



## Dissecting why superovulation and embryo transfer usually work on some farms but not on others

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### Abstract

Bovine embryo transfer is a well-established commercial industry that is often associated with veterinary practices. Practitioners offering embryo transfer services may possess a very high standard of technical expertise; however, success in the production of embryos and the impregnation of recipients cannot be achieved unless the cattle are healthy and maintained in a well-managed cattle operation. In addition to appropriate gonadotropin treatments of donor cattle, the use of highly fertile semen, known to have been properly stored and handled is required for success. Recipient cattle must be managed with the same attention to detail as donors. Traditionally, PGF has been used for the synchronization of recipients. However, PGF is limited in its effectiveness early and late in the bovine estrus cycle. Recipient estrus synchronization with progesterone releasing intravaginal inserts has been successful and high pregnancy rates have resulted following embryo transfer.

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### 1. Introduction

Since bovine embryo transfer became commercialized in the early 1970s, there have been millions of bovine embryos transferred internationally. According to the International

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Embryo Transfer Society (IETS) Data Retrieval Committee, an average of about 500,000 cattle embryos are transferred annually on a global basis [1]. Although the number of donors collected and embryos recovered are relatively easy to obtain from embryo transfer practitioners, accurate conception rates for transferred embryos are more difficult to acquire. For example, in the USA, many recipients are placed with a bull shortly after embryo transfer and it is only after parturition that it can be determined whether the calves were the result of transferred embryos or natural matings. In the authors' experience, results are seldom reported to the practitioner who transferred the embryos. However, in most well-managed dairy and beef cattle operations, embryo transfer conception rates are routinely determined as a means to measure and improve management practices. Experienced embryo transfer practitioners can usually identify farms where donor females consistently produce high numbers of high quality embryos and where high conception rates are achieved in recipients. Conversely, low embryo production rates and below average embryo transfer conception rates are usually associated with poorly managed farms. In some cattle operations, high numbers of embryos are produced by superovulated donors, but recipient management is suboptimal, or vice versa. The objective of this paper is to identify some of the specific causes, both positive and negative, for these effects. By doing so, inexperienced embryo transfer practitioners and researchers in this industry can understand the complexities of producing acceptable results from donor and recipient females on a consistent basis.

## **2. Materials and methods**

### *2.1. Farms*

The data utilized in this study were collected on farms where the authors conducted commercial embryo transfer procedures. There were no experimental manipulations involved with either donor or recipient cattle. For the comparison of superovulation results and embryo transfer pregnancy rates, two farms were identified on which embryo transfer procedures were conducted by the authors for a period of 2–3 years. Farm A, a cattle program that has been in operation for 25 years, is located in Oklahoma and maintains several hundred head of beef cows on pasture consisting primarily of native grasses. The cattle are supplemented at a moderate level with standard protein-energy-mineral supplements. The authors' experience with embryo transfer on this farm covered a 3-year period. Consistently good results with superovulation and recipient pregnancy rates have been achieved on this farm. Farm B is a beef cattle operation that has only been in business for about 3 years. The management on this farm is also much less experienced than at Farm A. The authors have provided embryo transfer services on this farm for approximately 2 years, which overlapped in time with the service provided to Farm A.

In addition, data on conception rates for cows and heifers bred by AI and a combination of cows and heifers to which embryos had been transferred were provided by managers of other farms with reliable record keeping systems. These farms are Farm A, described above, plus three additional farms designated as Farms C, D, and E.

## 2.2. Semen

At Stroud Veterinary Embryo Services, detailed records have been kept on all semen delivered for use in breeding client-owned embryo donors. The records include whether the semen was shipped directly from or by a field-representative of an artificial insemination center, or whether it was delivered from the inventory of a client, or some other second-hand source.

Prior to AI of donor cattle at Stroud Veterinary Embryo Services, a consistent volume of semen is removed from all thawed straws and examined on a warm slide with a phase-contrast microscope at 100× and 400× magnifications. Each semen sample is scored for the following parameters, with subclasses of quality as follows. Concentration: high, medium, low; percent motile: 1–10%, 11–20%, 21–30% and >30%; rate of forward movement: rapid, moderate, slow; direction of movement: forward, circular, asymmetrical; morphology: excellent, good, fair or poor, with corresponding percentages of abnormalities. Overall semen scores represented a combined score of excellent, good, fair, poor, or unacceptable considering all the above factors.

