Effect of synchronizing ovulation in cattle administered a norgestomet ear implant in association with eCG and estradiol treatments on pregnancy rate after fixed-time embryo transfer

R.M. Ferreira[1,4], C.A. Rodrigues[2], H. Ayres[3], R.F. Mancilha[2], P.H. Franceschini[1], C.R. Esper[1], P.S. Baruselli[3,4]

[1]Department of Preventive Veterinarian Medicine and Animal Reproduction, FCAV/UNESP, Jaboticabal, SP, Brazil.
[2] SAMVET, São Carlos, SP, Brazil.
[3] Department of Animal Reproduction, FMVZ/USP, São Paulo, SP, Brazil.

Abstract

This study evaluated the effect of changing the day of administration of eCG and d-cloprostenol (PGF) and the replacement of estradiol benzoate (EB) with estradiol cypionate (EC) in fixed-time embryo transfer (FTET) protocols in order to simplify embryo recipient management. Three-hundred cycling heifers (Bos taurus x Bos indicus) were allocated to one of three groups. On a random day of the estrous cycle (D0), all groups received 2 mg of EB and an ear implant containing 3 mg of norgestomet, which was removed on D8. The control group (G-C; n = 100) received 400 IU of eCG and 150 μg of PGF on D5 and 1 mg of EB on D9. Group EB (G-EB; n = 100) received a similar protocol, but D5 treatments were postponed until D8. Group EC (G-EC; n = 100) received the same treatments as G-EB, except for the replacement of EB on D9 with 0.5 mg of EC on D8. Ultrasonographic determination of the number and area of corpora lutea (CLs) was performed on D17 for all groups and was followed by embryo transfer. Recipients with multiple CLs or a single CL $\geq$ 15 mm in diameter received an in vitro produced embryo. Pregnancy was diagnosed by ultrasonography 23 d after embryo transfer. Transferred-to-treated rate was similar among groups (G-C = 92.0%, G-EB = 93.0%, and G-EC = 96.0%). However, pregnant-to-transferred and pregnant-to-treated rates were higher (P = 0.03) in G-EC [61.5% (59/96) and 59.0% (59/100)] than in G-C [45.6% (42/92) and 42.0% (42/100)] and G-EB [45.2% (42/93) and 42.0% (42/100)]. As expected, multiple ovulation rate (multiple ovulation-to-treated) in G-C (31.0%) was higher (P = 0.002) than in G-EB (11.0%) and G-EC (13.0%). In conclusion, the modifications to the conventional protocol of FTET reduced animal handling and increased pregnancy rates.

Keywords: fixed-time embryo transfer, norgestomet, pregnancy rate, eCG, estradiol cypionate.

Introduction

Recipients play an important role in the success of embryo transfer (ET) programs. However, the high cost of maintaining recipients and the time and labor to accomplish all treatments in a fixed-time embryo transfer (FTET) protocol limit the widespread application and the success of this technology (Bó et al., 2002).

Previous studies have shown that by using the Ovsynch protocol (Baruselli et al., 2000b) or a progesterone (P4)-releasing intravaginal device (Tríbulo et al., 2000; Baruselli et al., 2000a; 2001; Bó et al., 2001) it is possible to precisely manipulate follicular and luteal dynamics, thus abolishing the need for estrus detection for artificial insemination (AI; Baruselli et al., 2002; Martinez et al., 2002; Bó et al., 2003) and for ET. One approach to increase the overall pregnancy rate is to elevate circulating progesterone concentrations by single or multiple ovulations induced by administration of eCG in association with a P4-releasing intravaginal device during an ovulation synchronization protocol (Fuentes and De la Fuente, 1997; Baruselli et al., 2000a; 2001; Tríbulo et al., 2002). This procedure is supported by several studies that found a positive correlation between serum progesterone concentrations and pregnancy rates in cattle (Binelli, et al., 2001; Thatcher et al., 2001; Bó et al., 2002). Recently, Looney et al. (2006) reported various protocols using eCG or hCG treatments to increase plasma progesterone concentrations and improve pregnancy rates. Higher progesterone concentrations have also been associated with increased embryo development and the capacity of the conceptus to produce interferon-τ (Mann et al., 1999), thus improving conception rates (Fuentes and De La Fuente, 1997; Baruselli et al., 2001; Santos et al., 2000; Marques et al., 2003). A luteotrophic effect of eCG was also reported in Bos indicus x Bos taurus embryo recipients by increasing both progesterone concentrations and pregnant-to-transferred rates (Baruselli et al., 2000a).

