Embryo transfer (ET)

Embryo transfer (ET) is the process of removing one or more embryos from the reproductive tract of one female to another. Transfers may be conducted by either surgical or nonsurgical procedures and may involve transferring the embryo(s) from either the oviduct or uterus of one female, known as a donor, into the oviduct or uterus of another female, known as a recipient. Embryo transfer also may involve the transfer of laboratory-produced embryo(s), such as those made by *in vitro* or cloning procedures, into the reproductive tract of a recipient.

Advantage of embryo transfer:

1- Genetic improvement
2- Disease control: less transport of live animals, thereby reducing risks of disease transmission.
3- increase in the number of offspring per female : Embryo transfer is one option that can increase a cow's reproductive efficiency, allowing her to have numerous calves per year. While the average cow produces six to seven calves in her lifetime
4- easier and more rapid exchange of genetic material between countries than with live animals.
5- Obtain offspring from old or injured animals incapable of breeding or calving naturally.
6- Increased farm income through embryo sales.

Steps of Embryo transfer:

1- **donor and recipient selection**
2- **estrus synchronization**
3- **Superovulation**
4- **Insemination**
5- **Embryo Recovery**
6- **Embryo Handling and Evaluation**
7- **Storage**
8- **Embryo Transfer**

1- Donor and recipient selection :

Donor : It has been suggested that prospective donor cows in embryo transfer programs must be selected on the following criteria:

1) Regular estrous cycles commencing at a young age
2) A history of no more than two breeding per conception
3) Previous calves with approximately 365-day intervals
4) No parturition difficulties or reproductive irregularities
5) No conformational or detectable genetic defects.

Recipient : Proper recipient herd management is critical to embryo transfer success. Cows that are reproductively sound, that exhibit calving ease and that have good milking and mothering ability are recipient prospects. They must be on a proper plane of nutrition.

2- Estrus synchronization:

Over the years, much evidence has accumulated on the importance of synchrony between donor and recipient in terms of their cycle stage. Exact synchrony should be the aim, but recipients out of phase by ± 1 day are generally regarded as acceptable, although some reduction in pregnancy rate is to be expected; cattle that are out of synchrony by as much as 2 days would not normally be used because of the reduced pregnancy rates. Some workers have looked at ways of making synchrony as exact as possible.

3- Superovulation

The species covered in this review include almost exclusively monotocous (horses), usually monotocous (cattle), twin-bearing (sheep and goats), polytocous with small or moderate litters (dogs and cats), and polytocous with large litters (pigs). When females of any of these species are used as embryo donors, *superovulation*, or the induction of the maturation and ovulation of more ova than normal, is usually induced with the injection of a gonadotrophin. The gonadotropin most frequently used is follicle stimulating hormone (FSH) purified from porcine or ovine pituitary glands. In some cases, equine chorionic gonadotropin (eCG), formerly known as pregnant mare serum gonadotropin (PMSG), is used, although it is less popular due to its long half-life.

4- Insemination

Donor females are inseminated either artificially (AI) or by natural service, depending on the species and the specific situation. For example, frozen semen is used almost exclusively for trans cervical artificial insemination in cattle. In contrast to cattle, trans cervical AI is very difficult in sheep and goats and donors are inseminated primarily by a laparoscopic approach.

5- Embryo Recovery

A number of different methods are employed for embryo recovery. The use of a catheter inserted trans cervically into the uterus is relatively easy and is routine in cattle and horses. Smaller species require either laparoscopic surgery or full surgical exposure to access the uterus.

Most embryos are collected six to eight days after estrus. (why?)

1- **Nonsurgical methods** for collecting bovine embryos were first developed more than 40 years ago, but because recovery rates were poor,

2- Surgical methods continued to be used for many years. However, even under ideal conditions for surgery, scar tissue sometimes forms in the reproductive tract, causing infertility and even sterility in some cases. In addition, surgical collections must be performed in specialized facilities with expensive equipment and supplies.