## 2.3. Statistical analyses

A one-way analysis of variance (ANOVA) was used to analyze differences in quantitative data, e.g. superovulation results, whereas Chi-square analysis was applied to pregnancy rates and semen factors.

## 3. Results and discussion

### 3.1. Superovulation and donor management

As seen in Table 1, the donors on Farm A produced, on average, 15.8 ova/embryos and 9.8 viable or transferable embryos per collection. The mean number of transferable embryos collected on this farm is almost three more per donor than the mean for the more

Table 1  
Ova/embryo production following superstimulation of beef cattle on two ranches in south central USA; comparison with AETA tabulated data from the same geographic region

Location	No. donor collections	Total (mean) ova/embryos	Total viable (mean) viable embryos	Percentage of viable embryos
Ranch A	172	2719 (15.8) a	1681 (9.8) a	63.4 c
Ranch B	63	593 (9.4) b	283 (4.5) b	48.2 d
AETA data <sup>e</sup>	10643	NA <sup>f</sup>	73182 (6.9) <sup>g</sup>	NA <sup>f</sup>

(a, b) Mean values differ significantly ( $P < 0.001$ ; ANOVA). (c, d) Percentages differ significantly ( $P < 0.001$ ; Chi-square analysis).

<sup>e</sup> AETA, not published.

<sup>f</sup> Not available.

<sup>g</sup> Not included in statistical analysis.

than 10,000 superstimulated beef donors reported to the American Embryo Transfer Association (AETA, unpublished data) for the south central region of the USA for the same time period. In contrast, Farm B produced more than five fewer embryos per donor than Farm A, over a contemporary time period.

More than 99% of dairy and most beef embryos collected in commercial embryo transfer programs are from superovulated females (AETA, unpublished data). However, superovulation is an unnatural process for cattle. Naturally cycling cattle normally ovulate one ovum per estrus cycle, while multiple follicles that would normally undergo atresia are stimulated to mature and ovulate as a result of the superstimulation process [2]. Several billion fresh sperm are normally deposited in the vagina of single-ovulating cattle that are naturally mated. In single-ovulating cows that were not superstimulated, fertilization rates of 85–90% [3] and, on occasion, nearly 100% [4] have been reported. In superovulated cattle, fertilization rates have been reported to range from 50 to 70% [5].

Superstimulation in the cow requires a 3- or 4-days period of gonadotropin treatment, followed by estrus detection and AI. All of these steps provide opportunities for error. Some of the most important factors leading to a high level of success with superovulation are as follows: (1) donor management experience and expertise; (2) donor genetics; (3) donor nutrition; (4) donor age; (5) donor lactational status; (6) quality of semen; and (7) timing of insemination.

In the authors' opinions, donor management experience is the single most important variable affecting superovulation results, since it encompasses all of the other disciplines listed. Successful donor managers both understand and implement the factors listed above on a routine basis. Poorly qualified managers do not have full understanding of these factors and therefore have difficulty utilizing them.

### 3.2. Donor management

When Farm A began utilizing embryo transfer 25 years ago, donor collections yielded extremely variable embryo numbers (personal communication). As a result, collections that produced high numbers of high quality embryos resulted in more female embryo transfer offspring than those with low numbers. Consequently, more females from collections producing high numbers of embryos were selected for retention in the herd. The management on this farm has expressed the opinion that embryo production has increased and become more consistent over the years. Experience with individual cattle operations for more than 25 years has led the authors to believe that cows which superovulate well tend to produce daughters that also superovulate well. However, this is in contrast to the conclusions of Tonhati et al. [6], who studied records on 5387 embryo collections in Brazil and described a low level of heritability for superovulatory response. Similarly, the examination of superovulation records among four breeds of Danish dairy cattle showed heritability coefficients of 0.25 for total ova and 0.15 for numbers of embryos [7].

