* cows were similar. These results were reached because of the 24 h earlier EC administration used in the ovulation synchronization protocol. The experimental hypothesis was confirmed, since fertility between groups was similar. The main advantage of EC is the reduced need to handle these animals to perform the TAI.

LH peaks occurred 19.6 and 50.5 h after the administration of EB and EC, respectively. These results were consistent with previous studies in which the administration of EB to beef cows (*Bos taurus*) or EC to crossbred beef heifers promoted LH surges at approximately 20.4 [12] or 54.6 h [11] after treatment, respectively. This is result of the distinct pharmacokinetics of the estradiol esters used. The EC is formed by esterification of estradiol by cyclepentano propionic acid, resulting in low solubility in water and consequent slower release from the administration site and prolonged biological activity compared to estradiol benzoate [16]. In addition to LH peak anticipation, differences in the pharmacokinetics of the estradiol esters are responsible for the greater LH release and shorter LH peak duration of animals treated with estradiol benzoate. The recognition of these differences is important to determine the appropriate time to administer the ovulation inducer in TAI protocols in which estradiol and progesterone are used. Despite the greater LH peak synchronization of animals receiving EB as the ovulation inducer, ovulation synchronization between the two experimental groups (EB and EC) was similar.

Presently, progesterone and estradiol based TAI protocols are highly efficient in synchronizing ovulation in *Bos indicus* cows [17,18]. In the present study, >80% of the cows responded to the synchronization protocol and in only 6.7% a DF was not detected at P4-device removal. Other authors reported that 4.7% [19] and 6.3% [20] of females synchronized with estradiol and progesterone based protocols did not exhibit a new wave of follicular growth.

Another important characteristic of TAI protocols is the ovulatory follicle diameter (OFD), because it is directly related to CL size during the subsequent diestrus [21]. The CL diameter is highly correlated to the progesterone it synthesizes [22] which is essential for

Table 3
Risk factors for P/AI following two estradiol/progesterone-based synchronization protocols in suckled *Bos indicus* cows.

Variable	P/AI, % (n/n)	Adjusted OR (95% CI)[a]	*P*
Ovulation inducer			
EB	57.5 (277/482)	Referent[b]	0.22
EC	61.8 (291/471)	1.18 (0.91–1.53)	
Farm			
A	58.7 (229/390)	Referent[b]	0.15
B	60.2 (339/563)	1.24 (0.93–1.65)	
BCS category			
Low (2.0–2.5)	54.9 (218/397)	Referent[b]	0.01
Moderate to high (>2.5)	63.0 (350/556)	1.39 (1.07–1.81)	

[a] OR, odds ratio; CI, confidence interval.
[b] Referent, reference group for adjusted risk ratio.

post-fertilization embryonic development [23]. In the present study, OFD did not differ between ovulation inducers or between treatments and diameter of the ovulatory follicle was >13 mm at TAI. Satisfactory pregnancy rates were observed in cows with OF diameters >13 mm at TAI [24].

Besides their efficiency in synchronizing the LH peak, both estradiol esters induced high ovulation rates (~80%), approximately 70 h after P4-device removal. Ovulation synchronization allows cows to be inseminated at a predetermined time point, thereby avoiding the need for estrus detection [1]. Ovulation synchronization is important in situations in which the flexibility in relation to the time of AI is low, as in cows inseminated with sex-sorted semen [18].

The moment of ovulation in relation to the P4-device removal was similar between estradiol esters because of the 24-h delay on the EB administration, since the LH peak in this group occurred 30 h earlier than in EC group. This delay allowed a similar final growth of the ovulatory follicle between cows receiving either EB or EC. In a previous study, EB administration at progesterone device removal resulted in smaller ovulatory follicle diameters and lower pregnancy rates in cows inseminated 54 h after P4-device removal [17].

Fertility was not influenced by estradiol esters in this study. Similar results were observed by Meneghetti, et al. [25] in which no pregnancy rate differences were detected between cows treated with EB (50.8%) 24 h after P4-device removal or EC (51.9%) at P4-device removal. The estradiol esters studied had similar efficacy in inducing ovulation and fertility for TAI in beef cows.

Body condition scores lower than 2.5 (1–5 scale) led to lower TAI pregnancy rates in beef cows [2]. In the present study, the risk for P/AI was higher in cows of moderate to high BCS in both treatments. In various studies with beef cows, it was reported that low BCS at the beginning of TAI synchronization protocols may reduce fertility [4,25,26]. Thus, management techniques aiming at maintain or improving BCS before and, mainly, after parturition are important to guarantee the future fertility of cattle herds.

In conclusion, despite pharmacologic differences between the two ovulation inducing agents studied (EB and EC), both were effective in inducing an LH peak which resulted in synchronized ovulations, adequate fertility in suckled *Bos indicus* beef cows submitted to an estradiol/progesterone-based synchronization protocol. However, the use of EC allows for reduction on the number times cows must be handled without reduced fertility. This is also a desirable characteristic for increasing the adoption and spread of TAI.

