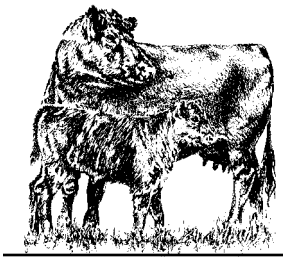

Western Beef Resource Committee *Fourth Edition*

Cattle Producer's Handbook

Reproduction Section 408

Embryo Transfer: A 2010 Perspective

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Background

Embryo transfer (ET) began in 1890 when Walter Heape, a German scientist, successfully transferred two Angora rabbit embryos into a recipient Belgian doe. The first bovine embryo transfer was in 1949, and the first report of a calf resulting from embryo transfer occurred in 1951. Since that humble beginning, millions of cattle embryos have been collected and transferred, currently resulting in the birth of hundreds of thousands of calves throughout the world each year.

The initial driving force of commercial embryo transfer was the introduction of dual-purpose European cattle breeds into the U.S., Australia, and New Zealand during the early to mid 1970s. Embryo transfer eliminated the need to purchase and import breeding stock subject to lengthy and costly quarantine periods. The high demand for embryos resulted in the rapid development of practical methods of superovulation, embryo collection, cryopreservation (freezing), and transfer at reduced costs.

While originally performed solely by surgical methods at central clinics, the development of procedures by which embryos could be non-surgically collected and transferred during the 1970s resulted in a large increase in ET at reduced costs. A second advance, which took place in the 1990s, the "direct" thawing and transfer of frozen embryos, has had a dramatic impact on the ease with which embryo transfer is performed. A third, more recent advance, production of *in vitro* produced (IVP) embryos, has the potential to greatly increase the number of embryos available for transfer. These developments, and many others, have resulted in a technology that now influences, and in the future will continue to affect, a useful role in the production of beef in many countries.

Embryo Transfer—Why?

Embryo transfer offers several advantages for the beef industry. ET can amplify the reproductive rate of valuable females. Without embryo transfer, an outstanding female will have only one calf per year and usually 8 to 10 calves in her lifetime. However, she has thousands of oocytes or "eggs" in her ovaries that have the potential to develop into calves. By subjecting a cow to superovulation and embryo collection, the number of calves produced in a lifetime can be multiplied many fold. As an unusual example, Brigham Young University (BYU) owned a Holstein cow several years ago that was the dam of over 200 calves. Such success, however, is unusual.

Embryo importation/exportation has provided beef producers throughout the world with opportunities to improve the genetic base of their herds, increase variability within the gene pool of a breed, or introduce new breeds into their countries. A big advantage offered by embryo transfer over importation/exportation of semen is that the resultant offspring will be purebred when embryo transfer is employed.

Through oocyte collection and *in vitro* fertilization (so-called *in vitro* production), infertility that is the result of age, disease, or injury can be overcome. However, one must keep in mind that success rates with IVP embryos remains lower than that derived from *in vivo* produced embryos. In addition, genetic infertility should not be propagated.

Twinning has been proposed as a means of increasing economic returns for beef producers. Embryo transfer can be an integral part of such a management system.

Identification and screening for genetic markers associated with traits of economic importance will offer many advantages to the beef industry in the future. The full exploitation of these advantages will result from genetic screening and subsequent transfer of embryos carrying (or as appropriate, not carrying) the markers.

Embryo Transfer—Why Not?

The commitment to collect and transfer beef embryos should be well-planned and based on sound financial reasoning. Too many beef producers have gotten involved in ET in the past only to suffer significant financial setbacks. Be aware that ET is a costly, supplies-consuming, technique-intensive process that yields highly variable results with regards to number and quality of embryos collected and the percentage of embryos transferred that results in pregnancies.

Embryo collection and transfer is an expensive process. The minimal equipment needed for embryo collection and transfer includes a good quality dissecting microscope that could cost well over $1,000. If embryos are to be frozen for later thawing and transfer, a liquid nitrogen tank and embryo freezer are needed, with minimal costs being in the $2,000 range.

