Bovine Embryo Transfer and the Veterinary Practitioner

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Once embryo transfer techniques become familiar to the veterinary practitioner, embryos can be efficiently collected from four to five donors and transferred nonsurgically into recipients in less than half a day provided excellent facilities are available. However, considerable initial effort is required to learn the techniques of recovery, recognition, manipulation, and transfer of embryos. It usually takes a full year for an experienced bovine practitioner to become proficient in the technique of embryo transfer.

Ovum Recovery Techniques

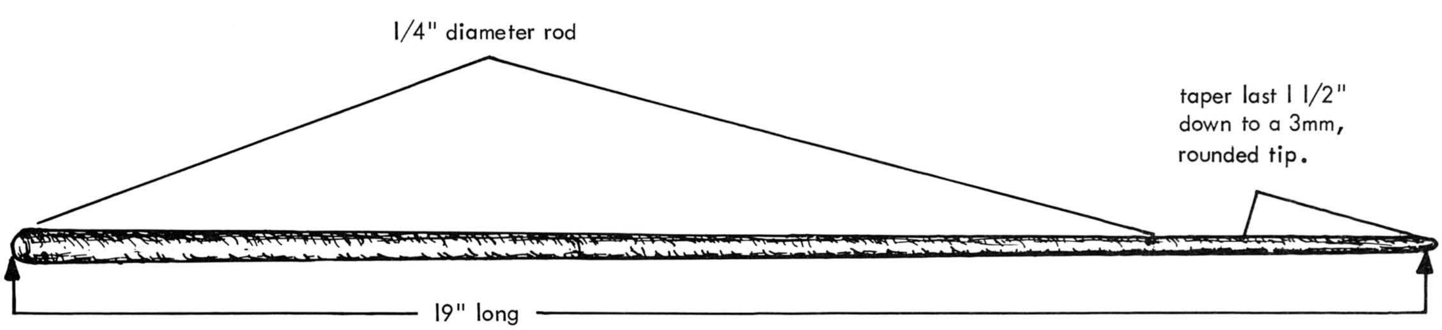
Ova are washed out of the uterus by infusing a balanced salt solution, such as Dulbecco's phosphate-buffered saline (PBS), into the uterus and allowing the fluid to flow out under some pressure. This technique has been described in detail (Mapletoft, 1981; Elsden, 1981) and only some of the problems will be discussed.

Difficult situations encountered are: 1) heifers with small cervices, for example, some Holsteins and most *Bos indicus* breeds; 2) large Holstein cows with cervices and tracts hanging over the pelvic rim into the abdominal cavity; 3) fat cows, often infertile beef animals (deposits of abdominal fat increase the difficulties of palpation and accurate deposition of instruments in the uterus; additionally, epidural anesthesia is much more difficult to attain in fat cows; 4) donors with long vaginae; 5) donors with cervical adhesions; and 6) inflated rectal walls following epidural anesthesia. The lumen of small cervices can usually be enlarged with cervical dilators (See Figure 1). Careful manipulation of the cervical expander will not cause significant damage to the cervix, in fact, we have used this instrument for more than 40 collections in some animals. A great deal of patience has to be exercised in trying to pass catheters through some small or tortuous cervices; however, with practice rarely does a cervical lumen prove impassable.

Large donors present other problems. The front feet should be elevated (1 to 2 feet) to facilitate manipulation of the uterus. Cervical retractors are extremely useful for retaining a large uterus in the pelvic cavity after it has been retrieved from the abdomen. The practitioner should attach the retractor firmly to the caudal ring of the cervix following epidural anesthesia, and with the hand in the rectum, grasp the transfixed cervix and pull it into the pelvic cavity, where it is retained during collection by an assistant holding the retractor. Using this technique, one can palpate all of the reproductive tract, and embryos can be collected efficiently. Before a catheter can be inserted successfully into a fat cow's uterus, it is essential to achieve epidural anesthesia. Frequently, a second or even third injection proceeding caudally from the tailhead will achieve the desired result. Proceeding cranially usually will not work, and if it does, may result in dorsal recumbency.

