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Embryo Transfer in Cattle in U. S. A.

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The first calf produced by embryo transfer was born in 1951. The calf was the result of work by researchers at the University of Wisconsin. This work was first duplicated by researchers at the University of Minnesota in 1960. This author was fortunate to be a member of that Minnesota research team.

Commercial embryo transfer first started in U.S. in 1972 and at that time both embryo collections and transfers were done surgically. Relatively few transfers were done in the early years. Figure 1 shows the estimated growth of the embryo industry of the number of pregnancies produced in the U.S.

Estimated number of pregnancies produced by embryo transfer in the U.S. (Figure)

1978	9000+
1979	16500
1980	20 - 25,000
1981	over 30,000
1983	50,000 +
1985	over 100,000

Progress has been made in a number of areas of technology relating to embryo transfer. First techniques were developed to non-surgically collect embryos from the donor. This eliminated the adhesions and scar tissue associated with surgical recovery which dramatically limited the times a donor might be used for embryo collection. Usually two or three embryo collections could be done per donor and still have them be in condition to be able to get pregnant and carry a calf naturally. Non-surgical collection allows for repeated donor collections with out impairing donor fertility.

Subsequently non-surgical transfer techniques were perfected. Initially pregnancy rates were lower with non-surgical transfers compared to those that were being achieved with surgical transfers. However as techniques have been modified and more transfers were done, success rates have improved and in most cases are comparable to those achieved with surgery.

The general steps involved in transfer are:

1. Donor selection
2. Superovulation
3. Breeding
4. Collection'
5. Examination & evaluation of ova and embryos
6. Transfer and/or Freezing of embryos

Superovulation is one area that still remains subject to a great deal of variability and inconsistency in response. Superovulation can be accomplished by the administration of Pregnant Mare Serum Gonadotrophin (PMSG) or the pituitary derived Follice Stimulating (FSH) Hormone. PMSG requires a single injection while FSH is given twice daily for a period of 4 or 5 days. Currently FSH is the most extensively used drug for superovulation.

The objective of superovulation is to increase the production of ova matured by the ovary at a single estrus so that multiple embryos can be recovered to produce pregnancies. In this way we can increase the number of pregnancies per reproductive cycle of valuable donor cows. Cows which normally have 1 calf per year or reproductive cycle, it is the goal to increase this to 4 or 5 up to 15-20 calves.

For superovulation, the donor cow should be 50-60 days post partum and have had a couple of estrous cycles. Her reproductive tract should be involuted normally and ready to carry a calf naturally. The superovulation procedure is started 10 to 14 days after estrus. The cow is always examined rectally prior to starting the treatment tobe sure she has a normal reproductive tract and has a mature corpus luteum.

An example of a superoulation schedule is shown in Fig. 2.

Typical Superovulation Schedule (Figure 2)

Donor In Estrus

,

,

,

10 - 14 days

,

Palpate donor for mature Corpus luteum

		PMSG	FSH
Treatment day	1	x	x A.M. & P.M.
	2		X A.M. & P.M.
	3		X A.M. & P.M.
	4	PGF	X A.M. & P.M. & PGF
	5		X A.M. & P.M.
	6	Breed	Breed
	7	Breed	Breed

PMSG requires only one injection. This is given on Day 1 of treatment and is followed on day 4 by prostaglandin, as can be seen in Figure 1. The donor usually shows standing estrus 48 hours later.

Follicle stimulating hormone requires twice daily injections for 5 days with prostaglandin being given on the 4th treatment day. FSH is the most commonly employed for superovulation at present because of the difficulty in obtaining PMSG and it is thought that FSH gives a more consistent suerovulation response. Elsden et al. (1978) obtained a higher number of fertilized eggs when FSH was used for superovulation vs. PMSG. Sidel et al. (1971) attemped to improve response to PMSG by adding varying proportions of FSH:LH but this resulted in very little if any benefit.

Unfortunately there is a high degree of variability in response to superovulation between animals and also within the same animal from superovulation to sperovulation. The ideal degree of response would be 4 to 6 ovulations on each ovary. The total ovulations may however range form 0 to 40 or 50. The extreme responses present several problems. First of all three appears to be a general decline in quality of the ova recovered when there are more than 20 ovulations. Also, with the greater degree of response there is often a greater percentage of follicles that fail to ovulate which may contribute to pooer quality ova.

The variability occurs from cow to cow and within the same cow from stimulation to stimulation using the same treatment regieme and hormone batch. A number of factors affect variability of response to superovulation. Table 1 illustrates the effect of reproductive status of the donor on superovulatory response (Hasler et al., 1983).

