BOVINE EMBRYO TRANSFER: ARE EFFICIENCIES IMPROVING?

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Abstract

Embryo transfer (ET) in cattle has been commercially practiced in a number of countries for nearly four decades. Numerous technologies have been introduced and adopted by the industry during this period, including non-surgical embryo recovery and transfer, closely timed hormonal synchronization of donor and recipient estrous cycles, reliable cryopreservation of embryos and direct transfer following thawing, embryo splitting, embryo sexing, production of in vitro-derived embryos, and cloning. However, actual success rates within the ET industry, as measured by the mean number of embryos recovered per donor or by conception rates following embryo transfer, have changed very little over the years. Nevertheless, there are fundamental principles related to donor and recipient management and also to ET procedures that do profoundly influence the level of success.

Introduction

Commercial bovine ET is a well established, mature industry in its fourth decade of existence in many countries. The history of the development of the industry has been previously described (Betteridge, 1981; Betteridge, 2000). As used henceforth, ET will refer individually or collectively to the collection, handling and transfer of embryos. Research in the field of ET is very expensive and many of the basic procedures were established some years ago. Consequently, little academic research currently is being conducted that is likely to immediately benefit the commercial industry. Because success rates in well-managed cattle herds are generally quite high, most recent improvements involve rather small, albeit important, increments. A valuable approach to understanding how various factors in ET influence success is to analyze well kept, accurate commercial records. A good share of the data presented in this review involves just such an approach.

Commercial ET programs vary widely in terms of factors that may be beyond the control of practitioners. For example, selection of specific donor cows and service sires are ultimately chosen by the owner. Long term weather problems or storms during the superovulation/recipient synchronization process are beyond the control of anyone and can wreck havoc with ET success. Travel problems sometimes means traveling to a farm a day or two late, which mandates working with older embryos than planned. Probably the single most important variable affecting success in ET is the level of donor and recipient management. Many factors go into cattle management and there are sometimes opportunities for ET practitioners to make a meaningful contribution to improving or changing certain management programs. In some cases, however, change either is not welcome or not possible and then the practitioner must make the best of the situation at hand.

Factors affecting donor embryo production rates

In a 1992 review article, Hasler (1992) stated “There have been no significant improvements in techniques for the superovulation of cattle in the last 15 years”. Eighteen years later that statement remains largely true. Data provided by both the American and Canadian Embryo Transfer Associations indicate that mean embryo yields per donor are in the range of 5-7 and basically have not changed for many years. The embryo means above are a composite average of individual means of both beef (6.6) and dairy cattle (5.7) flushed by the certified members of the AETA.

Table 1. Number of bovine flushes and embryos recovered yearly in the USA between 2002 and 2008

Year	No. flushes	No. embryos	Mean embryos/flush
2002	28,109	172,118	6.1
2003	34,896	205,441	5.9
2004	40,701	248,469	6.1
2005	48,233	305,129	6.3
2006	51,802	319,984	6.2
2007	54,080	332,486	6.1
2008	52,804	329,171	6.2

(Compliments of the American Embryo Transfer Association)

Although beef breeds produce on average more embryos than do dairy cattle, the differences are small, averaging approximately one embryo per collection. Looney (1986) reported a mean of 6.6 embryos from 2,048 superovulations of beef cattle and Hasler et al. (1983) reported a mean of 5.1 good embryos from nearly 1,000 different Holstein cows. It is obvious that the mean number of embryos collected from both beef and dairy cattle has not substantially improved in the years since the above cited studies.

While implantable devices for the release of progesterone (CIDR) were not available in the early days of the ET industry, it is currently possible to more closely regulate superovulation procedures with the use of these devices. Embryo production is similar whether donor superovulation is initiated during mid-cycle or following the insertion of a CIDR at a random stage of the estrous cycle. In addition, the use of CIDRs has led to the successful shortening of the inter-superovulation interval, allowing the production of more embryos per unit time.

For many years it was a widely accepted premise in the ET industry that for best results donor females should be allowed to have at least two estrous cycles between superovulations. With this approach, donors in the Em Tran, Inc. program previously were superovulated approximately every 65-70 days. However, starting in the late 1990s, we routinely employed a superovulation protocol that significantly reduced this interval. This protocol involved injecting prostaglandin and inserting a progesterone implant immediately following nonsurgical embryo recovery. Ten days later the implant was removed and superovulation was again initiated following the next estrus. If estrus was not observed within 3 days after implant removal, another implant was inserted and superovulation initiated 5 days later. The results of the repeated superovulation using this system on a group of 24 Red Angus cows are shown in Table 2. These cows were all primiparous, their calves had been weaned and they had been superovulated twice on-farm prior to entering the boarding facility at Em Tran, Inc.

