Embryo Transfer In Cattle

Cooperative Extension Service • Division of Agriculture • Oklahoma State University No. 3158

Glenn Selk
OSU Extension Animal Reproduction Specialist

History of 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 1970's and 1980's. 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. She went on to produce a mixed litter of Belgian and Angora bunnies. Embryo transfer in food animals began in the 1930's using sheep and goats, but it was not until the 1950's 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 1970's. Initially, embryos were recovered from valuable donors and transferred to recipient animals using surgical procedures. It was not until non-surgical methods were developed in the late 1970's that embryo transfer grew in popularity.

Why Consider 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 female and countless billions of sperm produced by each male. By natural breeding, only a fraction of the reproductive potential of an outstanding individual could be 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 numbers of sperm produced by a genetically superior bull. However, the reproductive potential of the female has been largely un-utilized. She will produce an average of eight to 10 calves in her entire lifetime under normal management programs. Like artificial insemination has done for the bull, embryo transfer is a technique that can greatly increase the number of offspring that a genetically important cow can produce.

How Is Embryo Transfer Performed On Cattle?

Virtually all commercial embryo transfer done today uses non-surgical 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 the selection of the donor cow. Beef producers will differ in their opinions as to what is the criteria for selecting a genetically outstanding cow. Whether the criteria be performance records, showring appeal, or both, consideration must be given to potential dollar value of her calves. As you will see later, considerable expense can be incurred to achieve a successfully transferred pregnancy. Therefore, the sale value of the newborn calf should be high enough to warrant the added expense of this procedure. Because dairy cattle are selected more routinely on one major trait (milk production), the decisions concerning donor cows are actually 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 maximal results. This means that she should have a normal reproductive tract on rectal palpation and have a normal postpartum history, especially with regard to cycle 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:

1) Regular heat cycles commencing at a young age.
2) A history of no more than two breedings per conception.
3) Previous calves were born at approximately 365 day intervals.
4) No parturition difficulties or reproductive irregularities.
5) No conformational or detectable genetic defects.

She should be maintained at the level of nutrition 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 cow be in an appropriate body condition score at the time of embryo transfer. (See OSU Extension Circular E-869 to learn appropriate body condition for beef cattle and OSU Extension Leaflet L-221 for dairy cattle.)

2) Superovulation of the donor cow

"Superovulation" of the cow is the next step in the embryo transfer process. Superovulation is the release of multiple eggs at a single estrus. Cows or heifers that are properly treated can release as many as 10 or more viable egg cells during one estrus. Approximately 85 percent of all normal fertile donors will respond to superovulation treatment with an average of five transferable embryos. Some cows are repeatedly treated at 60 day intervals with a slight decrease in embryo numbers over time. The basic principle of superovulation is to stimulate extensive follicular development through the use of a hormone preparation given intramuscularly or subcutaneously. The hormone preparation will have follicle stimulating hormone (FSH) activity. Commercially available preparations of FSH are injected twice daily for four days, eight to 14 days following estrus, while a functional corpus luteum (CL) is on the ovary. A prostaglandin injection is given on the third day of the treatment schedule, which will cause CL regression and a heat or estrus to occur approximately 48 to 60 hours later.

3) Insemination of the cow

Because of the release of many ova from the multiple follicles on the ovaries over a period of several hours, there is a greater than normal need to be certain that viable sperm cells 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 that has been used successfully is to inseminate the superovulated cow at 12, 24, and 36 hours after the onset of standing heat. 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. Semen is placed either in the body of the uterus or at the entrance into each uterine horn.

4) Flushing the embryos

To collect the embryos non-surgically, 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 harvest the embryos seven or eight days after estrus. This collection procedure is relatively simple and can be completed in 30 minutes or less without harm to the cow. The donor is given an epidural block and 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 operators use a smaller volume and flush one uterine horn at a time. Each uterine horn is filled and emptied five to 10 times with 30 to 200 ml of fluid each time, according to size of the uterus. The embryos are flushed out with this fluid into a large graduated cylinder. After about 30 minutes, embryos settle and can be located under a stereomicroscope by searching through an aliquot from the bottom of the cylinder. Filters with a pore size of 60 to 70 microns are also utilized to concentrate the embryos.

