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Advances in Bovine Reproduction and Embryo Technology

Timed embryo transfer programs for management of donor and recipient cattle

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Abstract

Currently, timed ovulation induction and fixed-time artificial insemination (FTAI) in superstimulated donors and synchronization protocols for fixed-time embryo transfer (FTET) in recipients can be performed using GnRH or estradiol plus progesterone/progestin (P4)-releasing devices and prostaglandin $F_{2\alpha}$ (PGF2α). The control of follicular wave emergence and ovulation at predetermined times, without estrus detection, has facilitated donor and recipient management. However, because *Bos taurus* cows have subtle differences in their reproductive physiology compared with *Bos indicus* cattle, one cannot assume that similar responses will be achieved. The present review will focus on the importance of orchestrating donor and recipient management to assure better logistics of procedures to achieve more desirable results with embryo collection and transfer. In addition, this will provide clear evidence that the use of FTAI in superstimulated donors and FTET in embryo recipients eliminates the need to detect estrus with satisfactory results. These self-appointed programs reduce labor and animal handling, facilitating the use of embryo transfer in beef and dairy cattle.

Keywords: Superovulation; Bos indicus; Repeat breeder; Dairy cattle; Beef cattle

Contents

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1. Introduction

In general, superovulation (SOV) followed by AI, can be used to obtain numerous embryos from valuable donors. These techniques, which are associated with the embryo transfer (ET) to recipients, are powerful tools to disseminate high-quality genetics. However, there is an ongoing need to simplify bovine SOV and ET protocols, specifically by reducing the number animal handlings, without compromising embryo production and pregnancy rates. Advances in the control of ovarian follicular wave emergence and ovulation with self-appointed treatments and without estrus detection have facilitated donor and recipient management. However, these procedures can be influenced by several factors related to the animals and their management [1–5]. For instance, *Bos taurus* and *Bos indicus* have several differences in their reproductive physiology. Specifically, *B. indicus* cattle have a greater sensitivity to gonadotropins, a shorter duration of estrus, and more often express estrus during the night [6]. Also *B. indicus* cows, compared with *B. taurus*, have more ovarian follicles recruited per follicular wave (30 to 60 versus 15 to 33, respectively [7]), a smaller diameter of the dominant follicle (DF) both at deviation (6.0 versus 8.5 mm [8,9]), and at acquisition of ovulatory capacity after LH challenge (7 to 8.4 versus 10 mm [9,10]). Furthermore, a smaller maximum diameter of the DF (10 to 12 mm for *B. indicus* versus 14 to 20 mm for *B. taurus*) has been associated with a smaller CL (17 to 21 mm for *B. indicus* versus 20 to 30 mm for *B. taurus* [6]). These differences between *B. taurus* and *B. indicus* must be considered when planning SOV and ET programs.

Continuous research has been done in South America aimed at reducing the number of animal handlings and improving the efficiency of protocols using estradiol or GnRH and progesterone/progestin (P4)-releasing devices to control follicular wave emergence and ovulation for SOV and ET in *B. taurus* and *B. indicus* cattle [2–4,11,12]. Herein, we discuss some key points to improve the efficacy of the protocols for SOV (e.g., the number of FSH treatments, its replacement with eCG, time of ovulation induction for FTAI and superstimulatory treatments during the first follicular wave, and avoiding the use of estradiol) and fixed-time embryo transfer (FTET; e.g., reduction of handlings required to synchronize the recipients, use of ET as a tool to improve both the conception rate during the summer heat stress, and in repeat breeder dairy cows, and synchronization treatments using GnRH, and avoiding the use of estradiol). Furthermore, we highlight the importance of orchestrating donor and recipient management to improve both logistics and results.

2. Superovulation of *B. Taurus* and *B. indicus* donors without estrus detection

Traditional SOV protocols have some limitations, including the necessity of numerous animal handling events and detecting estrus to establish "marker heat", an inability to start superstimulatory treatments at the beginning of the ovarian follicular wave, and the necessity of detecting estrus to determine the time of AI. However, recent protocols have been designed to control follicular wave emergence and ovulation and allow initiation of superstimulatory treatments and the insemination of donors at a self-appointed time [2]. Protocols for SOV without estrus detection are especially important when working with *B. indicus* donors and high-production dairy *B. taurus* cows, due to the inherent difficulties with estrus detection with these animals [3,13].

Thus, three important aspects should be considered when developing SOV protocols: 1) control of ovarian follicular dynamics and the follicular wave emergence to initiate gonadotropin treatments; 2) time of ovulation induction and AI in superstimulated donors; and 3) type (FSH or eCG), dosage, and frequency of gonadotropin treatments used for SOV.