Ovarian size may play a role in superovulatory response. The authors have observed during transrectal ultrasound scans, transvaginal ultrasound-guided oocyte aspirations, and slaughterhouse ovary oocyte aspirations that ovaries with a larger volume usually contain more small follicles. Indeed, Singh et al. [8] reported that superovulatory response was

highly correlated with the number of follicles entering a follicle wave. It is likely that cattle with larger numbers of follicles will also have larger ovaries.

Donor nutrition has been reported to affect embryo production and quality. Beef heifers fed a citrus/beet pulp concentrate ad libitum produced significantly fewer ova and embryos following superstimulation than those fed 3 kg per day of the concentrate [9]. In the opinion of the authors, good quality forage is the single most important nutritional factor associated with response to superstimulation and viable embryo production in beef cattle. Donor females grazing on highly palatable, green pastures usually have a higher superovulatory response than those on poor-quality pasture, hay, or silage. Other nutritional factors such as minerals are difficult to scientifically evaluate in commercial settings. Controlling conditions, such as maintaining adequate numbers of closely related genetic lines of donor females that are similar in age, lactational status, and overall health is difficult in research settings. In the field, many donor managers are convinced as to the superiority of their mineral and supplement programs, but those opinions are almost always based on unquantified observations, and not on scientifically controlled studies. In an experiment that evaluated the effect of organic or inorganic supplementation on superovulatory response, heifers that received organic minerals tended to have more transferable embryos than those receiving inorganic minerals [10]. However, the number of embryos produced by the heifers in the control group was intermediate and not different from either treatment.

The authors' have observed that over-conditioned, overweight donor females tend to produce fewer viable embryos per collection. The most frequently encountered overweight cattle are heifers that are fed for show and mature donor cows that have not been lactating for extended periods of time. Again, the authors have observed that overweight females seem to be more likely to develop cystic ovarian disease and to produce more unfertilized ova when superstimulated.

Donor age is also an important factor that affects the number of viable embryos collected from superovulated cattle [11]. Virgin heifers tend to produce fewer embryos than mature cows. Dairy donors that have reached the age of approximately 10 years tend to have smaller responses following superstimulation [11]. Meaningful data relating age to superovulatory response are not available for beef donors, but the authors agree that embryo production in beef cows 10 years or older begins to decline.

Inexperienced cattle owners and managers frequently purchase older donor cows that possess name recognition or desirable phenotypes and highly accurate indices such as, total performance index (TPI), and expected progeny differences (EPDs); however, many of those aged donors are poor embryo producers. Conversely, experienced cattlemen select younger females with sound genetic indices and their reproductive lives ahead of them for embryo donors.

### *3.3. Insemination of donors*

Variation in the level of management on farms is also evident with an examination of conception rates following AI (Fig. 1). Although superstimulated donors are occasionally mated naturally to a herd sire, the vast majority of donors are inseminated with frozen-thawed semen. Unfortunately, most AI technicians neither have access to a microscope nor

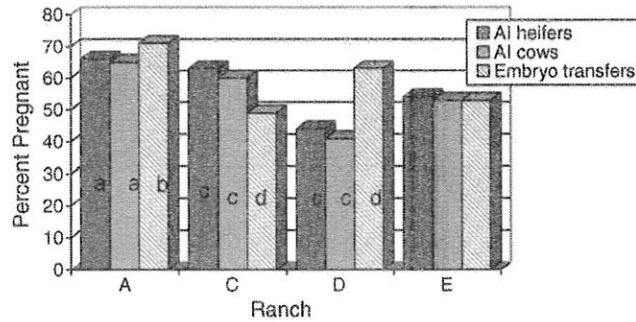


Fig. 1. Comparison of beef cow and heifer AI pregnancy rates and embryo transfer pregnancy rates on four different ranches (data collected over a 2- to 15-year period on individual ranches). Sample sizes for cow AI, heifer AI, and embryo transfer for the four ranches were as follows: Ranch A: 1125, 2350, 355, respectively; Ranch C: 618, 1366, 403, respectively; Ranch D: 320, 785, 224, respectively; Ranch E: 280, 312, 550, respectively. Within ranches, percentages differ significantly; <sup>ab</sup> $P < 0.025$ ; <sup>cd</sup> $P < 0.001$  (Chi-square analysis).

