

Bovine embryo transfer recipient synchronisation and management in tropical environments

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Abstract. Numerous studies have shown that it is possible to manipulate follicular and luteal dynamics, thereby eliminating the need for oestrus detection in embryo transfer (ET) programmes. Fixed-time ET (FTET) protocols are based on the use of gonadotrophin-releasing hormone (GnRH) and prostaglandin (PG) F or progesterone/progestogen (P4)-releasing devices and oestradiol. The FTET protocols increases the proportion of recipients transferred, and therefore pregnancy rates, compared with the use of PGF followed by ET 7 days after oestrus. Furthermore, the addition of equine chorionic gonadotrophin (eCG) to the P4 and oestradiol-based FTET protocols results in an even higher proportion of recipients transferred, and thus higher pregnancy rates. The beneficial effect of eCG treatment may be related to increased growth of the dominant follicle and increased plasma P4 concentrations during the subsequent luteal phase. In *Bos taurus* × *Bos indicus* recipients, pregnancy rates were positively correlated with the diameter of the corpus luteum (CL) and the number of CL at ET. When repeat-breeder Holstein cows were used as recipients, FTET protocols increased number of recipients transferred and pregnancy rates compared with the traditional PGF-based synchronisation protocols. In conclusion, the use of FTET protocols eliminates the need for the detection of oestrus and results in a greater proportion of recipients transferred and satisfactory pregnancy rates. Thus, FTET optimises the use of recipients, reducing labour and animal handling and facilitating the use of ET.

Additional keywords: ovulation, progesterone, repeat breeder.

Introduction

Bovine embryo transfer is widely used. It has been reported that, in 2007, 823 160 bovine embryos (*in vivo* derived + *in vitro* produced) were transferred (Thibier 2008). This included 285 387 embryos transferred in South America, representing approximately 35% of global activity.

Recipients play a critical role in the success of embryo transfer (ET) programmes. However, the high cost of recipient maintenance and the low efficiency of traditional prostaglandin (PG) F-based programmes for oestrus synchronisation in recipients limit the widespread application and success of ET technology (Bó *et al.* 2002). Hormonal protocols using gonadotrophin-releasing hormone (GnRH) and PGF or progesterone/progestogen (P4)-releasing devices and oestradiol have been used successfully to control follicular and luteal dynamics and to synchronise ovulation, allowing for ET without the need for oestrus detection; this is called fixed-time embryo transfer (FTET; Baruselli *et al.* 2000, 2001; Bó *et al.* 2001, 2002).

The main purpose of the present paper is to review the use of synchronisation protocols for FTET and to discuss how they may impact on the effectiveness and application of ET programmes

in tropical environments. Because most protocols for FTET used in South America are based on the association between P4 and oestradiol, the paper includes several strategies to improve the reproductive outcomes during FTET programmes using this hormonal association.

Effect of plasma progesterone concentrations on conception rates in embryo recipients

Several studies have reported a positive correlation between serum P4 concentrations, embryonic development, the capacity of the conceptus to secrete interferon- τ (Mann *et al.* 1999) and pregnancy rates in cattle (Baruselli *et al.* 2001; Binelli *et al.* 2001; Thatcher *et al.* 2001; Bó *et al.* 2002; Nasser *et al.* 2004). Many cows that fail to maintain a pregnancy tend to have low concentrations of P4 in the early and mid-luteal phases. In these animals, it has been suggested that the corpus luteum (CL) may not be functioning optimally, resulting in substantial losses in reproduction (Rajamahendran and Sianangama 1992).

In a study involving the transfer of frozen embryos into 542 *Bos taurus* × *Bos indicus* recipients with a CL > 15 mm in diameter, as detected ultrasonographically at the time of ET,

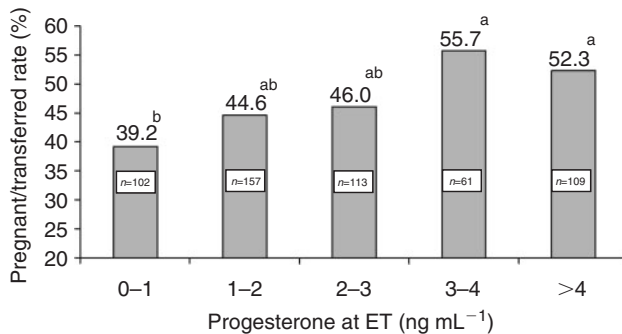


Fig. 1. Conception rates (no. recipients pregnant/transferred) relative to plasma progesterone concentrations at the time of embryo transfer in *Bos indicus* × *Bos taurus* recipients ($n = 542$; adapted from Reis *et al.* 2004).

