Anim Reprod, v.9, n.3, p.312-317, Jul./Sept. 2012

Increasing pregnancies following synchronization of bovine recipients

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Abstract

The success of a commercial embryo transfer program depends on the production of high numbers of viable embryos from donor cows that result in high numbers of calves born when the embryos are transferred to suitable recipients. In recent years, a great deal of effort has been devoted to the development of treatment protocols that permit the efficient use of recipients and result in high pregnancy per recipient synchronized, especially in recipients managed on pasture. One of the most successful alternatives to increase the number of recipients utilized in embryo transfer programs is the use of protocols that allow for embryo transfer without the need for estrus detection, usually called fixed-time embryo transfer (FTET). Pregnancies to FTET have been reported to be similar to those after detection of estrus, but the overall proportion of recipients pregnant over those synchronized are higher because these treatments have increased the proportion of recipients that receive embryos. Treatments that increase progesterone concentrations and pregnancies per embryo transfer have also been investigated in recent years. Most of these treatments have resulted in increased pregnancy per embryo transfer in recipients with *Bos indicus* influence, while benefit were not so obvious in *Bos taurus* recipients managed under more optimal conditions. While factors such as the reproductive history of the recipients and the stage and quality of embryos may affect pregnancy per embryo transfer, other factors such as estrus detection and the time interval from thawing to transfer do not seem to affect the proportion of recipients pregnant with embryos frozen in ethylene glycol and transferred at a fixed-time.

Keywords: eCG, fixed-time embryo transfer, GnRH, hCG progesterone.

Introduction

The main objective of implementing embryo transfer in beef operations is to produce genetic progress in the herd. Among the main factors that affect the extensive use of these technologies are those related to nutrition, management and efficiency in the detection of estrus. An alternative to increase the number of synchronized recipients receiving an embryo in an embryo transfer program is to apply protocols that synchronize ovulation and allow for embryo transfer without the need for detection of estrus, usually referred to as fixed-time embryo transfer (FTET). Other treatments aiming to increase pregnancy per embryo transfer have reached variable success. The objective of this manuscript is to briefly review some of the protocols that are used to synchronize ovulation of recipient cows, and discuss treatments and factors that may impact on the effectiveness and application of commercial embryo transfer in beef cattle.

Treatments that synchronize estrus and ovulation

Although prostaglandin F2α (PGF) has been the most commonly used treatment for synchronization of estrus for embryo transfer (reviewed in Odde, 1990), poor estrus detection efficiency and the variability of the interval from treatment to estrus and ovulation makes this treatment very inefficient on many farms, with only 50% of the treated recipients receiving an embryo 7 days after estrus (reviewed in Bó *et al.*, 2002). This situation may be even worse if the recipients have *Bos indicus* influence, where the percentage of recipients receiving an embryo over those treated with PGF has been reported to be as low as 30% (reviewed in Bó *et al*., 2004).

In order to avoid problems associated with estrus detection, treatments that synchronize the time of ovulation, which were originally developed for fixed-time AI, have been utilized more recently for FTET. These treatments are generally divided into those that are GnRH-based and those that are estradiol-based. The treatment protocol that utilizes GnRH and PGF for fixed-time AI in cattle is often referred to as Ovsynch (Pursley *et al.*, 1995). The Ovsynch protocol consists of an injection of GnRH followed by PGF 7 days later, and a second injection of GnRH 48 to 56 h after PGF treatment with fixed-time AI 16 h later.

GnRH-based protocols have also been used to synchronize ovulation in recipients that received *in vivo*- (Hinshaw *et al.*, 1999; Baruselli *et al.*, 2000) or *in vitro*- (Ambrose *et al.*, 1999) derived embryos. In these studies, more recipients received embryos because the GnRH-based protocol was not dependent on estrus detection. Although pregnancy per embryo transfer of

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Received: May 20, 2012
Accepted: July 16, 2012

GnRH-based protocols has often been lower than PGF-treated recipients, the overall proportion of recipients pregnant over those synchronized has been higher.

Recent studies have shown that contrary to the original study (Pursley *et al*., 1995), the first GnRH results in ovulation in only 44 to 54% of dairy cows (Bello *et al*., 2006; Colazo *et al*., 2009), 56% of beef heifers (Martinez *et al*., 1999) and 60% of beef cows (Small *et al*., 2009). It has also been demonstrated that the emergence of a new follicular wave was synchronized only when treatment caused ovulation (Martinez *et al*., 1999). If the first GnRH does not synchronize follicular wave emergence, ovulation following the second GnRH may be poorly synchronized (Martinez *et al*., 2002), and recipients may be asynchronous with the stage of embryo development at the time of transfer. Prevention of the early ovulations by addition of a progestin-releasing device to a 7-day GnRH-based protocol has improved pregnancy per fixed-time AI in heifers (Martinez *et al*., 2002) and cows (Lamb *et al*., 2001), and the addition of a norgestomet implant to a GnRH-based protocol for FTET resulted in similar proportion of recipients pregnant/treated to those synchronized with GnRH plus PGF 7 days later and transferred 7 days after observed estrus (Hinshaw, 1999).

