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Embryo Transfer in Cattle

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Introduction

Embryo transfer is one step in the process of removing one or more embryos from the reproductive tract of a donor female and transferring them to one or more recipient females. Embryos also can be produced in the laboratory via techniques such as *in vitro* fertilization or somatic cell cloning. But the actual transfer of an embryo is only one step in a series of processes that may include some or all of the following: superovulation and insemination of donors, collection of embryos, isolation, evaluation and short-term storage of embryos, micromanipulation and genetic testing of embryos, freezing of embryos and embryo transfer.

Embryo transfer in cattle has recently gained considerable popularity with seedstock dairy and beef producers. Most of the applicable embryo transfer technology was developed in the 1970s and 1980s; however, the history of the concept goes back much farther. Embryo transfer was first performed and recorded by Walter Heape in 1890. He transferred two Angora rabbit embryos into a gestating Belgian doe. The Belgian doe produced a mixed litter of Belgian and Angora bunnies. Embryo transfer in food animals began in the 1930s with sheep and goats, but it was not until the 1950s that successful embryo transfers were reported in cattle and pigs by Jim Rowson at Cambridge, England.

The first commercial embryo transfers in this country were done in the early 1970s. Initially, embryos were recovered from valuable donors and transferred to recipient animals using surgical procedures. It was not until nonsurgical methods were developed in the late 1970s that embryo transfer grew in popularity.

Potential Value of Embryo Transfer in Cattle

The reproductive potential of each normal newborn calf is enormous. There are an estimated 150,000 potential "eggs" or ova in the cow and billions of sperm produced by each bull. By natural breeding, only a fraction of the reproductive potential of an outstanding individual is realized. The average herd bull will sire 15 to 50 calves per year, and the average cow will have one calf per year. With artificial insemination, it is possible to exploit the vast number of sperm produced by a genetically superior bull; however, the reproductive potential of the female has been largely unutilized. Under normal management programs, a cow produces an average of eight to ten calves in her lifetime. Like artificial insemination has done for the bull, embryo transfer is a technique that can greatly increase the number of offspring a genetically important cow can produce.

Steps for Embryo Transfer in Cattle

Virtually all commercial embryo transfers use nonsurgical recovery of the embryos rather than surgical techniques. The process involves several steps and considerable time as well as variable expense.

1) Selection of the Donor Cow

The first step is selecting a donor cow. Beef producers will differ in their opinions regarding the criteria for selecting a genetically outstanding cow.

Whether the criteria is performance records, show ring appeal or both, consideration must be given to potential dollar value of her calves. There is considerable expense incurred in achieving a successfully transferred pregnancy. Therefore, the sale value of the newborn calf should be high enough to warrant the added expense of the embryo transfer procedure. Because dairy cattle are selected more routinely on one major trait (milk production), the decisions concerning donor cows are somewhat less complicated than in beef cattle. However, the economic considerations are equally important. Embryo transfer is not a "cure-all." It does not make average cattle good or good cattle better. It is suitable for a limited number of seedstock producers with beef or dairy cattle that can be breed or species "improvers" for one or more economically important traits.

The potential donor cow should be reproductively sound to produce the desired results. The cow should have a normal reproductive tract and postpartum history, especially with regard to estrous lengths of 18 to 24 days. Both beef and dairy cows should be at least 60 days postpartum before the transfer procedure begins. It has been suggested that prospective donor cows in embryo transfer programs be selected on the following criteria:

- Regular estrous cycles commencing at a young age
- A history of no more than two breedings per conception
- Previous calves with approximately 365-day intervals
- No parturition difficulties or reproductive irregularities
- No conformational or detectable genetic defects

The cow should be maintained at a nutrition level appropriate for her size and level of milk production. Both the very obese cow and the thin cow will have reduced fertility, so it is important that the donor be in an appropriate body condition score at the time of embryo transfer.

