Costs and Effects of Embryo Transfer Programs Kollege of Knowledge XI

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Definition of ET

Embryo transfer is a procedure where an embryo is collected (flushed) from one female (donor) and then transferred to the uterus of another female (the recipient) for the duration of gestation. The procedure is commenced by superovulation of the donor by administering the gonadotropin, follicle-stimulating hormone (FSH) both morning and evening for four consecutive days. Two to three days after the beginning the FSH treatment prostaglandin (Lutalyse or Estrumate) injections are given to initiate estrus, and then are immediately artificially bred usually with frozen semen. Approximately seven days later, the collection is performed by first administering an epidural anesthesia to the donor cow and removing the fertilized ova by a per rectum manual procedure of inserting a Foley catheter thru the cervix into the uterus. Several intermittent flushes of a 30-60 ml of saline solution are placed into each horn and then pulled out by either suction or gravity flow. The solution recovered from the uterus is collected in a filter then the concentrated ova are searched using a stereomicroscope and those identified as viable are loaded into a 1/4cc semen straw then placed into an embryo transfer gun. The recipient cow is synchronized usually with prostaglandin and seven days into the estrous cycle is matched to the embryo donor. Just prior to embryo transfer an epidural 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 containing the straw holding the embryo is carefully passed through the cervix. The tip of the rod is then guided into the uterine horn on the same side of the ovary with an active corpus luetum (ovulation point) and the embryo discharged.

Advantages of ET

Since the first successful transfer of a rabbit embryo in 1890 it was known that a surrogate mother could raise another’s embryo. Today this technology is commonplace but only utilized with purebred cattle because of the high cost.

Because purebred cattle can be sold for higher prices than commercial cattle ET is utilized in most operations in the USA.
Embryo transfer can be used to produce a large number of offspring from a donor cow in a shorter time period than possible with normal reproduction.
Since a cow's average lifespan is only 6-7 years, and usually produces 5-6 calves, the chances of obtaining a herd sire is limited. However, with superovulation and ET the chances are increased greatly.
In beef cattle, replacements for both sexes can be selected on their own performance prior to reaching reproductive age. A highly selective ET scheme could double the rate of genetic improvement. Some commercial cattlemen have used ET technology to rapidly change to a purebred herd by transferring purebred embryos to their commercial herd.
ET Technology can also be utilized to 1) allow for genetics to become easily exported or imported opening up international trade, 2) treatment of infertility. Either collection or transfer techniques can be utilized to assist breeders in extending the reproductive lives of cattle whom may have become infertile usually of environmental reason rather than of genetics, 3) Disease control. Many diseases can't be transferred with the embryo and washing and enzymatic treatments can render the embryo sterile to some bacterial and viral diseases. By freezing embryos and using long term storage in liquid nitrogen genetics are safe from catastrophic disease outbreaks like FMD or BSE that could wipe out the entire herd. 4) Embryo transfer allows for faster genetic testing of bulls and cows for progeny tests and accurately screening for genetic recessives that would be detrimental.5) Embryo transfer can be used in research trials to decrease variability in experiments in animal science areas and 6) the production of twins for increased calf production.

Costs of Embryo Transfer Services

A comparison of service fees collected for ET procedures vary by region and by company providing the service. The first commercial ET programs in the USA where developed here in Texas. During these early days of the Exotic Boom most of the procedures were performed surgically in imported breeds from Europe. In the early development of ET many of the fees were charged after the calf was produced because the producer needed some assurance that the procedures would be successful. The first commercial ET programs charged and enrollment fee for the donor cow ($500) and only a pregnancy fee of $3000 which included the recipient cow. At that time the commercial cows would cost $300 per head. As the success rates of the procedures improved the fees decreased. Fees normally charged today represent each procedure such as embryo collection fee, transfer fee, embryo freezing fee, etc. Normally, the costs of embryo transfer fees when based on a per embryo basis range from $125-150. Costs for recipient pregnancies reflect the commercial cow market price. Typically, you can count on the cost of

pregnancies purchased from an ET center to be $400 to 700 higher than the top price paid for high quality cows. Many ranchers elect to manage their own recipients improve efficiency.

Recent Improvements

Over the years researcher and practioneers have made many improvements to the technology. One of the recent improvements in the area of superovulation and estrous control has been the use of CIDR (Controlled Internal Drug Releasing) vaginal inserts in combination with Progesterone/Estradiol and Prostaglandin injections. The functions of these treatments are to control follicular dynamics so that timed Artificial Insemination can be utilized and start superovulation treatments without regard to what day of the estrous cycle when FSH treatment begins. The use of ultrasound for early pregnancy determination and fetal sexing has been another recent improvement in the ET technology. In addition, the ultrasound can be utilized in evaluation the ovarian structures of donors and recipients with much greater accuracy than just hand palpation. Researchers have determined that without ultrasound more than 30% of ovarian evaluations for ovulation are incorrect. In-line filters with a built-in searching dish have been developed to eliminate losses of embryos and prevent contamination during the flushing procedure. Recent improvements in embryos cryopreservation make it practical to freeze embryos for transfer later. Many producers collect donors during the non-breeding season and freeze embryos for storage until the spring or fall breeding season then transfer. Embryos frozen in 1.5 M Ethylene Glycol can be thawed like semen and directly transferred (DT) into recipients. We have improved the freezing protocols by refining the superovulation program so that embryos that are more readily frozen are collected and also by using an improved cryoprotectant equilibration period with freezing parameters designed to minimize freeze thaw stress on the embryo.