These donors were superovulated every 40 days, an average of 13.8 times, with individual cows superovulated as few as 11 or as many as 16 times in the 18 month period. The mean number of embryos per superovulation (5.5) includes 20 donors that did not exhibit estrus plus another 39 that produced no embryos (17.8%).

Table 2. Performance of Red Angus donor cows repeatedly superovulated at a 40 day interval over a 16-month period

No. donors	24
Treatment period	18 months (548 days)
Total no. superovulations (mean per donor) (range)	332 (13.8) (11-16)
Mean interval between superovulations	39.7 days
Total no. embryos (mean per donor) (range)	1812 (76) (26-162)
Mean no. embryos per superovulation (range)	5.5 (0-35)

The number of embryos produced on average for each sequential flush is shown in Table 3. It is obvious that repeated, short-interval superovulations did not have a detrimental effect on embryo production over the 18-month period.

Table 3. Mean numbers of embryos produced in 15 sequential superovulations of 24 Red Angus donor cows

	Sequence of Superovulations														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
No. Donors	24	24	24	24	24	24	24	24	24	24	24	23	21	17	7
No. Embryos	5.5	5.7	3.7	5.8	4.5	6.2	4.6	4.9	5.8	4.3	8.5	5.3	3.9	7.2	6.9

Superovulation efficacy has not been greatly influenced by the numerous different FSH preparations that have been employed over the years. Superovulation success is related to parity, with more variability observed among virgin heifers and fewer embryos recovered on average. Superovulated cattle produce fewer embryos after reaching age 8 to 10 and heavy lactation in dairy cattle may decrease the response. Season has little effect on superovulation efficacy in temperate climates, but elevated heat and humidity are detrimental, especially when lactating dairy cattle are utilized as donors. Individual variation among females in response to superovulation remains the largest and least understood variable. Some females consistently produce large numbers of embryos in response to superovulation, while other females of similar age, breed, management, etc. perform poorly. The heritability of response to superovulation in cattle is not well understood.

Due to the short half life of FSH in the bovine circulatory system (~5 hours), traditional superovulation protocols usually involve 8 separate injections of FSH administered twice daily over a 4 day period. Recent work with a slow release formulation (SRF) compound used to reconstitute Folltropin® FSH demonstrated that satisfactory superovulation could be achieved with just two injections of FSH administered at a 48 h interval. In this study, 29 beef cows were randomly assigned to one of three treatment groups and superovulated three times in a cross-over design. The treatment groups consisted of controls injected 8 times and FSH injected twice into two treatment groups in which either 0.5% or 1.0% SRF diluent was utilized.

Follicle development and superovulatory response as measured by the numbers of ovulations did not differ among groups. As shown in Table 4, although the mean number of ova/embryos was higher in treatment groups 2 and 3 than in group 1, the number of fertilized ova and transferrable embryos did not differ among groups.

Table 4. The mean number of ova and embryos recovered from beef donors superovulated in a traditional 8 injection protocol (controls) compared to the same donors superovulated with two injections of FSH in two different concentrations of slow release formulation diluent in a cross over design.

Treatment	Number	Ova/embryos	Fertilized	Trans. embryos
1 – control	29	10.2 ± 1.8[a]	6.7 ± 1.3	4.0 ± 0.8
2 – 0.5% SRF	29	14.3 ± 2.1[b]	9.3 ± 1.9	6.1 ± 1.3
3 – 1.0% SRF	29	14.4 ± 2.0[b]	8.9 ± 1.4	5.0 ± 0.9

[ab]Values differ P<0.05 (unpublished, compliments of Bo, et al.)

The influence of the male on the outcome of embryo transfer is evident at two levels. First and most obvious, is the percentage of ova that are fertilized and the number of good quality embryos produced. It clearly has been shown that sperm quality, sperm number, and the timing of insemination are closely correlated with fertilization success in superovulated cattle. Most superovulated donors are inseminated with one unit of semen at 12 and at 24 h post onset of estrus. Of equal importance to fertilization is the ability of embryos to establish pregnancies following transfer into recipients. Even after fertilization and development into morphologically good-quality embryos, differences in pregnancy rates relative to sperm quality and among different sires have been demonstrated.

Factors affecting conception rates

In recent years, *conception rate* has been adopted as the percentage of animals pregnant following estrus detection and actual AI or ET, whereas *pregnancy rate* refers to the percentage of animals that are pregnant out of a group available for AI or ET, whether or not they were actually observed in heat. The author has arbitrarily chosen to group the many factors that potentially affect bovine ET success rates into four main categories as follows: donors, embryos, recipients and practitioner, with an almost limitless number of factors within each of these categories.

