Introduction

Embryo transfer is a process by which an embryo is collected from a donor female and transferred into a recipient female where the embryo completes its development.
Through the use of embryo transfer, a genetically superior female produces more offspring than she could by natural reproduction, thus maximizing her genetic abilities.
Embryo transfer is profitable for producers of registered purebred animals and is used in several species of domestic animals, especially cattle, horses, goats, and sheep. It has also been used in non-domestic species, such as deer, elk, bison, and wildcats.
The first embryo transfer was performed in 1890 at the University of Cambridge using rabbits.

It was not until 1951 that the technique was successfully used in cattle and has since become a more popular procedure for other animal species.
As long as embryo transfer remains a key step in many of the developing technologies, such as prenatal sex selection, the commercial embryo transfer industry will continue to grow.
PROCEDURE FOR EMBRYO TRANSFER

Superovulation of donor with gonadotrophins

Artificial insemination (5 days after initiating superovulation)

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

Foley catheter for recovery of embryos

Isolation and classification of embryos

Storage of embryos indefinitely in liquid nitrogen or at 37°C or room temperature for 1 day

Transfer of embryos to recipients surgically or nonsurgically

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

Birth (9 months after embryo transfer)
Donor, Superovulation, and Insemination

The producer should consider the following factors when developing an embryo transfer program:

• Selection of donor females,
• Superovulation, and
• Insemination.
Donor Female Selection

The donor female selected for embryo transfer should have outstanding genetics.

She should also be healthy and reproductively sound.
Superovulation

Superovulation refers to the release of many oocytes (eggs) during a single estrus period.

Normally, only one follicle ruptures in cows and mares releasing one ovum and two follicles rupture in ewes releasing two ovum.
Superovulation can be achieved through treatment with gonadotropins.

Most embryo transfer donors are treated with follicle stimulating hormone (FSH) to induce the maturation and ovulation of a larger than normal number of oocytes.
For example, a single treatment of a cow results in 0 to 20 more embryos, with an average of seven normal embryos.
When attempts are made to recover a single ovum from a non-superovulated cow at every estrus, the 12-month yield averages 5 calves.

In contrast, when a donor is superovulated three times, the yield ranges from 9 to 12 calves.
Following embryo transfer, superovulated ova result in normal offspring with success rates similar to those achieved with normally ovulated ova.
Adequate procedures exist for superovulation of laboratory and livestock species, except for horses.

A mare does not respond satisfactorily to superovulatory treatment, thus maximization of reproduction in mares must be achieved by collecting embryos at each estrus.
Insemination

Because superovulation of the donor female causes the release of a large number of eggs over a 24-hour period, many producers choose to inseminate the donor several times to achieve optimal fertilization.
Embryo Recovery

Success of embryo recovery depends on the age of the embryos, as well as, the technical procedure used and the skill level of the technician.
With superovulated donors, many follicles never ovulate; others may develop as if they had ovulated, but they never release oocytes.
Furthermore, if the ovaries enlarge greatly in response to superovulatory treatment or if many ovulations occur, the fimbriae apparently do not gather all of the oocytes into the oviducts.
Radically altered endocrine levels may occur as a result of superovulatory treatment.

This causes the uterus to become an unfavorable environment for embryonic development.

The incidence of degenerate embryos may increase during days 7 and 8 after estrus.
It is difficult to count the number of ovulations in superovulated donors, even with surgical examination.

With non-surgical recovery methods, the number of ovulations can only be estimated by palpation.
Non-Surgical Recovery

Advances in technology have made embryo recovery a non-surgical procedure with cattle and horses.

A specially designed catheter (Foley catheter) is used when collecting embryos using non-surgical methods.
Steps in non-surgical procedure for embryo recovery:

1. Clean the donor’s genital area.

2. Inject a local anesthetic in the rump or hip.

3. After anesthetic takes effect, insert the Foley catheter into the vagina and through the cervix.
4. Flush out the embryos by forcing fluid from the catheter into the uterus and collecting it in a special container, which filters out the embryos.
Surgical Recovery

Unfertilized oocytes for specialized applications, such as in-vitro fertilization, must be collected near the time of ovulation from the follicles, surface of the ovary, or oviduct.
For most applications, embryos are collected anytime between fertilization and implantation, but usually after they migrate to the uterus.
In cattle and horses, embryos for commercial purposes are usually recovered 6 to 9 days after estrus.

Before this time, non-surgical recovery is ineffective.

After 9 days, recovery and pregnancy rates are slightly reduced, at least with surgical transfer of bovine embryos.
Surgical recovery can be done in all species and is the method of choice for sheep, goats, and hogs.