Expected Results

Since ET technology results rest on biological responses, therefore vast variability is expected. Variable responses to ET technology can be attributed to breed of donor and recipient, parity, stage of lactation or post-partum interval, body condition, environmental inputs such as nutrition and temperature and age. Also experience level of the embryo transfer practioneers can have tremendous effect on ET results. Typically, superovulation results average 12.7 total ova and embryos with 6.5 (51%) viable embryos (freezable and transferable quality) and 2.4 (19%) degenerate embryos (fertilized embryos that stop developing before collection) and 3.8 (30%) unfertilized ova (UFO). Once embryos are deemed viable they can be

transferred fresh or frozen. Since embryos are already fertilized results are usually reported as pregnancy rates (No. Pregnant / No. Transferred X 100). Fresh pregnancy rates are usually higher than frozen and range from 50 to 70%, while frozen rates range from 40-50%. However, it is not uncommon for frozen rates to equal fresh rates especially when in practice normally high quality embryos recently collected are frozen leaving the lower quality embryos for fresh transfer. Besides embryo quality, synchrony with the donor (embryo) and recipient and transfer ease and depth affect pregnancy rates. Pregnancy rates for fresh embryos by quality grade along with frozen DT embryos by grades and stage prior to freezing are given in the tables below. Pregnancy rates were determined by ultrasound 30-70 days of gestation by skilled technicians. Results are shown for all breeds of donors and recipients in both in clinic and client's ranches in the USA and Mexico using at least four transfer technicians.

Table 1. Fresh Embryo Pregnancy Rate

Embryo Quality	No. Transferred	Pregnancy rate
1	2,338	68%
2	3,427	58%
3	1,098	47%
Overall	6,863	58%

Table 2. Post-thaw Pregnancy rates by Pre-freeze Embryo grade

Pre-freeze grade	No. transferred	Pregnancy rate
1	1890	58%
2	1060	50%
3	138	28%
Overall	3788	53%

Table 3. Post Thaw Pregnancy rate by Pre-freeze Development stage

Developmental Stage	No. transferred	Pregnancy rate
Compact Morula	2395	63%
Early Blastocyst	963	59%
Blastocyst	398	49%
Expanded Blastocyst	32	28%
Overall	3788	53%

Future Developments

Embryo Sexing--- Embryos can be sexed by identification of the male and female chromosomes. The procedure entails taking a small biopsy of 3-5 cells of the

embryo proper that necessitates insulting the integrity of the zona pellicuda (acellualar shell around the embryo). The procedure requires DNA Amplification using PCR technology and is slow and detrimental to the embryo survival. Recently, progress in embryo sexing has been improved, especially reducing the tedium and time required in the DNA identification. Embryo sexing greatest limitation is the need to biopsy the embryo by micromanipulation which necessitates the use of fresh embryos recently collected and whereas sexed embryos can't be frozen and frozen-thawed embryos don't work well either. The technology that is needed is a method that uses a simple and fast antibody test to react to the male chromosome, which is non-invasive.
Other DNA probes will be soon developed for identification of coat color, polledness and other economically important traits. The ability to make breeding decisions at the embryo stage saves time and money.

In Vitro Fertilization--- The number of potential ova in the ovaries of a heifer exceeds 75,000. The process of fertilizing the oocytes (egg) in a test tube provides the producer to take advantage of this potential large resource of oocytes. Researchers have developed harvesting techniques that can repeat every 7 days and methods where each oocyte can be fertilized individually to different bulls. Harvesting can be accomplished in prepuberal heifers, cycling heifers and cows, pregnant heifers and cows, and post-partum cows.

Cloning and Transgenesis--- since several other laboratories in many other livestock species including cattle, goats and pigs have now demonstrated the pioneering work with Sheep at the Roslin Institute cloning using somatic body cells. Although there are wide variations between experiments, efficiencies appear to be improving with 10% better survival rates of transferred blastocysts being achieved in cattle. At least three commercial companies in the United States are offering cloning procedures for cattle. Costs per live cloned calf at 30 days of age range from $25,000 to $10,000. Cloning requires tissue being collected from the parent either from skin or internal organs such as ovaries, muscle or mammary epithelia at necropsy. Fetal tissue also can be grown in as primary cell cultures in the laboratory. Cell lines can be frozen for deferred cloning, DNA testing and used for disease testing. Clones gender is entirely predictable and genetics are identical. Some differences are noted in hair coat in multicolored cattle like Holstein or Simmental. Also differences are expected due to environmental inputs such as recipient milking ability and disposition.

Summary

Embryo transfer is a segmented process that all require maximum attention to detail for success. Management, selection of the donor, recipients and offspring are

all-important as well as the collection, handling, preservation and transfer or the embryos. It has been proven may times that hiring the professional team to provide the ET service with their experience and knowledge will pay dividends towards success of the program.

The decision to utilize ET technology depends on the profitability and marketability of the offspring produced. Let Ovagenix assist you in your ET and breeding program.