In crossbred Bos taurus x Bos indicus recipients, a conventional synchronization protocol consists of insertion of a P4-releasing intravaginal device plus administration of 2 mg of estradiol benzoate (EB) i.m. on a random day of the estrous cycle (designated D0) to synchronize follicular wave emergence, eCG to stimulate follicular growth, d-cloprostenol (PGF) on D5 to induce luteolysis, P4-releasing intravaginal device withdrawal

on D8, 1 mg of EB 24 h later to synchronize ovulation, and FTET on D17 (Baruselli *et al*., 2000a). Rodrigues *et al*. (2004) found that the norgestomet ear implant and EB treatment on D0 synchronized follicular wave emergence and ovulation in heifers destined for FTET.

One attempt to improve ovulation rate was the administration of estradiol 24 h after device withdrawal, in order to induce a synchronized LH peak (Hanlon *et al*., 1996; Macmillan and Burke, 1996; Martínez *et al*., 1999). The administration of EB increased, hastened, and synchronized ovulation of treated *Bos indicus* x *Bos taurus* heifers (Marques *et al*., 2003). Colazo *et al*. (2003) reported that estradiol cypionate (EC) administration at the time of P4-releasing intravaginal device removal induced a synchronous ovulation of the dominant follicle when the follicular wave was synchronized with estradiol-17β (E-17β) on D0.

Preliminary studies on follicular dynamics in *Bos indicus* cattle (Reis *et al*., 2004; Martins *et al*., 2005) showed that EC administration at the time of device removal promoted synchronized ovulations approximately 70 h later, similar to EB administration 24 h after device withdrawal. This finding reinforced the notion of the ability of EC to synchronize ovulation. Penteado *et al*. (2005) also compared the use of EC or EB to synchronize ovulation in fixed-time artificial insemination (FTAI) programs using P4-releasing intravaginal devices and achieved higher pregnancy rates using EC. Marques *et al*. (2004), in similar study, and Ayres *et al*. (2006), having used a norgestomet ear implant, obtained similar results with both compounds.

Despite advances in conventional recipient synchronization protocols in FTET programs, recipient synchronization still requires frequent animal handling, which may reduce program efficiency. Therefore, the aim of the present study was to compare the use of a norgestomet ear implant in addition to EB or EC treatment and to evaluate the effect of delaying the administration of eCG and PGF from D5 to D8 in FTET protocols. The purpose of these modifications was to reduce the handling and labor required.

Null hypotheses were: (1) recipients that receive eCG treatment on D5 or D8 (in a protocol using a norgestomet ear implant) would have the same efficiency (transferred-to-treated, pregnant-to-transferred, and pregnant-to-treated rates) and (2) the replacement of EB on D9 with EC on D8 would not affect efficiency.

Materials and Methods

Farm and animals

This experiment was conducted at a commercial farm in southwest Brazil (22° 01' 27" S and 47° 53' 19" W) during August 2005. Crossbred *Bos indicus x Bos taurus* heifers (n = 300) without previous service, from 24 to 32 mo of age, having a mean body weight of 315 kg, with ovarian cyclicity (presence of a corpus luteum confirmed by ultrasonography), free from brucellosis and tuberculosis, and vaccinated against leptospirosis, foot and mouth disease, clostridiosis, IBR, BVD, PI₃, and BRSV, were selected. The experiment was performed in three replicates (n = 126, n = 120, and n = 54, respectively). All animals were kept on pasture, supplemented with good quality mineral salt, and had *ad libitum* access to water.

Ovulation synchronization and treatments

At the beginning of each replicate, heifers were randomly allocated to one of three treatment groups (n = 100/group). At unknown stages of the estrous cycle (D0), all heifers received a norgestomet ear implant (Crestar®, Intervet, Netherlands) plus 2 mg of EB i.m. (Estrogin, Farmavet, Brazil) given concurrently. On D5, the control group (G-C) animals received 400 IU of eCG i.m. (Folligon®; Intervet, Netherlands) and 150 μg of d-cloprostenol i.m. (PGF; Preloban®; Intervet, Netherlands). On D8, the ear implant was removed and 1 mg of EB (i.m.) was administered 24 h later (D9; Fig. 1). Group EB (G-EB) received the same protocol used for G-C, except for the administration of eCG and PGF at the time of ear implant removal (D8). Group EC (G-EC) received the same protocol as G-EB, but the EB given on D9 was replaced by the administration of 0.5 mg of EC i.m. (E.C.P.®; Pfizer, Brazil) on D8.