peripheral P4 levels influenced conception rates (i.e. the number of recipients pregnant of those transferred; Reis *et al.* 2004). As shown in Fig. 1, conception rates were lower in recipients with P4 concentrations below 1 ng mL^{-1} compared with those with P4 concentrations $>3 \text{ ng mL}^{-1}$; recipients with P4 concentrations between 1 and 3 ng mL^{-1} exhibited intermediate conception rates.

There are other reports regarding the effect of P4 on conception rates in the literature. For example, Spell *et al.* (2001) did not observe any differences in plasma P4 concentrations between *B. taurus* recipients that did or did not become pregnant after ET. Furthermore, Nogueira *et al.* (2004) found a decline in conception rates in recipients with P4 levels above 6 ng mL^{-1} .

These differences may be due to nutrition management and genetics (e.g. *B. indicus* v. *B. taurus*). There are several physiological differences between Nelore (*B. indicus*) and Holstein (*B. taurus*) cows: the dominant follicle at the time of deviation is smaller in *B. indicus* than in *B. taurus* (6.0 v. 8.5 mm, respectively; Ginther *et al.* 1996; Sartorelli *et al.* 2005; Gimenes *et al.* 2008); the onset of ovulatory capacity of the dominant follicle after LH challenge occurs at a smaller diameter in *B. indicus* compared with *B. taurus* (7 – 8.4 v. 10 mm, respectively; Sartori *et al.* 2001; Gimenes *et al.* 2008); and the maximum diameter of the dominant follicle (10 – 12 v. 14 – 20 mm) and the CL (17 – 21 v. 20 – 30 mm) is smaller in *B. indicus* than in *B. taurus* (for a review, see Bó *et al.* 2003). These differences have important practical implications, because the CL is more difficult to palpate in *B. indicus* cattle. Previous studies have also shown that the P4 content of the CL and serum P4 concentrations are lower in *B. indicus* than in *B. taurus* cows (Segerson *et al.* 1984). Therefore, recipient conception rates relative to P4 levels in tropical countries, primarily involving *B. indicus* genetics on pasture, may be quite different than in *B. taurus* females maintained in cold-temperate areas with adequate nutrition.

Effect of the size and number of CL on progesterone concentration and conception rate in embryo recipients

It is well established that larger CL secrete more P4 and this may have a positive effect on pregnancy recognition and consequently pregnancy rates in ET programmes. However, both CL size and

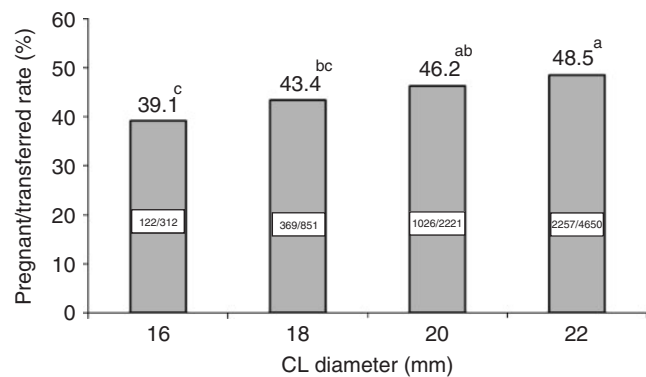


Fig. 2. Conception rates (no. recipients pregnant/transferred) rate relative to the diameter of the corpus luteum (CL) in *Bos indicus* × *Bos taurus* recipients ($n = 8034$) receiving *in vitro*-produced embryos (different letters show that they differ significantly, $P < 0.0001$; adapted from Nasser *et al.* 2009).

the P4 concentration at the beginning of the oestrous cycle have been shown to have variable effects on conception rates. In synchronised *B. indicus* × *B. taurus* embryo recipients, a positive association was found between the area of the CL and plasma concentrations of P4 (Baruselli *et al.* 2000). In this same study, the mean plasma concentration of P4 increased as the size of the CL increased from <1.5 to $>2.0 \text{ cm}^2$. These data are in agreement with previous results indicating that the ultrasonographic assessment of the CL can be used to predict plasma P4 concentrations and luteal function (Kastelic *et al.* 1990). Ambrose *et al.* (1999) found a linear relationship and a positive correlation ($r = 0.46$; $P < 0.01$) between plasma P4 concentrations and CL quality. Specifically, in lactating Holstein cows, mean plasma concentrations of P4 increased as the quality of the CL improved from Category 0 (2.99 ng mL^{-1}) to 1 (4.62 ng mL^{-1}), to 2 (5.44 ng mL^{-1}) and to 3 (7.13 ng mL^{-1}).