Factors related to embryos

Embryos from superovulated versus non-stimulated, single embryo donors

Hasler, et al. (1987) reported a conception rate of 71% following transfer of 146 embryos recovered from cycling (non-superovulated) Holstein donors into Holstein heifers versus 73%

for 2061 embryos transferred from superovulated Holstein donors with large responses of 16 or more ova/embryos.

Size of superovulatory response

Neither the size of the Superovulatory response nor the percentage of ova fertilized affected the conception rate of 7,000 transferred embryos (Hasler, et al., 1987). Also, neither the day of the estrous cycle (8-14) when superovulatory treatment was started nor the percentage of ova fertilized affected conception rate (Hasler, et al., 1987).

Donor characteristics

There are indications that some donor factors do potentially affect conception rates following ET. For example, there is abundant anecdotal evidence that embryos from Jersey donors do not survive freezing-thawing as well as the embryos from Holstein cattle. Recently, significantly lower pregnancy rates for Jersey embryos compared to Holstein embryos frozen either in ethylene glycol or glycerol were reported (Steel and Hasler, 2004). There is also a widespread perception that both fresh and frozen embryos from *Bos indicus* result in lower pregnancy rates compared to embryos from *Bos taurus* cattle, although there are no published data providing conclusive proof. Among *Bos taurus* breeds, embryos from dairy cattle resulted in a similar pregnancy rate to embryos from beef breeds (Hasler, 2001; Hasler et al., 1987). In one of these studies it was shown that embryos from cows over 15 years of age resulted in lower pregnancy rates following transfer than did embryos from younger animals (Hasler et al., 1987).

A higher conception rate (68%) was achieved following transfer of embryos from healthy (68%) compared to infertile (58%) donors into recipient heifers (Hasler et al., 1983). In the same study, the lactational status and age of Holstein donors had no effect on the conception rate of transferred embryos (Hasler et al., 1987). In contrast, however, in a recent study examining embryo quality, a significantly lower percentage of the embryos from lactating dairy cows were of excellent quality compared to those from dairy heifers or non-lactating beef cows (Leroy, et al., 2005). Embryo quality was based on morphology and the degree of embryo darkness or density.

Sire of embryos

It is well known that service sires can have a very profound effect on conception rate following natural service or AI. This is usually considered to be related to fertilization efficacy. In one commercial ET program, however, an influence of sire on conception rate was detected even after the embryo was judged suitable for transfer (Coleman, et al., 1987). It also has been shown that AI bulls of low fertility have higher rates of embryonic loss than do bulls of high fertility (Courot and Colas, 1986). Stroud and Hasler (2006) presented data that semen quality was directly related to fertilization rate in superovulated donors. Striking, however, was the significant, positive relationship between semen quality and the percentage of excellent-quality embryos. Of the total embryos in a collection, the percentage of embryos of excellent quality was directly related to semen quality. Another factor involving fertilization is the phenomenon described by Saacke, et al. (2000), in which the greater the number of accessory sperm found in the zona pellucida (ZP), the better the quality of the embryo.

Embryo morphology

In the early days of the ET industry, prior to the widespread utilization of cryopreservation, embryos often were collected as early as day-5 after estrus and as late as day-9 or 10. However, embryos survive cryopreservation best if they are approximately 7 days of age. Cryopreservation is now very widely practiced, with 64% of embryos collected in the US in 2005 cryopreserved (Thibier, 2006). Today most donors are collected between 6 ½ and 7 ½ days after estrus and, as a result, the majority of embryos collected range from late, compact morulae (stage 4) to mid-blastocysts (stage 6). The three most important criteria related to embryo morphology are age, stage and quality. Embryo stage and quality are usually based on the descriptions published by the International Embryo Transfer Society (IETS) (Stringfellow and Seidel, 1998). Based on a large number of transfers, no differences were noted in the pregnancy rate of embryos recovered between days 5 and 8 following standing estrus (Hasler, 2001; Hasler et al., 1987). On days 9 or later, however, when most bovine embryos have undergone hatching, a lower pregnancy rate was achieved (Hasler et al., 1987). Since embryo age corresponds rather closely to stage of development, it can be stated rather conclusively, based on a large number of fresh in vivo-derived embryo transfers, that embryo stages ranging from late morulae (stage 4) to expanded blastocysts (stage 7) result in comparable pregnancy rates, whereas following hatching (stage 8), lower pregnancy rates can be expected (Hasler et al., 1987). In contrast, conception rates of IVP embryos are more stage sensitive than are in vivo-derived embryos. Higher conception rates were achieved following transfer of expanded blastocysts compared to morulae and earlier stage blastocysts (Hasler, 1998; Lamb, 2005).