2.1. Synchronization of follicular wave emergence to initiate gonadotropin treatments

Follicular wave emergence for SOV can be controlled mechanically (follicle ablation [14]) or pharmacologically (GnRH [15], LH, hCG, or estradiol plus P4 [16]). In general, the elective treatment to induce follicular wave emergence uses estradiol and P4, especially in *B. indicus* cattle, due to their prolonged anestrous and consequently poor response to GnRH treatment at random stages of the estrous cycle [17]. The efficacy of estradiol and P4 administration, followed by initiation of FSH treatment at the expected time of follicular wave emergence (i.e., 4 d later), has been demonstrated in several studies with *B. taurus* (reviewed by Bó et al [2]) and *B. indicus* [18] cattle. Regardless of the stage of the estrous cycle, estradiol benzoate (EB) treatment at P4 administration (either a norgestomet ear implant or P4-releasing intravaginal device) induces a synchronous follicular wave emergence approximately 3 to 4 d after treatment (reviewed by Baruselli et al [3]). Therefore, co-treatment with estradiol and P4 has been considered the most successful hormone therapy to synchronize follicular wave emergence in cattle [19].

2.2. Time of ovulation induction and AI in superstimulated donors

Although the control of follicular wave emergence allows for self-appointed initiation of gonadotropin treatments for superstimulation, the need to detect estrus to perform AI in superstimulated donors remains an important problem. Therefore, several studies have been conducted to investigate the pharmacological control of the time of ovulation in superstimulated *B. taurus* and *B. indicus* cattle, thus enabling FTAI and embryo collection. The time to ovulation can be controlled through delaying the removal of the progestin/progesterone implant and administration of GnRH/LH at the end of SOV protocol [18,20]. Moreover, postponing the LH peak in relation to PGF2α treatment allows the development of more follicles that acquire the capacity of ovulation, thereby resulting in more embryos [21,22].

Studies have been directed toward the development of an SOV protocol that allows for FTAI in *B. taurus* and *B. indicus* cattle treated with P4-releasing devices and EB on the first day of the protocol (Day 0) [2,3]. Protocols are named according to the time from the first PGF2α treatment to the time of P4 source removal, which occurs before the induction of ovulation to avoid the deleterious effect of high P4 concentration on embryo quality during the ovulation period [11,18]. Therefore, when the PGF2α is given on Day 6 AM and the P4 device is removed on Day 7 AM, the treatment is called "P-24" (i.e., a 24-h interval between PGF2α and P4 device removal), whereas when the P4 device is removed on Day 7 PM, the treatment is called "P-36" [18]. No significant differences in the number and quality of transferable embryos have been detected between the P-24 and P-36 treatments [3]. Therefore, both treatments can be used to superovulate *B. taurus* and *B. indicus* cattle with FTAI.

Follow-up studies were conducted to determine the appropriate time to induce ovulation for FTAI in superstimulated *B. indicus* (Nelore) and *B. taurus* (Holstein) donors. Because the diameter of the dominant follicle at deviation and the diameter at which the DF acquire ovulatory capacity are smaller in Nelore than Holstein cows [9,10], it is understandable that the appropriate time to induce ovulation may differ. In previous studies, when the administration of porcine luteinizing hormone (pLH) was postponed from 12 to 24 h after the last FSH treatment, the SOV response was improved in *B. taurus* cattle [2,23,24], but reduced in *B. indicus* cattle [25]. Therefore, treatment with GnRH or pLH to induce ovulation for FTAI in superstimulated *B. indicus* and *B. taurus* donors should be done at 12 and 24 h, respectively, after the last FSH treatment [2,3].

2.3. Superovulation during the first follicular wave induced by GnRH to avoid the use of estradiol

In previous studies, it was possible to superovulate *B. taurus* and *B. indicus* cows during the first follicular wave [26–28]. Recently, a series of experiments were conducted with the overall objective of developing a protocol for SOV during the first follicular wave using P4-releasing devices that are not associated with estradiol [29–32]. The developed protocol was based on previous reports that indicated that ovulatory response to GnRH could be increased by the administration of PGF2α, which regresses the CL, at the time of insertion of a P4-releasing device that would remain in place for 7 to 10 d; ovulation and wave emergence occurred 1 to 2 d after administration of GnRH [33].

The protocol is easy to follow, and embryo production is comparable to that of the estradiol and P4 protocol [34]. The recommended protocol consists of the administration of PGF2α concurrent with P4 device insertion (Day 0), followed by the administration of GnRH on Day 7 AM. Treatment with gonadotropins is then initiated on Day 8 PM (36 h after GnRH), with twice daily administration of FSH until Day 12 AM. In this protocol, PGF2α is administered on Days 11 PM and 12 AM, and the P4 device is removed on Day 12 AM. Donors are given GnRH or pLH on Day 13 AM, with FTAI 12 h and 24 h later. Finally, embryos are collected on Day 20 (Fig. 1). If a practitioner prefers to use a 5 d instead of a 4 d FSH treatment protocol, the last FSH and PGF2α treatments and P4 device removal are done on Day 13 AM (instead of Day 12 AM), pLH or GnRH is given on Day 14 AM, with FTAI 12 h and 24 h later, and embryos are collected on Day 21 [34].

