25 Embryo Transfer

Glenn Selk

Objectives

- Discuss the history of embryo transfer
- Learn the benefits of embryo transfer
- Learn how to transfer embryos
- Discuss the potential costs of embryo transfer

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 1970s and 1980s; however, its history goes back much further. 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 1930s using 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 completed 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.

Benefits for 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 can be realized. The average herd bull sires 15 to 50 calves per year and the average cow will have one calf per year. With artificial insemination, it is possible to utilize the vast numbers of sperm produced by a genetically superior bull; however, the reproductive potential of the female has been largely unutilized. She will produce an average of eight to 10 calves in her entire lifetime under normal management programs. As artificial insemination has done for the bull, embryo transfer can greatly increase the number of offspring that a genetically important cow can produce.

How to Perform Embryo Transfer on Cattle

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

Selection of the Donor Cow

Beef producers differ in their opinions as to the criteria for selecting a genetically outstanding cow. Whether the criteria are performance records, show-ring appeal, or both, consideration must be given to the potential dollar value of her calves. As seen 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 cattle that can serve as 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. Prospective donor cows in embryo transfer programs should be selected on the following criteria:

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

A donor cow should be maintained at the level of nutrition appropriate for her size and level of milk

All Web addresses given in this chapter are subject to change. The links to these Web sites will be updated regularly at the Master Cattleman Web site, http://agecon.okstate.edu/cattleman/manual_chapters.asp 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 (BCS 5 to 6) at the time of embryo transfer (Chapter 15).

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 properly treated can release as many as 10 or more viable egg cells at one estrus. Approximately 85% 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. Superovulation stimulates extensive follicular development through the use of a follicle stimulating hormone (FSH) that should be given intramuscularly or subcutaneously. Commercially available preparations of FSH 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 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.

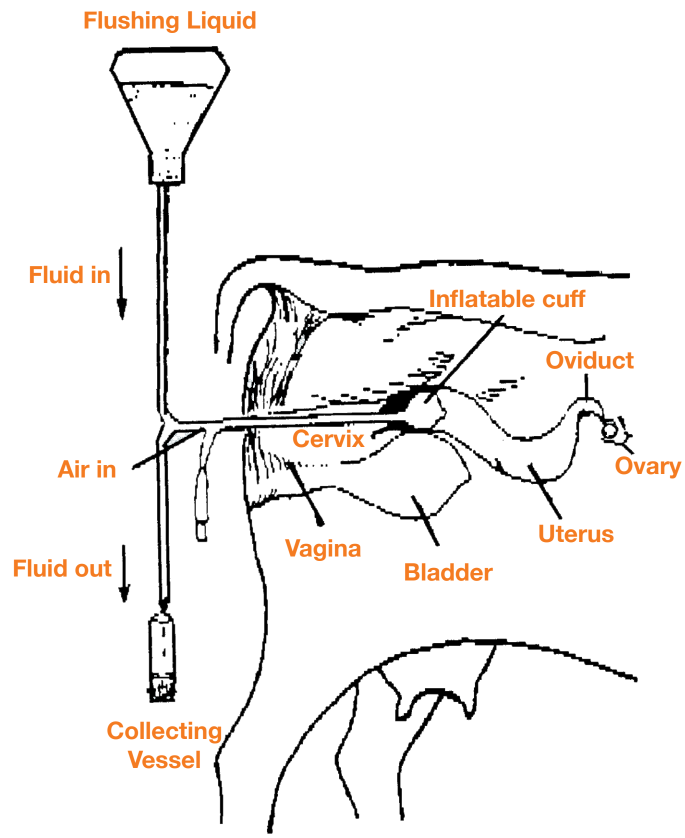

Figure 25.1 – Flushing the embryos. Source: Selk.

Insemination of the Cow

Because of the release of many ova from the multiple follicles on the ovary, 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 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. Inseminators tend to pass the rod too deep and deposit the semen into one of the uterine horns, thereby reducing fertility if ovulations are taking place at the opposite ovary.

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 harvest the embryos seven or eight days after estrus (Figure 25.1). This collection procedure is relatively simple and can be completed in 30 minutes or less without harm to the cow. A pre-sterilized 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 ml 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.

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 (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)

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 (Figure 25.2). These stages are also numbered:
Stage 1: Unfertilized
Stage 2: Two 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 that they are transferred to 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 are typically reduced. In a recent Louisiana study involving 1,116 beef and dairy cows of 15 breeds, 58% of all embryos were transferable, 31% were unfertilized, and 11% were degenerated.