In the absence of estrus detection, D10 was considered the day of estrus. Embryo transfer of an *in vitro* produced embryo was performed for all groups on D17 for recipients with a corpus luteum (CL).

Ultrasonographic examinations

On D17 (day of ET), both ovaries were examined using transrectal ultrasonography (Aloka SSD-500 console equipped with a 5 MHz linear transducer; Tokyo, Japan) to detect the presence of CLs, number of CLs, and the area of a single CL. In heifers with a single CL, the image with the largest CL diameter was frozen, and its area (including the cavity, when present) was estimated using software integrated in the scanner. In heifers with more than one CL, area of the CL was not measured. Only heifers with more than one CL or a single CL ≥ 15 mm in diameter were selected to receive an embryo. Ultrasonographic pregnancy diagnosis was done 23 d after ET.

In vitro embryo production and transfer

Embryos were produced *in vitro* by a commercial Brazilian company, following standard protocols as previously reported (Dayan et al., 2002). Twenty-one donors and semen of four bulls were used (21 different in vitro fertilization processes) to produce three-hundred embryos which were transferred during the experiment. The same sires were used in all replicates, and only Grade-1 blastocysts were used. All embryos were transferred non-surgically into the uterine horn ipsilateral to the CL by the same veterinarian on D17.

Group control (G-C):
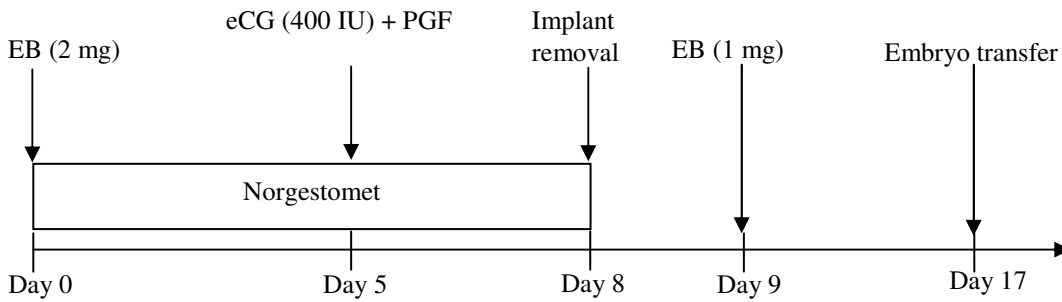
Group estradiol benzoate (G-EB):
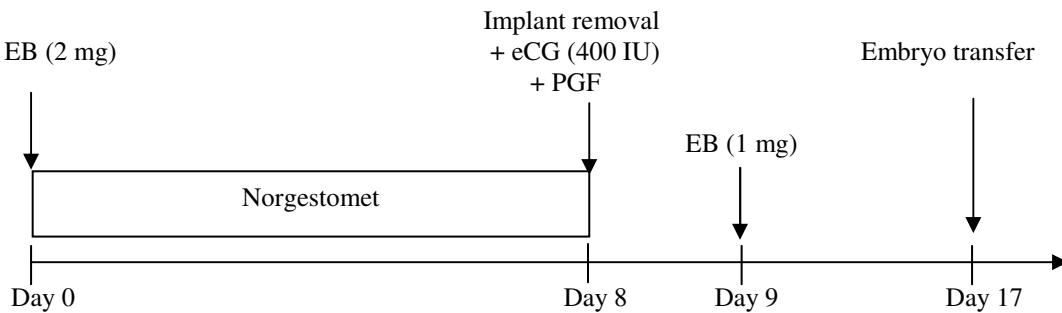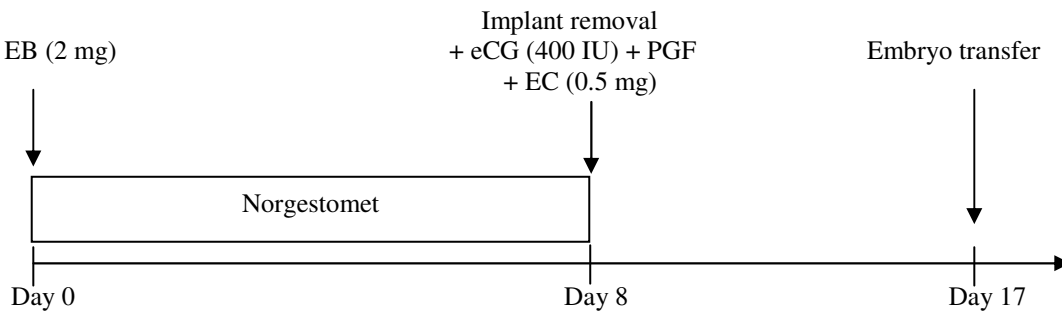