the skill to evaluate semen immediately post-thaw, prior to insemination. In addition, many AI technicians and farmers assume that the semen they thaw is highly fertile and unchanged from the day it was frozen at a commercial artificial insemination center. Table 2 shows that semen shipped directly from artificial insemination centers and thawed and evaluated at Stroud Veterinary Embryo Services had a very high level of acceptable motility and morphology, and no semen with zero motility. In contrast, semen delivered or shipped to Stroud Veterinary Embryo Services by farm or ranch personnel had a high percentage of straws with either dead sperm or very poor sperm viability. These data suggest that semen stored in owner's liquid nitrogen tanks had a greater chance of being mishandled, resulting in exposure damage. However, there are also several reports of semen of poor-quality being distributed by commercial artificial insemination centers [12–14]; consequently, one cannot assume that semen is of high quality, especially if the embryo transfer practitioners has not had experience with the bull or batches in question.

Damage to semen can occur from improperly moving straws between tanks or by partial-thawing resulting from raising canes too high and for too long a period of time into the neck of the nitrogen tank [15]. Also, warming and resulting damage to straws often occurs as a result of reading straw codes or moving straws from goblet to goblet without the use of a Dewar flask.

Table 2

Comparison of semen quality in straws shipped directly from commercial artificial insemination centers compared to straws provided by breeder clients

Classification of semen at thawing	Source of semen	
	Direct from AI center	Provided by client
No. of shipments	1278	981
No. shipments with poor-quality semen (%)	22 (1.7) a	66 (6.7) b
No. shipments with semen with no motility (%)	0 (0) a	24 (2.4) b

(a, b) Percentages in the same row differ significantly ( $P < 0.001$ ; Chi-square analysis).



Table 3  
Effect of semen quality on fertilization rate and embryo quality in superstimulated cattle (227 bulls used on 742 donors)

Semen quality <sup>e</sup>	Fertilized ova (%) (n = 9732)	Excellent embryos (%) (n = 4035)
Excellent	82.1 a	61.2 a
Good	67.6 b	55.7 b
Fair	58.3 c	53.9 c
Poor	51.8 d	33.7 d

(a, b, c, d) Percentages within columns differ significantly ( $P < 0.002$ ; Chi-square analysis).

<sup>e</sup> Semen quality combined the following factors: concentration, percent motile, rate of forward movement, direction of movement and morphology.

Microscopic examination of semen by an experienced practitioner prior to insemination of embryo transfer donors allows the inseminator to reject dead or poor-quality semen. It also gives the inseminator the opportunity to evaluate a number of semen parameters that relate to fertilization of multiple ova in superovulated donors [16]. We have demonstrated a highly significant relationship between semen quality and the percentage of fertilized ova and transferable embryos in superovulated donors (Table 3). Most striking is the positive relationship between semen quality and the percentage of excellent-quality embryos. This observation supports the work of DeJarnette et al. [17], who showed that embryo quality was positively correlated with accessory sperm numbers, which in turn were positively correlated with fertilization rates.

In addition to semen quality, the timing of insemination is important for consistent results in embryo recoveries from superovulated females. The most reliable indicator of when to inseminate a superstimulated donor is the first mount during estrus. High fertilization rates usually result when superstimulated beef donors are inseminated once at 12–14 h after they first stand to be mounted followed by a second insemination 17–24 h after the first mount. In cases where the AI technician is limited to only one unit of frozen semen due to cost or availability, we recommend that females be inseminated in a 16- to 20-h window after the first mount. The authors' observations on insemination of superstimulated cattle in commercial embryo transfer are supported by well-controlled laboratory studies [18,19].

Although it is often taken for granted or trivialized, conscientious and competent observation for estrus in superstimulated donors can be a major component of success. The best sentinels for estrus detection are other females in or coming into estrus, gomer bulls, and young bull calves 3–6 months of age. Professional management will have sentinel animals in the presence of a superstimulated donor at the appropriate time and will actually observe the first mount or will record it by use of an electronic heat detection device.

