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Effect of fixed-time embryo transfer on reproductive efficiency in high-producing repeat-breeder Holstein cows

C.A. Rodrigues[a], A.A. Teixeira[b], R.M. Ferreira[b], H. Ayres[b], R.F. Mancilha[a], A.H. Souza[b], P.S. Baruselli[b,*]

[a] SAMVET Embriões, Rua Getúlio Vargas 300, CEP 13560-000, São Carlos, SP, Brazil
[b] Departamento de Reprodução Animal, FMVZ-USP, Rua Prof. Dr. Orlando Marques de Paiva 87, CEP 05508-000, São Paulo, SP, Brazil

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ABSTRACT

The aim of the present study was to compare a synchronization of time of ovulation protocol for fixed-timed embryo transfer (FTET) with the usual administration of a single dose of prostaglandin associated with detection of estrus. Also, the effect of the presence of CL at the beginning of FTET protocol was evaluated. Lactating Holstein cows ($n = 651$) with three previous artificial inseminations were classified according to presence or absence of a corpus luteum (CL). Cows with a CL were randomly assigned to two additional treatments and submitted to embryo transfer after detection of estrus (PGF-Estrus) or FTET (FTET-CL). Cows without CL were allocated to the FTET-NoCL treatment. On a random day of the estrous cycle (Day 0), cows in the PGF-Estrus treatment ($n = 229$) were treated with 150 μg d-cloprostenol (PGF) i.m. followed by detection of estrus from Day 1 through Day 5 after PGF. Embryos were transferred 6–8 days after estrus detection. Cows in the FTET-CL ($n = 208$; presence of CL) and FTET-NoCL ($n = 214$; absence of CL) treatments received a norgestomet ear implant plus 2 mg estradiol benzoate (EB) and 50 mg progesterone i.m. on Day 0. On Day 8, the implant was removed and 400 IU eCG, 150 μg d-cloprostenol and 1 mg estradiol cypionate i.m. were administered. No detection of estrus was performed and Day 10 was arbitrarily considered as the estrus day. Ultrasonographic exams were performed in all recipients and only cows with a single CL ≥ 15 mm or multiple CL received a fresh or frozen–thawed embryo on Day 17. Pregnancy was diagnosed by ultrasonography at 30 and 60 days of pregnancy. When FTET and PGF-Estrus were compared, the proportion of cows receiving an embryo (recipients transferred-to-treated rate) was greater in the FTET-CL (75.0% (156/208) than in PGF-Estrus (34.5%, 79/229; $P < 0.0001$) treatment. Pregnancy rate (60 days) was also greater in FTET-CL (29.3%, 61/208) when compared to PGF-Estrus (16.2%, 37/229; $P = 0.001$). However, no differences were found in pregnancy loss [PGF-Estrus = 11.9% (5/42), FTET-CL = 9.0% (6/67); $P = 0.62$] and circulating progesterone concentration at embryo transfer [PGF-Estrus = 4.02 ± 0.52 ng/mL ($n = 25$), FTET-CL = 3.33 ± 0.32 ng/mL ($n = 27$); $P = 0.25$] among these treatments. The presence of CL at the beginning of FTET protocol resulted greater transferred-to-treated rate [FTET-CL = 75.0% (156/208) vs. FTET-NoCL = 61.2% (131/214); $P = 0.003$], but showed no effect on pregnancy rate at 60 days [FTET-CL = 29.3% (61/208) vs. FTET-NoCL = 22.9% (49/214); $P = 0.13$], pregnancy loss [FTET-CL = 9.0% (6/67) vs. FTET-NoCL = 2.0% (1/50); $P = 0.15$] and circulating progesterone concentration at ET [FTET-CL = 3.33 ± 0.32 ng/mL ($n = 27$) compared

* Corresponding author. Tel.: +55 11 3091 7674; fax: +55 11 3091 7412.
E-mail address: barusell@usp.br (P.S. Baruselli).

