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History

- 1890** Transfer of rabbit embryos showed there was no genetic influence on the offspring by the recipient.
- 1930** Collection of first bovine embryo
- 1951** First successful bovine embryo transfer (ET)
- 1964** First non-surgical collection
- 1983** In-vitro fertilization of a bovine ovum (egg)

Definition of Terms

- Embryo Transfer** The process by which an embryo is collected (flushed) from one female (the donor) and transferred to another female (the recipient) to complete the gestation period.
- Embryo** A fertilized ovum which will eventually develop into the offspring. After 9 to 10 days of age it will hatch and break out of its protective "shell-like" coating (the zona).
- Superovulation** The result of treatment of a donor with gonadotropin (especially follicle stimulating hormone, FSH) to produce more than a single ovum.
- Synchronization** Matching the estrous cycle of a donor and a recipient by the injection of prostaglandin (PGF_{2α}) to stimulate the onset of estrus (heat).

Commentary

Since the first successful transfer of fertilized rabbit ovum in 1890 it was known that a surrogate mother could raise another's embryo. Today this technology is becoming commonplace. It is estimated that 100,000 pregnancies will be produced in United States cattle in 1984.

Currently, ET is profitable only for registered purebred cattle which can be sold for breeding stock. Originally most transfers were on so-called exotic beef breeds (Charolais and Limousin). Today purebred Holsteins, Jerseys and Simmentals are responsible for nearly half of all ET's in the United States.

Embryo transfer can be used to produce a large number of offspring per female in a shorter time than possible with normal reproduction. Since a producer's life span covers only 6 or 7 generations of cows, the chances of obtaining a super bull from a superior cow are limited. By the use of superovulation and ET, the number of opportunities is increased.

In beef cattle, replacements for both sexes can be selected on their own performance prior to reproductive age. A highly selective ET scheme could double the rate of genetic improvement.

In dairy cattle, genetic improvement (Fact Sheet IRM-16) for milk production is limited. Embryo transfer fees would have to be less than \$100 per pregnancy if the cost of the ET was to be paid exclusively by an increase in milk production.

Many dairy producers are finding markets for animals with strong pedigrees, superior type and increased production potential. Thus more and

more cows are being flushed. Embryo transfer allows dairy producers to increase the number of offspring from cows believed to be genetically superior. Each cow can be selectively bred to three or four superior bulls in a given year. The identification of a qualified donor is critical, since the marketability of her offspring is the important factor.

Usually the success rate should be measured in terms of marketable calves. If one must count on a 50:50 sex ratio for a profit (e.g. extra bull calves may be of marginal economic value), one can get into financial trouble. For example, the odds of getting seven or more bulls from 10 pregnancies are over 17%.

Purpose

- Increase the number of offspring, either male or female, from a genetically superior female.
- Allow ease of import/export.
- Genetic testing of bulls for inherited defects. Mating a bull to six or eight of his superovulated daughters will exhibit the recessive genes a bull carries more accurately and in much less time than by testing the population at large.
- Produce twins, although 12-15% of the offsprings will be freemartins (i.e. infertile females).
- Decrease variability in research subjects—for the study of the physiology, pathology and immunology of reproduction or other traits.
- Long-term storage by freezing.
- Disease control. Many diseases present in the dam will not be transmitted by the embryo.
- Treatment of infertility. In general, reproductive traits are lowly heritable. However, producers should consider possible decreases in fertility of subsequent generations before using infertile donors extensively. Also, ET may or may not work on infertile cows depending on the cause of infertility.
- Rapid genetic change within a small population (ex., from grade to purebred herd).

Procedure

When a donor is selected, her estrous cycles are recorded. The producer decides whether a single fertilized ovum is going to be recovered or if superovulation is desired. See Fig. 1.

If a single fertilized ovum is desired, the donor can be flushed 6-8 days after a normal estrus and breeding. The recipient must be in the same stage of the estrous cycle as the donor. Therefore, unless a large pool of recipients is available it will be necessary to synchronize estrus in the donor and recipient (Fact Sheet IRM-8).

When superovulation is the choice, the cow is treated with the gonadotropin, follicle stimulating hormone, between day 9 and 14 of the estrous cycle (day 0 = day of estrus). FSH treatment is given twice a day for 4 or 5 days resulting in the development of multiple follicles on the ovaries of the donor.

