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FACTORS AFFECTING AN EMBRYO TRANSFER PROGRAM

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Summary

A commercially viable cattle embryo transfer (ET) industry was established during the early 1970s. Initially, techniques for recovering and transferring cattle embryos were exclusively surgical. However, by the late 1970s, most embryos were recovered and transferred nonsurgically. Successful cryopreservation of embryos was widespread by the early 1980s, followed by the introduction of embryo splitting, in vitro procedures, direct transfer of frozen embryos and sexing of embryos. The widespread adoption of ethylene glycol as a cryoprotectant has simplified the thaw-transfer procedures for frozen embryos. The number of embryos recovered annually has not grown appreciably over the last 10 years in North America and Europe; however, there has been significant growth of commercial ET in South America, especially Brazil. Although a number of new technologies have been adopted within the ET industry in the last decade, the basic procedure of superovulation of donor cattle has undergone little improvement over the last 20 years. The effects of a large number of factors on the pregnancy rates of fresh and frozen cattle embryos were examined over a period of years at several different locations. For fresh embryos, overall pregnancy rates were 68.3% (n=9023) and 77.1% (n=2650) at different locations and time periods. Frozen-thawed embryo pregnancy rates were 56.1% (n=3616) in The Netherlands and 58.4% (n=5297) and 68.7% (n=774) for two studies in the United States. Pregnancy rates of surgical versus nonsurgical transfers were very similar. There were no differences in the pregnancy rates of beef versus dairy embryos, but the pregnancy rate was greater in dairy and beef heifers and beef cows than in dairy cows. Estrous asynchrony between plus and minus 24 h did not affect pregnancy rate for frozen-thawed or fresh embryos. Neither breed nor parity of recipients affected the influence of asynchrony on pregnancy rates. Embryo grade was a significant factor in pregnancy rate for both fresh and frozen-thawed embryos, but neither embryo stage nor age was a significant factor. Pregnancy rate was not affected by holding embryos after flushing for up to 3 h prior to freezing. Therefore, there are numerous potential donor and recipient factors that may alter pregnancy rates for an embryo transfer program. This review will focus on these factors.

Introduction

The primary use of embryo transfer in cattle has been to amplify reproductive rates of valuable females. Ideally, embryo transfer can be used to enhance genetic improvement and to increase marketing opportunities with purebred cattle. Because of their relatively low reproductive rate and long generation interval, embryo transfer is especially useful with cattle (Seidel, 1991). Once transferable embryos are collected from a donor cow, the decision is made as to which of the available recipients should receive embryos to achieve the greatest number of pregnancies (Wright, 1981). Suitability of recipients is dependent on the timing of estrus and the

presence of a functional corpus luteum (CL). Most embryo transfer technicians rely on palpation per rectum to identify and characterize the size and integrity of the corpus luteum. Ultrasonography can be used to measure luteal structure and to more accurately define luteal characteristics (Kastelic et al., 1990; Singh et al., 1997). The success of embryo transfer depends on factors associated with the embryo, the recipient or an interaction among factors of the embryo and recipient. Many studies have focused on these factors. However, differences in techniques, small sample sizes and other elements limited the applicability of the results of those studies to embryo transfer as it is practiced today. Therefore, the purpose of this review is to characterize the effects to the donor and recipient that affect success of the embryo transfer program.

Donor Factors

Many factors may influence how donors respond to superstimulation and generate a high number of fertilized good to excellent quality embryos. Outside of genetics, nutrition probably is the single greatest factor that influences the response of donor cows to superstimulation. It is important to ensure that cows are maintained on a positive plain of nutrition and are fed a diet that meets maintenance requirements.

Throughout the embryo transfer industry, the current dogma exists that feeding an organic source of mineral prior to superovulation of donors will enhance the total number and quality of transferable embryos. Until now, no published study has demonstrated that feeding organic mineral enhances response of donors to produce greater quantities of transferable embryos. One previous unpublished study has demonstrated that donors receiving organic mineral may yield a greater quantity of embryos, but this report failed to demonstrate that organic mineral enhanced the quality or quantity of embryos. Therefore, we conducted a study to determine whether trace mineral supplementation prior to embryo collection affected embryo production and quality.

In this study (Table 1; Lamb et al., 2004), among all heifers, the total number of recovered embryos was similar among treatments. The number of unfertilized embryos was greater for Inorganic than Organic heifers, whereas Control heifers were intermediate. In addition, Control heifers had a greater number of degenerate embryos than Organic or Inorganic heifers. Organic heifers produced a greater number of transferable embryos than Inorganic and Control heifers remained intermediate. Although the appearance occurs that Organic heifers produced more transferable embryos than inorganic heifers, there is not an explanation for not noting differences in the Control heifers. Therefore, we concluded that mineral source probably does not influence embryo quality or number.