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to FTET-NoCL = 3.44 ± 0.40 ng/mL (n = 2 9); P = 0.82]. In conclusion, the protocol for synchronization of time of ovulation using norgestomet ear implant, EB and eCG increased recipients transferred-to-treated and pregnancy rates in high-producing repeat-breeder Holstein cows. Also, recipients without CL at the beginning of the time of ovulation synchronization treatment resulted in similar pregnancy rate as recipients with CL submitted to FTET protocol. Thus, the suggested protocol allowed the performance of FTET, without the need for detection of estrus, simplifying the reproductive management and increasing the reproductive efficiency in repeat-breeder Holstein recipients.

1. Introduction

Repeat-breeder cows are usually defined as sub-fertile animals without any anatomic or infectious abnormality and that require three or more services to become pregnant. These cows are characterized by poor fertilization rates (Graden et al., 1968; O'Farrell et al., 1983) and/or early embryonic loss (Linares, 1981; Gustafsson and Larsson, 1985). Thus, embryo transfer (ET) can be potentially used to minimize the eventual effects of uterine environment and lactation on early embryonic development, promoting greater pregnancy rates and, consequently, avoiding early embryonic death.

Success of embryo transfer depends on the efficiency of detection of estrus in recipients. However, only up to 50% of the cows in estrus are detected (Lyimo et al., 2000; Van Eerdenburg et al., 2002). Poorer results may be achieved when high-producing lactating cows are used as recipients, because of the deleterious effects of high milk yield increasing liver metabolism of estradiol and progesterone and consequently reducing expression of estrus and pregnancy rates (Sangsritavong et al., 2002; Lopez et al., 2004; Mann and Lamming, 2000). Several studies also reported a positive association between serum progesterone concentrations, embryonic development, capacity of the conceptus to secrete interferon-τ (Mann et al., 1999), and pregnancy rates in cattle (Santos et al., 2000; Baruselli et al., 2001; Binelli et al., 2001; Thatcher et al., 2001; Bó et al., 2002; Nasser et al., 2004).

One approach to overcome these problems associated with recipients reproductive efficiency is the use of hormonal protocols designed to control both follicular and luteal dynamics, abolishing the need for estrus detection for ET (Baruselli et al., 2000a,b, 2001; Tríbulo et al., 2000; Bó et al., 2001; 2002; Ferreira et al., 2006) Additionally, Rodrigues et al. (2007a) reported in a retrospective study that conception rates in repeat-breeder Holstein cows were greater after ET (41.7%; 1609/3858) than after artificial insemination [AI; 17.9% (1019/5693)], indicating that ET may be an effective alternative to achieve satisfactory conception rates throughout the year, especially during periods of heat stress.

Therefore, the aim of the present study was to compare the use of a single dose of PGF plus estrus detection with a FTET protocol in lactating Holstein repeat-breeder cows. The hypotheses were: (1) recipients treated for FTET would have greater transferred-to-treated and pregnancy rates than cows treated with a single dose of PGF associated with detection of estrus; (2) recipients submitted to FTET without CL at the beginning of the protocol would have similar reproductive efficiency (transferred-to-treated and pregnancy rates) of recipients with CL.

2. Materials and methods

2.1. Farm and animals

This experiment was conducted in a commercial farm in southwest Brazil (22°01′27″S/47°53′19″W) during the autumn (March through June) and winter (June through August), 2006. Lactating Holstein (*Bos taurus*) cows (n = 651; 206 primiparous and 445 multiparous) housed in free stall facilities were used. All recipients were considered repeat-breeders (having ≥three artificial inseminations) averaging 358.7 ± 152.6 (average ± S.D.) days in milk and milk production of 25.5 ± 8.2 kg/day (average ± S.D.). Cows were milked three times daily at approximately 8 h intervals and fed with silage as forage and a corn and soybean meal-based concentrate, sufficient to exceed the nutritional requirements of lactating dairy cows (NRC, 2001). All procedures, including injections, timed AI, ovarian ultrasonography and embryo transfer were approved by the Bioethic Commission of the School of Veterinary Medicine and Zootechny of University of São Paulo, São Paulo.