When *B. indicus* × *B. taurus* recipients ($n = 140$) were classified according to the area of the CL as CL1 ($>2.0 \text{ cm}^2$), CL2 (1.5 – 2.0 cm^2) or CL3 ($<1.5 \text{ cm}^2$), heifers with larger CL showed higher conception rates (58.4% , 41.5% and 31.8% , respectively; Baruselli *et al.* 2000). Supporting these results, Nishigai *et al.* (1998) found that pregnancy rates for favourably developing CL (55.3%) tended to be higher than for poorly developing CL (44.4%) in Japanese Black recipients. However, Looney *et al.* (2006) reported no differences in pregnancy results as the CL increased in diameter above 10 mm in diameter. This is in agreement with earlier reports that failed to indicate any correlations between CL size and pregnancy rates in *B. taurus* recipients (Remsen and Roussel 1982; Hasler *et al.* 1987). Furthermore, Spell *et al.* (2001) did not observe differences in CL size or plasma P4 concentrations between recipients that did or did not become pregnant after ET. It should be noted that the conception rates in that study were $>70\%$, indicating adequate management. Consequently, these favourable conditions may have contributed to the absence of a detectable effect of P4 on conception rates. Recently, a retrospective analysis of 8034 *B. indicus* × *B. taurus* recipients synchronised with a P4 and oestradiol-based protocol for FTET and receiving *in vitro*-produced embryos showed

a positive correlation between CL diameter (measured by ultrasonography) and pregnancy rates (Nasser *et al.* 2009; Fig. 2). In the same study, pregnancy rates were also affected by the number of CL at the time of ET: conception rates in recipients with one CL were lower than in recipients with two or more CL (45.8% (1880/4109) v. 51.3% (328/639), respectively). These data are supported by other reports showing an improved pregnancy rate in *B. indicus* × *B. taurus* recipients with multiple CL and increased plasma concentrations of P4 (Baruselli *et al.* 2001; Bó *et al.* 2002).

Despite the conflicting data regarding the effect of the size of the CL on pregnancy outcomes between *B. taurus* and *B. indicus* cattle, results presented herein supported the notion that, in some situations, it is advantageous to use recipients with larger CL, especially when working with *B. indicus* × *B. taurus* kept on pasture.

Refining the FTET protocol using P4 devices, oestradiol and equine chorionic gonadotrophin

Oestradiol and P4 treatments have been used increasingly in recent years for FTET (Bó *et al.* 2002). In general, treatments are very similar to those used for fixed-time artificial insemination except that the PGF treatment is given earlier. Therefore, recipients receive a P4-releasing device and i.m. injections of 2 mg oestradiol benzoate (EB) and 50 mg P4 on Day 0; PGF is given on Day 5 (1 day after wave emergence), P4 devices are removed on Day 8 and 1 mg, i.m., EB is given on Day 9. Day 10 is considered the day of oestrus; therefore, embryos are transferred on Day 17 in all recipients with a CL. The advances in PGF treatment were made due to the results of two experiments in which giving PGF early (Day 5) increased the diameter of the dominant follicle (13.2 ± 0.2 v. 11.5 ± 0.2 mm; $P < 0.05$), plasma P4 concentrations at the time of FTET (6.9 ± 0.8 v. 5.2 ± 0.6 ng mL⁻¹; $P = 0.08$), the proportion of recipients selected for transfer (70.5% v. 52.7%; $P < 0.02$) and the overall pregnancy rate (41.1% v. 21.5%; $P < 0.004$), compared with PGF administration at device removal (Day 8; Bó *et al.* 2002).