#### 3.4. Recipient management

Recipient selection and management are major contributors to high conception rates following embryos transfer. Morphologically high quality embryos result from a donor environment that allows folliculogenesis, oocyte maturation, multiple ovulation, sperm transport, fertilization, and subsequent early embryonic development to occur in biological

harmony. Within a few hours of recovery, embryos must be either frozen or returned to a similar environment within recipients to maximize the chances of establishing pregnancies. Selection of suitable recipients, much as was described for donors, is an important management decision. Beef and dairy recipients are broadly categorized by parity, age, and body condition. There is an advantage to using virgin heifers for recipients, but they will produce smaller calves and are more likely to experience dystochia [20]. Embryo transfer conception rates, especially in dairy cattle, are higher in heifers than in mature, lactating cows [21]. This is an especially important economic decision because superovulated dairy females typically produce fewer transferable embryos per collection than beef donors (AETA, unpublished). Pregnancy rates in beef heifers and lactating beef cows have not been shown to differ [21].

In addition to parity, factors such as reproductive history should be considered before a recipient female is selected for estrus synchronization. In dairy cows, cystic ovaries, retained placentae, and lactation number are important selection criteria. Similarly, all reproductive factors available should be taken into account prior to selection of beef cattle recipients. It may take months of planning, especially for a new beef breeder, to properly select females for recipients. It is not uncommon for beef breeders to purchase recipients from a sale barn, have them palpated per rectum for pregnancy, and synchronize the open animals all within 1 week of purchase. In addition to not knowing the history of sale barn cattle at time of purchase (other than lactation status), it should be assumed that they were very likely exposed to bull(s) until immediately prior to the sale. The transfer of embryos into synchronized recipients that have aborted a nonpalpable conceptus shortly after PGF treatment is not an effective approach to embryo transfer. Also, the use of nonpregnant females that originate from a herd with normal or high pregnancy rates as recipients is a poor approach; experienced recipient managers generally avoid such scenarios. A better option is to purchase late-term females, calve them out, palpate them for reproductive soundness, and initiate estrus synchronization no sooner than 60 days post-partum. Also, cow-calf pairs can often be purchased if the calves are less than 60 days of age. Such females should be treated with PGF as soon as possible to avoid any unwanted pregnancies.

### *3.5. Recipient estrus synchronization*

Once recipients have been selected, they must be managed specifically for use in embryo transfer. Traditionally, PGF has been used for the synchronization of recipients. However, PGF is limited in its effectiveness both early and late in the bovine estrus cycle [22]. As a consequence, approximately one-half of a group of randomly cycling females will not respond to treatment with PGF. Therefore, very effective synchronization protocols involving the use of progesterone releasing insert (e.g., CIDR) have been developed (for review see [23]).

There is one physiological issue that can have a negative effect on conception rates following estrus synchronization and embryo transfer, especially when virgin heifers are used as embryo transfer recipients. Estrus in virgin heifers is often synchronized with progesterone releasing inserts without regard to their reproductive maturity. Although pre-pubertal (anestrus) heifers will often exhibit estrus and ovulate following removal of progesterone releasing inserts, conception rates of transferred embryos are usually lower



Table 4

Conception rates following embryo transfer in pre-pubertal vs. pubertal beef recipient heifers in which estrus had been synchronized with a CIDR

Heifer status <sup>a</sup>	No. received CIDR	No. estrus detected (%)	No. received embryo (%) <sup>b</sup>	No. pregnant (%) <sup>b</sup>
Pre-pubertal	428	161 (37.7) a	135 (83.9) a	48 (35.6) a
Pubertal	477	403 (84.5) b	380 (94.3) b	278 (73.2) b

(a, b) Percentages in the same column differ significantly ( $P < 0.001$ ; Chi-square analysis).

<sup>a</sup> Puberty status determined by palpation per rectum and ultrasound at time of CIDR insertion.