2.2. Experimental design

Non-pregnant females were evaluated by rectal palpation and classified according to the presence or absence of a CL. Cows with CL were randomly assigned to one of two treatments and submitted to embryo transfer after detection of estrus (PGF-Estrus) or FTET (FTET-CL). Cows without CL were allocated to the FTET-NoCL treatment and submitted to FTET. At a random day of the estrous cycle (Day 0), cows in the PGF-Estrus treatment (PGF-Estrus; n = 229) were treated with 150 μg of d-cloprostenol (PGF; Preloban®, Intervet, Brazil) i.m. followed by detection of estrus from Day 1 through Day 5 after PGF. Embryos were transferred 6–8 days after detected estrus. Cows in the FTET-CL (n = 208; presence of CL) and FTET-NoCL (n = 214; absence of CL) treatments received a norgestomet ear implant (Crestar®, Intervet, Brazil), 2 mg of estradiol benzoate (EB; Estrogin®, Farmavet, Brazil), and 50 mg of progesterone (Progesterona, Index Farmacêutica, Brazil) i.m. on Day 0. On Day 8, the implant was removed and 400 IU of eCG (Folligon®, Intervet, New Zeeland), 150 μg of d-cloprostenol (PGF; Preloban®, Intervet, Brazil) and 1 mg of estradiol cypionate (EC; E.C.P.®, Pfizer, Brazil) i.m. was administered. No detection of estrus was performed and

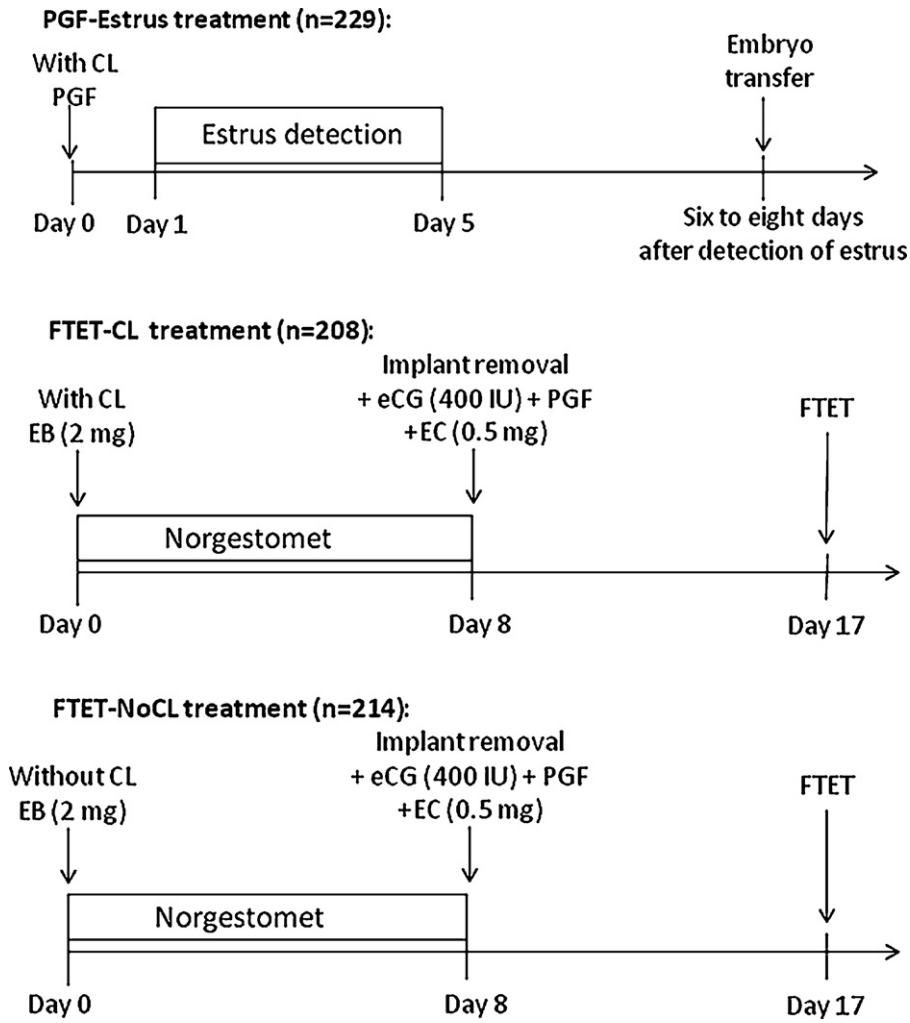

Fig. 1. Experimental design.