Table 1. Embryo production in heifers receiving inorganic, organic or no mineral after superovulation with follicle stimulating hormone (Lamb et al., 2004).

Item	Treatments[a]			P-value
	Control	Inorganic	Organic	
	----------n ± SE----------			
All treated heifers[b]:				
No. of heifers	49	51	51	
Total embryos recovered	4.24 ± 0.60	3.64 ± 0.60	3.29 ± 0.58	0.5219
Degenerate/cleaved	0.93 ± 0.24^{x}	0.26 ± 0.23^{y}	0.25 ± 0.23^{y}	0.0632
Unfertilized	1.31 ± 0.37^{x}	2.32 ± 0.36^{y}	0.82 ± 0.36^{x}	0.0135
Transferable	2.01 ± 0.39^{xy}	1.07 ± 0.38^{y}	2.18 ± 0.38^{x}	0.0900
Grade 1	1.43 ± 0.33	0.82 ± 0.32	1.43 ± 0.32	0.2955
Grade 2	0.56 ± 0.14^{xy}	0.23 ± 0.13^{x}	0.68 ± 0.13^{y}	0.0494
Grade 3	0.00 ± 0.03	0.02 ± 0.03	0.06 ± 0.03	0.1948

[a] Heifers received either 0.11 kg of organic mineral, 0.11 kg inorganic mineral, or no mineral for the 23 days prior to embryo collection.
[b] All heifers receiving FSH.
[x,y] Uncommon means within a row differ ($P < 0.05$).

Other more important factors tend to influence donor response more than mineral nutrition. For example, Bos Indicus cows respond differently to Bos Taurus cows in terms of quantity of hormone administered or the timing of hormonal treatment. Because Brazil focuses on Bos Indicus cattle, this section will focus on superstimulation protocols found to enhance embryo production in those cattle.

Multiple ovulation and embryo transfer (MOET) is one of the reproductive technologies that will facilitate genetic improvement of Zebu cattle. Unfortunately, high variability in the ovarian follicular response to gonadotropin stimulation continues to be a major problem in commercial MOET programs (Adams, 1994) despite considerable research in this area (Hyttel et al., 1991; Stock et al., 1996).

After the characterization of follicular dynamics in Bos Indicus (Castillo et al., 2000) it was possible to develop hormonal treatments to control the follicular growth and the time of ovulation, in order to allow fixed time artificial insemination. Similarly, the follicular development and ovulation can be pharmacologically manipulated to improve MOET programs in Zebu cattle (Barros et al., 2000). Brazilian veterinarians have reported an apparent higher inconsistency after superstimulation treatment in their cattle than their Bos Taurrus counterparts (Gradela et al., 1996). The reasons for these variable results in Zebu type cattle are numerous, such as higher difficulty in detecting estrus, genetic background, higher susceptibility to stress, and physiological or anatomical peculiarities of the Zebu type cattle.

In Zebu cattle, several studies compared the superovulatory response to reduced doses of a product with relatively high LH:FSH ratio (Pluset, Serono, Italy) with higher doses of Pluset commonly used for superstimulation of Bos Taurus donors. The production of transferable embryos was not altered significantly (2.1 to 10.0 vs 2.8 to 10.1) after reducing the total dose

from 350 to 500 to 200 to 300 units I.V., respectively (Visintin et al., 1996; Gradela et al., 1998; Pinto Neto et al., 2000). These studies indicate that Zebu cattle may require less FSH than Bos Taurus cows to achieve optimal superovulatory response. However, lower doses of a purified porcine FSH (pFSH, Folltropin-V, Bioniche Animal Health, Canada) with about 80% of the LH removed did not decrease the superovulatory response of 15 Bos Indicus heifers, which produced 9.37 and 9.60 transferable embryos, after using 200 and 160 mg of pFSH, respectively (Fernandes and Santos, 2000). Experiments using decreasing concentrations of FSH (300, 200 and 100 mg, Folltropin-V) and comparing simultaneously the superovulatory response of Bos Indicus versus Bos Taurus donors, are currently under way to test the hypothesis that Zebu cattle are more sensitive to FSH treatment than European breeds.

The use of recombinant bovine somatotropin (bST) is a promising alternative to increase follicle recruitment before superovulation. Bovine somatotropin can stimulate the synthesis of insulin-like growth factor I (IGF-I) in many tissues, including the ovary. Spicer and Geisert (1992) have shown that small follicles contain lower quantities of IGF-I than medium or large follicles. Katz et al. (Katz et al., 1993) suggested the presence of an intraovarian system for IGF-I, with receptors and binding proteins. Considering that bST can stimulate IGF-I synthesis, which stimulates granulosa cells, it is expected that bST may influence the superovulatory response. Although some reports have shown increase in both ovarian superstimulation and number of embryos (Herrler et al., 1994), not all of them observed positive response (Gray et al., 1993). Moreira et al. (2001) reported an increase in pregnancy rates in recipients treated with bST 1 d after estrus and re-injected every 14 d or receiving embryos from donors injected with bST at insemination.