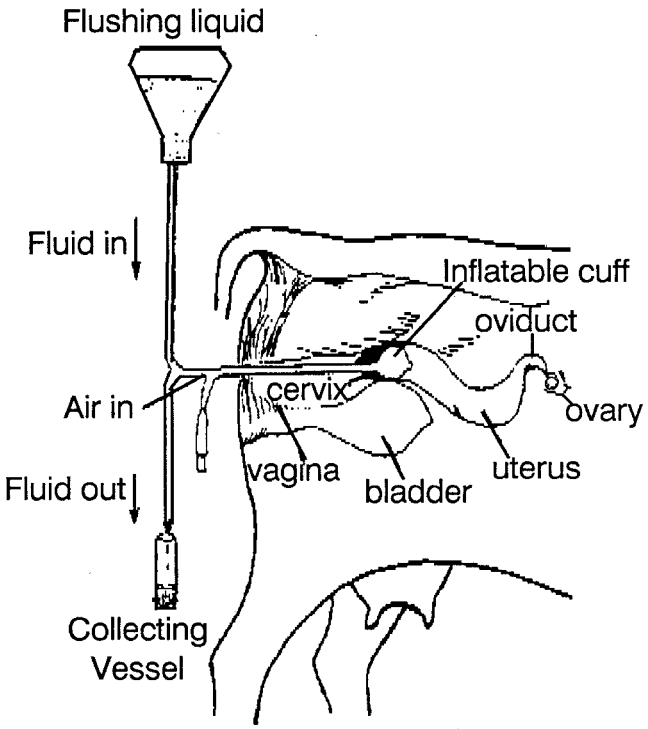

Figure 1. Diagram of the embryo flushing and recovery procedure.

5) Evaluation of the embryos

As the individual embryos are located using the 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
Overall diameter of the embryo
Presence of extruded cells
Regularity of the zona pellucida
Presence of vesicles

Using these subjective criteria embryos are classified 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

Photographs of bovine embryos in different stages are shown in Figure 2.

There is apparently no difference in pregnancy rates of fertilized cells in different stages of development, assuming that they are transferred to a recipient female in the appropriate stage of the estrous cycle. Stage 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 are generally considered the only ones to freeze. Grade 2 embryos can be frozen and thawed, yet pregnancy rates typically are reduced. In a recent Louisiana study involving 1116 beef and dairy cows of 15 breeds, 58 percent of all embryos were transferable, 31 percent were unfertilized, and 11 percent were degenerated.