Day 10 was considered as the day of estrus. Embryos were transferred on Day 17 (Fig. 1).

2.3. Embryo production, freezing and transfer

Embryos were produced by a Brazilian embryo transfer company (SAMVET Embriões, São Carlos, São Paulo, Brazil). Non-lactating Holstein cows were submitted to a superovulation protocol and flushed (non-surgical technique) to produce and collect the embryos. All procedures were performed by the same technician. Recovered embryos ($n = 366$) were kept in cell culture dishes containing holding solution TQC® (AB Technology, Nutricell, Brazil) during embryo evaluation (stage of development and grad) under stereomicroscope (50×). Embryos were graded according to the Manual of the International Embryo Transfer Society (Robertson and Nelson, 1998). Only morulas, early blastocysts and blastocysts that were grades 1 and 2 were selected for embryo transfer or to be frozen. Both fresh and frozen–thawed embryos used in this trial were produced by the previously described method and homogenously distributed among the treatments. Embryos were frozen in ethylene glycol TQC® (AB Technology, Nutricell, Brazil), using an automatic freezing machine (TK 2000®, program P1-01, BOV/E/O1; TK and Nutricell, Brazil). When the procedure was completed, the straws were transferred to liquid nitrogen (−196 °C), where they were stored until required for embryo transfer. To thaw the embryos, straws were removed from the liquid nitrogen, kept at air temperature for 10 s and then submerged in water at 25 °C for 10 s more. Recipients were submitted to epidural anesthesia using lidocaine chloridrate 2%. All embryos were transferred non-surgically into the uterine horn ipsilateral to the CL, by the same veterinarian, on Day 17 for cows in the FTET treatments and 6–8 days after estrus detection for cows in Group PGF-Estrus.

2.4. Blood samples and progesterone assay

On the day of ET a subset of cows from both treatments ($n = 81$) had blood samples arbitrarily collected by coccygeal venipuncture, using vials with heparin

Table 1
Effect of different treatments for embryo transfer (PGF + detection of estrus compared to FTET) on fertility rates of high-producing Holstein repeat-breeder recipients with CL at the beginning of the treatment.

	Treatments		
	PGF-Estrus	FTET-CL	*P* value
Estrus detection rate (%)	59.4 (136/229)	–	
Transferred-to-treated rate (%)	34.5 (79/229)	75.0 (156/208)	<0.0001
Pregnant-to-transferred rate at 30 days (%)	53.2 (42/79)	42.9 (67/156)	0.14
Pregnant-to-transferred rate at 60 days (%)	46.8 (37/79)	39.1 (61/156)	0.26
Pregnant-to-treated rate at 30 days (%)	18.3 (42/229)	32.2 (67/208)	0.001
Pregnant-to-treated rate at 60 days (%)	16.2 (37/229)	29.3 (61/208)	0.001
Pregnancy loss (%)	11.9 (5/42)	9.0 (6/67)	0.62

PGF-Estrus = PGF i.m. (Day 0), detection of estrus (Day 1 to Day 5), embryo transfer (6–8 days after detection of estrus).
Nor-CL = norgestomet ear implant + 2 mg estradiol benzoate (Day 0), implant removal + 400 IU eCG + 1.0 mg estradiol cypionate + PGF (Day 8), fixed-time embryo transfer (Day 17).