2.4. Type (FSH or eCG), dosage, and number of gonadotropin treatments used for superovulation

A series of studies was conducted aiming to reduce the number of animal handlings that were required to accomplish SOV in *B. indicus* donors [35]. In the first experiment, 24 Nelore cows received the previously described P-36 SOV. Four days after EB and P4 device insertion, donors were assigned into three groups in a cross-over experimental design [36]. All donors received a total dose of 133 mg porcine follicle-stimulating hormone (pFSH, Folltropin-V, Bioniche Animal Health, Belleville, ON, Canada): donors in group 2FSH received two doses of pFSH 48 h apart (70% on Day 4

	Donors		Recipients
Treatment Day	AM	PM	AM
0	P4 device insertion + $PGF_{2\alpha}$		
4			P4 device insertion + GnRH
7	GnRH/LH		
8		FSH (20%)	
9	FSH (20%)	FSH (15%)	
10	FSH (15%)	FSH (10%)	
11	FSH (10%)	FSH (5%) + $PGF_{2\alpha}$	P4 device removal + $PGF_{2\alpha}$
12	P4 device removal + FSH (5%) + $PGF_{2\alpha}$		
13	GnRH/LH	FTAI	GnRH/LH
14	FTAI		
20	Flushing		FTET

Fig. 1. Timed embryo transfer programs using GnRH plus progesterone combinations in *B. taurus* donor cows and embryo recipients.

and 30% on Day 6 AM); donors in group 4FSH received four decreasing doses of pFSH 24 h apart (40% on Day 4, 30% on Day 5, 20% on Day 6, and 10% on Day 7 AM); and donors in group 8FSH (Control) received eight decreasing doses of pFSH 12 h apart from Day 4 to Day 7. The 4FSH and 8FSH group had a similar number of CL at flushing (8.8 ± 0.6 vs 10.8 ± 0.6, respectively) and transferable embryos (6.3 ± 0.5 vs 7.7 ± 0.5, respectively). However, the number of CL (7.3 ± 0.6) and transferable embryos (5.4 ± 0.6) decreased in the 2FSH group compared with the 8FSH group.

In the following trial, the use of two or three pFSH treatments was evaluated in *B. indicus* donors [36]. Donors in group 3FSH were treated on Day 4 AM, Day 5 PM, and Day 7 AM (40, 40, and 20% of the dose, respectively). Donors in group 2FSH received a half dose (50%) of pFSH on Day 4 AM and a half dose (50%) on Day 5 PM. The Control group was 8FSH, similar to the previous experiment. The 3FSH and the 8FSH groups had a similar number of follicles > 8 mm at ovulation induction with pLH (15.8 ± 0.9 vs 16.1 ± 1.1, respectively), number of CL at flushing (11.8 ± 0.8 vs 12.8 ± 0.7, respectively) and transferable embryos (7.0 ± 0.6 vs 7.2 ± 0.5, respectively). However, the number of follicles > 8 mm (8.9 ± 0.5), CL at flushing (7.1 ± 0.6) and transferable embryos (4.8 ± 0.6) decreased in the 2FSH group relative to the other groups. Recently, an experiment [37] using the same experimental design in Brahman donors also showed that the 3FSH treatment had the same efficiency [number of follicles > 8 mm at LH injection (17.7 ± 7.8 vs 17.5 ± 6.5), CL at flushing (14.1 ± 7.6 vs 15.3 ± 5.9) and transferable embryos (8.4 ± 8.3 vs 8.2 ± 5.9)] as the 8FSH treatment.

Based on the studies reviewed above, it was possible to reduce the number of animal handlings to complete the SOV protocol by reducing the number of FSH treatments in *B. indicus* donors. However, results may be different in *B. taurus* cows, which are less sensitive to exogenous gonadotropins than *B. indicus* cattle [3,38].

Recently, alternatives to induce a consistent SOV response with a single injection of FSH were evaluated. Associations between the pituitary extract with agents that cause the hormone to be released slowly over several days have been suggested [12]. These agents are commonly known as polymers, which are biodegradable and non-reactive in the tissue, facilitating their use in animals [39]. A series of experiments was conducted to evaluate the superovulatory response in donors in which Folltropin-V was diluted in a slow release formulation (SRF, Bioniche Animal Health) and administered in a single intramuscular (im) treatment. In the first experiment [40], a single im injection of Folltropin-V diluted in SRF (n = 29) was compared with the traditional twice daily im injection protocol over 4 d (n = 29) in Red Angus donors. The mean number of total ova/embryos collected (12.3 ± 1.5 vs 13.7 ± 2.1), fertilized ova (7.2 ± 1.1 vs 8.4 ± 1.4), and transferable embryos (4.9 ± 0.8 vs 6.4 ± 1.3) did not differ between the twice daily and single injection groups. These results were confirmed in additional experiments conducted in several different breeds of donors (reviewed in Bó et al [34]).

Additional studies were performed to evaluate the possibility of reducing the concentration of SRF from 50 to 25% to simplify the dilution of the Folltropin-V lyophilized powder. The lower concentration of SRF was less viscous and easier to mix, but the use of a single im injection resulted in lower superovulatory response. However, increasing the administration of Folltropin-V diluted in SRF to two im treatments given 48 h apart (Split Treatment) resulted in similar numbers of transferable embryos (Group 25% SRF: 6.1 ± 1.3; Group 50% SRF: 5.0 ± 0.9) than when FSH was given in twice daily im treatments (4.0 ± 0.8) [12].