Supplies for embryo collection and transfer are an additional expenditure. As an example, during the 1990s embryo collection, evaluation, and transfer were performed by "on the farm" personnel (herdsmen) working at the BYU farm. A conservative cost estimate for each embryo collection was ~$120 for pharmaceuticals and supplies. This excludes the cost of semen, which could easily double this expense for each flush.

Embryo transfer is really the culminating event of a process that may involve several techniques—estrus synchronization of donors in order to detect a "marker heat," superovulation, artificial insemination, embryo collection, embryo evaluation, embryo freezing, estrus synchronization of recipients, and, finally, embryo transfer into recipient cattle. The need for highly trained personnel to perform all of these tasks adds to the cost of ET. For example, a common charge for personnel trained to perform ET is $150 per donor flushed plus $50 for each embryo transferred or frozen. Ranchers should plan on each collection costing a minimum of $250 to $1,000, depending on the number of embryos collected. This excludes the costs of semen and labor performed by ranch personnel. Also, a return visit to transfer frozen then thawed embryos could cost as much as $75 per embryo, with the price decreasing as more embryos transferred.

Probably the greatest frustration experienced by everyone involved in ET is the tremendous variation in results attained. Recent scientific research has examined ways in which this variation might be minimized. The bottom line, however, is that variation in results continues to plague the ET industry today. Our results to date lead us to the conclusion that everyone who does ET on a regular, continuing basis will probably experience this variation.

Embryo Transfer—How?

It is impossible to adequately describe in detail in this report the procedures involved in superovulation, embryo collection, evaluation, and freezing. Several good books have been written on these subjects. Our purpose here is to give a brief overview of each of these techniques.

Superovulation—Cattle are generally considered to be a monovulatory species. That is, they usually only release one egg during each estrous cycle. Superovulation is the process of hormonally tricking the ovary into releasing many eggs during a cycle. In the U.S., follicle stimulating hormone (FSH), produced and released naturally in the body, is the hormone most commonly given to donor cattle to induce superovulation.

Research conducted during the 1970s and 80s was interpreted to suggest that FSH should be given over a 3- to 5-day period, and, that the best results were obtained when treatment began between 8 to 12 days after observed estrus. A common treatment schedule is to give FSH in decreasing amounts as outlined in Table 1.

Prostaglandin $F_{2\alpha}$ (PGF) is given to the donor cow to destroy the corpus luteum and thereby set up the hormonal environment needed to induce the cow to come into heat. In our experience, superovulated donor cows usually come into heat between 36 to 60 hours after PGF.

Typically, donors are inseminated with two straws of semen; one straw at 12 hours after and a second straw at 24 hours after observed heat. The use of as many as 3 or 4 straws of semen is not uncommon.

Table 1. Common treatment schedule of FSH* and PGF* for donor cows. The percentages refer to amount of recommended dose for each product.

Day of cycle	Amount of FSH	Amount of PGF
Day 9 p.m.	100%	0%
Day 10 a.m.	100%	0%
Day 10 p.m.	80%	0%
Day 11 a.m.	80%	0%
Day 11 p.m.	60%	0%
Day 12 a.m.	60%	100%
Day 12 p.m.	40%	100% (optional)
Day 13 a.m.	40%	0%

*FSH = Follicle stimulating hormone; PGF = Prostaglandin $F_{2\alpha}$

Embryo Collection—Embryos are typically non-surgically collected 6 to 8 days after observed heat. The first step is to palpate the ovaries of donor cows to estimate the number of corpora lutea (CL) present. Since each CL represents an ovulation, this is also an estimate of how many embryos might be collected. In our experience, the ability to accurately estimate CL numbers by palpation (or by ultrasonography) decreases as the number of CL/ovary increases. Only donors that have responded with two or more ovulations are usually candidates for embryo collection.