(Vacutainer®, Becton-Dickinson, Franklin Lakes, NJ, USA). Tubes were centrifuged and plasma was set apart and immediately stored at −20 °C for subsequent analysis. Plasma concentrations of progesterone were determined by a solid-phase RIA kit containing antibody-coated tubes and 125 I-labelled progesterone (Coat-A-Count; Diagnostic Products Corporation, Los Angeles, CA, USA) as described previously (Gümen and Wiltbank, 2005). The assays were performed at the Hormonal Dosage Laboratory at University of São Paulo (São Paulo, Brazil). The intra-assay coefficient of variation was 3%.

2.5. Ultrasonographic examinations

On day of ET, cows had their ovaries examined by transrectal ultrasonography (Aloka SSD 500®, 5 MHz linear transducer, Tokyo, Japan) in order to establish the presence of CL, the number and area of single CL. In cows with a single CL, the image with the largest CL diameter was frozen and the area was estimated using software integral to the scanner. In cows with more than one CL, the area of the CL was not determined. Only cows with multiple CL or a single CL ≥ 15 mm diameter were selected to receive an embryo. Transrectal ultrasonography was used to diagnose pregnancy at 23 and 53 (30 and 60 days of pregnancy) days after embryo transfer.

2.6. Statistical analysis

Continuous variables were analyzed by MIXED procedure of SAS (Littell et al., 1996) and binomially distributed data (i.e. transferred-to-treated, pregnant-to-transferred, pregnant-to-treated and multiple ovulation rates) were analyzed by logistic regression using the GLIMMIX proce-

Table 2
Effect of the presence of CL at the beginning of the protocol for FTET on fertility rates of high-producing Holstein repeat-breeder recipients.

	Treatments		
	FTET-CL	FTET-NoCL	*P* value
Transferred-to-treated rate (%)	75.0 (156/208)	61.2 (131/214)	0.003
Pregnant-to-transferred rate at 30 days (%)	42.9 (67/156)	38.2 (50/131)	0.41
Pregnant-to-transferred rate at 60 days (%)	39.1 (61/156)	37.4 (49/131)	0.77
Pregnant-to-treated rate at 30 days (%)	32.2 (67/208)	23.4 (50/214)	0.04
Pregnant-to-treated rate at 60 days (%)	29.3 (61/208)	22.9 (49/214)	0.13
Pregnancy loss (%)	9.0 (6/67)	2.0 (1/50)	0.15

Nor-CL = norgestomet ear implant + 2 mg estradiol benzoate (Day 0), implant removal + 400 IU eCG + 1.0 mg estradiol cypionate + PGF (Day 8), fixed-time embryo transfer (Day 17). All cows with CL on Day 0.
Nor-NoCL = norgestomet ear implant + 2 mg estradiol benzoate (Day 0), implant removal + 400 IU eCG + 1.0 mg estradiol cypionate + PGF (Day 8), fixed-time embryo transfer (Day 17). All cows without CL on Day 0.

Table 3
Effect of different treatments for embryo transfer (PGF + detection of estrus compared to FTET) on corpus luteum and plasma concentration of progesterone (P4) of high-producing Holstein repeat-breeder recipients with CL at the beginning of the treatment.

	Treatments		
	PGF-Estrus	FTET-CL	*P* value
Average number of CL	1.51 ± 0.06 (*n* = 79)	1.58 ± 0.05 (*n* = 156)	0.41
Average area of CL (cm^2)	4.23 ± 0.26 (*n* = 40)	3.73 ± 0.14 (*n* = 78)	0.06
Multiple ovulation rate (%)	50.6 (39/79)	50.0 (78/156)	0.93
Plasma concentration of P4 at embryo transfer (ng/mL)	4.02 ± 0.52 (*n* = 25)	3.33 ± 0.32 (*n* = 27)	0.25

Only animals with concentration of progesterone above 1 ng/mL were use in the present analysis.
PGF-Estrus = PGF i.m. (Day 0), detection of estrus (Day 1 to Day 5), embryo transfer (6–8 days after detection of estrus).
Nor-CL = norgestomet ear implant + 2 mg estradiol benzoate (Day 0), implant removal + 400 IU eCG + 1.0 mg estradiol cypionate + PGF (Day 8), fixed-time embryo transfer (Day 17).