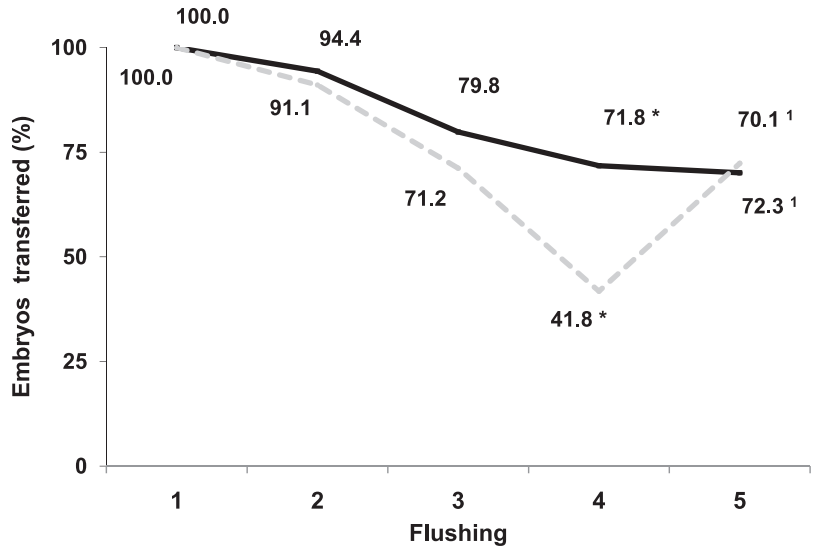

Fig. 2. Percentage of transferable embryos (Grades 1, 2, and 3) collected from Nelore (*B. indicus*) donors superovulated four consecutive times, 35 d apart, using 133 mg FSH (solid line; n = 10) or 2000 IU eCG (dashed line; n = 10). Percentages of transferable embryos relative to number obtained at the first superovulation (flushing 1 = reference).

*$P < 0.05$.

[1]In the fifth superovulatory treatment, all cattle were treated with FSH.

Other studies were conducted to test the hypothesis that it is possible to obtain satisfactory embryo production using a single dose of eCG in the P36 protocol with FTAI in Nelore and Holstein donors (reviewed by Baruselli et al [35]). A "cross-over" design was used in two experiments to study the dose of eCG (1,500, 2,000 or 2,500 IU) on the SOV response and embryo production of *B. indicus* donors subjected to FTAI [24,34]. All donors received a P4 device with 2 mg EB on the first day of the SOV protocol (Day 0), and the eCG-treated donors received a single eCG treatment on Day 4. In Experiment 1, 2,000 and 2,500 IU of eCG were tested, whereas in Experiment 2, 1,500 and 2,000 IU were used. In both experiments, the donors from the FSH group (Control) were treated with Folltropin-V (eight decreasing doses 12 h apart), following the P-36 SOV protocol, with LH administration 48 h after PGF2α (Day 8 AM). Collectively, the treatment with eCG (1,500, 2,000 or 2,500 IU) produced similar number of transferable embryos compared with the group treated with eight decreasing doses of FSH. However, 2,500 IU of eCG resulted in several anovulatory follicles in Nelore cows. Thus, in general, lower doses of eCG to SOV *B. indicus* cows are recommended to avoid excessive superovulatory responses. Another advantage of using the eCG treatment is the possibility of reducing donor handling.

In *B. taurus* dairy cows (Holsteins; reviewed by Baruselli et al [35]), the effect of eCG on superovulatory response was also evaluated. A total of 12 high-producing dairy cows were allocated into three groups: 200 mg FSH, 2,000 IU eCG, and 2,500 IU eCG. The same cross-over experimental design previously described for Nelore was utilized. The number of transferable embryos were not significantly different among groups given FSH (7.9 $\pm$ 1.1) and either 2,000 IU (6.7 $\pm$ 1.1) or 2,500 IU (8.1 $\pm$ 0.7) of eCG.

To assess the efficacy of successive treatments with eCG on embryo production, the effect of four consecutive SOV treatments, 35 d apart, was evaluated in Nelore (*B. indicus*) donors (reviewed by Baruselli et al [35]). Similar embryo production was found as the cows repeatedly received eCG treatments during three consecutive SOV protocols, compared with cows superovulated with a protocol using FSH (Fig. 2). However, embryo production of eCG-treated donors was reduced during the fourth SOV protocol; therefore, they were submitted to an extra SOV protocol (fifth) with FSH (similar as the control group) to evaluate the success of re-establishing original production. The donors treated with eCG produced similar number of embryos than those cows in the control group when they were treated with FSH (Fig. 2). Based on these data, we concluded that it was possible to superovulate donors using a single dose of eCG for three consecutive times without decreasing embryo production. However, for the fourth consecutive SOV protocol, FSH should