Within each embryo stage, morphological quality is also closely associated with pregnancy rate, as reported in a number of studies (Donaldson, 1985b; Hasler, 2001; Hasler, et al., 1987; Lindner and Wright, 1983; Putney et al., 1988; Putney al., 1989). Farin, et al. (1995) showed that agreement among 6 experienced embryo evaluators was higher for in vivo- compared to in vitro-derived embryos. In addition, there was a relatively high degree of agreement on evaluating excellent and degenerated (poor) embryos, but a lower degree relative to good and fair embryos.

Conditions of embryo storage

Both temperature and the length of time that embryos are stored potentially can affect embryo viability. Although elevated temperature can potentially be very injurious, bovine embryos can be maintained successfully for the short-term at a wide range of temperatures. Hasler et al. (1987) found no difference in pregnancy rates following transfer of embryos that were stored in PBS containing 10% serum at ambient temperature for 2-7, 8-18 or 19-24 hours. A commonly held view in commercial embryo transfer is that chilling bovine embryos increases the length of time that they can be held in a liquid medium. However, when embryos were held at 5°C versus 22°C in commercial holding media, there was no difference, regardless of the interval from collection to freezing, in the proportion of embryos that developed in culture after being frozen and thawed (Jousan et al., 2004). In contrast, embryos that were held for 12 h before freezing were less likely to survive freezing than embryos held for only 2 h prior to freezing. In another study that involved the export of embryos that were frozen as late as 180 min following collection, there was no effect of storage time at ambient temperature on pregnancy rate (Hasler, 2001).

Embryo Source

The highest conception rates in bovine ET are achieved with fresh, in vivo-derived embryos. Frozen and manipulated embryos and embryos resulting from various in vitro systems, including cloned and transgenic embryos, are all associated with lower conception rates. Conception rates under controlled conditions comparing embryos produced from different sources are not readily available in many cases. However, some data are available on conception rates of embryos from different sources within one program: For Grade 1 embryos, conception rates of 76% for fresh in vivo, 64% for frozen in vivo, 56% for fresh IVF-derived and 42% for frozen IVF-derived embryos were reported (Hasler, 1998). Similar results for in vivo-derived embryos were reported from The Netherlands, with 77.8% of fresh Grade 1 embryos and 66.6% of frozen Grade 1 embryos resulting in pregnancies (Otter, 1994).

Conception data from a large ET program in Brazil involving fresh, frozen and IVP embryos all transferred at one facility recently became available to the author (Table 5). The embryos originated from a number of different ET programs and all were transferred by a small number of practitioners into one herd of cross-bred *Bos taurus* x *indicus* heifers. Almost all transfers were into heifers observed in heat following natural, PG or CIDR-induced estrus. These data clearly show that conception rates varied with the source of the embryo. Also, early pregnancy losses were lower for fresh in vivo-derived embryos compared to frozen-thawed or IVP embryos.

Table 5. ET conception rates and pregnancy losses in one program in Brazil

Embryo Source	No. Transfers	% Preg. (30 d)	% Preg. (60 d)	% loss
In Vivo				
Fresh	1,483	46	42	7
Glycerol	93	42	37	13
EG	2,056	38	34	10
IVP	10,094	32	27	16

(compliments of Waldyr Da Costa Neto)

In an early report on embryo splitting, demi embryos produced via micromanipulation and then placed back into a ZP resulted in a 60% pregnancy rate per half, while intact embryos in the same commercial program resulted in a 70% pregnancy rate when transferred non-surgically (Takeda et al., 1986). In the late 1980s, when it became clear that the ZP was not necessary in order to achieve acceptable pregnancy rates with split embryos, the commercial embryo transfer industry largely discontinued the practice of putting split embryos back into zonae. Kippax, et al. (1991) achieved pregnancy rates of 57% with demi embryos transferred both with and without ZP.