Table 4
Effect of the presence of CL at the beginning of the protocol for FTET on corpus luteum and plasma concentration of progesterone (P4) of high-producing Holstein repeat-breeder recipients.

	Treatments		
	FTET-CL	FTET-NoCL	*P* value
Average number of CL	1.58 ± 0.05 (*n* = 156)	1.60 ± 0.07 (*n* = 131)	0.83
Average area of CL (cm^2)	3.73 ± 0.14 (*n* = 78)	3.58 ± 0.15 (*n* = 61)	0.45
Multiple ovulation rate (%)	50.0 (78/156)	46.6 (70/131)	0.54
Plasma concentration of P4 at embryo transfer (ng/mL)	3.33 ± 0.32 (*n* = 27)	3.44 ± 0.40 (*n* = 29)	0.82

Only animals with concentration of progesterone above 1 ng/mL were use in the present analysis.
Nor-CL = norgestomet ear implant + 2 mg estradiol benzoate (Day 0), implant removal + 400 IU eCG + 1.0 mg estradiol cypionate + PGF (Day 8), fixed-time embryo transfer (Day 17). All cows with CL on Day 0.
Nor-NoCL = norgestomet ear implant + 2 mg estradiol benzoate (Day 0), implant removal + 400 IU eCG + 1.0 mg estradiol cypionate + PGF (Day 8), fixed-time embryo transfer (Day 17). All cows without CL on Day 0.

dure of SAS. The experimental unit "cow" was included in the statistical model as a variable of random effect. Variables initially considered for inclusion in the models were treatment, season of the year, days in milk, parity (primiparous compared to multiparous), milk production, number of previous inseminations, synchrony between donor and recipient (±24 h), total number of CL, area of single CL, embryo quality grade, embryo stage and embryo condition (fresh or frozen–thawed) and interactions among these variables. Also, the presence of CL was included in the models when FTET-CL compared to FTET-NoCL treatments were analyzed. The final logistic regression model removed non-significant variables by a backward elimination based on the Wald statistics criterion, when $P > 0.20$. Probabilities with $P < 0.05$ were considered as significant.

3. Results

No effect of interaction between season of the year and treatment ($P > 0.10$) was found for any analyzed variable. Thus, results for both seasons were grouped and analyzed together. Also, no difference among treatments was detected for days in milk (358.7 ± 6.0 days on average), milk production (25.5 ± 0.3 l of milk per day), number of lactations (2.3 ± 0.1) and number of previous services (5.3 ± 0.1, mean ± S.E.M.).

The detection of estrus rate found for cows in PGF-Estrus treatment was approximately 60% (136/229; Table 1); however, the proportion of cows receiving an embryo (transferred-to-treated rate) was lower (34.5%, 79/229) than in FTET-CL [75.0% (156/208); $P < 0.0001$] treatment (Table 1). Also, pregnancy rates at both 30 and 60 days were greater ($P = 0.001$) in FTET-CL than PGF-Estrus cows (Table 1).

The absence of CL on Day 0 of the protocol resulted in a 14% decrease ($P < 0.01$) in recipients transferred-to-treated rate in FTET recipients [FTET-CL = 75.0% and FTET-NoCL = 61.2%; Table 2) and reduced pregnancy rate at 30 days [FTET-CL = 32.2% and FTET-NoCL = 23.4%; $P = 0.003$; Table 2). However, the presence of CL before starting the hormonal treatments seemed not to affect pregnant-to-transferred at 30 and 60 days and pregnancy rates (pregnant-to-treated) at 60 days (Table 2). Additionally, pregnancy loss was similar among cows in PGF-Estrus and FTET-CL treatments (Table 1) and among cows with or without a CL on Day 0 of the FTET protocol (Table 2).