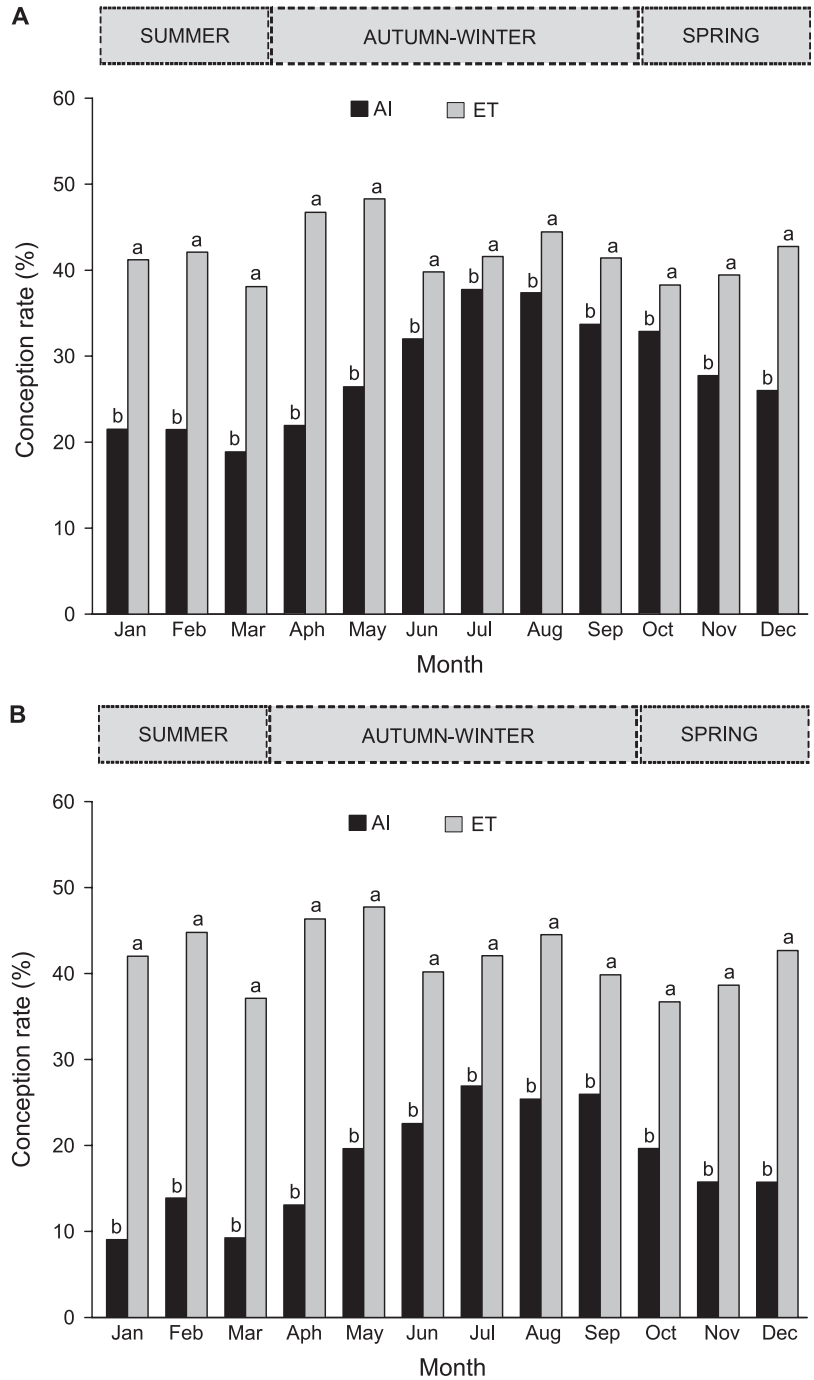

Fig. 3. Conception rates of high-producing Holstein cows submitted to AI (black bars) or embryo transfer (ET; gray bars): (A) non-repeat breeders (AI, 18,568 and ET, 4,871) and (B) repeat breeders (≥ 4 services; AI, 5,693 and ET, 3,858; adapted from Rodrigues et al [46,47]).

be used to maintain embryo production. Other experiments should be conducted to confirm these observations and to study the possible immunological response developed against eCG after repeated high dosages of this hormone in cows, as previously described [41].

3. Transfer of embryos to *B. Taurus* and *B. indicus* recipients without estrus detection

The most important advantages of using ET are: acceleration of the dissemination of desirable genetics