Nuclear transfer cloning of cattle has evolved into a commercial endeavor in North America, with several companies offering cloning services. The efficiency of producing clones from somatic cells has improved a great deal since the initial success with Dolly the sheep, but it is still extremely variable (Wells, et al., 1999). Commercially, most clones are produced using fibroblasts that are cultured from a skin biopsy provided from the donor animal. Initial pregnancy rates following transfer of cloned embryos are often surprisingly high. Nevertheless, calving rates are low due to the high rate of abortions that occurs throughout gestation in recipients carrying cloned embryos. In one report (Lanza et at., 2001), 45% of 247 recipients were pregnant 35-40 days after transfer. Eighty of these spontaneously aborted (73% compared with 7 to 24% for IVF-derived pregnancies). Faber et al. (2003) reported pregnancy rates up to 86% on day 40 for recipients carrying clones; calving rates, however, ranged up to only 28%. In addition, it was reported that 21% of cloned calves failed to survive the first week after birth. Faber et al. (2006) subsequently reported day-40 conception rates ranging from 36 to 51% for >1,000 cloned embryos produced from donors ranging from 1 to 10+ years of age. Later, pregnancy rates at 210 days had dropped to a range of 13 to 16% for this group of recipients.

Factors related to recipients

Breed

No difference was reported in the conception rate of dairy heifers and beef heifers whether the embryos were fresh or frozen (Hasler, 2001). In contrast, Putney et al. (1988) reported that the overall mean pregnancy rate was higher for dairy heifers compared to beef heifers, but there were no differences between dairy and beef cows. Also, there were no differences within beef cattle between Brahman and European breed-type recipients. However, it is widely accepted within the ET industry that conception rates are lower in recipients of *Bos indicus* background, whether pure or cross-bred, compared to *Bos taurus* recipients. In addition to possible differences in innate fertility, *indicus* females are more difficult to handle, are less likely to clearly exhibit behavioral estrus and it generally is more difficult to pass a transfer gun through the cervix compared to *taurus* females.

Parity

In one commercial ET program, *Bos taurus* beef cow recipients sustained a pregnancy rate that was very similar to both beef heifers and dairy heifers (Hasler, 2001; Hasler, et al., 1987). However, the pregnancy rate of dairy cow recipients was lower following transfer of either fresh or frozen embryos than that of dairy heifers or beef cows or heifers. There are numerous additional reports of lower pregnancy rates in Holstein cows compared to heifers following transfer of fresh in vivo embryos (Putney et al., Dochi et al., 1988, Sauvé, unpublished). Pregnancy rates of IVP embryos were reported to be significantly lower in Holstein cows (33.1%) than in Holstein heifers (44.0%) (Aoki et al., 2004). First parity cows also had lower conception rates (39.6) versus heifers (47.5) following the transfer of IVP embryos (van Wagtendonk-de Leeuw et al., 1997).

Age

The author is unaware of any specific data on conception rate versus the age of recipients. Logic would suggest, however, that conception rates would tend to drop with advancing age of recipients. With the increased use of synchronization systems involving the use of CIDRs, pre-pubertal heifers are sometimes utilized as recipients. Stroud and Hasler (2006) showed that when pre-pubertal heifers were synchronized with a CIDR, a lower percentage of them were detected in estrus compared to pubertal heifers. A more serious problem, however, was that a much lower percentage (36%) of pre-pubertal were pregnant following ET compared to pubertal heifers (73%).

Lactational status

Wright (1981) did not report a difference in conception rates between lactating and non-lactating beef cattle used as recipients for embryo transfers. There are no published comparisons between lactating and dry dairy cows when used as recipients within the same ET program. However, there are numerous anecdotal data indicating that dairy cows sustain lower conception rates following transfer during the early period of their lactational curve compared to a later period or compared to dry cows. However, interpretation of data of this type from commercial ET programs is often problematic because dairy cows that are used late in lactation or during the dry period may be available as recipients specifically due to an infertility problem.

Reproductive history

It is generally assumed that recipients with a history of reproductive problems or infertility are not good candidates for use as embryo transfer recipients. However, practitioners are often presented with cattle that are reproductively unsound, especially dairy cattle, to be used as recipients. Since embryo transfer bypasses the steps of fertilization and early embryonic development, pregnancy rates are sometimes surprisingly high in cattle that are considered to have infertility problems. In a study utilizing dry Holstein cows purchased as repeat-breeders, a 70% conception rate was achieved following transfer of fresh in vivo-derived embryos compared to 82% in normal control recipient cows (Tanabe, et al., 1985). The use of anestrus beef cows versus cycling beef cows as ET recipients was shown to result in much lower conception rates following synchronization with CIDRs™ (Stroud and Hasler, 2006).

Nutrition

Nutrition is often emphasized as an important factor regarding success rates in ET recipients. However, very little information is available from controlled field trials. Obviously, low body condition scores should be avoided whenever possible when choosing recipients (Broadbent et al., 1991). In at least one study (Mapletoft et al., 1986), body condition significantly affected conception rate following ET. However, although a number of exotic mineral and trace element additives are touted as improving pregnancy rates in recipients, there are virtually no published data demonstrating an improvement in pregnancy rates as a direct result of using feed additives in animals that have access to a well balanced, traditional ration.