Treatment with equine chorionic gonadotrophin (eCG) on Day 5 was subsequently included in the protocol (for a review, see Bó *et al.* 2002). eCG is a glycoprotein secreted by the endometrial cups of the pregnant mare that has both LH and FSH activity in cattle (Murphy and Martinuk 1991). Studies with beef cows and heifers (Baruselli *et al.* 2004a, 2004b), as well as in lactating dairy cows (Souza *et al.* 2009), have shown that animals receiving 400 IU eCG have greater plasma P4 concentrations during the subsequent luteal phase. In *B. taurus* × *B. indicus* recipients, eCG treatment resulted in increased CL diameter compared with control (18.5 ± 0.4 v. 17.7 ± 0.4 mm, respectively; $P < 0.05$), as well as increased conception rates (76/132 (57.6%) v. 53/127 (41.7%), respectively; $P < 0.05$). Furthermore, plasma P4 concentrations in recipients treated with eCG that had two or three CL ($n = 8$) or only one CL ($n = 18$) at time of embryo transfer were significantly higher than concentrations in recipients not treated with eCG ($n = 29$; 30.2 ± 8.2 , 7.5 ± 0.7 and 5.7 ± 0.4 ng mL⁻¹, respectively; $P < 0.01$; Bó *et al.* 2002).

Although previous studies have demonstrated the efficacy of this treatment protocol, it requires running the recipients through the chute at least five times for treatments and ET. Therefore, a series of studies was designed to simplify the protocol by reducing the number of days required for treatment. A study was designed to evaluate the effect of delaying the administration of eCG and PGF from Day 5 to Day 8 (time of removal of the P4 device) in order to avoid handling the recipients on Day 5. Another objective was to evaluate the effect of different doses of eCG (400 v. 500 v. 600 IU) on pregnancy rates in *B. indicus* × *B. taurus* recipients receiving fresh *in vitro*-produced embryos (Reis *et al.* 2004). The results presented in Table 1 demonstrate that the dose of eCG does not significantly affect the efficiency of this protocol. However, the day on which eCG is administered significantly affects the results, with recipients treated on Day 5 having higher pregnancy rates than those treated on Day 8. Similar results have been reported by Nasser *et al.* (2004), with pregnancy rates higher for recipients treated with eCG on Day 5 compared with Day 8 (47.0% (71/151) v. 40.7% (61/150), respectively). These studies demonstrate that postponing eCG administration adversely affects the results of this protocol. This could be related to changes in follicular growth between Days 5 and 8. Because most recipients used in these studies had a CL at the time of insertion of the P4 device, delaying the administration of eCG and PGF to Day 8 may have resulted in overexposure to high circulating concentrations of P4 (i.e. from the CL plus the P4-releasing device), which may have adversely affected LH pulsatility and follicular growth (Savio *et al.* 1993; Carvalho *et al.* 2008). Thus, follicular growth could have been already compromised when eCG was administered on Day 8.

In another study, two hypotheses were tested: (1) recipients that receive eCG treatment on Day 5 or Day 8 (in a protocol using an ear implant containing 3 mg norgestomet) would have the same conception rates; and (2) replacement of the second EB administration on Day 9 with oestradiol cypionate (EC) on Day 8 (i.e. at the time of implant removal) would not affect reproductive outcomes (Ferreira *et al.* 2006). The purpose of these modifications was again to reduce the labour and animal handling required associated with the original protocol. The norgestomet ear implant was chosen to minimise the suppressive effects of P4 on follicular growth in *B. indicus* cattle (Torres-Júnior *et al.* 2005). Recipients received a norgestomet ear implant (Crestar; Schering-Intervet, São Paulo, Brazil) plus 2 mg, i.m., EB on Day 0. On Day 5, recipients in the control group (five treatments) received 400 IU, i.m., eCG and 150 µg, i.m., D-cloprostenol. On Day 8, the ear implant was removed and 1 mg, i.m., EB was administered 24 h later (Day 9). Recipients in the EB group (four treatments) were subjected to the same protocol used for in the control group, except that eCG and PGF were given at the time of ear implant removal (Day 8). Recipients in the EC group (three treatments) were subjected to the same protocol as those the EB group, except that the second EB administration was replaced by administration of 0.5 mg, i.m., EC at the time of implant removal (Day 8). The results given in Table 2 demonstrate that, when norgestomet ear implants are used, it is possible to reduce the number of handlings without affecting the overall efficiency of the synchronisation protocol.