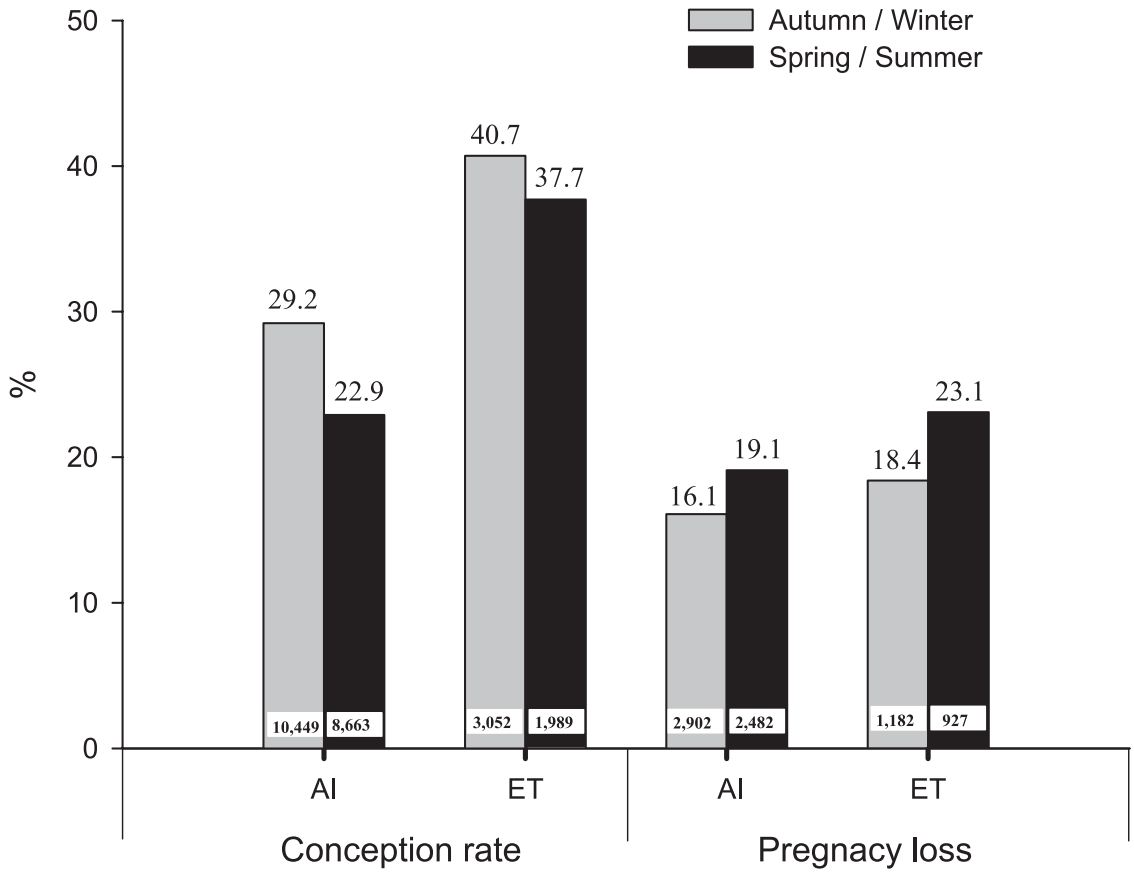

Fig. 4. Conception rate and pregnancy loss (between 30 and 60 d) of high-producing Holstein cows subjected to AI (n = 19,112) or embryo transfer (ET; n = 5,364) during hot (gray bars) and cool (black bars) seasons. There were differences for breeding technique (AI versus ET; P = 0.001), season (hot versus cool; P = 0.001) and an interaction of breeding technique and season (P = 0.003) on the conception rates. Pregnancy loss was influenced by breeding technique (P = 0.001) and season (P = 0.001), but there was no interaction (P = 0.55).

Adapted from [48].

by increasing the number of offspring obtained from donors with high genetic value; improvement of reproductive efficiency of repeat breeders; and reduction of fertility problems caused by heat stress at breeding and the first few days of pregnancy. Previous studies focused on several particularities inherent to *B. indicus, B. taurus* and cross-breed *B. taurus* cattle during FTET programs; various strategies to reduce the number of times the embryo recipients are handled during FTET protocols; and strategies to improve the efficiency of FTET programs, therefore increasing pregnancy rates in recipients [4,19]. Thus, the main purpose of the following part of this review is to briefly present some of our most recent data associated with FTET programs in lactating dairy cows, especially repeat breeders.

Clearly, ET is a potential tool to minimize the deleterious effects the uterus can have on early embryo development (during the first 7 d of gestation); this reduction avoids early embryonic death and thus leads to greater conception (i.e., pregnant-to-transferred) and pregnancy rates [42–45].

A retrospective study using data from a large number of high-producing dairy cows in Brazil confirmed that ET can be successfully used as a tool to improve conception rate during summer heat stress, especially in repeat breeder cows (Fig. 3; [46,47]). Moreover, pregnancy loss (from 30 to 60 d) was compared among high-producing Holstein cows submitted to AI or ET during the hot (spring/summer) and cool (autumn/winter) seasons of the year (Fig. 4; [48]). Conception rates and pregnancy loss of repeat breeder (≥ 4 services) and non-repeat breeder Holstein cows subjected to AI or ET were also compared (Fig. 5). During periods of heat stress, the overall conception rate decreases for cows submitted to both AI and ET. However, when ET is performed, conception rates are always higher com-