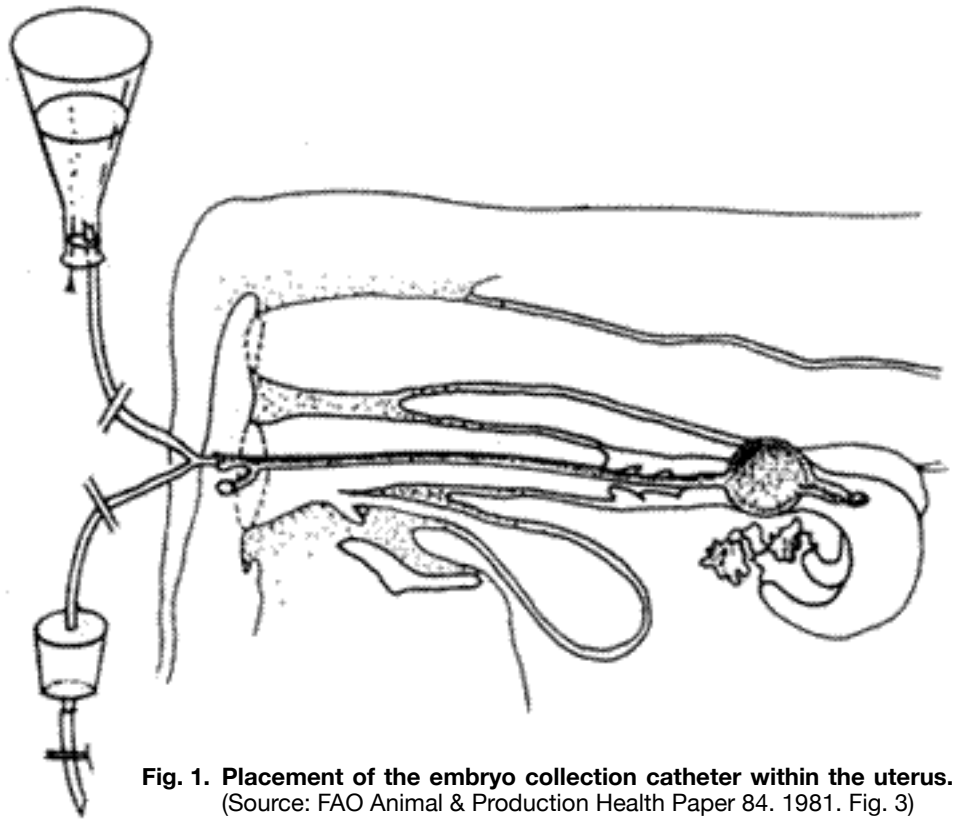

Fig. 1. Placement of the embryo collection catheter within the uterus. (Source: FAO Animal & Production Health Paper 84. 1981. Fig. 3)

Next, responsive donors are given an epidural anaesthetic to allow the reproductive tract to be manipulated without fighting against rectal contractions. A catheter, with an inflatable balloon cuff, is placed into the uterus (Fig. 1). Once in the appropriate position inside the uterus, the balloon is inflated to keep the catheter in place, and the uterus is repeatedly flushed with fluid.

The fluid may be collected into a cylinder and the retained fluid microscopically searched for embryos. However, more commonly, the fluid collected from the uterus is passed through an in-line filter that retains collected embryos. The filter is then rinsed into a dish and the dish is subsequently searched for embryos.

Embryo Evaluation—Once collected embryos have been located, they are transferred into a dish containing fresh fluid. They are then visually evaluated to determine their stage of development and their quality.

Embryos are usually rated excellent (grade 1), good (grade 2), fair (grade 3), or poor (grade 4). Unfertilized (UFOs) eggs may also be present. Embryos of grades 1 to 3 may be transferred immediately upon collection ("fresh transfer"). Grade 4 embryos and UFOs are not suitable for transfer into recipients.