Table 1. Number of corpora lutea, progesterone concentration and pregnancy rate in *Bos indicus* × *Bos taurus* recipients treated with different doses of equine chorionic gonadotrophin on Day 5 or Day 8 (adapted from Reis *et al.* 2004)

Mean (\pm s.e.) values or proportions within rows with different superscript letters differ significantly (^{a,b} $P < 0.05$; ^{x,y} $P = 0.07$). eCG, equine chorionic gonadotrophin; CL, corpus luteum; ET, embryo transfer; P4, progesterone/progesterone

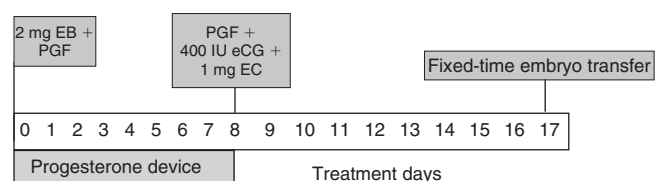
	eCG dose (IU)			Day of eCG treatment	
	400	500	600	Day 5	Day 8
No. transferred/treated (%)	165/201 (82.1)	165/197 (83.8)	171/196 (87.2)	260/299 (87.0 ^a)	241/295 (81.7 ^b)
No. pregnant/transferred (%)	85/164 (51.8)	71/161 (44.1)	84/170 (49.4)	132/255 (51.8 ^x)	108/240 (45.0 ^y)
No. pregnant/treated (%)	85/201 (42.3)	71/197 (36.0)	84/196 (42.9)	132/299 (44.1 ^a)	108/295 (36.6 ^b)
No. CL at ET	1.17 \pm 0.03 ^b	1.33 \pm 0.06 ^b	1.82 \pm 0.12 ^a	1.74 \pm 0.09 ^a	1.13 \pm 0.03 ^b
No. recipients >2 CL at ET/treated (%)	26/165 (15.8 ^b)	32/165 (19.4 ^b)	56/171 (32.7 ^a)	91/260 (35.0 ^a)	23/241 (9.5 ^b)
Plasma P4 (ng mL ⁻¹)	2.96 \pm 0.22 ^b	3.45 \pm 0.38 ^b	4.69 \pm 0.55 ^a	4.68 \pm 0.43 ^a	2.73 \pm 0.17 ^b

Table 2. Pregnancy rates, multiple ovulation rates, number and area of the corpora lutea in recipients treated with norgestomet ear implants and oestradiol benzoate (EB) on Day 0, equine chorionic gonadotrophin and PGF on Day 5 (control group) or Day 8 (EB group) and a second EB treatment on Day 9 (adapted from Ferreira *et al.* 2006)

Recipients in the EC group received oestradiol cypionate on Day 8 instead of the second treatment with EB on Day 9. Mean (\pm s.e.) values or proportions within rows with different superscript letters differ significantly

	Control group (five treatments)	EB group (four treatments)	EC group (three treatments)	<i>P</i> value
No. transferred/treated (%)	92/100 (92.0)	93/100 (93.0)	96/100 (96.0)	>0.05
No. pregnant/transferred (%)	42/92 (45.6 ^a)	42/93 (45.2 ^a)	59/96 (61.5 ^b)	0.03
No. pregnant/treated (%)	42/100 (42.0 ^a)	42/100 (42.0 ^a)	59/100 (59.0 ^b)	0.02
Mean no. CL	1.51 \pm 0.89 ^b	1.11 \pm 0.32 ^a	1.19 \pm 0.64 ^a	0.0002
Mean CL area (cm ²)	3.55 \pm 0.99 ^b	3.14 \pm 0.86 ^a	3.21 \pm 0.94 ^a	0.03
No. recipients >2 CL at ET/treated (%)	31/100 (31.0 ^b)	11/100 (11.0 ^a)	13/100 (13.0 ^a)	0.002