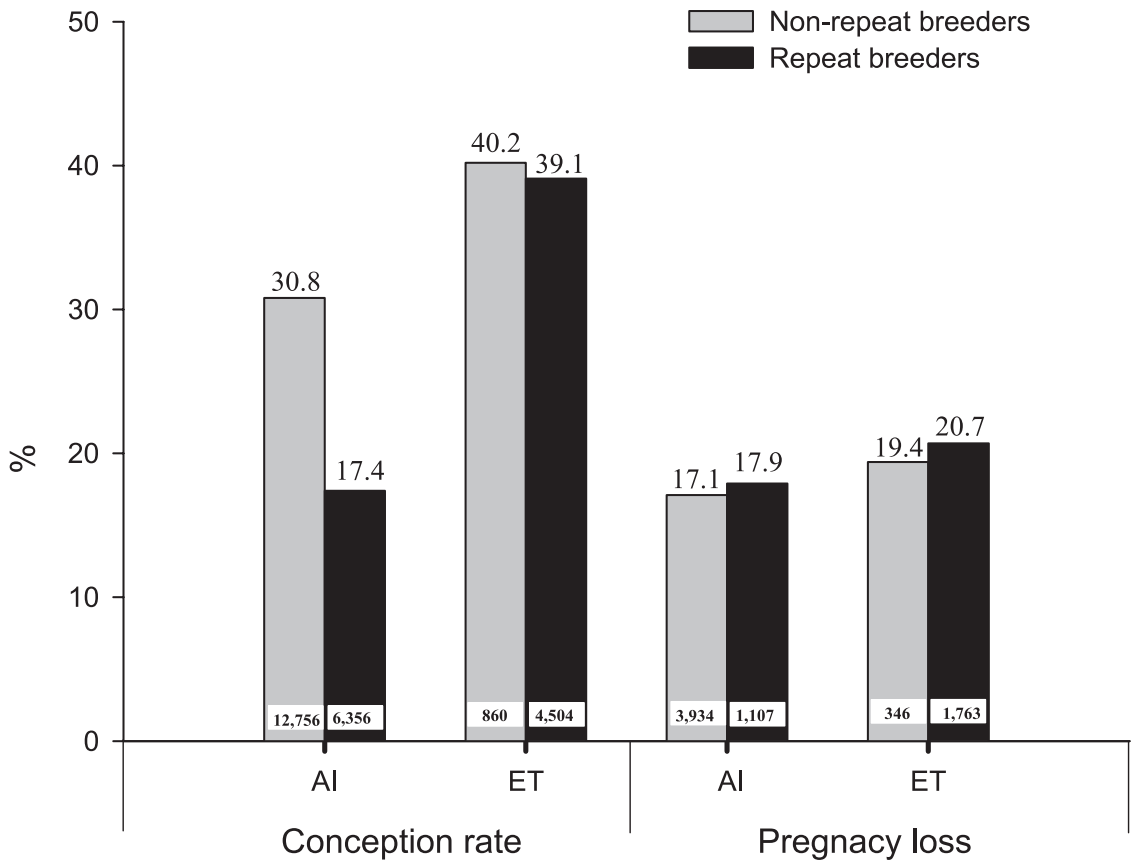

Fig. 5. Conception rate and pregnancy loss of high-producing repeat breeders (≥ 4 services; gray bars) and non-repeat breeder Holstein cows (black bars) submitted to AI (n =19,112) or embryo transfer (ET; n = 5,364). There were effects for breeding technique (AI or ET; P = 0.001), animal category (repeat breeder or non-repeat breeder cows; P = 0.001) and their interaction (P = 0.001) on conception rates. Pregnancy loss was not influenced by the breeding technique (P = 0.08) or animal category (P = 0.39), and there was no interaction (P = 0.87).

Adapted from [48].

pared with AI, mostly during the hot season. Heat stress also increases pregnancy loss between 30 and 60 d of pregnancy in animals subjected to AI or ET (Fig. 4). In repeat breeder cows, conception rates to AI are lower than in non-repeat breeder cows. However, only ET increases conception rates in repeat breeder cows, enabling the achievement of values near to those obtained in non-repeat breeder cows subjected to AI or ET (Fig. 5). Furthermore, pregnancy losses are similar among repeat breeders and non-repeat breeder cows, regardless of the breeding technique (AI or ET), reinforcing the hypothesis that the fertility problem off repeat-breeders may be associated with oocyte quality and/or failure of early embryo development.

In a follow-up study, we hypothesized that the lower fertility of repeat breeder Holstein cows is associated with oocyte quality and that this negative effect would be enhanced during summer [49]. Heifers, peak-lactation and repeated breeder cows were subjected to ovum pick-up (OPU) during the summer and the winter to assess oocyte recovery, *in vitro* embryo developmental rates, and blastocyst quality (comparing TUNEL-positive cells and total cell number). Summer heat stress prejudiced the physiological parameters of peak-lactation and repeat breeder cows, and consequently, *in vitro* embryo production was affected (Fig. 6).

Although cleavage rate was similar among animal categories, the blastocyst rate was severely compromised by heat stress, especially in repeat breeder cows (Fig. 6). Moreover, the number of copies of mitochondrial DNA was reduced, and the fragmentation rate of blastocysts was enhanced in repeat breeders during the summer (compared with winter and other categories), suggesting that the association between repeat breeder fertility problems and summer heat stress may potentially impair oocyte quality. Based on our findings, we inferred that the