<sup>b</sup> Percentage based on previous column.

for heifers that have ovulated for the first time than for those that have already reached puberty (Table 4). These data clearly show that a larger percentage of post-pubertal heifers respond to progesterone releasing inserts by coming into estrus, and that a higher percentage will become pregnant following embryo transfer. Careful screening of heifer recipient candidates prior to synchronization with a progesterone releasing insert, either by estrus detection, reproductive tract scores or by palpation per rectum and/or ultrasound, will eliminate most pre-pubertal females ultimately receiving embryos. When heifers cannot be pre-screened for puberty, experienced managers often elect to use PGF for estrus synchronization rather than progesterone releasing inserts, since PGF will not induce an anestrous female to show estrus.

Multiparous, post-partum, anestrous females are basically no different than pre-pubertal heifers in their response to progesterone releasing inserts and conception rates following transfer of embryos. Some will exhibit estrus following treatment with progesterone releasing inserts, but conception rates following embryo transfer are lower than for cycling recipients (Table 5). It is also noteworthy that progesterone releasing inserts will more consistently synchronize recipients within  $\pm 24$  h of donors than PGF [23]. When asynchrony is much beyond  $\pm 24$  h, recipient conception rates following embryo transfer have been shown to be reduced [21,24]. Before a synchronization protocol is chosen, it is necessary for a recipient manager to know the goals of ownership and the reproductive status of the donors and recipients; management must take into consideration whether the goal is to maximize conception rates or maximize the total number of embryos transferred, and thus, total numbers of pregnancies. In this regard, fixed-time embryo transfer protocols will facilitate the optimal utilization of recipients [23].

Nutritional management of recipients is probably even more important than for donors. Again, good quality forage is the primary consideration, but protein and energy

Table 5

Conception rates following embryo transfer in anestrous vs. cycling beef recipient cows

Cow status <sup>a</sup>	No. received CIDRs	No. estrus detected (%)	No. received embryo (%) <sup>b</sup>	No. pregnant (%) <sup>b</sup>
Anestrous	321	161 (50.1) a	140 (87.0)	56 (40.0) a
Cycling	376	338 (89.9) b	311 (92.0)	206 (66.2) b

(a, b) Percentages in the same column differ significantly ( $P < 0.001$ ; Chi-square analysis).

<sup>a</sup> Determined by palpation per rectum and ultrasound at time of CIDR insertion.

<sup>b</sup> Percentage based on previous column.

supplementation during drought and nongrowing seasons are necessary for establishing and maintaining pregnancies in recipients. It is very important to not let recipients go through negative energy deficits, especially during the first 45 days after embryo transfer. Recipients are often managed as second-class, crossbred females that are merely temporarily hired as surrogates for the upper-class purebreds. Inexperienced management may relegate them to the poorest quality pastures, providing inadequate levels of protein and energy to maintain pregnancy. Experienced management sees them for what they are, incubators for the most valuable calves on the farm, and treat them accordingly. In the experience of the authors, however, most ranch managers do not utilize body condition scores. Nevertheless, conception rates following embryo transfer were significantly higher in recipients with body condition scores that were above average [25]. When one looks at statistics on well-managed beef operations, there are some noteworthy and important similarities. Conception rates following AI of single-ovulating virgin beef heifers tend to be only slightly higher than for single-ovulating beef cows, and embryo transfer conception rates are not different than AI conception rates in the purebred herd. This makes it relatively easy to troubleshoot problems that arise in any one of these areas.

In Fig. 1, conception rates from four different beef ranches in the south central USA reveal certain tendencies which help to identify specific problem areas. Data from Ranch A suggest that management was excellent based on comparable conception rates across all three classes of females. There appears to have been a problem in the recipient herd at Ranch C. The AI conception rates were quite acceptable, while the conception rates following embryo transfer were significantly lower. Embryo transfer by-passes potential problems with fertilization in recipients and, as a consequence, may lead to higher conception rates than AI. Thus, if one assumes that a competent embryo transfer practitioner was employed on Ranch C, factors such as recipient selection, nutrition, synchronization protocols, estrus detection, and the range of synchrony between donors and recipients should be evaluated.

