

Review Article

Implication of Embryo Transfer Technology in Livestock Productivity

Dhaval Kumar Patel, Nilufar Haque*, Gaurang Kumar Patel, Ashvinkumar Chaudhari,
Mahesh Madhavatar, Nikita Bhalakiya, Natvarbhai Jamnesha and Pankaj Patel

Sardarkrushinagar Dantiwada Agricultural University, SKNagar, Gujarat, India

*Corresponding author

ABSTRACT

In animal husbandry, embryo transfer has become the most powerful tool for animal scientists and breeders to improve genetic construction of their animal herds and increase quickly elite animals. It is a technique by which embryos are collected from a donor female and are transferred to recipient females. The applications of this method are numerous in the field of research, but its main economic interest is in bovine selection programs. The embryo transfer technique can also be used for detecting recessive traits, the production of twins in beef cows, salvage of genetic material from terminally ill cows or infected herds. The technology of embryo transfer has been benefited from the establishment of newer, more efficient procedures for the superovulation of donors, embryo retrieval and transfer through low-invasive methods. The use of embryo transfer has still some limitation i.e. high cost of embryo production, poor freezability, abnormal fetuses and calves with altered sex ratios. It is recommended to have enough process for production, selection of donor and recipients, and transfer procedures of embryos as well as in recipient management will be needed before embryo transfer can be an effective and economical method to improve fertility in herds with reasonable fertility.

Keywords

Ashwagandha,
Alternaria alternata,
management,
fungicides, organic
manure,
biofertilizer

Introduction

Embryo transfer is a process by which an embryo is collected from a donor female and then transferred into a recipient female where the embryo completes its development. Embryo transfer is profitable for producers of registered purebred animals. Through the use of embryo transfer, a genetically superior female produces more offspring than she could by natural reproduction. The increased number of offspring thus maximizes the donor female's genetic. Embryo transfer is used in several species of domestic animals, namely cows, horses, goats, and sheep. Research indicates its use in certain non-domestic species, such as deer, elk, bison, and wildcats. In the current period ET beyond

doubt is being used in many developed and developing countries confined to certain research stations with success, however, still needs precautions in handling of embryos and evaluation of genetics progenies. Though this technique aims to improve the genetic progress of the herds and utilize the merit of the superior animals, nevertheless shadowed by the well documented adverse effects on the survival and wellbeing of the offspring.

History of embryo transfer

Embryo transfer in cattle has recently gained considerable popularity with seed stock dairy and beef producers. Most of the

applicable embryo transfer technology was developed in the 1970s and 1980s; however, the history of the concept goes back much farther. Embryo transfer was first performed and recorded by Walter Heape in 1890. He transferred two Angora rabbit embryos into a gestating Belgian doe. She went on to produce a mixed litter of Belgian and Angora bunnies. Embryo transfer in food animals began in the 1930s with sheep and goats, but it was not until the 1950s that successful embryo transfers were reported in

cattle and pigs by Jim Rowson at Cambridge, England.

The first commercial embryo transfers in this country were done in the early 1970s. Initially, embryos were recovered from valuable donors and transferred to recipient animals using surgical procedures. It was not until non-surgical methods were developed in the late 1970s that embryo transfer grew in popularity. (<http://osufacts.okstate.edu>).

Events	Species	Scientist	Year
First successful ET	Rabbit	Walter Heape	1890
First successful ET	Rat	JS Nicholas	1933
First successful SOV	Cattle	Casida	1940
First successful ET	Sheep and Goat	BL Warmick & RO Berry	1949
First successful ET	Pig	AV Kvensnickii	1951
First ET reported in cattle		Umbaugh	1949
First successful ET	Cattle	EL willett	1951
Baby girl born through ET		Steptoe & Edwards	1979
Calf -Frozen thawed Embryo	Cattle	Wilmot & LEA Rowson	1973
Calf born by ET	Buffalo	Drost	1983
Calf born by IVF	Buffalo	Madan et al	1990
Calves through surgical and non-surgical ET in Asia/India	Buffalo	Misra et al	1988
Calf born by frozen thawed embryo		Misra <i>et al.</i> ,	1991