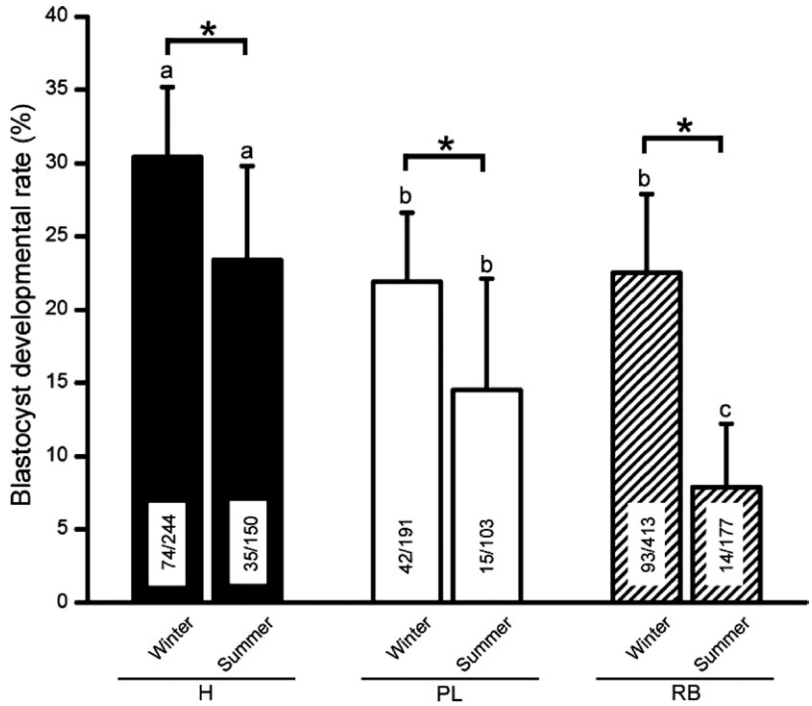

Fig. 6. Blastocyst rate 7 d post-in vitro insemination of Holstein cattle oocytes of various animal categories during summer and winter [Heifers (H; n = 150 and 244, respectively), high-producing cows in peak lactation (PL; n = 103 and 191, respectively), and repeat-breeder cows (RB; n = 177 and 413, respectively)]. Interaction season-group ($P < 0.0001$); mean (± SEM) values within season ($a \neq b \neq c$) and within group (*) differ ($P < 0.0001$). Adapted from Ferreira et al [49].

oocytes of repeat breeders were more susceptible to heat stress than those of cattle with normal fertility.

In a recent study, we compared the use of FTET with the usual administration of a single dose of PGF2α and the detection of estrus during the ET programs in lactating repeat breeder dairy cows [50]. Moreover, the effect of the presence of a CL at the beginning of FTET protocol was evaluated. We concluded that FTET increases the proportion of cows receiving an embryo (transferred-to-treated) than cows receiving a PGF2α treatment following estrus detection and ET (75% (156/208) and 34.5% (79/229), respectively; $P < 0.0001$). Pregnancy rate at 60 d was also greater ($P = 0.001$) in FTET (29.3%, 61/208) than PGF2α-estrus cows (16.2%, 37/229). Furthermore, the presence of a CL at the first day of FTET protocol increased the transferred-to-treated rate [FTET-CL = 75.0% (156/208) vs FTET-No CL = 61.2% (131/214), respectively; $P = 0.003$], but there was no significant effect ($P = 0.13$) on pregnancy rate at 60 d between cows with or without a CL at the first day of the synchronization protocol [FTET-CL = 29.3% (61/208) vs FTET-No CL = 22.9% (49/214)]. Therefore, we inferred that the protocol for synchronization of ovulation allowed for the use of FTET, regardless of the presence of a CL and without the need to detect estrus, simplifying management of donors and recipients, and increasing reproductive efficiency in repeat breeder Holstein cows.

Another study was performed to evaluate the efficiency of FTET protocols in high-producing Holstein cows using estradiol (n = 394) or GnRH (n = 390) in association with norgestomet implants during the summer and winter. Treatments with estradiol and GnRH resulted in similar pregnancy rates during the winter; however, during the summer, the pregnancy rate was reduced when estradiol was used [51]. Thus, it is possible to efficiently synchronize follicular wave emergence and ovulation for FTET in Holstein cows using protocols without estradiol, which can be applied in all countries.

4. Conclusion

Programs for ET estradiol plus P4 or GnRH plus P4 for *B. indicus* and *B. taurus* donors and recipients are proposed (Fig. 1 and 7). These reproductive programs are based on several studies discussed above and highlight the importance of orchestrating handling of donors

	Donors		Recipients
Treatment Day	AM	PM	AM
- 2			P4 device + EB (2 mg) + $PGF_{2\alpha}$
-1			
0	P4 device + EB (2 mg)		
4	FSH (20%)	FSH (20%)	
5	FSH (15%)	FSH (15%)	
6	FSH (10%) + PGF2α	FSH (10%)	P4 device removal + EC (1 mg) + $PGF_{2\alpha}$ + eCG (400 IU)
7	FSH (5%)	FSH (5%)+ P4 device removal	
8	GnRH/LH*	FTAI	
9	FTAI		
15	Flushing		FTET

Fig. 7. Timed embryo transfer programs using estradiol plus progesterone combinations in *B. indicus* donor and embryo recipients.

*In *B. taurus* donors cows, the GnRH or LH treatment should be given 24 h after the P4 device removal (Day 8 PM), followed by FTAI both 12 h (Day 9 AM) and 24 h (Day 9 PM) later; embryos are then collected (flushing) on Day 15 PM.

and recipients to assure better logistics and management of the animals, resulting in higher pregnancy rates. Furthermore, both SOV and FTET programs can be easily incorporated in a daily basis into farm management and can be performed by farm workers.

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