Climate and Season

There are few comparisons of pregnancy rates relative to climate within a single embryo transfer program. Putney et al. (1989) found no differences in pregnancy rate of Holstein recipients maintained either below or above a mean temperature of 32°C. Perhaps a more relevant influence of climate is when recipients do not cycle normally and in some cases do not exhibit estrus when environmental temperatures are extremely low or high. It has also been widely observed, although published studies are lacking, that the onset of sudden weather changes, for example severe winter storms, can profoundly affect bovine estrous cycles. In various areas of temperate North America, season did not affect the pregnancy rate in recipients following embryo transfer (Coleman et al., 1987; Hasler, 2001; Hasler et al., 1987; Putney et al., 1988). In fact, in a study involving more than 9,000 fresh embryos transferred throughout the North East USA during a four year period, conception rates varied by only 1 percentage point (Hasler, 2001). In The Netherlands, neither the season of embryo recovery nor the season of frozen embryo transfer affected conception rates following ET (Holland Genetics, unpublished).

Estrus induction
Recipients induced into estrus with PG exhibited a higher conception rate following embryo transfer than recipients coming into estrus naturally in one study (Hasler, et al., 1987) but not in others (Coleman et al., 1987; Wright, 1981). Also, data from Holland Genetics (unpublished) indicated a non-significant trend for higher conception rates for recipients induced with PG (60%) versus those coming into estrus spontaneously (56%). The higher conception rate from one study (Hasler, et al., 1987) could have been due to more accurate heat detection in synchronized animals because estrus was expected. It might also be due to an enhancement of fertility by PG as discussed previously (Hasler, et al., 1987). Macmillan and Day (1982) in New Zealand reported that regulating estrus in dairy cows with PG resulted in a conception rate of 69% in 2,000 cows compared to 60% in a similar number of untreated herd mates. In addition, the interval from PG injection to onset of estrus and the number of previous PG injections (1-13) did not affect pregnancy rate (Hasler, et al., 1987).

The availability of controlled intra-vaginal releasing devices (CIDR) led to the development of a number of different estrous synchronization protocols. Some of these protocols allow ET in a timed format without the detection of estrous behavior, sometimes called fixed-time embryo transfer (FTET).

Estrous synchrony between recipient and age of embryo
Schneider et al. (1980) reported that conception rates, following surgical ET, were higher when recipients were in estrus 12 h ahead of or synchronous with the donor compared to 12 h behind the donor. Based on approximately 7,000 flank surgical transfers to Holstein heifers, Hasler et al. (1987) reported that the conception rate was not affected by asynchrony between the donor (embryo) and recipient within the range of plus 36 h and minus 12 h. Plus synchrony is defined as the recipient in estrus before the donor. There was a slight but significant decrease in conception rate when synchrony was minus 24 h or more. This difference in the effect of plus and minus synchrony may be due in part to the observation that embryos from superovulated cattle are slightly advanced in development relative to those from normally cycling animals. Extremely similar synchrony results were also reported from another commercial ET program utilizing Holstein heifers (Coleman et al., 1987). In a later synchrony study, Hasler (2001) reported that asynchrony between plus 24 h and minus 24 h for both fresh and frozen embryos transferred non-surgically did not affect pregnancy rates. Also, this study showed for the first time that synchrony requirements were no different for dairy heifer, dairy cow and beef cow recipients.

Synchrony involving IVF-derived embryos involves the potential for confusion due to the convention of referring to the day of estrus in recipients as day 0, whereas in IVF culture systems the day of fertilization is designated as day 0. Consequently, zero synchrony actually involves transferring day 7 IVF-derived embryos into day 8 recipients. Thus, if day 6 recipients are used, they are actually minus 48 h in synchrony and not minus 24 h. As a consequence, the use of day 6 recipients resulted in a significantly lower pregnancy rate compared to day 7 or 8 recipients (Hasler, 1998). Similarly, Aoki et al. (2004) reported a higher pregnancy rate in day 8 recipients than in both day 6 and 7 recipients that received IVF-derived embryos. In a large study of IVP embryo transfers in Brazil, Lamb (2005) showed that there was a distinct advantage of using day 8 versus day 6 recipients for expanded blastocysts but no advantage for early or full blastocysts.

In some programs, synchrony is not calculated on a precise difference between the onset of donor and recipient estrus. For practical reasons, many practitioners merely determine whether a recipient is day 6, 7, or 8, etc. of the cycle. Since most embryos are collected day 7, this provides an approximate estimate of synchrony. Unpublished data from a number of practitioners have shown that very acceptable pregnancy rates can be achieved using this approach. It is also evident that there is not a consistent difference in whether day 6 or 8 is advantageous or disadvantageous to day 7 recipients. Using this approach to determining synchrony can result in a wider deviation than 24 h because, depending on the actual age of the embryo, asynchrony can be as much as 1 ½ days or more.