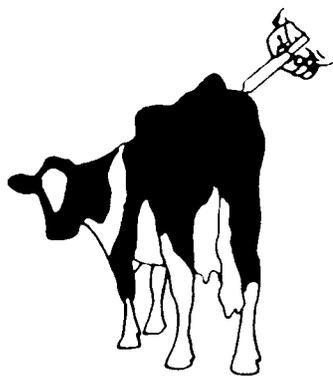
Two or three days after beginning the FSH treatment, prostaglandin injections are given to both the donor and the selected recipients to initiate a synchronized estrous period 2 to 3 days later. At least 10 recipients should be in the same stage of the estrous cycle as the donor cow. Therefore 14-18 recipients will need to be synchronized.

When in standing estrus or shortly thereafter, the superovulated donor is bred with frozen semen of one or more choice bulls. (Parentage can be determined by blood type.) Usually multiple inseminations at 12, 24 and 36 hours after the onset of estrus are recommended. Preferably, more than one vial or ampule of high-quality semen is used per insemination. The resulting embryo(s) is (are) flushed from the donor's uterus 6 to 8 days later.

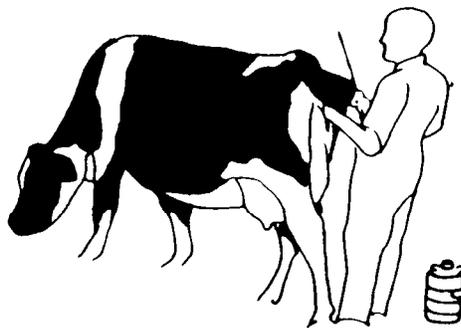
Flushing is accomplished by inserting a catheter with an inflatable balloon into the donor's uterus and washing a limited area with continuous or intermittent flushes of 30-200 ml. of a saline solution or other suitable culture media. Each uterine horn is flushed separately. The embryos are flushed out in the saline solution, which is collected.

After 30 minutes, embryos settle and can be located using a stereoscopic microscope. Small amounts of fluid decanted from the bottom of the collection cylinder are searched for the embryo. When an embryo is found, it is washed and transferred to fluid containing bovine serum.

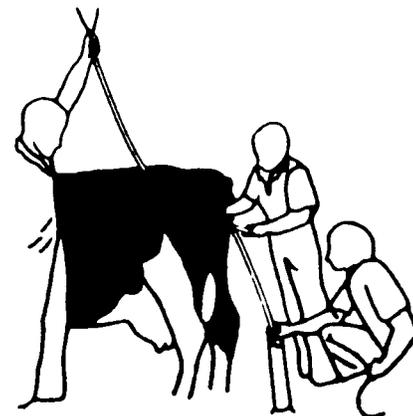
The embryo is evaluated for stage of development and quality. Healthy embryos can be transferred to synchronized recipients (in estrus \pm 1 day from the donor). The embryo may be surgically or non-surgically transferred into the uterus of the recipient.



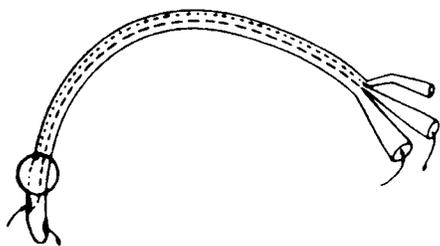
Superovulation of donor with gonadotropins



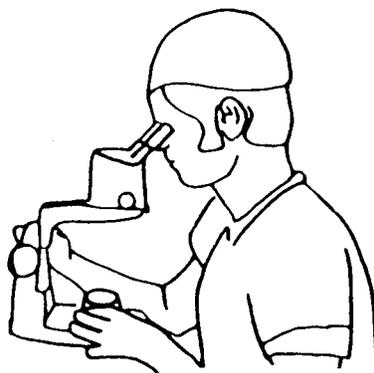
Artificial insemination (5 days after initiating superovulation)



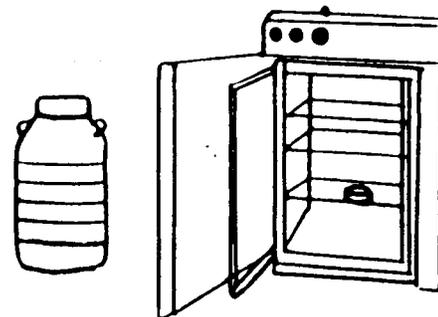
Nonsurgical recovery of embryos (6 to 8 days after artificial insemination)