2) Superovulation of the Donor Cow

Superovulation of the donor cow is the next step in the embryo transfer process. Superovulation is the release of multiple eggs at a single estrus. Cows or heifers properly treated can release as many as ten or more viable eggs at one estrus. Approximately 85 percent of all normal fertile donors will respond to superovulation treatment with an average of five transferable embryos. Some cows that are repeatedly superovulated at 60-day intervals may produce fewer number of eggs over time. The basic principle of superovulation is to stimulate extensive follicular development through the use of follicle-stimulating hormone (FSH). Superovulation protocols may differ among embryo technicians, but generally, FSH preparations are injected twice daily for four days at the middle or near the end of a normal estrous cycle, while a functional corpus luteum (CL) is on the ovary. A prostaglandin injection given on the fourth day of the treatment schedule will cause CL regression and estrus to occur approximately 48 hours later.

3) Insemination of the Cow

Because of the release of many ova from multiple follicles, there is a greater need for viable sperm cells to reach the oviducts of the superovulated females. Therefore, many embryo transfer technicians will choose to inseminate the cow several times during and after estrus. One scheme is to inseminate the superovulated cow at 12, 24 and 36 hours after the onset of standing estrus. Using high-quality semen with a high percentage of normal, motile cells is a very critical step in any embryo transfer program. The correct site for semen placement is in the body of the uterus. This is a small target (1/2 to 1 inch) just in front of the cervix.

4) Flushing the Embryos

To collect the embryos nonsurgically, a small synthetic rubber catheter is inserted through the cervix of the donor cow, and a special medium is flushed into and out of the uterus to collect the embryos seven days after estrus (Figure 1).

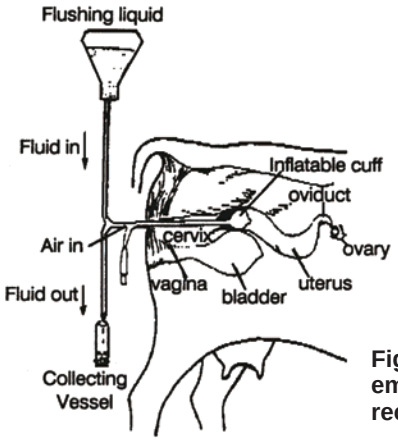

Figure 1. Diagram of the embryo flushing and recovery procedure.

This collection procedure is relatively simple and can be completed in 30 minutes or less without harm to the cow. A presterilized stylet is placed in the lumen of the catheter to offer rigidity for passage through the cervix into the body of the uterus. When the tip of the catheter is in the body of the uterus, the cuff is slowly filled with approximately 2 mL of normal saline. The catheter is then gently pulled so that the cuff is seated into the internal os of the cervix. Additional saline is then added to the cuff to completely seal the internal os of the cervix. A Y-connector with inflow and outflow tubes is attached to the catheter. A pair of forceps is attached to each tube to regulate the flow of flushing fluid. The fluid is sequentially added and removed by gravity. The fluid in the uterus is agitated rectally, especially in the upper one-third of the uterine horn. The uterus is finally filled with medium to about the size of a 40-day pregnancy. One liter of fluid is used per donor. Many embryo transfer technicians use a smaller volume and flush one uterine horn at a time. Each uterine horn is filled and emptied five to ten times with 30 to 200 mL of fluid each time, according to size of the uterus. The embryos are flushed out with this fluid and collected in a filter with the fluid. The pores in the filter are smaller than the embryos, so excess fluid drains out

of the filter without losing the embryos. Embryos are separated from the flush media and examined under a microscope to determine their quality and stage of development.