One of the most promising strategies is the use of hormonal treatments to synchronize the follicular wave in such a way that the beginning of a new follicular wave coincides with the beginning of FSH administration. Bo et al. (1991, 1996) tested the association of estrogel (estradiol 17-13 or estradiol valerate) with progestin (progesterone, CIDR-B; or norgestomet, Synchromate-B) to induce follicle turnover and synchronize a new wave. The progestin is kept for 6 to 7 days and the estrogen (2.5 to 5.0 mg for estradiol benzoate) is administered one day after progestin administration or alternatively it is injected simultaneously with progesterone (i.m. 50 to 100 mg) at the time that donors receive the intravaginal device (CIDR-B) or the ear implant (norgestomet). They observed that estrogen when associated with progestin induced the synchronized growth of a new follicular wave, approximately 4 to 5 days after its administration. Therefore, the superovulatory treatment (single s.c. injection or 8 injections every 12 h, total dose 400 mg NIH-FSH-PI Folltropin-V) is initiated 4 to 5 d after estrogen injection, coinciding with the beginning of a new follicular wave. A luteolytic agent (PGF) is administered 48 h after the beginning of the superovulatory treatment and the progestin source is removed 12 h after PGF administration. Artificial insemination is performed 12 and 24 h after the beginning of estrus, around 60 and 72 h after the PGF injection. These treatments resulted in a number of transferable embryos similar or superior to those found on conventional superovulatory treatments performed between day 8 and 12 of the estrous cycle (Bo et al., 1991, 1996). One of the advantages of this protocol is that it facilitates the management of the donors, since it can be initiated at random stages of the estrous cycle.

In summary, even though, progress has been made in manipulating the bovine follicular development in order to facilitate donor management, the high variability in the ovarian follicular response to gonadotropin stimulation continues to be major problem in embryo transfer programs and warrants further research.

Recipient Factors

In a large field study we (Spell et al., 2001) estrus synchronized 763 potential recipients. Cows and heifers were observed for signs of behavioral estrus at least four times daily. Based on estrus detection, 526 recipients were presented to be evaluated for embryo transfer. At the scheduled time of embryo transfer one technician performed transrectal ultrasonic examinations of the ovarian structures. In addition to ultrasonic measurements of CL in recipients, a quality score was applied to the CL of each recipient after palpation per rectum. Blood samples were collected from all recipients presented for embryo transfer.

Each recipient deemed suitable for embryo transfer based on ultrasonographic evaluation received a single fresh or frozen-thawed embryo using a standard embryo transfer technique in accord with the International Embryo Transfer Society (Savoy, IL). Embryos had been collected from 43 Angus donors and were sired by 10 different Angus bulls. Embryos were transferred 6.5 to 8.5 d after detection of estrus.

The procedure of removing an embryo from its natural uterine environment increases the level of stress experienced by that embryo. When embryos experience additional external stress, such as freezing and thawing, the result is a decreased survival rate for those embryos. Our findings of a decrease in pregnancy rate from 83% with fresh embryos to 69% with frozen-thawed embryos are similar to the 10 to 15% decrease in pregnancy rates reported previously (Table 2; Spell et al., 2001). The results in this study reflect no difference in pregnancy rates among cows receiving a Grade 1 or Grade 2 embryo. Previous reports (Coleman et al., 1987; Hasler et al., 1987; Schneider et al., 1980; Wright, 1981) noted a decrease in pregnancy rate with each corresponding decrease in quality score.

Table 2. Effects of embryo type, recipient-donor synchrony, embryo grade, and recipient parity on pregnancy rates of transferred embryos (Spell et al. 2001).

Item	No. ofTransfers	Pregnancy rate, no. (%)	P value[d]
Embryo Type			
Fresh	122	101 (82.8)[x]	0.01
Frozen-thawed	326	225 (69.0)[y]	
Synchrony[a]			
-24	4	2 (50.0)	0.43
-12	76	54 (71.0)	
0	205	144 (70.2)	
+12	135	103 (76.3)	
+24	27	22 (81.5)	
Embryo Stage[b]			
3	2	1 (50.0)	0.62
4	282	207 (73.4)	
5	145	106 (73.1)	
6	19	12 (63.2)	
Embryo Grade[c]			
1	182	135 (74.1)	0.34
2	266	191 (71.8)	

[a] Hours of first detected estrus of recipient relative to the donor.
[b] 3 = early morula, 4 = compact morula, 5 = early blastocyst, 6 = blastocyst.
[c] 1 = excellent, 2 = good.
[d] Probability of differences among levels of each main effect.