An alternative to reduce P4 concentrations during the synchronisation treatment without increasing handling days is to administer PGF at the time of insertion of the P4-releasing device. This protocol was based on preliminary studies in which advancing the administration of PGF from the time of removal of the P4 device (Day 8) to the time of device insertion (Day 0) increased the growth rate of the dominant follicle and the size of the ovulatory follicle (Cutaia *et al.* 2004; Carvalho *et al.* 2008). *Bos indicus* × *B. taurus* cross-bred heifers received a CIDR device (Pfizer Animal Health, São Paulo, Brazil) and 2 mg, i.m., EB on Day 0 and were randomly assigned to receive a half dose of PGF on Days 0 and 8 or a full dose of PGF on Day 8 only. All recipients received 1 mg EC and 400 IU eCG on Day 8 and those with a CL received an *in vitro*-produced embryo on Day 17. In recipients treated with PGF on Day 0, the diameter of the dominant follicle was larger compared with those receiving PGF on Day 8 only (14.7 \pm 0.4 v. 12.6 \pm 0.5 mm, respectively; $P < 0.05$), and the former group also had a larger CL at ET (19.3 \pm 0.5 v. 18.1 \pm 0.4 mm, respectively) and a higher pregnancy rate (50.5% (50/99) v. 39.4% (39/99), respectively). These data are in agreement with those of previous studies (Peres *et al.* 2007) that showed no differences in pregnancy rates in recipients treated with PGF on Day 0 and eCG at implant removal

**Fig. 3.** Proposed new protocol for fixed-time embryo transfer (FTET) in cattle (three treatments) consisting of: (1) insertion of a progesterone-releasing device, 2 mg, i.m., oestradiol benzoate (EB) and prostaglandin (PG) F, i.m., on Day 0; (2) PGF, 400 IU equine chorionic gonadotrophin (eCG), 1 mg oestradiol cypionate (EC), i.m., and device removal on Day 8; and (3) embryo transfer on Day 17 (no need for oestrus detection). If a norgestomet ear implant is used, PGF treatment on Day 0 is not necessary.

compared with recipients treated with PGF and eCG on Day 5 (40.3% (123/305) v. 41.3% (129/312), respectively).

Finally, Nasser *et al.* (2004) tested the hypothesis that administration of 2 mg, i.m., EB without 50 mg progesterone at the beginning of FTET protocol would not decrease pregnancy rates. Progesterone treatment had no effect on pregnancy rate (45.3% (68/150) v. 42.4% (64/151) for EB alone v. EB plus progesterone, respectively). Therefore, a new protocol, with only three

Table 3. Pregnancy rates in Holstein repeat-breeder cows transferred 7 days after observation of a prostaglandin F-synchronised oestrus or on Day 17 of a fixed-time embryo transfer synchronisation protocol (adapted from Rodrigues *et al.* 2009a)

All recipients had a corpus luteum (CL) at the beginning of the treatment. PGF-Oestrus, prostaglandin (PG) F i.m. on Day 0, detection of oestrus on Days 1–5 and embryo transfer 6–8 days after the detection of oestrus; FTET, norgestomet ear implant + 2 mg oestradiol benzoate on Day 0, implant removal + 400 IU equine chorionic gonadotrophin + 1.0 mg oestradiol cypionate + PGF on Day 8 and fixed-time embryo transfer on Day 17

	Treatment		P value
	PGF-Oestrus	FTET	
Oestrus detection rate (%)	136/229 (59.4)	–	
No. transferred/treated (%)	79/229 (34.5)	156/208 (75.0)	<0.0001
No. pregnant/transferred at 30 days (%)	42/79 (53.2)	67/156 (42.9)	0.14
No. pregnant/transferred at 60 days (%)	37/79 (46.8)	61/156 (39.1)	0.26
No. pregnant/treated at 30 days (%)	42/229 (18.3)	67/208 (32.2)	0.001
No. pregnant/treated at 60 days (%)	37/229 (16.2)	61/208 (29.3)	0.001
No. pregnancy loss between 30 and 60 days/ pregnant at 30 days (%)	5/42 (11.9)	6/67 (9.0)	0.62

Table 4. Effect of the presence of a corpus luteum at the time of norgestomet ear implant insertion (Day 0) on pregnancy rates in high-producing Holstein repeat-breeder recipients transferred at a fixed-time (adapted from Rodrigues *et al.* 2009a)
CL, corpus luteum

	With a CL	Without a CL	P value
No. transferred/treated (%)	156/208 (75.0)	131/214 (61.2)	0.003
No. pregnant/transferred at 60 days (%)	61/156 (39.1)	49/131 (37.4)	0.77
No. pregnant/treated at 60 days (%)	61/208 (29.3)	49/214 (22.9)	0.13
No. pregnancy loss between 30 and 60 days/ pregnant at 30 days (%)	6/67 (9.0)	1/50 (2.0)	0.15

handlings, has been proposed for FTET (Fig. 3) that is easier for farm personnel to perform, particularly when large numbers of recipients are synchronised at the same time.

