Società Italiana di Embryo Transfer

Notiziario Luglio 2011

Cari colleghi,

come ogni anno qualche considerazione estiva. Ci sono parecchie novità degne di nota che il consiglio direttivo sta vagliando. Prima fra tutte è la decisione di affidare la parte amministrativa della Siet per l'anno 2012 alla MV congressi del collega Pietro Montanari. La gestione "fai da te" tentata quest'anno si sta dimostrando difficoltosa e soprattutto rischiosa. Dal punto di vista scientifico il congresso del 2011 è ormai confezionato. Il programma prevede la presenza di ospiti molto blasonati: Fernando Lopez Gatius Università di Lleida, Spagna e a grande richiesta, ormai amico della nostra società, Pietro Baruselli. Come si nota dal programma allegato, la qualità degli interventi è molto elevata e conferma l'intenzione della società di affrontare temi che riguardano principalmente la riproduzione bovina. Anche l'interesse della Sib, Società Italiana di Buatria, ad organizzare eventi comuni è la prova che la Siet in questi anni ha lavorato bene. Questa collaborazione non vuole precludere nel modo più assoluto possibili eventi culturali organizzati con altre società mantenendo così la nostra indipendenza e identità. Proprio in questi giorni abbiamo con piacere ricevuto notizie dal Ministero della Salute; è da più di un anno che stiamo cercando di collaborare per risolvere problemi dovuti alla "famigerata" legge 30. Il Ministero dovrebbe emanare a breve delle direttive alle Regioni per snellire gli obblighi legislativi dei veterinari che trapiantano embrioni. Il sottoscritto, con Roberto Landriscina, e l'appoggio di Fnovi, ha speso tempo e risorse ma alla fine qualcosa, forse, si sta ottenendo per il bene di tutti. E' ormai operativo il software di raccolta dati gratuito per i soci in regola con la quota societaria. Il software lavora via web e, per ora, in locale solo per utenti Mac ed è possibile accedervi dal sito www.embryotransfer.it ma bisogna prima richiedere, per chi non l'avesse ancora fatto, tramite mail alla Siet username e password. La raccolta e l'elaborazione scientifica dei dati è un passo fondamentale per la crescita professionale; è per questo motivo che mi sono molto impegnato per ottenere uno strumento utile ed efficiente. Per quest'autunno c'è anche in programma un corso completo di embryo transfer patrocinato dalla Siet con la stretta collaborazione organizzativa e scientifica del Dr. Gianni Gnemmi. Maggiori informazioni verranno pubblicate sul nostro sito e il programma inviato via mail ai soci. Un arrivederci a Parma, sede universitaria, per il prossimo congresso il 30 Settembre e 1 Ottobre, sperando nel solito clima di amicizia e crescita professionale che contraddistingue i nostri congressi.

Pierluigi Guarneri

Timed embryo transfer programs in cattle

P.S. Baruselli*, G.A. Bo, M.F. Sá Filho

*Corresponding author: barusell@usp.br
Departamento de Reprodução Animal, FMVZ-USP, Rua Prof. Orlando Marques de Paiva, 87, CEP 05508-000, São Paulo-SP, Brazil

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. The present article 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.

Superovulation of 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 [Bo et al., 2006]. Protocols for SOV without estrus detection are important due to the inherent difficulties with estrus detection [Lopez et el., 2005, Baruselli et al., 2006].

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.

Synchronization of follicular wave emergence to initiate gonadotropin treatments

Follicular wave emergence for SOV can be controlled mechanically (follicle ablation [Bergfelt et al., 1997]) or pharmacologically (GnRH [Kohran et al., 1998], LH, hCG, or estradiol plus P4 [Bo et al., 1995]). In general, the elective treatment to induce follicular wave emergence uses estradiol and P4, due to the poor response to GnRH treatment at random stages of the estrous cycle [Baruselli et al., 2003]. 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 Bó *et al.* 2006).

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 donors, 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 [Docchio et al., 1997]. 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 [Vos et al., 1994].

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 [Gimenes et al., 2008, Sartori et al., 2001], 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 [Rodrigues et al., 2005], but reduced in *B. indicus* cattle [Martins et al., 2005]. 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 [Bo et al., 2006, Baruselli et al., 2006].

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 [Nasser et al., 1993, Nasser el al., 2011]. 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 [Carbalho et al., 2009]. 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 [Small et al., 2009].

The protocol is easy to follow, and embryo production is comparable to that of the estradiol and P4 protocol [Bo et al., 2010]. 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 [Bo et al., 2010].

Transfer of embryos to recipients without estrus detection

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

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. 2; Rodrigues et al., 2007a, b).

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 [Rodrigues et al., 2010]. 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.

Conclusion

ET programs for donors and recipients are proposed (Fig 1). These reproductive programs are based on several studies discussed above and highlight the importance of orchestrating handling of donors 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.

	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.