Work done in India

India is the fastest growing country with changing trends in economy and as per

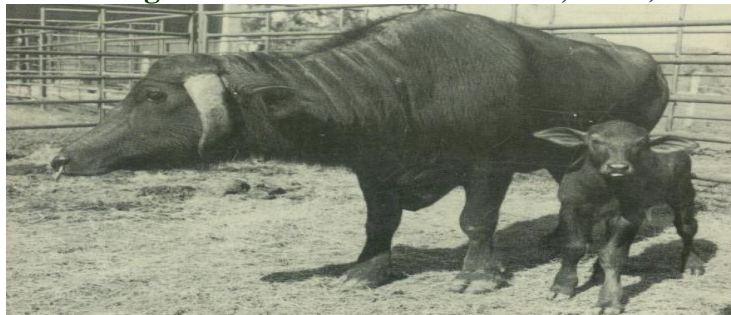
information from different sources more than 60 percent people are earning directly or indirectly from agricultural and animal husbandry. The contribution of agricultural

and animal husbandry in Indian national GDP is equal (Patel, 2011). In India, initial isolated attempts of ET in dairy animals were made at the National Dairy Research Institute (NDRI), Karnal, Central Institute for Research on Buffalo (CIRB), Hisar, Indian Veterinary Research Institute (IVRI), Bareilly, and State Agricultural Universities. Simultaneously, the National Dairy Development Board (NDDB), Anand, initiated a pilot project in 1986 to investigate the utility of the ET technology for buffalo improvement and its encouraging results lead to the launching in 1987 of llational science and technology project on Cattle Herd Improvement for Increased Productivity Using Embryo Transfer Technology, implemented by the Department of Biotechnology (DBT), Ministry of Science and Technology. The NDDB was designated as the lead implementing agency of the project and NDRI, Karnal, National Institute of Immunology (NII), New Delhi, CIRB, and IVRI were the collaborating agencies. The NDDB established its main ET Laboratory at Sabarmati Ashram Gaushala (SAG), near Ahmadabad and 4 regional laboratories at Animal Breeding Centre, Salon (UP), Shree Nasik Panchwati Panjarapole, Nasik (Maharashtra), Buffalo Breeding Centre, Nekarikallu (Andhra Pradesh) and Central Frozen Sernen Production Station and Training Institute, Hesserghatta(Karnataka). The NDDB also established 14 State ET

centers at Bangalore, Bhubaneswar, Ernakulam, Erode, Jaipur, Caira, Kolhapur, Lalkuan, Ludhiana, Mehsana, Ongole, Pondicherry, Trivandrum and Ujjain. In the project, NDDB was assigned the role of creation of infrastructure of labs, skilled manpower and the pool of superior germplasm in terms of live calves and frozen embryos whereas collaborating agencies were primarily entrusted with the research in the areas related to folliculogenesis, superovulation, *in vivo* fertilization, etnbryo sexing, embryo cloning etc. Subsequently, between 1992- 96, DBT supported the NDDB project on Embryo Transfer- Downstream Activities and Embryo Transfer-Upstream Activities of NDR1, NII and IVRI. DBT also supported the NDDB project on "Open Nucleus Breeding System" in Cattle Using Multiple Ovulation and Embryo Transfer (Misra *et al.*, 2005).

In India, due to ban on cow slaughter, almost all the embryos are produced either *in vivo* following superovulation and non-surgical embryo collection or *in vitro* following ultrasound guided trans-vaginal aspiration of oocytes (ovum pick-up) and subsequent *in vitro*, maturation (IVM), fertilization (IVF) and culture (IVC). In buffalo, however, most of the embryos are produced *in vitro* from the abattoir derived oocytes.

(First calf using vitrified embryo Live animal (OPU/IVF)- Neglia *et al.*, 2004 Ovaries of slaughtered buffaloes- Duran *et al.*, 2004)



Applications of Embryo Transfer

1. Genetic Improvement

Embryo transfer is now commonly used to produce AI sires from proven donor cows and bulls in AI service. Although economics would not at this time support the use of embryo transfer techniques for anything but seed stock production, the commercial cattle industry can benefit by the use of bulls produced through well-designed Multiple Ovulation embryo Transfer (MOET) programs (Lohuis,1995).

2. Planned Mating

By far the most common use of embryo transfer in animal production programs is the proliferation of so-called desirable phenotypes. Embryo transfer provides the opportunity to disseminate the genetics of proven, elite females. Embryo transfer also permits the development of herds of genetically valuable females, most of which may be sibs if not full-sibs. Embryo transfer has also been used to rapidly expand a limited gene pool. As AI has led to the very valuable bull, embryo transfer has resulted in the very valuable female (Betteridge, 1981).

3. Genetic Testing

With this approach, it is possible to genetically test a bull in 3.5 years as opposed to 5.5 years using traditional progeny testing. Although accuracy may have been sacrificed, the shorter generation intervals can result in greater overall genetic gains.