Recent studies have also suggested that reducing the period of follicle dominance (by removing the progestin device 5 days after insertion) and increasing the time from progestin device removal to GnRH and fixed-timed AI may improve pregnancy per AI in beef and dairy cattle treated with GnRH-based protocols (Bridges *et al*., 2008; Santos *et al*., 2010; Lima *et al*., 2011). We have preliminary information indicating that this treatment results in a comparable proportion of recipients receiving an embryo and recipients pregnant per embryo transfer to the estradiol/progestin FTET protocol. Additional studies, with larger numbers of recipients, are needed to critically compare the 5-day GnRH-based protocol with the 7-day GnRH-based protocol in embryo transfer programs. However, as the 5-day protocol presumably influences oocyte quality as opposed to corpus luteum (CL) quality, one might speculate that there will be no difference in pregnancy per embryo transfer.

Estradiol and progestin devices are the most commonly used treatments to synchronize follicle wave emergence and ovulation in beef and dairy recipients in South America (Baruselli *et al*., 2010). The protocol consists of insertion of a progestin-releasing device and the administration of 2 mg estradiol benzoate (EB) on day 0 (to synchronize follicular wave emergence), and PGF either 5 days later, or at the time of insertion and removal of the progestin device (to ensure luteolysis). The progestin device is usually removed on day 8 and ovulation is induced by the administration of 0.5 or 1 mg of estradiol cypionate (ECP) at the time of progestin device removal or 1 mg of EB 24 h after progestin device removal (reviewed in Bó *et al*., 2002; Baruselli *et al*., 2010, 2011). As estrus detection is usually not performed, day 10 is considered to be the day of estrus. All recipients with an apparently functional CL on day 17 receive an embryo; pregnancy per embryo transfer have been comparable to those obtained with embryo transfer 7 days after observed estrus (Bó *et al*., 2002, 2012).

Treatment with equine chorionic gonadotropin (eCG) to improve pregnancy per recipient synchronized and/or transferred

The most common strategy used to increase the proportion of recipients pregnant over those synchronized in pasture-managed beef cattle in South America is the addition of 400 IU of eCG on either day 5 or day 8 of the estradiol/progestin treatment protocol. Overall, 75 to 85% of the recipients treated with this protocol receive an embryo (compared to 50% or less with PGF synchronization), progesterone concentrations are high at the time of embryo transfer, and pregnancy per embryo transfer usually exceeds 50%, when both embryos and recipients are of high quality (reviewed in Bó *et al*., 2002; Baruselli *et al*., 2010, 2011).

The FTET treatment protocol utilizing EB, progestin and eCG has been evaluated in different parts of the world. In a commercial embryo transfer program in Argentina, 1309 (84.9%) of 1542 recipients were suitable for embryo transfer and 692 (44.9%) became pregnant following Direct Transfer (Bó *et al*., 2004). In a commercial embryo transfer program involving *Bos taurus* x *Bos indicus* recipients in Brazil that received *in vitro*-produced embryos, pregnancy per embryo transfer was 41.1% during the winter and fall (448/1090) and 48.1% (1760/3658) during the spring and summer (Nasser *et al*., 2011). In another study involving 988 recipients of *in vitro*-produced, frozen-thawed embryos in China (Remillard *et al*., 2006), the proportion of recipients pregnant over those synchronized was significantly higher following eCG treatment than in untreated controls because of the higher proportion of recipients that received embryos. In a study in Mexico with 949 Brahman-influenced recipients, treatment with eCG increased the proportion of recipients receiving an embryo, with similar pregnancy per embryo transfer, resulting in a higher proportion of recipients pregnant/treated (Looney *et al*., 2010). In summary, results indicate that the administration of eCG in an estradiol/progestin-based synchronization protocol for FTET resulted in an increased proportion of recipients pregnant, especially in pasture-managed recipients or when the conditions are suboptimal (e.g. recipients receiving in vitro embryos in China).