5) Evaluation of the Embryos

As the individual embryos are located using a microscope, they are evaluated for their quality and classified numerically as to the potential likelihood of success if transferred to a recipient female. The major criteria for evaluation include:

- Regularity of shape of the embryo
- Compactness of the blastomeres (the dividing cells within the boundaries of the embryo)
- Variation in cell size
- Color and texture of the cytoplasm (the fluid within the cell wall)
- Overall diameter of the embryo
- Presence of extruded cells
- Regularity of the zona pellucida (the protective layer of protein and polysaccharides around the single-celled embryo)
- Presence of vesicles (small bubble-like structures in the cytoplasm)

Embryos are classified according to these subjective criteria as:

Grade 1: Excellent or good
Grade 2: Fair
Grade 3: Poor
Grade 4: Dead or degenerating

Embryos also are evaluated for their stage of development without regard to quality. These stages are also numbered:

Stage 1: Unfertilized
Stage 2: 2 to 12 cell
Stage 3: Early morula
Stage 4: Morula
Stage 5: Early blastocyst
Stage 6: Blastocyst
Stage 7: Expanded blastocyst
Stage 8: Hatched blastocyst
Stage 9: Expanding hatched blastocyst

There is apparently no difference in pregnancy rates of fertilized cells in different stages of development, assuming they are transferred to the recipient female in the appropriate stage of the estrous cycle. Stages 4, 5 and 6 embryos endure the freezing and thawing procedures with the greatest viability. Embryo quality is also of utmost importance in the survival of the freezing and thawing stress. Grade 1 embryos generally are considered the only ones to freeze. Grade 2 embryos can be frozen and thawed, yet pregnancy rates typically are reduced. In a Louisiana study involving 1,116 beef and dairy cows of 15 breeds, 58 percent of all embryos were transferable, 31 percent were unfertilized and 11 percent were degenerated.

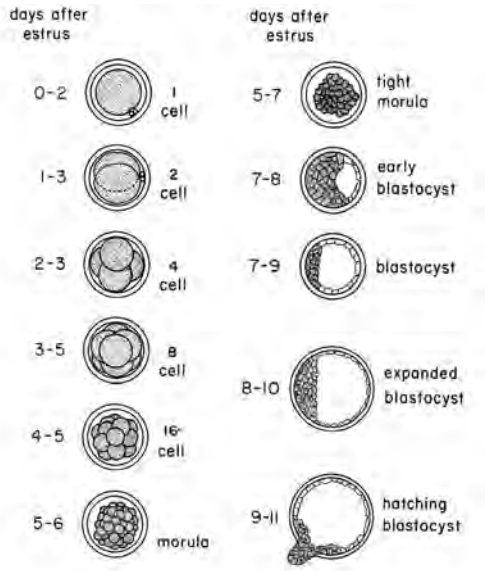

Figure 2. Morphologically normal bovine embryos recovered at various stages of development.

6) Selection and Preparation of Recipient Females

Proper recipient herd management is critical to embryo transfer success. Cows that are reproductively sound, that exhibit calving ease and that have good milking and mothering ability are recipient prospects. They must be on a proper plane of nutrition (body condition score 6 for beef cows and dairy body condition score 3 to 4). These cows also must be on a sound herd health program.

It is difficult to know how many recipient cows are needed per each donor flushed. To establish an average figure for the number of embryo transfer calves from a single donor cow in a year is difficult. Variations in conditions are wide, but if a cow is flushed every 90 days over a 12-month period and five pregnancies are obtained per collection, an average of 20 pregnancies per year could result. Some cows have produced more than 50 pregnancies per year by embryo transfer and probably could have produced more if economically feasible. In the Louisiana study previously mentioned, the average number of embryos found per cow was 7.4. With only 58 percent of these being transferable, the average was 4.3 transferable embryos per flush.

To maximize embryo survival in the recipient female following transfer, conditions in the recipient reproductive tract should closely resemble those in the donor. This requires synchronization of the estrous cycles between the donor and the recipients, optimally within one day of each other. Synchronization of the recipients can be done in a similar manner and at the same working time as the donor cows. There are a number of different estrous synchronization protocols with advantages and disadvantages for each protocol. The critical point regarding recipient cow estrous synchronization is the timing must match the time of insemination of the donor cow so that the donor and the recipients have a similar uterine environment seven days later when the

transfer takes place. Synchronizing products are more effective on recipient females that are already cycling. "Anestrus" or noncycling cows that are too thin or too short in days postpartum will not make useful recipients.