4. Disease Control

Risk of infectious disease transmission is less by in vivo-produced embryos, providing

embryo handling procedures were done correctly (Stringfellow *et al.*, 2004). Several large studies have now shown that the zona-intact, washed, bovine embryo will not transmit infectious diseases. Consequently, it has been suggested that embryo transfer may be used to salvage genetics in the face of a disease outbreak.

Criteria for donor selection

There are two broad criteria for selecting donors for most embryo transfer programs: (1) genetic superiority and (2) likelihood of producing large numbers of usable embryos. In the majority of embryo transfer programs, in both developed and less-developed countries, superiority is determined in practice by market forces. In some cases, the sole criterion for selection is scarcity, and embryo transfer is used to increase numbers of animals available. This may be required to determine whether a new type of animal fits the environment or to get enough animals to develop appropriate management systems. If the objective is to conserve germplasm of indigenous breeds by cryopreservation of embryos, one may wish to select a random sample of donors (and sires) or insure that a range of phenotypes within the breed is used.

Selection of Sires

Since half of the genes come from the male, it is extremely important to use genetically superior bulls.

In fact, selecting the male is usually more important than selecting the donor female because males will normally be bred to many females and can be selected more accurately than females. Likewise, it is necessary to select fertile bulls and fertile semen.

General procedural steps

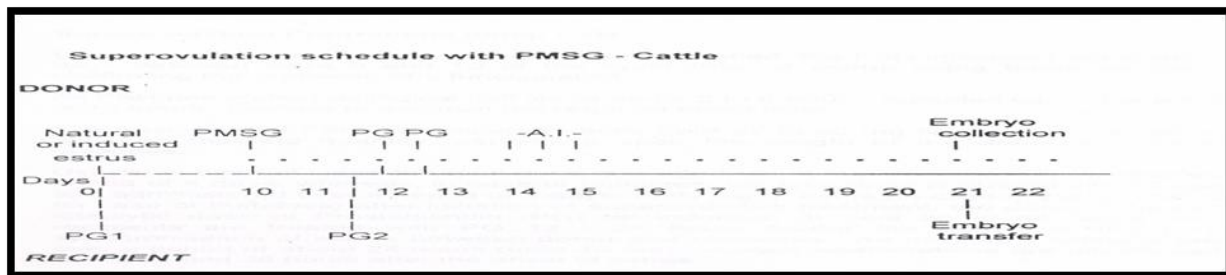
The donor may be inseminated naturally or artificially and embryos will be collected non-surgically six to eight days after breeding. Following collection, embryos must be identified, evaluated and maintained in a suitable medium prior to transfer. At this point, they may also be subjected to manipulations, such as splitting and sexing, if necessary and may be cooled or frozen for longer periods of storage. Hence, the procedural steps must begin with the discussion of donor superovulation, insemination and then embryo transfer.

1. Superovulation

“Superovulation” refers to the release of many oocytes (eggs) during a single estrus period (Mapletoft *et al.*, 2002). The objective of superovulation treatments is to obtain the maximum number of fertilized and transferable embryos with a high probability of producing pregnancies (Nogueira *et al.*, 2002). Superovulation is a very inefficient method of obtaining oocytes from ovaries and is likely to be replaced by

other approaches within the next decade. However, superovulation results in about ten times more embryos than single ovum recovery. Without superovulation, a usable embryo can be recovered about 60 percent of the time from normal donors by skilled technicians. There are various important factors associated with the success of this technique. These factors may include nutritional status, reproductive history, age, season, breed, ovarian status at the time of treatment and the effects of repeated superovulation.

The two generally accepted methods of superovulating cattle are based on two different gonadotrophins, although there are many minor variations of these methods. The simplest is to give an intramuscular injection of 1800-3 000IU (usually 2000-2 500IU) of pregnant mare's serum gonadotrophin (PMSG), more correctly designated equine chorionic gonadotrophin (eCG), followed by a luteolytic dose of prostaglandin F2 alpha or an analogue intramuscular injection two to three days later (Pursley *et al.*, 1995).