The effect of treatments on CL number and area are summarized in Tables 3 and 4. The area of single CL in PGF-Estrus cows tended ($P = 0.06$) to be greater than FTET-CL cows. However, similar CL area was observed in cows submitted to FTET with or without a CL on Day 0 of the protocol. Also, number of CL, multiple ovulation rate and, plasma concentrations of progesterone on embryo transfer day were similar among PGF-Estrus and FTET-CL and among cows with or without a CL submitted to FTET ($P > 0.05$; Tables 3 and 4).

No effects of stage of embryo development or embryo quality grade were observed on pregnancy rates at 30 and 60 days of pregnancy and on pregnancy loss ($P > 0.05$). Similarly, no effect of embryo condition (fresh or frozen–thawed) was detected.

4. Discussion

The first hypothesis of the present study was confirmed. Recipients in the FTET-CL treatment had greater pregnancy rates than cows in PGF-Estrus. However, the second hypothesis was partially confirmed. As expected, recipients without CL at initiation of the time of ovulation synchronization treatment had similar pregnancy rate as females with a CL that were submitted to the same protocol. However, transferred-to-treated rate was greater in recipients with CL at the beginning of the protocol.

The present study had a 59.4% detection of estrus rate after a single PGF treatment, which was considered above the average found for high-producing dairy cows. Previous studies showed that standing behavioral estrus is observed in less than 50% of the cows in estrus (Lyimo et al., 2000; Van Eerdenburg et al., 2002). In fact, in lactating Holstein cows, detection of estrus is difficult due to a number of factors including milk production. Lopez et al. (2004) verified that an increase on milk production produced a negative effect on characteristics of behavioral estrus, and also reduced plasma concentrations of estradiol on estrus day. The lesser plasma concentrations of estradiol were related to the greater hepatic metabolism found in these cows, and resulted poor expression of estrus (Sangsritavong et al., 2002).

In the present study, although the detection of estrus rate was greater than expected, transferred-to-treated rate in PGF-Estrus was less than rates found in FTET-CL cows. This result can be explained by errors on detection of estrus

due to false-positive cows (Reimers et al., 1985; Nebel et al., 1987; Grimard et al., 2006) or possible ovulation failures after estrus (López-Gatius et al., 2005; Vasconcelos et al., 2006).

Also, some researchers found CL maturity at the time of PGF treatment influenced the luteolytic response and that PGF administered 5–6 days after estrus was not able to induce luteolysis efficiently (Momont and Seguin, 1984). These data can also explain the low transferred-to-treated rate found for cows with CL receiving a single administration of PGF at random stages of the estrous cycle. Additionally, when PGF injection is used alone, cows can show estrous in 1–6 days interval after treatment (Macmillan and Henderson, 1984), and this large interval sometimes results in few number of recipients selected for embryo transfer (ET), due to an asynchrony between recipient and donor at ET day.

Recently, one approach to solve problems related to the detection of estrus and reduced transferred-to-treated rates was the use of hormonal protocols to control follicular and luteal dynamics and to promote synchronization of time of ovulation for embryo transfer without the need of detection of estrus (Baruselli et al., 2000a,b, 2001; Tríbulo et al., 2000; Bó et al., 2001; 2002; Nasser et al., 2004; Ferreira et al., 2006). Furthermore, the utilization of eCG in the current study was based in several previous studies in which the addition of eCG to the synchronization of time of ovulation protocol using progesterone device resulted greater proportion of selected recipients and satisfactory pregnancy rates after FTET (Fuentes and De la Fuente, 1997; Baruselli et al., 2001; Bó et al., 2002; Tríbulo et al., 2002).

The beneficial effect of eCG treatment on pregnancy is related to the increased plasma concentration of progesterone, which may stimulate the conceptus capacity of secreting interferon-τ and thus facilitate the maternal recognition of pregnancy (Mann et al., 1999). Some researchers used eCG (400 IU) at progesterone source removal aiming to stimulate follicular growth and increase the size of the ovulatory follicle (Sá Filho et al., 2004). Furthermore, eCG administration enhanced the steroidogenic capacity of the CL in crossbred Angus × Nelore (*Bos taurus* × *Bos indicus*; Marques et al., 2003), Nelore (*Bos indicus*; Baruselli et al., 2004) and high-producing Holstein cows (*Bos taurus*; Souza et al., 2009). This verified that cows treated with eCG had greater plasma concentrations of progesterone during the diestrus following synchronization of ovulation protocol.