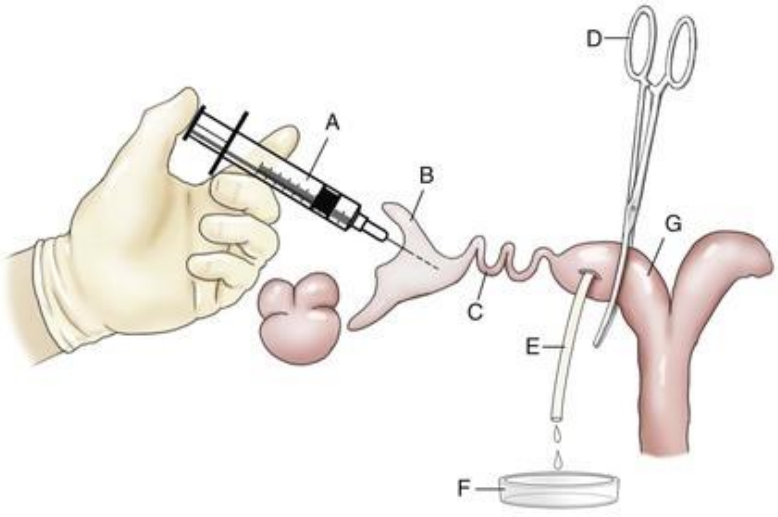
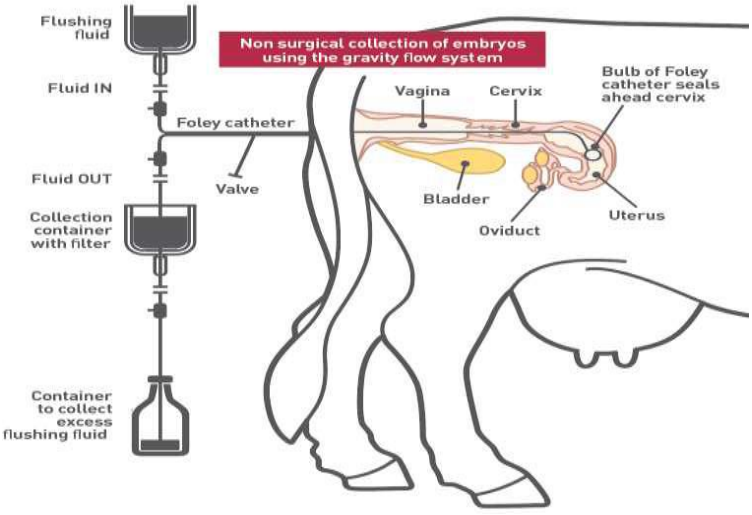


FIGURE 28-1 Illustration of the Surgical Embryo Collection Technique.**A,** Syringe with blunt needle. **B,** Infundibulum. **C,** Oviduct. **D,** Curved forceps. **E,** Pipette inserted in the uterine horn. **F,** Glass dish. **G,** Uterine horn.

In the mid-1970s much effort was put into improving the nonsurgical methods to avoid damaging valuable donors. Currently, virtually all embryos are collected by the nonsurgical method, which is often referred to as “flushing” embryos.

Steps of embryo flushing:

a- In preparation for non-surgically recovering embryos, a local anesthetic is administered by an epidural injection into the tail head.
b- A silicone catheter, temporarily made rigid with a removable metal stylette, is passed through the cervix into the uterus.
c- Some practitioners pass the catheter and inflate the balloon in one uterine horn, and following embryo collection of that side, deflate the balloon and move the catheter to the other horn, repeating the collection process. This is often referred to as **a horn flush**. Other practitioners prefer to inflate the catheter just inside the internal cervical os, thus collecting from both uterine horns and the uterine body simultaneously.
d- Flush fluid is introduced into the donor cow either by gravity flow or by injection with a large syringe.
e- In gravity flow collections, flush fluid exiting the uterus passes through a 70 micron stainless steel or nylon mesh in a plastic filter.
f- A number of isotonic media often based on Dulbecco's PBS, and containing antibiotics can be successfully used for recovering embryos of all the species. Embryos usually remain viable in this fluid for at least 24 hours.



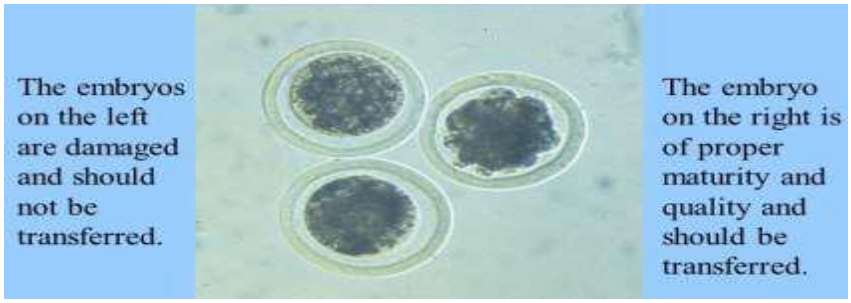
6- Embryo Handling and Evaluation

The mammalian oocyte is the largest cell in the body. Oocyte diameters of all the species covered in this review are similar and in the range of 150 to 180 microns. Aside from the similarity in the size of oocytes at ovulation, however, there also are significant differences in early embryonic development among these species. For example, on day 8 after estrus, the cow embryo normally has reached the expanded

blastocyst stage, with a diameter of approximately 250 microns, and hatches from the zona pellucida shortly thereafter.