Pregnancy rates were not compromised when the recipient was in estrus within 24 hours before or after the donor. Greater pregnancy rates were observed when recipients were in estrus coinciding with the donor or 12 hours before the donor. Pregnancy rates decreased in recipients in estrus 12 hours after the donor (Hasler et al., 1987; Schneider et al., 1980). Additional reports (Sreenan and Diskin, 1987) concur with results of our study (Table 2, Spell et al., 2001), reporting no significant difference in recipient-donor synchrony when synchrony between the donor and recipient was within 24 h. Perhaps embryos from superovulated cows could be more advanced in development relative to those from untreated controls (Hasler et al., 1987). In buffalo, the pregnancy rate was high when donors and recipients were synchronized within 12 h of each other (41%), whereas a compromise in pregnancy rates was noted when synchrony approached 12 h (18%). When synchrony exceeded 12 h, no embryos were conceived (Misra et al., 1999).

When concentrations of progesterone remained as low as 0.58 ng/mL or exceeded 16.0 ng/mL no differences in pregnancy rates were evident (Spell et al., 2001). The variability in progesterone concentrations reflects a combination of different rates of CL development and the fluctuation of progesterone secretion during the early luteal phase. The apparent optimum circulating concentration of progesterone to establish pregnancy was reported to range between 2.0 and 5.0 ng/mL (Niemann et al., 1985). This study reveals that the minimum threshold at which concentrations of progesterone essential for the establishment and maintenance of pregnancy on the day of embryo transfer may be lower than previously reported. In addition, a second study indicated that of 177 pregnant recipients, 8 recipients had concentrations of progesterone <0.5 ng/mL on d 10, 11, and 12 of the estrous cycle (Hasler et al., 1980). The mean values for luteal characteristics and plasma progesterone concentrations are reported in Table 3 (Spell et al., 2001). The diameter and luteal volume of the corpus luteum differed among recipients that received an embryo from 6.5 to 8.5 days after estrus. Corpus luteum diameter and luteal tissue volume increased as days post-estrus for the recipients increased. However, pregnancy rates did not differ among recipients receiving embryos 6.5 to 8.5 days after estrus.

Table 3. Mean characteristics of corpora lutea (CL) and plasma progesterone concentrations in pregnant or non-pregnant embryo transfer recipients.

Item	Overall mean	Range of values	Mean pregnant[a]	Mean nonpregnant[b]	P value[c]
CL diameter, mm	24.0	10.8 – 46.3	24.1	24.0	0.96
Luteal volume, cm^3	7.0	0.6 – 26.9	7.1	6.8	0.59
CL with cavity, % of recipients	79	NA	77	83	0.12
Plasma progesterone, ng/mL	4.0	0.6 – 16.9	4.1	3.9	0.44

[a] Mean value for recipients becoming pregnant after embryo transfer.
[b] Mean value for recipients that failed to become pregnant after embryo transfer.
[c] Probability values for pregnant and nonpregnant recipients differ.

The appearance of the CL may be used to estimate the stage of the bovine estrous cycle (Kastelic et al., 1990; Singh et al, 1997), yet differences in CL development decrease the accuracy of estimates. A greater percentage of corpora lutea have a fluid filled lumen in early diestrus than during late diestrus and advanced stages of pregnancy. Marciel et al. (1992) reported a high correlation among progesterone concentration and CL mass and volume. When using pixel values obtained by quantitative echotexture analysis of ultrasound images, a decrease in pixel values was noted from metestrus to mid-diestrus, and increased during proestrus (Singh et al., 1997). Pixel values of ultrasound images were highly correlated to plasma and luteal tissue progesterone concentrations to volume densities of luteal cells and stroma. In addition, Ireland et al. (1980) reported that 18% of cows verified to have a functional CL by rectal palpation had plasma progesterone at concentrations below those expected to maintain a pregnancy. In contrast, Perry et al. (1991) determined, with the use of transrectal ultrasonography, that cows with visible luteal tissue had serum concentrations of progesterone of greater than 0.5 ng/mL.

There are many variations found in today's embryo transfer industry. From these studies there is no reason to deviate from the normal practice of carrying out synchronous transfers where possible. However, allowing asynchrony of 24 hours between the recipient and donor does not appear to decrease conception rates. With the use of transrectal ultrasonography, a close evaluation of ovarian structures indicated that the best gauge of the suitability of a potential embryo transfer recipient is an observed estrus and a palpable corpus luteum, regardless of size or quality. Concentrations of progesterone at the time of embryo transfer were not predictive of pregnancy rates after embryo transfer.

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