Another consideration related to synchrony is whether there is an advantage to trying to match embryo stage, rather than embryo age, to the 'age' of the recipient. Of course, embryo stage is often fairly well correlated with age, but embryos of differing stages of development are often recovered from a donor. Data on conception rates resulting from transfer of embryos of different stages relative to day of the recipient estrous cycle suggest that late morulae are more tolerant of at least a full day of asynchrony than either stage 5 or 6 blastocysts (McDonald, unpublished). Stage 6 blastocysts are relatively intolerant of being transferred into day 6 recipients.

Corpus luteum quality

The quality of the CL at the time of embryo transfer, as determined by visual observation or by palpation, was not correlated with conception rates in a number of studies (Coleman et al., 1987; Donaldson, 1985a; Hasler, et al., 1987; Looney et al., 1984, Remsen and Roussel, 1982). Use of ultrasound to measure luteal volume also has not been predictive of subsequent ET conception rates (Spell, et al., 2001).

Site of transfer

It is well known that bovine embryos must be transferred into the uterine horn ipsilateral to the CL in order to maximize pregnancy rate. Less well documented is the issue of how far up the uterine horn the embryo should be deposited. In an analysis of 6,000 non-surgical transfers, a higher pregnancy rate was achieved when the embryo was place in the upper (69.6%) or middle 1/3 (68.8%) of the uterus compared to the lower 1/3 (59.8%) (Steel, unpublished). Interestingly, there was no influence of depth of transfer when the embryos were Quality 1. With Quality 2 or 3 embryos, however, the pregnancy rate was depressed when the transfer was in the lower 1/3 of the uterus compared to the middle or upper 1/3. In another study, transfers at a point adjacent to the external bifurcation of the uterine horns resulted in a 29.6% pregnancy rate, while deep transfers at a point more than two-thirds of the distance from the external bifurcation resulted in a 65.9% pregnancy rate (Beal et al., 1998).

Side of transfer

Cattle spontaneously ovulate more often on the right ovary (approximately 55%) than on the left (Reece and Turner, 1938). Studies involving non-surgical transfer (80), mid-ventral surgical transfer (Hasler et al., 1980) and flank surgical transfer (Remsen and Roussel, 1982) have not detected any difference in pregnancy rate between transfers to the left or right side. Sauvé (unpublished) reported no difference in pregnancy rate following non-surgical transfer of over 12,000 embryos by several practitioners. In a study involving the transfer of more than 12,000 IVP embryos, Lamb (2005) reported no difference in pregnancy rate relative to side of transfer. It should be noted that this study involved 23 different practitioners. For an individual, however, the side of transfer may affect success rate. In an analysis of approximately 8,000 transfers, Steel (unpublished) achieved a small but significantly higher pregnancy rate with transfers to the right horn (65.0%) versus the left horn (62.2%). Steel reported that he routinely placed his left arm in the rectum and that transfers to the left seemed slightly more difficult.

Method of transfer

Comparisons of pregnancy rates based on mid-ventral surgical, flank surgical and non-surgical transfer are usually tenuous because the different transfer methods usually were not performed contemporaneously. In a comparison in which different transfer methods did overlap in time, Hasler (2001) reported that that at one location pregnancy rates in heifers were not different between flank surgical and non-surgical transfers of large numbers of embryos, whether the embryos were fresh or frozen but at another location surgical transfer of fresh embryos (n=3716) resulted in higher conception rates (80%) than did non-surgical transfers (72%, n=1600) (Hasler, 2006). In the same study, the conception rate in cows was higher following surgical versus non-surgical transfer of fresh embryos at one location but not at the other.

Progesterone levels in recipients

Several studies have demonstrated no difference between serum progesterone levels at the time of embryo transfer in recipients that remained pregnant and those that did not (Chagas, et al., 2002; Ellington, et al., 1991; Hahn et al., 1977; Hasler, et al., 1980; Spell et al., 2001). Other studies have shown that progesterone levels above a certain range (5-8 ng/ml plasma) in ET recipients may be related to a lower pregnancy rate (Bierschwal and Murphy, 1985; Niemann et al., 1985; Northey et al., 1985).