Effect of FTET on reproductive efficiency in high-producing repeat-breeder Holstein cows

Repeat-breeder cows are usually defined as sub-fertile animals without any anatomical or infectious abnormalities that require three or more services to become pregnant. These cows are characterised by poor fertilisation rates (Graden *et al.* 1968) and/or early embryonic loss (Gustafsson and Larsson 1985; Albiñ *et al.* 1989, 1991). In many cases of infertility, ET can be used to minimise the effects of uterine environment and lactation on early embryonic development, promoting greater pregnancy rates and, consequently, avoiding early embryonic death (for a review, see Lucy 2001). Rodrigues *et al.* (2007) reported in a retrospective study that conception rates in repeat-breeder Holstein cows were greater after ET than after AI (41.7% (1609/3858) v. 17.9% (1019/5693), respectively), indicating that ET may be an effective alternative to achieving satisfactory conception rates throughout the year, especially during periods of heat stress. These data are in agreement with those of other experiments (Ambrose *et al.* 1999) with heat-stressed dairy cattle in which FTET using fresh *in vitro*-produced embryos improved pregnancy rates compared with timed AI.

In recent studies, a protocol for FTET was compared with the usual administration of a single dose of PGF after detection of oestrus (Rodrigues *et al.* 2009a). The FTET protocol consisted of the insertion of a norgestomet ear implant plus 2 mg, i.m., EB on Day 0, 400 IU eCG plus 1.0 mg EC plus PGF at the time of implant removal (Day 8) and FTET on Day 17.

The FTET protocol increased the proportion of recipients transferred and improved pregnancy rates in repeat-breeder high-producing dairy cows (Table 3). In addition, the presence of a CL at the time of implant insertion increased the proportion of recipients transferred, but had no effect on pregnancy rate at 60 days, pregnancy loss (Table 4) or circulating P4 concentrations at the time of ET (3.33 ± 0.32 v. 3.44 ± 0.40 ng mL⁻¹ for FTET with and without a CL, respectively). In conclusion, the FTET protocols for repeat-breeder Holstein cows increased the number of recipients transferred and pregnancy rates compared with the traditional PGF-based synchronisation protocols. Furthermore, FTET protocols increased the use of cows without a CL at the beginning of the protocol without the need for oestrus detection, simplifying reproductive management.

Effects of season on pregnancy rates and pregnancy losses in ET programmes in South America

In a recent retrospective study, Nasser *et al.* (2009) reported that season affected conception rate following the transfer of