Data which we have collected at our facility and is presented Table 2 agrees with the previous data. Donors classified as problem animals had been bred

Table 1. Effect of Reproductive Health on First Superovulatory Response

Item	Reproductive Status		
	Healthy	Infertile	Combined
Animals, number	666	318	984
Total ova, number	6828	1943	8771
Mean ova/donor	10.3	6.1	8.9
Mean fertilized ova/donor	6.7^{a}	2.6^{b}	5.4
Mean embryos/donor	6.4^{a}	2.4^{b}	5.1
Ova fertilized, %	66^{a}	42	61
Donors with No ova, %	5^{a}	21^{b}	10
Donors with no embryos. %	14^{a}	51^{b}	
Embryos transferred, no.	3707	604	4311
Pregnant recipients, %	68^{a}	58^{b}	67

a,b Values for healthy versus infertile animals with different superscripts were different, ($P < .05$)

Table 2. Summary of Holstein Donor Collections

Donor Group	*No. of Collections*	*No. of Ova Recovered*	*No. of Ova Transferred*	*No. Pregnant*
Single collection				
Normal Holsteins	79	65 (82%)	45 (69.2%)	33 (73.3%)
Problem Holsterins	207	104 (50.2%)	27 (26.0%)	13 (48.1%)
Superovulation				
Normal Holsteins	73 (62)[a] (85%)[b]	758 (X 10.4)	357 (47.1%)	235 (65.8%) (3.2)[c]
Problem Holsteins	232 (102)[a] (44%)[b]	1417 (X 6.1)	422 (29.8%)	228 (54%) (0.98)[c]

[a]Number of donors that produced at least one transferable embryo.
*b*Percentage of donors that produced at least one transferable embryo.
*c*Number of pregnancies per collection.

unsuccessfully several times and were currently not lactating and non pregnant.

Different batch of FSH have been regarded as a source of variability, however Looney (1986) reported omly slight differences in total ova and transferable embryo production between different lot numbers of FSH. Similar results were found by Lindsell (1986). The results are not as surprising as one might first think because of the large amount of variation due to donor females masks the differences due to the superovulatory agent.

Constant dosages and descending doseages of FSH during the superovulatory treatment have been compared (Looney, 1986). Donors superovulated with descending doses of FSH produced more total ova and transferrable embryos but responses were not statistically significant. Non response rates were found to be lower for donors superovulated with descending doses: 9.1% vs. 14.3%. French researchers found significantly better results with descending doses (Monnioux et al., 1983)

With superovulation we are trying to convert an estrous cycle which normally produces one pregnancy to one which will produce many potential pregnancies. Spicer and Echternkemp (1986) showed that more than one estrous cycle is required for small follicles to grow into large ones. Many follicles grow and undergo atresia. Presumably many of these are recovered by superovulation before irreversible atresia takes place.

Foot (1986) has suggested several possible methods for controlling superovulation:

a. Inhibin. This is a substance contained in follicles which has a negative feedback action inhibiting FSH release. He suggests that by preparing antibodies to inhibin or immunizing animals against inhibin an increase in the animals own FSH could be expected.

b. A follicle growth inhibitor has also been identified in follicles. This substance in the dominant follicle is perhaps the one way in which this follicle prevents growth of other follicles. By blocking the inhibitor, more of the animal's own FSH may be available.

c. Steriod hormone immunization of sheep against androstenedione increases ovulation rate (Bindon et al., 1986). Application of this principle might be used for superovulation.

Donaldson and Ward (1985) have reported an increase in good embryos obtained with highly purified FSH. With the potential of cloning of LH and FSH, this could result in production of substantial quantities of pure FSH and LH. Studies could then be conducted on formulating the ideal FSH:LH ratios for superovulation of donor cows.

We need to better understand the phenomena of follicular development in detail in the normal estrous cycle before we can definitively control superovulation

responses.

Recipient Animals An important aspect of a successful embryo transfer program is the quality of the recipients.

REcipients theoretically can be of any breed and color. All the genetic material has been laid down in the ovum at the time of fertilization so the recipient makes no genetic contribution to the embryo it is carrying.

One very important aspect of the recipient is that she must have estrus at the same time as the donor. This can be accomplished by natural synchronization by maintaining a sufficiently large pool of non-pregnant cycling animals. If one has 200-250 recipients, one should then have 8 to 12 heifers in estrus every day. Purchase and maintenance of such a large recipient herd is very costly.

The other method of synchronization involves the use of prostaglandin. Prostaglanding are effective only when there is a mature corpus luteum present on the ovary. With one injection only about 65-70 percent of the animals injected will respond and be synchronized. We prefer to give two injections of prostaglanding to recipients which are given 10 or 11 days apart. Virtually all animals should be at a stage of the estrous cycle to respond to the prostaglandin by the second injection and the estrous periods will be more closely synchronized then with a single injection.