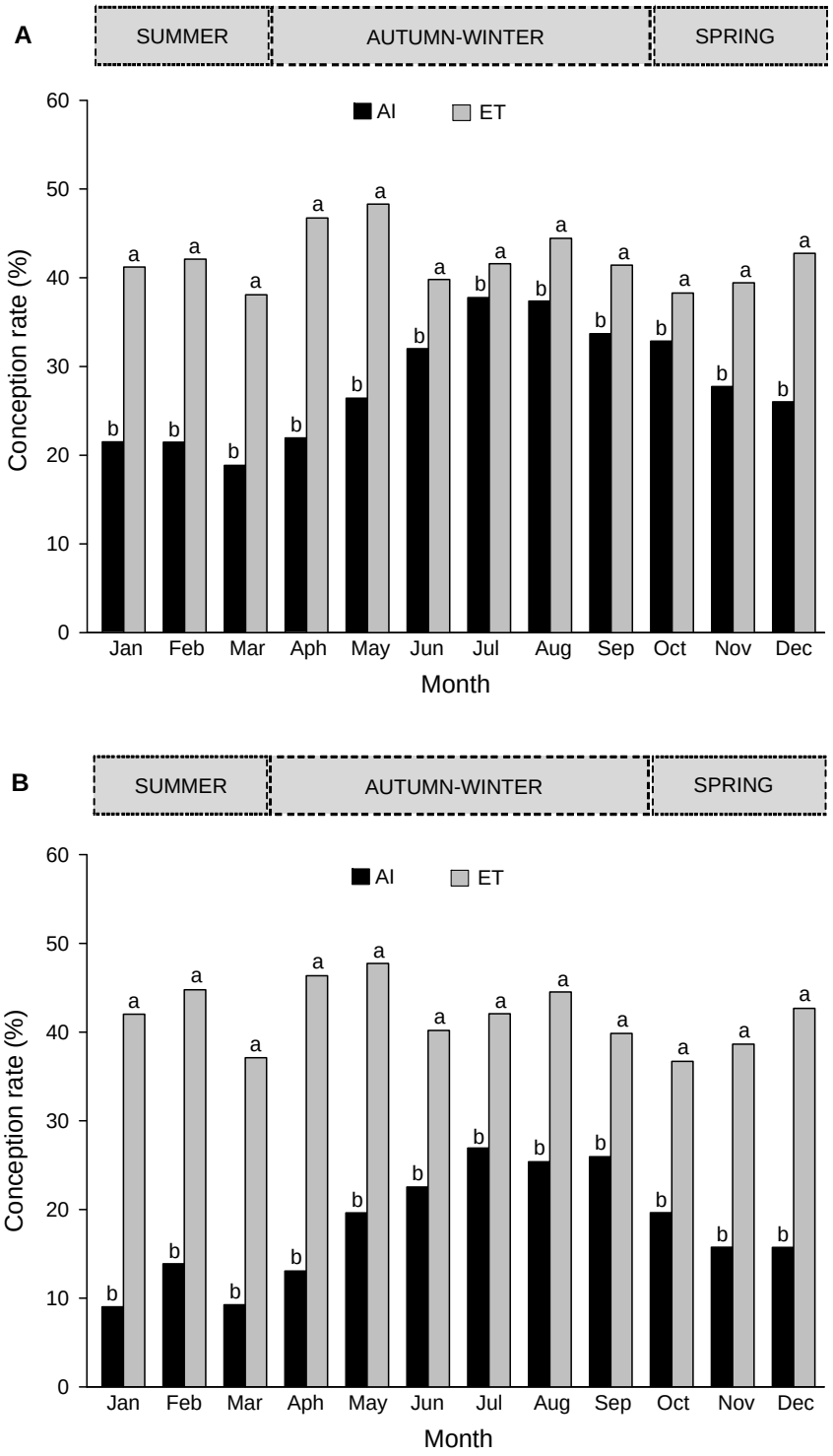

Fig. 2. Conception rates of high-producing Holstein cows submitted to AI (black bars) or embryo transfer (ET; gray bars): **(A) non-repeat breeders** (AI = 18,568 and ET = 4,871) and **(B) repeat breeders** (≥4 services; AI = 5,693 and ET = 3,858; adapted from Rodrigues *et al.* [46,47]).

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Comunicazioni ai soci

. Congresso SIET 2011
30 settembre - 1 ottobre 2011 Parma

Venerdì 30 settembre

Ore 8.30 Apertura segreteria
Ore 9,30. Saluto Accademico dall'Università degli Studi di Parma
Ore 10,00 Sincronizzazione dell'ondata follicolare. Applicazioni nelle pratiche di sincronizzazione/induzione dell'estro e superovulazione nella bovina (Pietro Baruselli, Brasile)
Ore 11.30 Coffee break
Ore 12.00 Gestione della ricevente di embrioni in bovine da carne e da latte (Pietro Baruselli, Brasile)
Ore 13.00 Pranzo
Ore 15.00 Effetti dell'infezione da BHV-4 sull'attività riproduttiva (Gaetano D'Onofrio, Parma)
Ore 15.30 Pericoli derivanti dalla trasmissione di malattie infettive mediante ET **(**Gaetano D'Onofrio, Parma).
Ore 16.00 Nuovi approcci per la gestione della paratubercolosi bovina (Giulietta Minozzi, Lodi)
Ore 16.45 Impiego del Ceftiofur nella prevenzione e terapia dell'endometriti nella bovina (Enrico Parmigiani, Parma)
Ore 17.30 La SIET un bilancio di 20 anni d'attività (Gigi Guarneri, Presidente)
Ore 18.00 Assemblea dei soci
Ore 20.30 Cena sociale

Sabato 1 Ottobre

Ore 9.00 Valutazione ecografica della mortalità embrionale nella bovina (F. Lopes-Gatius, Spagna)
Ore 10.30 Coffee break
Ore 11.00 Lopes-Gatius vs Baruselli. I nostri ospiti risponderanno a domande dei soci
Ore 13.00 Chiusura lavori

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