Drugs/Hormones

In addition to progesterone, a number of different drugs/hormones have been employed in embryo transfer programs with the goal of increasing conception rates in recipients. For the most part, treatments have not resulted in increased conception rates. In general, it appears that in those studies in which the administration of a drug or hormone led to an enhancement of conception rate, the control conception rate was lower than what would generally be considered normal. The effect of increasing progesterone concentrations on inducing and/or maintaining pregnancies, either with a progesterone releasing device or the induction of an accessory CL by the use of GnRH (Ellington et al., 1991; Nishisouzu et al., 2004; Smith and Grimmer, 2002), hCG (Greve and Lehn-Jensen, 1982; Hasler et al., 1995; Looney et al., 1984; Nishigai et al., 2002), LH (Small et al., 2004) or eCG (Nasser et al., 2004; Tribulo et al., 2005) has not been consistent. Recombinant bovine somatotropin (rbST) administered at the time of estrus prior to embryo transfer increased conception rates in lactating Holstein cows in one study (Moreira et al., 2001) but not in another involving Holstein heifers, where the rbST was given immediately after embryo transfer (Hasler et al., 2003).

The β-mimetic agent clenbuterol, utilized in order to maintain uterine quiescence, did not increase pregnancy rate when it was injected prior to non-surgical (Almeida, 1989; Walton et al., 1986) or surgical embryo transfer in one study (Barnes and First, 1985), but pregnancy rate increased when clenbuterol was used prior to surgical embryo transfer in other studies (Coulthard, 1982; Mapletoft et al, 1986). Ibuprofen (ibuprofen lysinate) is a non-steroidal anti-inflammatory drug that has a number of beneficial actions, including the reduction of prostaglandin synthesis, that has been attributed to the functional inhibition of both isoforms of cyclooxygenase (COX-1 and COX-2). When Ibuprofen was administered to heifers at 5 mg/kg of body weight one hour prior to the transfer of frozen embryos, the resulting pregnancy rate (82%) was significantly higher (n=50) than the pregnancy rate observed in control heifers (56%) (Elli et al, 2001).

Banamine (flunixin meglumine) is an effective inhibitor of $PGF_{2\alpha}$ release. As a consequence, it has been administered in several field trials, either immediately before or after transfer of embryos, in attempts to increase pregnancy rates. In one study, beef cows which received 10 ml of Banamine two to five minutes prior to transfer of both fresh and frozen embryos exhibited a higher overall pregnancy rate (63.8%) than did control animals (51.1%) (Schrick et al., 2001). In a field trial involving the transfer of a large number of frozen embryos (n=506) in Argentina, however, there was no difference in the pregnancy rates between Banamine-treated recipients (66.0%) and non-treated controls (66.4%) (Burry, unpublished). AL8810 (Embryo Armor®) is a prostaglandin antagonist that has been shown to bind to $PGF_{2\alpha}$ binding sites on bovine embryos. Embryos recovered with flush medium containing AL8810 resulted in higher conception rates following both fresh and frozen transfer into recipients (Scenna et al., 2008).

Stress

Stress is not easy to quantify in most ET situations and there are few references to stress in the context of ET in the literature. The author has observations with no quantified data involving the movement of animals by truck either just prior to or after embryo transfer. A negative effect on pregnancy rate was not observed when a few hours of trucking was involved. Yavas, et al. (1996) found no influence on pregnancy rate but an increase in cortisol levels in heifers following 1 h of trucking before or after artificial insemination. This study, along with others, also showed that there is a certain amount of stress, as indicated by cortisol levels, associated with AI and presumably with embryo transfer. Evidence also strongly suggests that changes in groupings of animals create stress in both the new animals and those in the existing group. Dobson, et al. (2001) suggested that the exact position of an animal in a social structure probably does not affect embryo transfer results but that stress-induced changes in hierarchical position are probably very important.

Factors related to the ET practitioner

A number of studies have shown that both non-return rates and pregnancy rates vary widely among experienced AI technicians operating under similar conditions. Lamb (2005) presented data showing that 23 ET technicians in Brazil achieved conception rates ranging from 0.4 to 48% following transfer of IVP embryos. Schneider et al. (1980) reported differences among conception rates relative to individual ET technicians. Also, in a large commercial embryo transfer program in The Netherlands, significant differences in conception rates achieved following embryo transfer were evident among different ET practitioners, all of whom were highly experienced (1997). In the same study, a higher conception rate was achieved when transfers were performed on Tuesdays (50.8%) compared to those performed on Fridays (42.2%).

Lastly, it should not be overlooked that *'luck'* plays a part in the outcome of some ET procedures. For example, in a situation where a conception rate of 50% is normally achieved, zero or 100% success rates are each to be expected 6% of the time when four embryos are transferred and a 50% pregnancy rate only 38% of the time (Seidel et al., 2003).

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