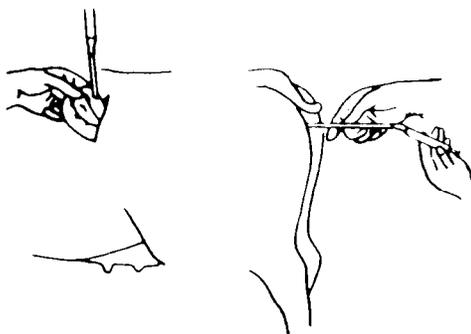
Foley catheter for recovery of embryos



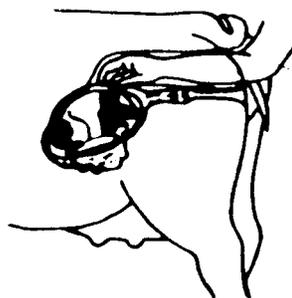
Isolation and classification of embryos



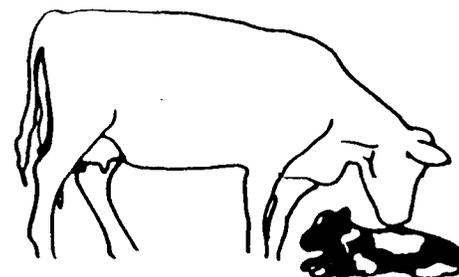
Storage of embryos indefinitely in liquid nitrogen at -196°C or room temperature for 1 day



Transfer of embryos to recipients surgically or nonsurgically



Pregnancy diagnosis by palpation through the rectal wall 1 to 3 months after embryo transfer



Birth (9 months after embryo transfer)

Fig. 1. Synopsis of bovine embryo transfer procedures. From Seidel, G. E., Jr., Superovulation and Embryo Transfer in Cattle, Science, Vol. 211, 23 Jan. 1981, p. 353.

Today, non-surgical transfers are becoming more common as success rates are approaching those obtained with surgery. An artificial insemination Cassou gun is used to deposit the embryo into the uterine horn. Care must be taken to be non-traumatic and to prevent the introduction of micro-organisms. Pregnancy rates following transfer range from 30 to 70%; depending on embryo quality, synchronizations, and method of transfer.

Embryos may be stored up to 24 hours with little trouble. This enables easy transport to other farms and even to other countries. Embryos kept at 37°C will continue to develop. When kept at low temperatures (0 to 10°C) development will halt. If long-term storage is required, embryos must be frozen. Freezing, however, will damage 30 to 50% of the embryos because of ice crystal formation within the embryo during freezing and thawing.

Recent developments with ET include **embryo splitting**. Using a device called a micromanipulator, it is possible to split or bisect the mass inside an early developing embryo.

When half of the cells are removed and put into an evacuated zona (the protecting shell-like coating), both embryos will continue to develop resulting in identical twins. This technique is successful, but equipment costs and the need for mastery of manipulative techniques have limited its usefulness.

Future Developments

Embryo Sexing

Embryos can be sexed by identification of the male and female chromosomes. This procedure is slow and detrimental to embryo survival. Recently, however, progress in embryo sexing has been made using a simple and fast antibody test to react with the male chromosome. The dairy industry eagerly awaits the perfection of this scientific advancement.

In Vitro Fertilization

The fertilization of an egg in a test tube is not commercially available for cattle, but it has been done. The number of potential ova in the ovaries of a heifer exceeds 75,000 when she is born. After maturation any number of these ova could be harvested for in vitro fertilization.

Collectively these procedures would make the impact of ET limitless.

Is Embryo Transfer for You?

Take a realistic approach. Success of ET depends on many factors from management and selection of the donor, recipients and offspring to the collection, handling, preservation and implantation techniques. The decision to use ET finally depends on the profitability and marketability of the offspring. Contact with other purebred breeders, the breed association, your marketing agent, bull studs and your veterinarian will help you determine the genetic value of your cow and the desirability of her proposed offspring. Others in your area who are presently involved with ET may share their experiences and recommend a competent team to facilitate a successful ET program.

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