The addition of eCG has also been tested in *Bos taurus* beef recipients synchronized with a GnRH/progestin protocol for FTET (Small *et al*., 2007). The percentage of recipients receiving an embryo did not differ whether cows did or did not receive a progestin device (93.4 *vs*. 85.5%) or eCG (91.0 *vs*. 87.8%). In addition, pregnancy per embryo transfer did not differ whether cows did or did not receive a progestin device (32.3 *vs*. 32.4%) or eCG (35.2 and 29.2%). However, the addition of eCG to a GnRH/progestin protocol significantly increased pregnancy per embryo transfer in a Colombian study (Mayor *et al*., 2008). In this study, *Bos indicus x Bos taurus* heifers were randomly allocated to one of three treatment groups. Heifers in the control group received a progestin device (1 g of progesterone, DIB, Syntex, Argentina) and 2 mg of EB on day 0 and PGF plus 400 IU eCG on day 5. Progestin devices were removed on day 8 and 1 mg of EB was administered on day 9. Heifers in the GnRH treatment group received a progestin device and GnRH on Day 0, PGF at progestin removal on day 7 and GnRH on day 9. Heifers in the GnRH+eCG group were treated similarly except that they also received 400 IU eCG on day 3. All heifers with a CL >16 mm in diameter 7 days after GnRH or 8 days after EB received a frozen-thawed embryo by Direct Transfer. The number of recipients selected/treated was higher in the EB+eCG (28/40, 70.0%) and the GnRH+eCG (29/40, 72.5%) groups than in the GnRH group (19/40, 47.5%). Pregnancy per embryo transfer and the proportion of recipients pregnant/treated were also significantly higher in recipients in the EB+eCG (16/28, 57.1% and 16/40, 40%) and GnRH+eCG (16/26, 61.5% and 16/40, 40%) groups than in the GnRH group (9/19, 47% and 9/40, 22.5%). In summary, the addition of eCG to estradiol- or GnRH-based protocols, which included the use of progestin devices, resulted in increased pregnancy per embryo transfer depending on the type of recipients. However, treatment with eCG may not improve pregnancy per embryo transfer in *Bos taurus* beef recipients managed under near optimal conditions.

Other treatments that increase circulating progesterone concentrations in recipients

There have been several studies investigating the relationship between circulating progesterone concentrations and pregnancy per embryo transfer in recipient cows (reviewed in Baruselli *et al*., 2010). In general, *Bos indicus* recipients seem to be influenced more than *Bos taurus* recipients by the size and number of CL in the recipient at the time of transfer (reviewed in Baruselli *et al*., 2010).

A strategy to increase circulating progesterone concentrations in recipients is to induce an accessory CL by induction of ovulation of the first wave dominant follicle around the time of embryo transfer. However, results have not been consistent. In *Bos indicus* recipients, treatment with 1500 IU of human chorionic gonadotropin (hCG) 7 days after estrus increased progesterone concentrations (Marques *et al*., 2002) and treatment with GnRH, hCG, pLH or a progestin device at the time of embryo transfer resulted in increased pregnancy per embryo transfer (Marques *et al*., 2003). However, the pregnancy per embryo transfer in non-treated (control) recipients in this study was lower than normally expected. The benefit of the administration of GnRH at the time of embryo transfer was confirmed by another experiment (Rodrigues *et al*., 2003) involving *Bos indicus* crossbred recipients. However, in another study involving *Bos indicus* x *Bos taurus* recipients synchronized with a progestin/estradiol plus eCG protocol (Tribulo *et al*., 2005), pregnancy per embryo transfer was not affected by treatment with hCG or GnRH at the time of FTET. Small *et al*. (2004) were also unable to improve pregnancy per embryo transfer in *Bos taurus* recipients treated with GnRH or pLH on days 5 or 7 after estrus. In a very recent study (Wallace *et al*., 2011), 719 beef recipients alternatively received 1,000 IU hCG or saline (control) at the time of embryo transfer. Serum progesterone concentrations at the time of pregnancy diagnoses (39 and 67 days) in pregnant recipients were higher after hCG treatment than in controls, and pregnancy per embryo transfer were 61.8 and 53.9% ($P < 0.05$) for hCG and control groups, respectively. The authors concluded that giving hCG at the time of embryo transfer increased the incidence of accessory CL, serum progesterone in pregnant recipients and pregnancy per embryo transfer. With the exception of the last report, the beneficial effects of increasing circulating concentrations of progesterone at the time of embryo transfer seem to be evident when the percentage of recipients pregnant in control (not treated) recipients were lower than expected. Furthermore, in studies with *Bos indicus* recipients, the induction of an accessory CL increased pregnancy per embryo transfer only when eCG was not used in the synchronization treatment protocol. One of the problems with the induction of an accessory CL at the time of embryo transfer is that the increase in circulating concentrations of progesterone occurs too late to have a significant effect on embryo development (reviewed by Lonergan, 2011). It has been suggested that elevated progesterone concentrations must occur shortly after conception in order to have beneficial effects on conceptus development. Insertion of a vaginal device impregnated with 1.55 g of progesterone (PRID, CEVA Animal Health Ltd, Chesham, UK) 3 days after AI, significantly elevated progesterone concentrations until day 8 and this was associated with a larger conceptus recovered at slaughter on days 13 or 16 (Carter *et al*., 2008). However, in a recent pilot study performed in our lab with 96 *Bos indicus* x *Bos taurus* cows, the insertion of a device containing 1 g of progesterone (DIB, Syntex) 4 days prior to embryo transfer (i.e., from days 13 to 17 in a FTET protocol using EB, progestin devices and eCG) resulted in decreased pregnancy rates compared to cows synchronized with the same FTET protocol but not treated with DIB devices 4 days prior to embryo transfer. The reasons for these disappointing results are still under investigation, but may be related to the negative effect of high progesterone concentrations early in the estrous cycle on CL development. Carter *et al*. (2008) inserted PRIDs on day 3 after AI, and not earlier, to avoid potential adverse effects of progesterone on CL development. Furthermore, Burke *et al*. (1994) reported that insertion of a CIDR device from days 1 to 5, but not from days 4 to 9, reduced the lifespan of the CL resulting in 'short' or 'shortened' cycles in beef heifers. Therefore, it is possible that insertion of the progestin-releasing device on day 13 of the FTET protocol, which based on our observations is around day 3 after estrus, was sufficiently early to affect the lifespan of the CL in the recipients.