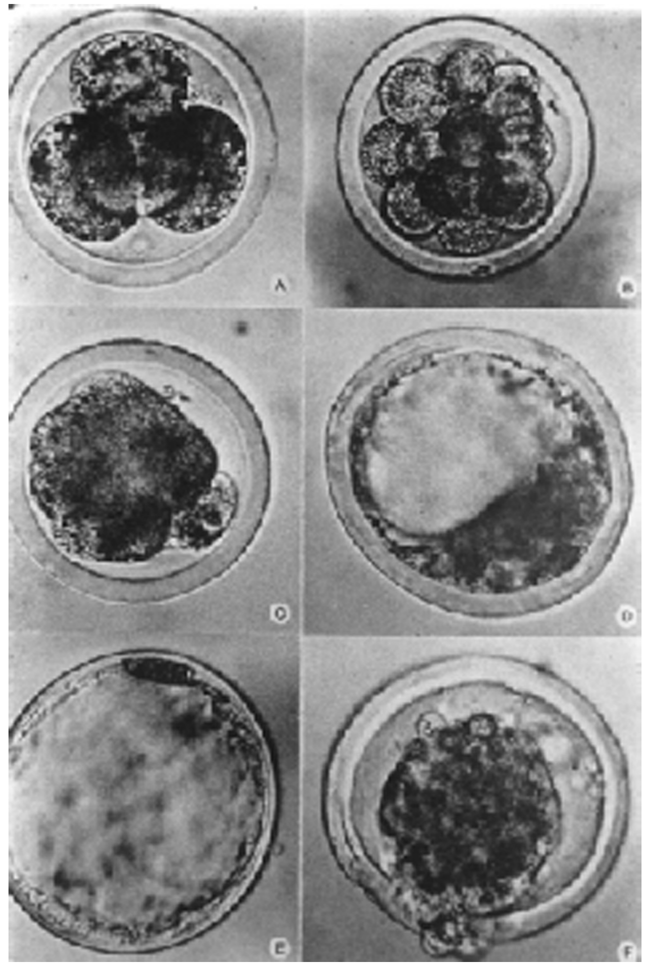

Figure 25.2 – Cattle embryos at various stages of development. Source: Betteridge.

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

Selection and Preparation of Recipient Females

Proper recipient herd management is critical to embryo transfer success. Cows that are reproductively sound, exhibit calving ease, and 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). They must also be on a sound herd health program.

How many recipient cows are necessary is a tough question to answer. 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 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% 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 of 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 the same working time as the donor cows. Many different estrous synchronization protocols are available and are used (Chapter 23). This timing again 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. Remember synchronizing drugs are effective only on recipient females that are already cycling. Anestrus, or noncycling, cows that are too thin or too short in postpartum days will not make useful recipients.

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 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 should be taken to prevent damage to the lining of the uterus. Inflammation and scarring greatly reduces 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 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 is also very intricate and usually results in an approximate 20% 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 in this country who believe that they own cattle with the genetic capability to be valuable in other nations may wish to contact the department of agriculture within their state and ask about regulations and marketability of frozen embryos from their herds. Different nations have different health requirements for cattle producing frozen embryos for import into their country. Therefore, individual inquiries are necessary to learn expected health and legal requirements.

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.

A thorough discussion with an embryo transfer technician should include an outline of all costs that will be incurred by the cow owner. The value of each embryo transfer calf must exceed the costs for embryo transfer to be a successful tool for the beef operation.

Beef producers considering using embryo transfer should first visit with their breed representative to determine the specific requirements needed for certification and registration of embryo transfer calves in that breed. This would also be an excellent opportunity to discuss the potential value of calves that result from the embryo transfer process.

Conclusion

Embryo transfer will cost producers money and time. However, it can also help improve the genetics of the herd when properly performed. A visit with the breed representative is important to determine requirements for embryo transfer for the breed.

References

Betteridge, K. J., Editor (1977) Embryo Transfer in Farm Animals. Agriculture Canada.

Seidel, G.E., Jr. and S.M. Seidel. (1991) Training Manual for Embryo Transfer in Cattle. FAO Animal Production and Health Paper 77. Retrieved from http://www.fao.org/DOCREP/004/T0117E/T0117E00.HTM

Selk, G. (2002) Embryo Transfer in Cattle, OSU Extension Fact Sheet ANSI-3158, Oklahoma Cooperative Extension Service, Oklahoma State University. http://osuextra.okstate.edu/