Techniques vary slightly with the species.
Embryo Evaluation

After collection, embryos are evaluated for quality using a stereoscopic microscope.

Embryos are graded on a scale from one (excellent) to four (poor).
Factors considered during the evaluation include:

- Shape,
- Color,
- Texture, and
- Size.
Embryos are also classified based on their stages of development.

Normal embryos will have between 2 and 64 cells.
Embryo Storage

Donor embryos can be transferred immediately into recipients or they can be stored for future use.
Procedures such as embryo transfer, in-vitro fertilization, sex determination, and cloning depend on maintaining the viability of embryos for hours to days outside of the reproductive tract.
For many applications, the storage system must not only maintain the viability of the embryo, it must also support continued development.
In some cases, it may be desirable to retard the growth of the embryo to a degree approaching suspended animation, so that development can be synchronized with later events.

For example, it may be necessary to store embryos until suitable recipients become available for transfer.
**Short-Term Storage**

Embryos can be stored at room temperature for one day for direct transfer from the donor to the recipients.
Embryos must be stored at 5°C in an appropriate medium, if kept 24 to 48 hours.

Most media and culture systems are adequate for maintaining the viability of the embryo between donor and recipient.
Long-Term Storage

If embryos are to be transported great distances or suitable recipients are not immediately available, a long-term storage system is essential.

Deep-freezing means that embryos are stored in liquid nitrogen for a long period of time.
Long-term storage through freezing usually results in damage of 30% to 50% of the stored embryos.

Damage is usually caused by ice crystal formation within the embryonic cell.
Although the average survival rate of frozen-thawed embryos is approximately 65%, it is profitable to maintain embryos in long-term storage.
Transfer of Embryos into Recipient Cows

Recipients to be implanted with the collected embryos should be healthy and reproductively sound.
To maximize success rate of the transfer, the recipient’s estrus should be in sync with that of the donor.

Estrus synchronization is important to establish a uniform uterine environment for the embryo; therefore, only one day or less difference in estrus between donor and recipient is acceptable.
Research indicates that there is little difference in pregnancy rates between natural estrus and induced estrus recipients.

Induced estrus for synchronization is accomplished with treatments of progestogens, which are synthetic progesterone products.
Non-Surgical Transfer of Embryos

For cattle and horses, technicians mainly use non-surgical techniques to recover and transplant embryos.
Flushed embryos that pass inspection are loaded into an AI straw.

An insemination rod is passed through the recipient’s cervix and into her uterus (similar to AI procedure).
The embryo is expelled into the uterine horn that is on the same side as the ovulated ovary (to optimize the embryo implantation rate).
Surgical Transfer of Embryos

Embryos in early stages of development must be deposited in the oviducts.

This procedure must be performed surgically.

Surgery is also required for uterine transfers in sheep, goats, and hogs.
Success of surgical transfer depends on interactions of a number of factors, including:

- Age and quality of embryos,
- Site of transfer,
- Degree of estrus synchronization between donor and recipients,
• Number of embryos transferred,
• In-vitro culture conditions,
• Skill of personnel, and
• Management techniques.
Conception rates are up to 5% greater with surgical transfer.

Surgical transfer of normal embryos compares to the first-service pregnancy rates using artificial insemination.
The reproductive tract can be exposed for surgical transfer of embryos.

Transfer occurs through a midline laparotomy (incision through the abdominal wall) with general anesthesia or through a flank incision with local anesthesia.
The midline approach is less traumatic to the reproductive tract and permits better exposure in some animals. The flank approach, on the other hand, is quicker, less expensive, and reduces risk by using minimum equipment.
Embryo Survival

The survival rate of the embryo, from implantation to term in the recipient female, ranges from 55% to 70%.

Embryo quality is an important determinant of transfer success.
The most accurate predictor of success is the stage of embryonic development.

Recipient females are palpated within one to three months after embryo transfer to diagnose stage of pregnancy.
New Technologies

Embryo transfer is a composite technology that requires expertise in many areas.
Present and emerging technologies that build upon current embryo transfer procedures include predetermination of sex, sex selection, in-vitro culture, production of identical twins, and cloning.
In addition, embryo transfer techniques proliferate a number of new industries, such as research and growth in gonadotropin purification, manufacturing of micromanipulators and other specialized equipment, international marketing of embryos, and specialized investment counseling.
Summary

Embryo transfer is a technology that can be used to maximize the production from superior females.

Embryo transfer requires skill in the management of reproductive cycles and skill in the performance of the procedure.