Group estradiol cypionate (G-EC):

Figure 1. Schemes of treatment protocols (G-C, G-EB and G-EC) for fixed-time embryo transfer recipients. (EB, estradiol benzoate; EC, estradiol cypionate).

Statistical analyses

Statistical analyses were performed using the SAS System for Windows (SAS, 2000). Effects of replicates, sires, and interactions between replicates and treatments were analyzed. Discrete Dependent variables (i.e. number of CLs and area of a single CL) were analyzed by ANOVA using the GLM procedure. A chi-square test was used to compare rates of transferred-to-treated, pregnant-to-transferred, pregnant-to-treated, and multiple ovulations (multiple ovulation-to-treated) among groups.

Results

There were no significant effects of replicate, sire, or an interaction between replicate and treatment. The effect of treatment on the number of CLs and CL area, transferred-to-treated, pregnant-to-transferred, pregnant-to-treated, and multiple ovulation rates are shown (Table 1). Heifers from Group G-C had the highest multiple ovulation response and consequently, the highest mean number of CLs. Treatment with eCG on D5 (G-C) produced a greater CL area compared to eCG administration on D8 (G-EB and G-EC, Table 1). The proportion of recipients that received an embryo to those treated was not influenced ($P > 0.05$) by the alternative treatments when compared to the conventional one. However, the overall pregnant-to-transferred and pregnant-to-treated rates were higher in recipients from group G-EC ($P < 0.05$, Table 1).

Table 1. Mean (± SD) outcomes in three groups of recipient heifers given a norgestomet ear implant and 2 mg of EB (Day 0).

	Groups [1]			P value
	G-C	G-EB	G-EC	
Transferred-to-treated rate (%)	92.0 (92/100)	93.0 (93/100)	96.0 (96/100)	> 0.05
Pregnant-to-transferred rate (%)	45.6 (42/92)[a]	45.2 (42/93)[a]	61.5 (59/96)[b]	0.03
Pregnant-to-treated rate (%)	42.0 (42/100)[a]	42.0 (42/100)[a]	59.0 (59/100)[b]	0.02
Mean number of corpora lutea	1.51 ± 0.89[b]	1.11 ± 0.32[a]	1.19 ± 0.64[a]	0.0002
Mean area of single corpus luteum (mm^2)	3.55 ± 0.99[b]	3.14 ± 0.86[a]	3.21 ± 0.94[a]	0.03
Multiple ovulation rate (%)	31.0 (31/100)[b]	11.0 (11/100)[a]	13.0 (13/100)[a]	0.002

[1] In group G-C, heifers were given 400 IU of eCG and PGF on Days 5 (implant removal on Day 8), and 1 mg of EB on Day 9. In the other two groups, Day 5 treatments were given on Day 8, with 0.5 mg of EC on Day 8 (in lieu of EB on Day 9) in group G-EC.
[a, b] Different letters within rows differ significantly.

Discussion

The impetus for this study was the possibility to simplify available protocols for FTET. Both null hypotheses were supported; changing the day of eCG administration from D5 (G-C) to D8 (G-EB) in *Bos indicus* x *Bos taurus* heifers treated with a norgestomet ear implant and replacing EB on D9 with EC on D8 had no effect on efficiency of FTET programs. These changes eliminated handling the cattle on two occasions. Furthermore, pregnancy rates were significantly higher in animals in which ovulation was synchronized using EC at the time of implant removal (G-C) than in those of the other two groups.