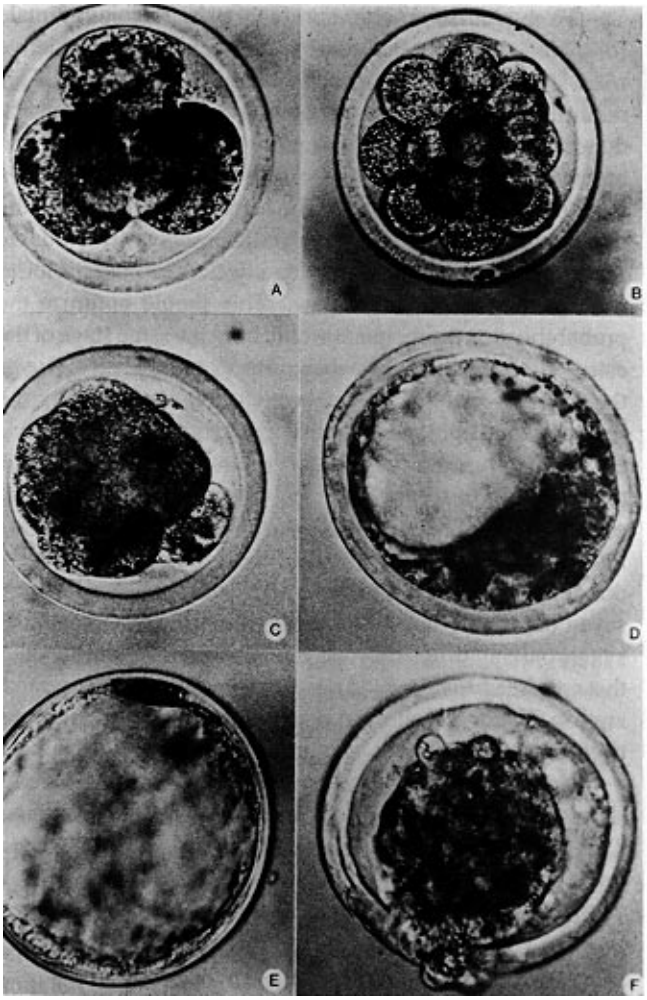

Figure 2. Cattle embryos at various stages of development.

A. 4-cell egg, day 3.
B. 16-cell egg, day 5.
C. Morula, day 6. Cells have compacted and lost individual outlines.
D. Early blastocyst, day 7.
E. Blastocyst, fully expanded within the zona pelucida, day 10.
F. Hatching blastocyst, day 10.

Photos reprinted from *Embryo Transfer in Farm Animals*. Monograph No.16. 1977. Agriculture Canada. Permission granted by K.J. Betteridge, editor.

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 for dairy breed recipients.) These cows also must be on a sound herd health program.

A tough question to answer is "How many recipient cows are necessary?" 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. 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. There are cows that have produced more than 50 pregnancies per year by embryo transfer and that probably could have produced more if it had been economically feasible. In the Louisiana study previously mentioned, the average number of embryos found per cow was 7.4. Remember, only 58 percent of these

were transferable for an average of 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 estrus 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 nearly the same working time as the donor cows. Injectable prostaglandin products are available from veterinarians and should be injected 12 hours prior to the injection of the donor cow. This should optimize the probability that the recipient would be in the same stage of the estrus cycle as the donor when transfer takes place. Use of the "Syncro-Mate-B" system which involves injecting the recipients and implanting them with a synthetic progesterone also has been used successfully. The implant is removed nine days after its insertion, and the cows will show standing estrus approximately 30 to 40 hours later. Again, this timing must match the time of estrus of the donor cow so that the donor and the recipients have a similar uterine environment seven days later when the transfer takes place. Remember synchronizing drugs only are effective on recipient females that are already cycling. "Anestrus" or non-cycling cows that are too thin or too short in postpartum days will not make useful recipients.

Heat detection is exceptionally important. Recipients should be properly identified, observed for heat 2 to 3 times daily, and adequate records made of date and time of estrus.

7) Transfer of the embryos

The transfer of the embryo into the recipient cow first requires "loading" of 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, an epidural anesthetic is given, and 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 guided 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.

Embryos can be transferred immediately upon recovery and evaluation, or may be stored frozen in liquid nitrogen and transferred at a later date. The freezing and thawing process also is very intricate and usually results in an approximate 20 percent reduction in pregnancy rates from those observed with fresh embryos.

Frozen embryos are a marketable commodity and have especially been useful in international sales of United States beef and dairy genetics. Producers in this country that believe that 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.

Costs of Embryo Transfer

The costs of embryo transfer are as variable as the costs of buying a new automobile. Many different options and packages are offered by embryo transfer technicians. Some technicians perform embryo transfer only on the farm or ranch where the donor cow is 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 do no include drug costs for superovulation and certainly do not include semen, registration, embryo transfer certificates, or blood typing of donor cows and ancestors. Most importantly, the cost of owning and maintaining recipient cows until the calves are weaned must be considered. Some embryo transfer centers may also provide recipients and charge $1200 to $1800 per pregnancy. Three to five straws of valuable semen will often be used for each breeding and cost $45 to $300. Proper nutrition, health care, and synchronization of the donor and the recipients 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 $1500 to $2000 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 detemine the specific requirements needed for certification and registration of embryo transfer calves in that breed.

Acknowledgement:

Thank you to Dr. Milton Wells of Reproduction Enterprises, Inc. in Stillwater, Oklahoma for technical advice.