Extended Foley catheters are often useful when collecting embryos from large cows with long vaginae. Commercial embryo-collection catheters are very expensive; however,

FIGURE 1.
Cervical Expander



the Foley can be easily extended temporarily with an extra 8 to 10 cm of tubing using glass or plastic connector on one-or two-way catheters. It is not necessary to extend the air passageway. In attempting to place lengthened catheters, some sensitivity at the tip is lost and even greater care has to be taken when inserting the instrument. Any slight trauma to the endometrium will cause hemorrhage and result in an opaque collection solution causing difficulty in locating embryos.

Cervices adhered to the pelvic wall can be extremely difficult to cannulate since the organ cannot be firmly held rectally. Excellent epidural anesthesia is required plus considerable patience and perseverence. Frequently when epidural anesthesia is attained, donors will suck in air and the rectal walls become board-like on palpation. Excess air can be removed in 30 seconds using a suction pump (e.g. vacuum cleaner). We hold an 8-mm plastic tube in the rectum, cupped in the hand, with the other end held in the vacuum cleaner hose. Once the air is removed, the rectal walls fall limply around the hand, and structures in the pelvic and abdomenal cavities can be easily palpated.

Transferring Embryos

Embryos can be transferred surgically by flank incision or nonsurgically through the cervix. Accurate palpation is required to identify corpora lutea and to manipulate a catheter approximately half way up the horn, to deposit an embryo nonsurgically. Initially, to establish reasonable pregnancy rates quickly, it may be opportune for veterinarians to transfer surgically. Problems with the flank method are: 1) difficulty in exteriorizing the uterine horn of large heifers, especially Holsteins and fat animals. Traction should only be placed on the broad ligament and never on the uterine Hoen itself; 2) two trained people are required, the surgeon and a trained embryologist to transfer the embryo; 3) frequently poor conditions on the farm or ranch, in which to perform elective surgery, thus reducing the efficiency of this method; 4) surgery often precludes the use of milking animals as recipients.

Problems with nonsurgical transfer are: 1) heifers with small cervices, which are difficult to cannulate; 2) tortuous cervices encountered in all *Bos indicus* breeds, 3) considerable practice required before pregnancy rates approximate those achieved using surgical transfer. Epidural anesthesia, well-constructed cervical dilators and the use of young cows as recipients will facilitate nonsurgical transfer of embryos.

Location, recognition and classification of embryos and unfertilized ova is one of the most difficult problems encountered by veterinarians. The only solution is viewing and handling as many embryos and ova as possible (Elsden, 1981). A high quality, stereoscopic microscope costing around $1000 is essential for searching and manipulating embryos. Magnification from 10X to 30X is essential. Magnification above 50X using a dissecting microscope does not help, and more detailed examination at 100X will only be achieved under a compound microscope, which many practitioners already own. Light of variable intensity should be reflected only from under the stage, a bulb directly in this area will heat the searching dishes too much and possibly damage embryos. Between recovery and transfer, embryos can be stored in culture dishes at room temperature without losing viability. When transporting embryos, small test tubes (e.g. Falcon tubes) containing 3 to 4 ml of PBS enriched with 10-20% serum, can be carried in a shirt pocket successfully for up to 24 hours.

Results

Approximately 80% of fertile donors will respond (>2 corpora lutea) to the first superovulatory treatment. We have observed a slight decrease in response in terms of embryos collected between the first and second superovulatory treatments (6.4 vs. 4.8), but there is a significant decrease in embryos after the fourth treatment (6.4 vs. 2.3). The donors that we superovulated in our clinic were reproductively sound and cycling regularly (at least two cycles) before they were treated. Usually beef donors superovulated on the ranch are treated in groups of 5 or more. The average recovery rate under these circumstances appears to be about three transferrable embryos per collection (unpublished results). This decrease in embryos may be due to unknon, and probably temporary, infertility of some donors. They are usually in the post-partum period, are not checked by palpation for reproductive soundness, and have not been observed for regular estrous periods. We have found no significant differences between cows and heifers following superovulation treatment in terms of transferrable embryos (4.8 vs. 5.8 respectively). When we compared breeds (dairy vs. British beef vs. Continental European beef) we observed no significant differences (6.5 vs. 5.0 vs 5.9 transferrable embryos, respectively). Under Coloradoan climatic conditions, were found no significant differences between seasons in numbers of transferrable embryos collected (Spring, 4.7 vs. Summer, 5.4 vs. Winter 5.8). However, there are reported decreases in collection rates during the summer in hotter climates and winter in colder climates. When lactating versus non-lactating donors were compared we again found no differences in recovered transferrable embryos (4.6 vs. 4.5, respectively). In fact, a lactating Holstein cow holds our record of 34 transferrable embryos from one collection. Approximately 60% of transferrable embryos will become 3- month pregnancies. From a survey (Elsden *et al.*, 1980) of 178 pregnant recipients owned by our clients, we found 2% aborted between 3 and 9 months of gestation, 4% died at birth, and another 4% died between birth and weaning. Thus, in well managed herds, 10% of the confirmed 90-day pregnancies were lost. There were 85 female and 89 male calves, a sex ratio of 51% males. Assistance was given to 58% of the recipients at parturition, and embryos from donors of the large breeds (e.g. Simmental) when transferred into recipients of small breeds (e.g. Simmental X Angus or Hereford recipients) resulted in significantly more problems. However, the assistance was often due to elective management practices and did not always represent dystocia.