In this study, we chose to use a norgestomet ear implant to minimize suppression of follicular growth. Norgestomet does not inhibit LH pulse frequency in contrast to a P4-releasing intravaginal device, which suppressed LH pulsatility and follicular growth as shown by the elegant findings of Kinder *et al*. (1996) and Rathbone *et al*. (2001). In a previous study of follicular dynamics that used cycling Nelore (*Bos indicus*) heifers treated with a norgestomet ear implant or P4-releasing intravaginal device (CIDR), follicular growth, maximum diameter of the dominant follicle, and ovulation rate were higher in norgestomet-treated heifers (Sá Filho *et al*., 2005). Furthermore, Torres-Junior *et al*. (2005) synchronized cycling heifers with a norgestomet ear implant with or without PGF administration on D0 and the data supported that of the aforementioned work. The addition or not of PGF did not significantly effect dominant follicle diameter on D8, dominant follicle maximum diameter, ovulatory follicle diameter, ovulation rate, or the interval between implant removal and ovulation. The authors suggested a lack of a suppressive effect of the norgestomet ear implant when the animals were treated either with or without the presence of CL.

Similar to our study, Reis *et al*. (2004) and Nasser *et al*. (2004) changed the day of eCG treatment from D5 to D8 but used a P4-releasing intravaginal device; they reported a reduction in transferred-to-treated, pregnant-to-transferred, and pregnant-to-treated rates. This reduction may have been due to higher progesterone concentrations reached during the treatment (P4-releasing intravaginal device plus a CL) resulting in suppression of the dominant follicle (Adams *et al*., 1992; Rathbone *et al*., 2001). This inhibitory effect of progesterone on follicular growth in synchronized heifers was confirmed by Carvalho (2004), and it was more pronounced in *Bos indicus* cattle; these animals, in particular, had higher concentrations of progesterone compared to *Bos taurus* cattle subjected to a similar treatment and management. Therefore, the success achieved in the present study following changing the day of eCG treatment from D5 to D8 was attributed to the use of norgestomet, but this is not recommended for progesterone-based protocols.

Estradiol cypionate treatment at the time of ear implant removal produced a satisfactory transferred-to-treated rate and a higher pregnancy rate compared to G-C and G-EB. The higher pregnancy rate obtained in the EC-treated group may be related to increased time of exposure to estradiol during the period of follicular development immediately preceding ovulation, which is critical to enhance luteal lifespan (Day *et al*., 1990). Attainment of a threshold concentration of estradiol during the pre-ovulatory period is necessary to allow adequate CL formation and maintenance. Potential sites of action for estradiol are numerous, including direct actions on follicular development, effects on uterine function during either the pre-or post-ovulatory period, and alteration of endocrine support for folliculogenesis (Day *et al*., 1990).

Based on studies of follicular dynamics, it does not seem to be relevant whether EC is given at device withdrawal or EB is given 24 h later; the efficiency to synchronize ovulation 70 h after progesterone source

removal was similar for both compounds (Reis *et al*., 2004; Martins *et al*., 2005). In a recent study using EC for FTAI in Nelore cows, similar pregnancy rates were achieved with EC at the time of implant removal (0.5 or 1.0 mg) or EB 24 h later (Ayres *et al*., 2006). Similarly, in the present study, EC at the time of implant removal was highly efficacious in a FTET protocol.

Estradiol cypionate was previously used to synchronize ovulation in heifers in FTAI protocols that used a P4-releasing intravaginal device (Colazo *et al*., 2003). In that study, synchronized follicular-wave emergence using a P4-releasing intravaginal device plus E-17β on D0 and induction of ovulation using 0.5 mg of EC, given at device removal or 24 h later, resulted in synchronous ovulation and acceptable pregnancy rates. These data were in agreement with Marques *et al*. (2003), who used the same protocol in Nelore cows. Furthermore, similar to our results, Penteado *et al*. (2005) reported higher pregnancy rates with 1.0 mg of EC at P4-releasing intravaginal device removal than with EB 24 h later in FTAI protocols using Nelore cows (49.4% vs. 41.4%, respectively). Despite such favorable results in the present study, further studies are necessary to confirm whether the improvement in pregnancy rate could be attributed to EC.

In summary, the protocol that used a norgestomet ear implant with EB at implant insertion and PGF, eCG, and EC at implant removal on D8 was successfully used for FTET in *Bos indicus x Bos taurus* recipients. This protocol resulted in a satisfactory proportion of recipients selected and higher pregnancy rates when compared to G-C and G-EB, thus optimizing the use of recipients and facilitating the utilization of ET technology. These changes not only reduced the frequency of animal handling but also increased pregnancy rates.

Acknowledgments

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