Embryo Freezing—The development of procedures to freeze embryos for later thawing and transfer has been a great boon to both the ET and the beef industries. For example, donor cattle may now be superovulated and embryos collected throughout the year. Embryos collected out of season can be frozen and then thawed and transferred into recipient cattle at the beginning of the breeding season.

Embryos rated grades 1 or 2 are suitable for freezing/thawing and subsequent transfer. Grade 3 embryos are usually not frozen. BYU researchers have collected and frozen embryos from donors outside the breeding season for several years and found this to be a profitable procedure under monitored management practices.

The freezing process is a fairly complex yet relatively easy one. Embryos are washed through a minimum of three changes of fluid (10 if embryos are to be exported). Embryos are then transferred into a freezing (cryoprotectant) solution and put into a .25 ml straw.

The temperature of the embryos is lowered to -6°C and ice crystal formation is initiated by touching the end of the straw with forceps cooled in liquid nitrogen. The temperature of the straws is gradually lowered to -30°C and they are then plunged into liquid nitrogen. They can be stored indefinitely in liquid nitrogen until needed for transfer.

Glycerol was used almost exclusively as the freezing solution for cattle embryos until the 1990s. The biggest drawback to use of glycerol, however, is the need to move embryos through at least two different solutions *after* thawing and *before* transfer into recipients. Of course, this means that a microscope is an absolute requirement for the transfer of such embryos.

Using ethylene glycol as a freezing solution is almost universally accepted as the method of choice in the ET industry today. Use of this solution eliminates the need for a microscope after thawing and before transfer.

Why? Because embryos frozen in ethylene glycol can be thawed using a procedure somewhat similar to that used for thawing semen and then transferred directly into the uterus *without* processing through any additional solutions ("direct transfer").

Embryo Transfer

Collected embryos can be either transferred "fresh" or frozen/thawed. The procedure for the actual transfer of an embryo into a recipient is the same regardless of the type of embryo transferred. However, embryo transfer is far more than the procedure of transfer itself; it is the culminating step in a process that begins with recipient selection and care.

Embryo Recipients—Selecting which cattle to use as recipients and managing those cattle once a selection is made are vital concerns in an ET program. Too often, ranchers go to great measures to ensure that they have the very best embryos possible, either from their own donors or someone else's, then do little more than use the cattle they or their neighbors aren't planning on AI'ing, or worse yet, using as breeding stock, as their recipients. Remember, using the "right" recipient has just as much impact on the bottom line (pregnancy success and financial profitability) as does using the "right" cow as a donor of the embryos.

Recipients should be in excellent health and must have the right genetics for the embryo they receive. They must be able to give birth to the calf without any difficulty and must produce enough milk and have the needed mothering ability to ensure that the calf will thrive before weaning.

Once recipients have been selected, they should have excellent care before transfer. They should be in moderate to good body condition and fed so they are gaining, or at the very least, maintaining condition before receiving embryos. They must be moved and handled so that stress is minimized, especially in the period immediately before, during, and after the transfer.

Cattle must have been in heat within 24 hours of the donor to be considered ideal candidate recipients. This means that if the donor is collected on day 7 of the cycle, recipients should receive embryos sometime between day 6 and 8.

Ovaries on recipient cattle are palpated to determine the presence or absence of a CL. BYU researchers routinely do this 1 day before collection, if embryos are to be transferred fresh. Transfer is *always done before thawing*, if embryos are to be frozen/thawed. The ovary bearing a CL and an estimate of the CL's quality is recorded, so it can be referred back to the source at the time of transfer. Recipients with a suitable CL should always be secured in a chute or headlock before loading or thawing an embryo for transfer.