7) Transfer of the Embryos

The transfer of the embryo into the recipient cow first requires "loading" the embryo into a 1/4-mL insemination straw. This is done under microscopic viewing with the aid of a 1-mL syringe and requires considerable practice, patience and dexterity. Degenerated embryos or embryos of very low grade need not be loaded and can be discarded. Just prior to embryo transfer, the ovaries of the recipient are palpated rectally to determine which ovary has ovulated. With the aid of an assistant to hold open the vulva of the recipient cow, the transfer gun or insemination rod is carefully passed through the cervix. The tip of the rod is then allowed to slide into the horn on the same side of the ovary with an active corpus luteum. The embryo is gently expelled in the forward tip of that uterine horn. Great care is taken to not cause damage to the lining of the uterus. Such inflammation and scarring would greatly reduce the probability of the pregnancy being established. Embryo flushing and embryo transfer are both done after an epidural anesthetic has been given to block contractions of the digestive tract and aid in the ease of manipulation of the cervix and the uterine horns. Embryos should be transferred as soon as possible after the flush (within 8 hours at least).

The freezing and thawing process is also very intricate and usually results in approximately 10 to 20 percent reduction in pregnancy rates from those observed with fresh embryos.

Frozen embryos are a marketable commodity and have been especially useful in international sales of United States beef and dairy genetics. Producers who believe they own cattle with the genetic capability to be valuable in other nations may wish to contact their state Department of Agriculture and ask about regulations and marketability of frozen embryos from their herd. Different nations have different health requirements of cattle producing frozen embryos for import into their country. Therefore, individual inquiries are necessary to learn what health and legal requirements are expected.

8) Expected Embryo Transplant Results

Embryo production varies greatly from donor to donor and flush to flush. Average production is approximately six freezable (excellent and good) and eight transferable (excellent, good, fair and poor) embryos per superovulation.

Pregnancy rates vary from flush to flush with fresh averages 60 to 70 percent and frozen 50 to 60 percent. Many factors affect pregnancy rates such as embryo quality, recipients, technical ability and donor. Some donors consistently produce embryos with higher pregnancy rates than others with embryos of similar grade. This last factor seems to be uncontrollable and unpredictable.

Costs of Embryo Transfer

The costs of embryo transfer are as variable as the costs of buying a new truck. Many different options and packages are offered by embryo transfer technicians. Some technicians perform embryo transfer only on the ranch where the donor cows are located. Others have facilities to house and board donor and recipient cows and perform embryo transfer under hospital-like conditions. Many technicians have the equipment and expertise to freeze and store embryos for later transplantation or shipment to other countries.

Minimum costs of $250 per pregnancy have been reported by embryo transfer technicians. These costs may not include costs for superovulation and certainly do not include semen, registration, embryo transfer certificates, blood typing of donor cows and ancestors and, most importantly, the cost of maintaining the donor cow until the calf is weaned. Three to five straws of valuable semen can be priced from $45 to $300. Proper nutrition, health care and synchronization of the donor and the recipient can add another $400 to $500 expense to each successful pregnancy. Consequently, many purebred operations conducting embryo transfer on a regular basis consider that each "ET" calf must have a market value of $1,500 to $2,000 greater than other naturally conceived and reared calves in the herd before embryo transfer is considered.

Beef and dairy producers considering using embryo transfer should first visit with their breed representative to determine the specific requirements for certification and registration of embryo-transfer calves in that breed.

Adapted from *Embryo Transfer in Cattle* (F-3158) by G. Selk, Oklahoma Cooperative Extension Service, Stillwater, OK.

Additional Sources

Davis, R. L. *Embryo Transfer in Beef Cattle*. Davis-Rairdan International.

Hasler, J. F. 2004. *Factors Influencing the Success of Embryo Transfer in Cattle*. 23rd World Buiatrics Congress, Québec, Canada.

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