Embryo transfer recipients that fail to become pregnant after receiving an embryo are often reused. So, we determined pregnancy rates (Nelson *et al.)* in previously failed recipients. Pregnancy rates of 59% (N=1,356) for first transfer, 46% (n=321) for second transfers, and 30% (n=23) from third transfers. When recipients were bred that failed to become pregnant after three tries with embryo transfer, their pregnancy rates were normal, which indicates that some animals will never be fertile recipients. Possibly the trauma of surgical transfer prevents some recipients from becoming pregnant.

When pregnancy rates were compared (Elsden, 1979) among superovulated fertile, diagnosed infertile, and undiagnosed infertile donors, the number of pregnancies per superovulation treatment were 3.6, 0.8, and 0.4, respectively. Results for diagnosed cases of endometritis (after treatment), mild adhesions of the upper reproductive tract, and cystic ovaries, were 1.1, 3.3, and 0.2 pregnancies per superovulation treatment. Relatively few viable embryos were recovered from donors with chronic cystic ovaries or from repeat breeders with problems of unknown etiology.

Mapletoft *et al.* (1980) also used embryo transfer techniques in an attempt to curcumvent infertility in cows and found low fertilization rates to be a problem. They observed a 43% fertilization rate of ova, recovered, and also noticed, as we have, that some of the previously barren donors became pregnant when bred after being superovulated and exposed to embryo transfer techniques. Unfortunately, the infertile cow is frequently presented to the practitioner as a last resort, and the results will most likely be discouraging.

Costs of Embryo Transfers

Costs to the practitioner of embryo transfer are relatively few, apart from the time involved. For recovery and transfer, a dissecting microscope is the only expensive item. Additional equipment such as medium, tubing, Foley catheters, 1-liter cylinders, searching and culture dishes, plus straws, sheaths and Cassou inseminating pipettes costs about $200. It is usually best to use disposable equipment, as proper washing and rinsing procedures are extremely time consuming. Supplies used in a day's work usually will cost under $100. If freezing is contemplated, then a machine will cost in the vicinity of $10,000; in addition, a technician will have to be employed to run the program.

For the owner of the donor, expenses are considerable. Seidel and Seidel (1982) illustrate the complexities of costing out embryo transfer. Conventional costs of raising purebred calves to 6 months of age is around $1000, and embryo transfer costs must be added to obtain total costs. To illustrate typical costs of embryo transfer when performed under practice conditions, two cases are described:

Case I. Recovery of embryos and surgical transfer on the farm with dairy cattle. Assume 12 heifers as recipients which can be synchronized for the donor, a 55% pregnancy rate, three pregnancies at 90 days' gestation, and the open heifers have wasted 90 days of reproductive life.

Case II. Nonsurgical recovery and transfer on the ranch with beef cattle. Assume ranch personnel are experienced in artificial insemination, 10 donors are superovulated and 80 recipients are synchronized, a 45% pregnancy rate and 25 calves born. The costs are summarized in Table I. (Adapted from Seidel and Seidel, 1982).