Once embryos have been loaded into a straw (fresh transfer), or the straw containing the appropriate embryo thawed (frozen/thawed), the straw is loaded into the transfer gun, covered and secured with a sheath, and the gun inserted into a plastic sleeve. A written record of the embryo development stage and quality, the donor cow and sire, and the recipient receiving the embryo is recorded before going out to the recipient.

An epidural anaesthetic may or may not be given to the recipient, and the embryo transfer gun (still covered with the protective plastic sleeve) is put into the vagina and gently advanced to the opening of the cervix. The plastic sleeve is then pulled away from the gun and the gun is manipulated through the cervix into the body of the uterus.

Extreme caution must be exercised once the gun is in the uterus. The lining of the uterus is extremely fragile and can be easily damaged. One of the most difficult tasks faced once the gun is in the uterine body is in getting the gun to slide up the correct uterine horn (the horn on the same side as the CL-bearing ovary). Patience and great care must be used.

Once the gun is in the correct horn, a segment of the horn is gently straightened and the gun carefully and slowly manipulated forward. This process is repeated until the target site or resistance is encountered. The embryo is then transferred out of the straw and into the uterus by slowly pushing the plunger of the gun.

Even when all conditions are optimized, some recipients will not get pregnant. A 60 to 65 percent pregnancy rate for embryos transferred fresh, and a 50 to 55 percent pregnancy rate for frozen/thawed embryos is acceptable and close to what researchers have experienced over the past 10 years at Brigham Young University's operation. Out in the field, under "real world" conditions, pregnancy rates of 45 to 55 percent are realized and probably more realistic.

Advantages of *In Vitro* Production

BYU researchers believe increased utilization of *in vitro* produced embryos will reduce one of the greatest risks associated with embryo transfer—the highly variable results obtained when donor cows are superovulated. Weekly *in vitro* aspiration of follicles present on ovaries allows for collection of a fairly consistent number of oocytes from donor cattle, without the need of incurring the expense associated with hormonal treatment.

One of the current primary drawbacks to incorporation of technology into embryo transfer programs is the lower pregnancy rates attained with *in vitro* derived, frozen/thawed embryos. However, pregnancy rates are improving as techniques are being refined. A recent experience transferring over 100 frozen/thawed, *in vitro* produced embryos on a large ranch in the southeastern United States resulted in a nearly acceptable 36 percent pregnancy rate. *In vitro* production of embryos accompanied by acceptable pregnancy rates after

transfer will increase the use of embryo transfer in some herds in the future.

Advantages of Genetic Screening

The ability to use molecular probes to screen embryos for genetic traits before transfer has been a research farm possibility for many years. The major drawbacks preventing incorporation by beef producers have been the high cost, the expertise needed, and the time required to carry out the screening process. In addition, the producer faces the challenge of what to do with screened embryos lacking the genetic trait desired.

BYU researchers recently performed on-farm collection, screening, and transfer of embryos screened for their sex. The goal was to increase the cow herd size in a rapid fashion without bringing in any females from outside the herd. Embryos were collected and screened for the presence or absence of a genetic marker associated with the Y chromosome using a relatively rapid (~3 hour) process. Female embryos were then (a) transferred fresh; (b) split in two and then transferred fresh as "half-embryos" into two recipients, (c) frozen whole, then thawed and transferred, or (d) split in two, with each half being frozen, thawed, and then transferred.

The pregnancy rate was highest with screened embryos transferred fresh (45%) as compared to results with screened embryos that were frozen-thawed and then transferred (~40%). Splitting screened embryos and then transferring fresh resulted in a ~30% pregnancy rate for each half transferred. Encouragingly, screened embryos that were split before being frozen/thawed, and then transferred did result in a few pregnancies under these field conditions.

While the genetic trait being screened for in this effort was a simple one (male or female) the techniques employed would be the same for any other trait that could be screened on the basis of the presence or absence of the desired genetic marker. As techniques are enhanced, BYU researchers feel confident that pregnancy rates will increase. Such improvements will pave the way for ET to have even greater value for some beef operations.

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