Although embryo transfer conception rates were acceptable on Ranch D, AI conception rates were somewhat lower than expected in both cows and heifers. It is possible that the AI technician is either inexperienced or not very skilled, or a problem with estrus detection or frozen semen handling exists. The time period of the low conception rates should be evaluated and if one or two sires are being heavily used over a breeding season, their semen should be evaluated or look to others breeders that have used the bull(s) in question. Care should be taken to record collection codes each time a female is inseminated, and semen handling techniques should be evaluated if all bulls within a breeding season produce poor conception rates.

Conception rates across all AI and embryo transfer groups were similar and very low on Ranch E. It initially appeared difficult to identify the problem(s) on this ranch. However, close examination revealed a problem with forage. This beef operation had nearly 1200 acres of Coastal Bermuda grass and no pastures with native grasses. The cattle consumed Bermuda grass during the growing season and Bermuda hay during the non-growing season, without any access to other forages. It became evident that there was no problem with newly purchased donors; they consistently produced a higher percentage of transferable embryos than resident donors. This problem was solved, as evidenced by improved superovulation results, by leasing sufficient native pasture to accommodate all nonlactating, bred females until approximately 2 weeks after calving.

### 3.6. Cattle facilities

It is not unusual for some cattle owners to spend tens of thousands of dollars on cattle, ranch signs, and entrances, yet fail to provide adequate facilities for their embryo transfer programs. Often, simple things, such as cattle chutes (squeezes) to secure recipients during embryo transfer can cause noticeable decreases in conception rates. Unsecured, nervous beef females that are free to move during embryo transfer can adversely affect conception rates. Some facilities have adequate chutes, yet lack sufficient access for the embryo transfer practitioner to work safely and comfortably behind the recipient.

## 4. Conclusions

The commercial embryo transfer industry has passed the end of its third decade of use in North America. During the early days of this industry, the pioneers in the field worried and speculated about scientific issues relative to successful superovulation, embryo recovery, and embryo transfer. Synchrony between donor and recipients, superovulation protocols, embryo quality, and many other factors that contribute to success have been exhaustively examined over the years. However, experienced embryo transfer practitioners also recognize the importance of suitable husbandry and management practices on success. Well-managed cattle operations can make an average embryo transfer practitioner appear very good, while poorly managed operations can humble the most experienced embryo transfer practitioner. It is evident that success in bovine embryo transfer requires a marriage of reproductive physiology, basic animal husbandry, and veterinary science to produce consistent and acceptable results. Breeding farms that utilize these resources with experienced personnel are consistently successful, while those that do not are often not successful. Interestingly, many new cattle breeders seem to think that technology coupled with skill on the part of the practitioner is all that is required for success. The importance of animal husbandry is often overlooked or under estimated. It is the responsibility of the embryo transfer practitioner to educate clients to the value of basic animal husbandry.

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## References

- [1] Thibier M. Stabilization of numbers of in vivo collected embryos in cattle but significant increases of in vitro bovine produced embryos in some parts of the world: a report from the IETS data retrieval committee. *Int Embryo Transfer Soc Newslett* 2004;12–9.
- [2] Romero A, Albert J, Brink Z, Seidel GE. Numbers of small follicles in ovaries affect superovulation response in cattle. *Theriogenology* 1991;35:265 [abstract].