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References

[1] Baruselli PS, Reis EL, Marques MO, Nasser LF, Bo GA. The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates. Anim Reprod Sci 2004;82/83:479–86.
[2] Bó GA, Baruselli PS, Martínez MF. Pattern and manipulation of follicular development in Bos indicus cattle. Anim Reprod Sci 2003;78:307–26.
[3] Baruselli PS, Marques MO, Carvalho NAT, Madureira EH, Campos Filho EP. Effect of different treatments for timed artificial insemination on the reproductive efficiency in lactating beef cows. Rev Bras Reprod Anim 2002;26:218–21.
[4] Sá Filho OG, Meneghetti M, Peres RFG, Lamb GC, Vasconcelos JLM. Fixed-time artificial insemination with estradiol and progesterone for Bos indicus cows II: Strategies and factors affecting fertility. Theriogenology 2009;72:210–8.
[5] Sales JN, Crepaldi GA, Girotto RW, Souza AH, Baruselli PS. Fixed-time AI protocols replacing eCG with a single dose of FSH were less effective in stimulating follicular growth, ovulation, and fertility in suckled-anestrus Nelore beef cows. Anim Reprod Sci 2011;124:12–8.
[6] Bó GA, Baruselli PS, Moreno D, Cutaia L, Caccia M, Tríbulo R, et al. The control of follicular wave development for self-appointed embryo transfer programs in cattle. Theriogenology 2002;57:53–72.
[7] Carvalho JB, Carvalho NA, Reis EL, Nichi M, Souza AH, Baruselli PS. Effect of early luteolysis in progesterone-based timed AI protocols in Bos indicus, Bos indicus x Bos taurus, and Bos taurus heifers. Theriogenology 2008;69:167–75.
[8] Colazo MG, Bo GA, Illuminati H, Meglia G, Schmidt EE, Bartolome J. Fixed-time artificial insemination in beef cattle using CIDR-B devices, progesterone and estradiol benzoate. Theriogenology 1999;51:404.
[9] Martínez MF, Kastelic JP, Adams GP, Cook B, Olson WO, Mapletoft RJ. The use of progestins in regimens for fixed-time artificial insemination in beef cattle. Theriogenology 2002;57:1049–59.
[10] Macmillan KL, Burke CR. Effects of estrus cycle control on reproductive efficiency. Anim Reprod Sci 1996;42:307–20.
[11] Colazo MG, Kastelic JP, Mapletoft RJ. Effects of estradiol cypionate (ECP) on ovarian follicular dynamics, synchrony of

ovulation, and fertility in CIDR-based, fixed-time AI programs in beef heifers. Theriogenology 2003;60:855–65.
[12] Martinez MF, Kastelic JP, Colazo MG. Effects of estradiol on gonadotrophin release, estrus and ovulation in CIDR-treated beef cattle. Domes anim. Endocrinologist 2007;33:77–90.
[13] Bolt DJ, Scott V, Kiracofe GH. Plasma LH and FSH after estradiol, norgestomet and Gn-RH treatment in ovariectomized beef heifers. Anim Reprod Sci 1990;23:263–71.
[14] Ayres H, Ferreira RM, Torres Junior JRS, Demétrio CGB, Lima CG, Baruselli PS. Validation of body condition score as a predictor of subcutaneous fat in Nelore (*Bos indicus*) cows. Livest Sci 2009;123:175–9.
[15] SAS. Sas/STAT1 user's guide (release 8.2), in SAS Inst. Inc., Cary, NC; 2001.
[16] Vynckier L, Debackere M, De Kruif A, Coryn M. Plasma estradiol-17β concentrations in the cow during induced estrus and after injection of estradiol-17β benzoate and estradiol-17β cypionate - a preliminary studyPlasma estradiol-17 beta concentrations in the cow during induced estrus and after injection of estradiol-17 beta benzoate and estradiol-17 beta cypionate—a preliminary study. J Vet Pharmacol Ther 1990;13:36–42.
[17] Ayres H, Martins CM, Ferreira RM, Mello JE, Dominguez JH, Souza AH, et al. Effect of timing of estradiol benzoate administration upon synchronization of ovulation in suckling Nelore cows (Bos indicus) treated with a progesterone-releasing intravaginal device. Anim Reprod Sci 2008;109:77–87.
[18] 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.
[19] Martínez MF, Kastelic JP, Bó GA, Caccia M, Mapletoft RJ. Effects of estradiol and some of its esters on gonadotrophin release and ovarian follicular dynamics in CIDR-treated beef cattleEffects of oestradiol and some of its esters on gonadotrophin release and ovarian follicular dynamics in CIDR-treated beef cattle. Anim Reprod Sci 2005;86:37–52.
[20] Bó GA, Adams GP, Pierson RA, Mapletoft RJ. Exogenous control of follicular wave emergence in cattle. Theriogenology 1995;43:31–40.
[21] Vasconcelos JL, Sartori R, Oliveira HN, Guenther JG, Wiltbank MC. Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. Theriogenology 2011;15:307–14.
[22] Thatcher WW, Moreira F, Santos JE, Mattos RC, Lopes FL, Pancarci SM, et al. Effects of hormonal treatments on reproductive performance and embryo production. Theriogenology 2001;55:75–90.
[23] Mann GE, Lamming GE, Robinson RS, Wathes DC. The regulatory of interferon-tau production and uterine hormone receptors during early pregnancy. J Reprod Fertil 1999;54:317–28.
[24] MSá 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.
[25] Meneghetti M, Sá 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.
[26] 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.