Another alternative to increase progesterone concentrations in recipients is to induce ovulation of a persistent ovarian follicle produced through prolonged progestin synchronization treatments. Although ovulation of persistent follicles resulted in to pregnancy per embryo transfer to similar those obtained in the control group in one study performed in North America (Wehrman *et al*., 1997), ovulation of persistent follicles resulted in lower pregnancy per embryo transfer than in the control group in two experiments performed in Brazil (Moura *et al*., 2001; Mantovani *et al*., 2005). Thus, the use of this approach to increase CL size and progesterone production must be done with caution.

Other factors that may affect pregnancy per embryo transfer

We have recently done logistic regression analysis to evaluate factors (other than the synchronization treatment) that affected pregnancy per embryo transfer following the FTET of 1333 embryos frozen in ethylene glycol and transferred in *Bos indicus x Bos taurus* recipients on the same farm (Bó *et al*., 2012). Pregnancy per embryo transfer was affected significantly ($P < 0.05$) by reproductive history (open in a previous FTET: 116/231 (50.2%) *vs*. no history: 347/606 (57.3%), embryo stage [morula: 259/468 (55.4%), early blastocyst: 275/463 (59.4%), blastocyst: 179/344 (52.0%) and expanded blastocyst 23/58 (39.7%] and embryo quality [grade 1: 693/1235 (56.1%), grade 2: 32/65 (49.2%) and grade 3: 11/33 (33.3%)]. The difference in pregnancy per embryo transfer obtained between recipients that were open following a previous FTET compared to those that were not previously used confirm results reported by Looney *et al*. (2006), and questions the usefulness of re-using recipients when high-valued embryos are transferred. It is also noteworthy that in this study, observation of estrus did not affect pregnancy per embryo transfer (517/924, 55.9% *vs*. 219/409, 53.5% for those seen or not seen in estrus 7 days prior to FTET, respectively; $P = 0.36$). It is also noteworthy that the time-interval from thawing to transfer did not affect pregnancy per embryo transfer. Percentage of recipients pregnant did not differ between transfers that were done <3 min (215/385, 55.8%), between 3 and 6 min (372/655, 56.8%) and between 6 and 16 minutes (42/82, 51.2%, $P = 0.42$) after thawing, questioning the notion of ethylene glycol toxicity. In a recent study, we have also shown that exposing embryos to ethylene glycol for up to 30 min prior to freezing did not affect post-thaw survival rates (Tribulo *et al*., 2012).

Summary and conclusions

Advances have been made in recent years to facilitate the implementation of embryo transfer programs in commercial beef cattle operations. Small modifications of the treatment protocols originally developed for fixed-time AI have been shown to be effective for their adaptation to embryo transfer programs without the necessity of estrus detection and without compromising results. Furthermore, the addition of eCG to the synchronization protocols have resulted in increased pregnancies per embryo transfer, especially in *Bos indicus*-influenced recipients. Other treatments designed to increase progesterone concentrations in recipients, such as an accessory CL by induction of ovulation of the first wave dominant follicle, seem to be beneficial when the conditions are suboptimal, especially when eCG has not been used. Conversely, the insertion of a progestin device in early metestrus before embryo transfer seemed to result in decreased pregnancy per embryo transfer. The selection of the optimal protocol will depend on several factors such as the availability of the necessary hormones, the breed, age, physiological and nutritional status of the recipients, as well as location of the farm, qualified personnel and availability and quality of pastures near the handling facilities.

Acknowledgments

The authors thank Bioniche Animal Health, Pfizer Animal Health, MSD Saude Animal and Syntex S.A. for the hormones used in the studies. Special thanks to our colleagues of IRAC, University of Sao Paulo and University of Saskatchewan for technical assistance.

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