- [3] Diskin MG, Sreenan JM. Fertilization and embryonic mortality rates in beef heifers after artificial insemination. *J Reprod Fertil* 1980;59:463–8.
- [4] Hawk HW, Tanabe TY. Effect of unilateral corneal insemination upon fertilization rate in superovulating and single-ovulating cattle. *J Anim Sci* 1986;63:551–60.
- [5] Hasler JF. Current status and potential of embryo transfer and reproductive technology in dairy cattle. *J Dairy Sci* 1992;75:2857–79.
- [6] Tonhati H, Lôbo RB, Oliveria HN. Repeatability and heritability of response to superovulation in Holstein cows. *Theriogenology* 1999;51:1151–6.
- [7] Liboriussen T. Genetic variation in response to superovulation in cattle. In: *Proceedings of the 5th World Congress on Genetics Applied to Livestock Production*, vol. 19; 1994. p. 234–6.
- [8] Singh J, Dominguez M, Jaiswal R, Adams GP. A simple ultrasound test to predict the superstimulatory response in cattle. *Theriogenology* 2004;62:227–43.
- [9] Yaakub H, O'Callaghan D, Boland MP. Effect of type and quantity of concentrates on superovulation and embryo yield in beef heifers. *Theriogenology* 1999;51:1259–66.
- [10] Lamb GC, Brown DR, Wasson JE, Larson JE, Dahlen CR, Di Costanzo A. Effect of trace mineral nutrition on follicular response, ovulation and embryo production in superovulated angus heifers. *J Reprod Fertil* 2003;16(12):289 [abstract].
- [11] Hasler JF, McCauley AD, Schermerhorn EC, Foote RH. Superovulatory responses of Holstein cows. *Theriogenology* 1983;19:83–99.
- [12] Witt AC, Witt GF, Witt FG. Relacion entre calidad embrionaria y dos cuadros espermaticos de un mismo toro. In: *Proceedings of the Cuarto Simposio Internacional de Reproduccion Animal*; 2001. p. 271 [abstract].
- [13] Nigro M, Burry E, Witt GF, Cutaia L, Bo GA. Importancia de la evaluacion de la calidad del semen congelado en un programa de produccion de embriones. In: *Proceedings of the Cuarto Simposio Internacional de Reproducción Animal*; 2001. p. 276 [abstract].
- [14] Miller DM, Hrudka F, Cates WF, Mapletoft RJ. Infertility in a bull with a nuclear sperm defect: a case report. *Theriogenology* 1982;17:611–21.
- [15] Saacke RG, Lineweaver JA, Aalseth EP. Procedures for handling semen. In: *Proceedings of the 12th Conference on Artificial Insemination in Beef Cattle*, Sponsored by the NAAB; 1978. p. 46–61.
- [16] Elliott FI. Semen evaluation. In: Salisbury GW, VanDemark NL, Lodge JR, editors. *Physiology of reproduction and artificial insemination of cattle*. W.H. Freeman and Co.; 1978. p. 400–27.
- [17] DeJarnette JM, Saacke RG, Bame JH, Vogler CJ. Accessory sperm: their importance to fertility and embryo quality, and attempts to alter their numbers in artificially inseminated cattle. *J Anim Sci* 1992;70:484–91.
- [18] Dalton JC, Nadir S, Bame JH, Noftsinger M, Nebel RL, Saacke RG. The effect of artificial insemination on fertilization status and embryo quality in superovulated cattle. *J Anim Sci* 2000;78:2081–5.
- [19] Saacke RG, Dalton JC, Nadir S, Nebel RL, Bame JH. Relationship of seminal traits and insemination time to fertilization rate and embryo quality. *Anim Reprod Sci* 2000;6061:663–77.
- [20] King KK, Seidel Jr GE, Elsdon RP. Bovine embryo transfer pregnancies. I. Abortion rates and characteristics of calves. *J Anim Sci* 1985;61:747–57.
- [21] Hasler JF. Factors affecting frozen and fresh embryo transfer pregnancy rates in cattle. *Theriogenology* 2001;56:1401–15.
- [22] Momont HW, Seguin BE. Influence of the day of estrus cycle on response to PGF<sub>2</sub>α products: implications for AI programs for dairy cattle. In: *Proceedings of the 10th International Congress on Animal Reproduction*, vol. 3; 1984. p. 336 [abstract].
- [23] Bo A, Baruselli PS, Moreno D, Cutaia L, Caccia M, Tribulo R, et al. The control of follicular wave development for self-appointed embryo transfer programs in cattle. *Theriogenology* 2002;57:53–72.
- [24] Hasler JF, McCauley AD, Lathrop WF, Foote RH. Effect of donor-embryo-recipient interactions on pregnancy rate in a large-scale bovine embryo transfer program. *Theriogenology* 1987;27:139–68.
- [25] Mapletoft RJ, Lindsell CE, Pawlyshyn V. Effects of clenbuterol, body condition and non-surgical embryo transfer equipment on pregnancy rates in bovine recipients. *Theriogenology* 1986;25:172 [abstract].