The morulae and early blastocysts are typical of the embryos recovered on day 7 post-estrus in cattle. The expanded blastocyst is a typical day-8 embryo. Following the embryo recovery procedure of a donor, embryos are normally located in the flush fluid with the aid of a stereomicroscope at 6× to 10× magnification, transferred to holding medium, and evaluated at 50× magnification.

Careful examination with a stereomicroscope of ova/embryos that are recovered from donors is necessary to ensure that viable embryos are not discarded, or on the other hand, unfertilized ova are not transferred or cryopreserved.



7-Storage :

As part of normal cattle ET operations, there is an obvious need for the short-term storage of embryos recovered from donor cattle until such time as they are either transferred to a waiting recipient or undergo cryopreservation.

Storage at ambient temperature:

The general view is that the viability of the bovine embryo, usually at the blastocyst stage, begins to decline after 12 h storage at normal room temperature (20–30°C) in an appropriate medium.

The choice of media for embryo collection and temporary storage has ranged from complex tissue culture media (e.g. TCM-199) to much simpler formulations such as Dulbecco’s phosphatebuffered medium with bovine serum (D-PBS).

For those operating under farm conditions, the requirement may simply be for PBS containing antibiotics and 2% fetal calf serum (FCS) for embryo collection and PBS with 10–20% FCS for temporary storage prior to transfer.

Cooled storage:

The conservation of equine embryos is regarded as technically more difficult than that of other farm mammal embryos. Equine embryos have been cultured in a variety of systems with variable results.

Cooling and storing embryos at 5°C temperature enables embryos to be shipped to a centralized station for transfer into recipient mares.

Embryos are collected on the farm and transported to recipient stations in a passive cooling device at 5°C either by commercial airline or by overnight carrier, working to a time schedule of 12–30 h between collection and transfer.

Freezing storage:

The freezing of mammalian embryos was first shown to be possible in 1971, when David Whittingham and colleagues in London obtained live mice pups after the transfer of frozen–thawed embryos that had been frozen using either glycerol or dimethyl sulphoxide (DMSO).

It is believed that sperm and embryos are capable of remaining viable at a temperature of -196°C (liquid nitrogen) for perhaps 1000 years or more.

7- Embryo Transfer

Induction of estrus in cows and horses is rather easy with the use of prostaglandin (PG). Controlled intrauterine drugreleasing devices (CIDR) frequently are also being used in cows. Today, cattle embryos are nearly always transferred to recipients nonsurgical. A few embryo transfer practitioners still use surgical procedures in rare situations.

Surgical methods:

Before the mid-1970s, most cattle embryos were **transferred surgically** while the recipients were secured on their backs under general anesthesia in a **surgical** facility. Embryos were transferred through a mid-line incision made between the udder and navel to expose the uterus. While this procedure resulted in excellent pregnancy rates, it was very labor intensive and required special facilities.

A simpler **surgical** approach, often called a flank transfer, was developed that involved administering a local anesthetic, making a flank incision, slightly exteriorizing the uterine horn, and transferring the embryo through a puncture wound in the uterine wall. This approach was used on cattle and horses, and still is used on horses in some cases.

Nonsurgical transfers

In cattle and horses are performed with a special transfer gun, similar to that used for AI. Nonsurgical pregnancy rates in dairy heifers were reported to be similar to those achieved with surgical flank transfers, but pregnancy rates were lower with nonsurgical compared to surgical transfers in diary cows. Most practitioners administer an epidural injection to relax the rectal muscles.

Although it is similar to artificial insemination, nonsurgical transfer of embryos is a more challenging procedure (why ?) because:

First, embryos usually are transferred approximately seven days after estrus. Because the cervix is closed at this stage of the estrous cycle, trans versing it with a transfer gun at this time is much more difficult than performing AI when a cow or heifer is in estrus.

Second, Sanitation is also more important, because penetrating the cervix one week after estrus is more likely to lead to uterine infection.

https://www.youtube.com/watch?v=Roh-goM-czo