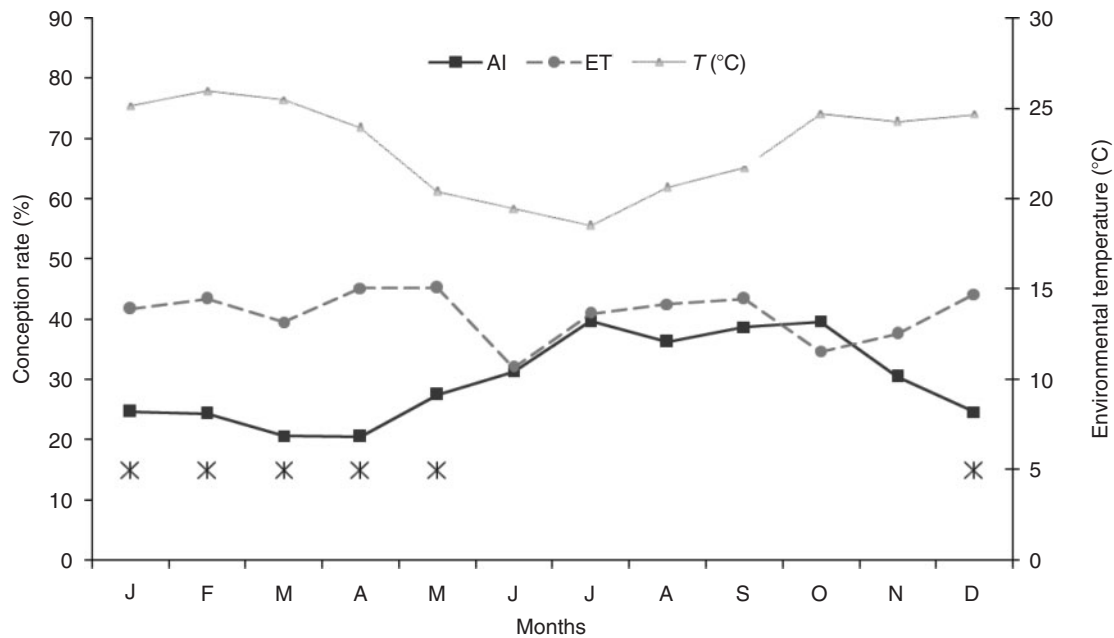


Fig. 4. Conception rates in lactating Holstein cows submitted to AI ($n = 7501$) or embryo transfer ($n = 2112$) throughout the year (adapted from Rodrigues *et al.* 2004).

fresh *in vitro*-produced embryos in *B. taurus* × *B. indicus* beef recipients. Lower pregnancy rates were observed during the autumn and winter compared with the spring and summer (41.1% (448/1090) v. 48.1% (1760/3658), respectively). These results are probably related to the dry weather and/or the lower availability/quality of forage during the autumn/winter period. In addition, the average rate of pregnancy loss (between 30 and 60 days) was 11.7% (258/2208) across all seasons. Pregnancy loss was not affected by CL diameter, the number of CL at the time of FTET, the season, the age of the recipients or the stage of embryo development. When parity was analysed, similar pregnancy rates (30 days) were reported between heifers and postpartum cows (45.8% (269/587) v. 48.1% (294/611), respectively). However, pregnancy loss was greater in heifers between 30 and 60 days than in cows (14.5% (39/269) v. 9.2% (27/294), respectively; $P = 0.05$). This is in contrast with reports in which the overall embryonic mortality was significantly higher ($P < 0.01$) and calving rate significantly lower ($P < 0.05$) in Holstein lactating cows than in heifers (Chagas e Silva *et al.* 2002).

A retrospective study was performed in high-producing Holstein cows (average milk production 28.4 ± 2.3 kg day⁻¹) submitted to ET ($n = 2112$) or AI (7501) during the period 2000–2003 (Rodrigues *et al.* 2004). Oestrus was detected in cows, which were subjected to AI 12 h later or ET 7 days later. Pregnancy rates were higher in those receiving embryos than those undergoing AI during the summer months, but no differences were observed during the colder months (Fig. 4).

Recently, pregnancy loss was compared among high-producing (average milk production 29.4 ± 0.6 kg day⁻¹) repeat-breeder Holstein cows (≥ 3 services) submitted to ET and AI during the period 2001–2007 (Rodrigues *et al.* 2009b). Again, oestrus was detected in cows, followed by AI 12 h later or ET

7 days later. Fresh or frozen–thawed *in vivo*-produced embryos were transferred. No differences were observed ($P = 0.24$) in terms of pregnancy loss (between 30 and 60 days) in recipients receiving fresh or frozen–thawed embryos (19.7% (193/981) v. 22.5% (83/369), respectively). Thus, these data were grouped and analysed together. Pregnancy loss among cows submitted to AI and ET was also similar (16.4% (147/896) v. 20.5% (277/1353), respectively; $P = 0.16$). However, pregnancy losses were higher ($P < 0.05$) during the spring and summer (18.5% (51/276) and 24.8% (131/529) for AI and ET, respectively) than during the autumn/winter period (15.5% (96/620) and 17.7% (146/824) for AI and ET, respectively). In conclusion, pregnancy loss from 30 to 60 days of pregnancy was similar among animals submitted to AI and ET. However, greater losses were observed during the months with elevated environmental temperatures and humidity (spring–summer).

Summary and conclusions

Although ET technology has been in use commercially for many years, the inefficiency in oestrus detection, especially in *B. indicus* cattle, has limited its widespread application and greatly increased the cost of commercial operations. The incorporation of techniques designed to control follicular wave dynamics and ovulation reduces the problem of oestrus detection and provides possibilities for the application of FTET programmes. The more recent changes to the protocol, such as the time when PGF and eCG are administered, make the technique easier for farm personnel to perform. ET can be used to improve the reproductive efficiency of both high-producing Holstein cows under heat stress and repeat-breeder Holstein cows. Despite conflicting data about the effects of P4 concentrations

and CL size on pregnancy rates, the data presented herein suggest that it is advantageous to transfer recipients with higher P4 concentrations and larger CL when working with *B. indicus* × *B. taurus* cattle raised on pasture.

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