Actual costs are increased by 10% loss of fetuses and calves from 90 days' gestation to weaning. One attractive advantage to most on-the-farm embryo transfer is much less cash outflow in that they do not purchase 90-day pregnant recipients. However, a few extra facilities usually have to be built, and extra labor is incurred for farm personnel. Most large dairy farms have sufficient open cows and heifers from the lower producing animals in the herd, unfortunately in the very large herds fertility is lower, reducing embryo transfer pregnancies.

Advice to Clients

Due to unpredictable results most of you will have three kinds of embryo transfer clients: 1) those experiencing poor results; 2) those with average results; and 3) those who believe you walk on water. We have one client with 91% pregnancy rate (35 transfers), however, we also have clients with very low pregnancy rates. As the veterinarian, you must impress upon your client the importance of well-managed fertile donors and recipients. Excellent quality semen and competent technicians to inseminate the donors are essential. Facilities must be adequate, farm and ranch staff must be conscientious, and believe in the value of accurate heat detection. They must be capable of following directions.

Advise your client to discuss possible tax advantages with his accountant. Many costs can be deducted from income as expenses, some donors may qualify for investment credit, and some profits can be considered as capital gains. Thus, many investors are attracted to the cattle industry and embryo transfer and no doubt contribute to the very high prices of top quality cows.

Advising a client on how to select his donors is probably difficult for the veterinarian; however, there are some guidelines to follow (adapted from Elsden, 1982).

Selection should be based on two main criteria, genetic superiority and reproductive ability. When selecting genetically superior beef cows as donors, the following traits should be considered: 1) percent calf crop weaned; 2) milking ability; 3) pre-weaning growth; 4) post-weaning growth; and 5) carcass cutability.

Until recently there has not been any national scheme to measure and compare the above traits. However, there is now a national sire evaluation program which measures, objectively, genetic superiority of beef bulls. The results are published as official sire summaries. The following records should be used to help select potential donors: 1) maternal breeding value; 2) weaning breeding value; and 3) yearling breeding value. All of these values are objective

Table I. Costs per pregnancy of bovine embryo transfer assuming 5 doses of semen, 6 transferrable embryos per donor, and calves raised to 6 months of age.

	CASE I Dairy cow collected on-farm, embryos transferred surgically on-farm to farmer's own heifers and synchronized recipients	CASE II Beef cows collected on-farm, embryos transferred non-surgically to rancher's own synchronized recipients
	55%	45%
Programming fee	35	0
Set-up fee	0	40
Pregnancy fee	600	500
Semen	35	40
Registration fee	50	50
Blood typing fees	35	35
Feed & board, recipient @ 1$/day	290	470
Feed, board, & delayed breeding of unused recipients	180[a]	165
Feed & board, calves	180	90
Veterinary fees, recipients	50	50
Veterinary fees, calves	10	10
Travel expenses	35	40
Collection fee	35	0
Interest expense	325	360
Owner's time@ $10/hour	100	100
Heat checking and synchronization	60	30
Extra facilities	25	15
Loss of recipient's own calf/net	100	100
Phone, postage, advertising, & misc.	50	50
Total cost	2195	2145
10% Calf losses	219	214
Required average value of calves	2414	2359

[a] Assuming an average of 6 heifers were unused and, thus, held open 30 days and 3 heifers used that did not remain pregnant, thus, were held open 90 days.

measurements based on pounds of gain. For optimum predictable genetic superiority, donors should be selected after producing two or more genetically superior calves from the same sire.

A dairy animal should be selected on the basis of a strong cow index (C.I.) This index includes the performance of the cow, the predicted difference of her sire and the C.I. of her dam. A cow index of +2000 lbs of milk is considered to be elite. The donor should have completed at least one lactation. The C.I. index becomes slightly more accurate with each additional lactation.

Additionally, the dairy cow should be classified very good or excellent in conformation. The bull selected should have predicted differences in milk, percent fat and fat greatly above the average for the breed; as an example, in the Holstein breed a predicted difference type above +.75 would be a good selection level.

For relatively small additional costs, the veterinary practitioner can add a new facet to practice with embryo transfer. In addition, experiences gained will familiarize the practitioner with reproductive physiology aid in diagnosing and treating some kinds of infertility. Palpation skills will be enhanced leading to improved accuracy in diagnosis. Additionally, there will be immense satisfaction in seeing embryo transfer calves for which you are partly responsible winning shows and improving the genetic base of your client's herd.

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