The importance of the recipient can not be overemphasized. There are several points to consider when selecting recipients for transfer:

1. The recipient has to be closely synchronized to the stage of the embryo that is being transferred into her (an embryo collected from the donor 7 days after breeding should be transferred to a recipient that was in estrus 7 days earlier). For maximum results recipients should be in estrus within 24 hours of donor.
2. Recipients must be in normal reproductive health. She can not have any uterine infection or be cystic. A repeat breeder does not make a suitable recipient.
3. The recipient must be in good general health. Recipients that are stressed by disease at the time of transfer or shortly thereafter will not produce good pregnancy results.
4. Recipients need to be in a good nutritional status. Animals that are stressed by poor nutrition will not cycle properly, will not have a good pregnancy rate and may have a higher abortion rate.

It is important to remember the recipient is going to be the incubator for the embryo transferred to her uterus. No matter how good the quality of the embryo transferred, if the conditions in the incubator are not proper, the embryo will not survive and result in a live calf.

Possibilities of Disease Transmission by Embryos. .

With the improved success rates in freezing embryos and their long term preservation, this has opened the market for international trading of embryos. This in tern has ingnited concern for potential disease spread by the embryos. Because of the nature of embryos, it is impossible to test each one for specific diseases as we do with live animals. It therefore becomes important to establish procedures which have proven to be effective in preventing the spread of diseases by embryos. This section with the paper will review research in this area and the techniques that are recommended and used to insure healthy disease-free embryos.

If disease were transferred by embryos, the disease organism would have to be carried on the embryo, in the media or on contaminated equipment. To avoid the spread of disease by embryos, two methods can be used: 1. healthy donors 2. treatment of embryos.

Only a few organisms have been detected in ova, but a considerable number have been found in semen (Eaglesome et al., 1980). Most organisms in semen are in seminal plasma and not associated with the sperm cell. Consequently these organisms have potential for infecting the female reproductive tract and not directly infecting the ovum. It is concluded that the majority of organisms causing infectious disease are not transmitted by gametes.

The other method which embryos potentially could become infected is through their environment. To be able to infect the embryo the organism must be able to penetrate the zona pellucida. The other potential means for spread of disease would be if the organ-

ism adhered to the zona pellucida. To study this embryos were exposed to infectious agents and then transferred to seronegative recipients. Embryos have also been collected from infected or seropositive donors and transferred to seronegative recipients.

A number of deseases have been studied for potential embryo-disease transmission. They include:

Bovine leukemia virus
Bluetongue virus
Infectious Bovine rhinotracheitis virus
Foot & Mouth virus
Akabane virus
Bovine viral diarrhea
Bovine parovirus
Brucella abortus

Most of these are viral diseases and these are the chief concern for disease transmission.

Singh (1986) reviewed factors that influence disease transmission of embryos. They include:

1. Presence of the zona pellucida: This structure has been found to be an effective pathogen barrier. Therefore, its presence or absence can greatly effect the disease transmission potential of embryos.
2. Limited exposure time for infection: Bovine embryos are generally transferred at day 7 or 8. This short period of time should severely limit the number of pathogens the embryo could be exposoed to in the donor's reproductive tract.
3. Dilution of any pathogens present in the donor's reproductive tract: When embryos are collected, the embryos are flushed form the uterine horns with several hundred milli]liters of fluid. If anything is present in the uterine tract it undergoes considerable dilution during embryo collection.
4. Washing or treating embryos: Embryo transfer techniques enable embryos to be processed under *in vitro* conditions. Thus, embryos can be washed *in vitro.* This technique has been shown to be effective in removing over 10^{7} of virus.
5. Possibility of introducing disease: One disadvantage of embryo transfer in terms of disease control involves the possibility of infecting or contaminating embryos between embryo collection and transfer. To avoid this, it is essential that sterile techniques and solutions be used throughout be used throughout embryo processing.

The results of studies on disease transmission possibilities have led recommended procedures for handling embryos.

1. All media, solutions, sera, enzymes and cryoprotective agents must be sterile and sterile techniques must be used.
2. Proper washing has been found to be effective in removing very high levels of most pathogens from embryos. The recommended procedure involves transferring embryos, in groups of ten or fewer through ten changes of medium. A fresh sterile micropipette must be used to transfer the embryos to each of the washes and each wash must consitiue a one-hundred fold dilution of the previous wash. Embryo washing must precede embryo freezing. Freezing can result in damage to the zona pellucida and thus any pathogens present must be removed prior to carry out freezing. Once the zona has been cracked and a virus has entered the embryonic cells, further washing would be to no avail.
3. All embryos must have a zona pellucida that is intact and free of adherent materia. This evaluation should take place after washing and before cryopreservation.

Elsden (1986) summarized several of the studies on disease transmission and reported that of 873 embryos experimentally infected or naturally contaminated with six different virsuses which after sanitary treatment and transfer into recipients did not transfer disease to their host. He concluded that from evidence presented, embryos are by far the most humane, economical and safest method of importing germ plasm.

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