In the present experiment, increased plasma concentrations of progesterone were not observed in cows receiving eCG (FTET-CL) when compared to cows that did not receive eCG (PGF-Estrus) which is inconsistent with previous findings. However, recipients not treated with eCG were not exposed to the same synchronization protocol, enabling such comparison. Despite progesterone concentrations were similar among treatments, cows detected in estrus showed greater CL area when compared to FTET-CL cows. This result may be related to the greater size of the ovulatory follicles in recipients that had ovulations with no induction (PGF-Estrus), compared to cows that were in FTET protocols and were exposed to exogenous hormones that induce ovulation. Although CL area was greater in PGF-Estrus cows, the similarity considering plasma concentrations of progesterone is probably related to eCG administration in cows from FTET-CL treatment. The luteotropic effect of this hormone could have lead to increased concentrations of progesterone (Baruselli et al., 2000a), reducing the effect of decreased CL area on progesterone production (Baruselli et al., 2003; Vasconcelos et al., 2001).

The reduced fertility in repeat-breeders can be explained by an endocrine disequilibrium between conceptus and oviduct and/or uterus, resulting in early embryo death (Albihn et al., 1989, 1991). Thus, embryo transfer can be considered as a potential tool to minimize the effects of uterus on early embryo development (during the first 7 days), leading to greater pregnant-to-transferred and pregnancy rates and avoiding early embryo death, as reviewed by Lucy (2001) in non-repeat-breeder cows and showed by Rodrigues et al. (2007a) in repeat-breeder cows. Supporting this evidence, the present study showed that using ET, lactating repeat-breeder cows showed pregnant-to-transferred rate around 40%, in all experimental treatments.

As explained, considering cows with CL at the beginning of the hormonal treatments (PGF-Estrus and FTET-CL), cows that received PGF and were submitted to estrus detection presented lesser transferred-to-treated rate when compared to time of estrus synchronized females. As pregnant-to-transferred rate was similar among the treatments, consequently, pregnancy rate was greater when FTET protocols were used (pregnancy rate = pregnant-to-transferred rate × transferred-to-treated rate). Also, the FTET protocol enabled the utilization of cows without CL as recipients. These cows would not respond to PGF administration and thus would not be included at the embryo transfer program.

Another problem related to reproductive efficiency of high-producing dairy herds is the considerable pregnancy loss, either in repeat-breeder cows (Ferreira, 2008) and non-repeat-breeders (Vasconcelos et al., 1997; Pursley et al., 1998). However, in the current experiment the pregnancy loss was considered low (∼10%) for high-producing repeat-breeders Holstein cows.

No difference on pregnant-to-transferred rate was observed in recipients receiving fresh and frozen–thawed embryos. However, previous studies with beef and dairy cattle showed great pregnant-to-transferred and pregnancy rates in cows receiving fresh embryos compared to frozen–thawed embryos (Hasler, 2001; Spell et al., 2001; Rodrigues et al., 2007b). Two possible reasons for the obtained result may be that all embryos were collected from dry cows, eliminating the negative effect of milk production on embryo quality (Chebel et al., 2008) or even the occurrence of a type II error.

5. Conclusion

Repeat-breeder cows have considerable harmful impact on commercial dairy farms income (Lafi and Kaneene, 1992) due to their decreased fertility. Thus, the use of biotechnologies as FTET could be justified by allowing a substantial improvement on reproductive efficiency of

repeat-breeder cows that would be submitted to embryo transfer.

The synchronization of time of ovulation protocol increased the proportion of recipients selected for FTET and improved the pregnancy rate in repeat-breeder high-producing dairy cows. Moreover, FTET protocols enable the utilization of cows that are not presenting CL with same efficiency and eliminate the necessity of detection of estrus, facilitating reproductive management by reducing labor and animal handling.

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