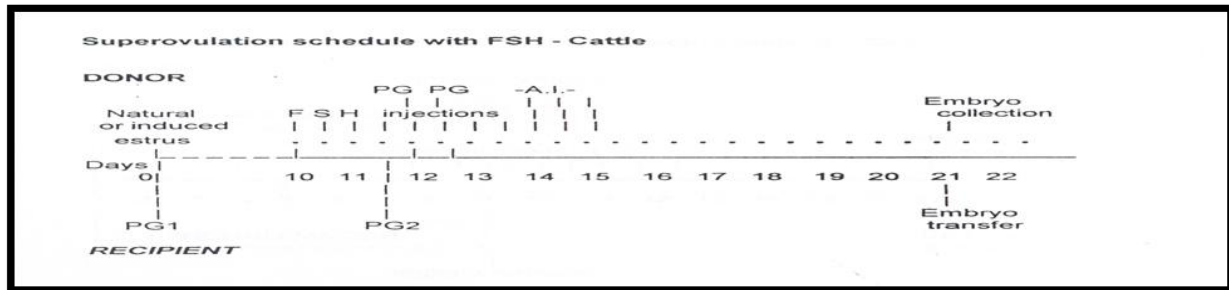


The second method of superovulation is to give eight to ten injections of follicle stimulating hormone (FSH) subcutaneously or intramuscularly. At half-day intervals, intramuscular injection is more reliable under field conditions. As with PMSG, prostaglandin F2 alpha is given 48-72 hours after initiation of treatment with the fifth, sixth, or seventh FSH injection. The most

common FSH regimen is 6,6,4,4,2,2,2 and 2 mg at half-day intervals with prostaglandin F2 alpha given with the sixth or seventh FSH injection. About 20 percent more gonadotrophin should be given to cows weighing over 800 kg. Sometimes, higher doses are used for the first two days; others give 5 mg for each injection. There are few studies with adequate numbers of donors per

treatment group in which constant and decreasing doses have been compared, so reliable conclusions cannot be drawn

regarding efficacy of such regimens (Baruselli, 2006).



2. Insemination

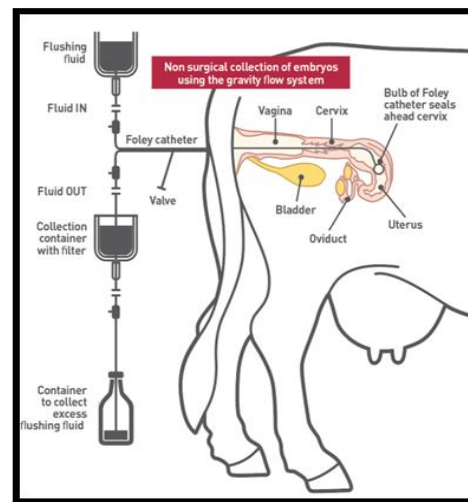
Because of the release of many ova from multiple follicles, there is a greater need for viable sperm cells to reach the oviducts of the superovulated females. Therefore, many embryo transfer technicians will choose to inseminate the cow several times during and after estrus. One scheme is to inseminate the superovulated cow at 12, 24 and 36 hours after the onset of standing estrus. Using high quality semen with a high percentage of normal, motile cells is a very critical step in any embryo transfer program. The correct site for semen placement is in the body of the uterus. This is a small target (1/2 to 1 inch) just in front of the cervix.

3. Recovery of embryos

i. Non-surgical recovery of embryos

The first step in non-surgical recovery is to palpate the ovaries per rectum to estimate the number of corpora lutea. Ultrasonography provides more accurate information about responses than palpation (Kunkel, 2002). Epidural anaesthesia is recommended for non-surgical recovery procedures. The tailhead should be clipped, then scrubbed with iodine soap and swabbed with 70 percent alcohol to prevent infection of the spinal column. It is recommended to inject 5 ml of a sterile 2 percent solution of

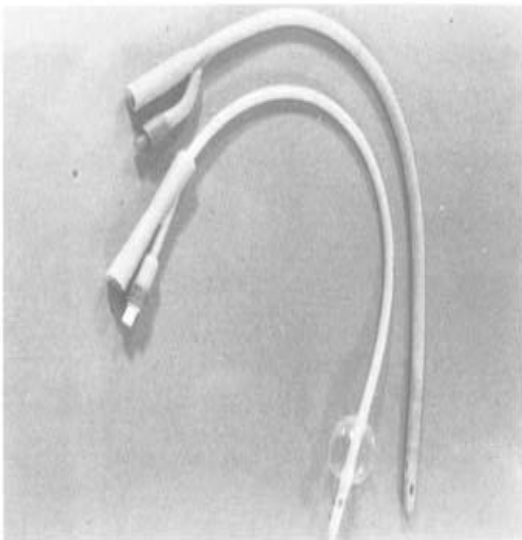
procaine in water using a new 18-gauge needle each time. Recovery procedures are carried out by manipulation per rectum. To collect the embryos non surgically, a small synthetic rubber catheter (Foley catheter) is inserted through the cervix of the donor cow, and a special medium is flushed into and out of the uterus to collect the embryos seven days after estrus (Hasler, 2004).



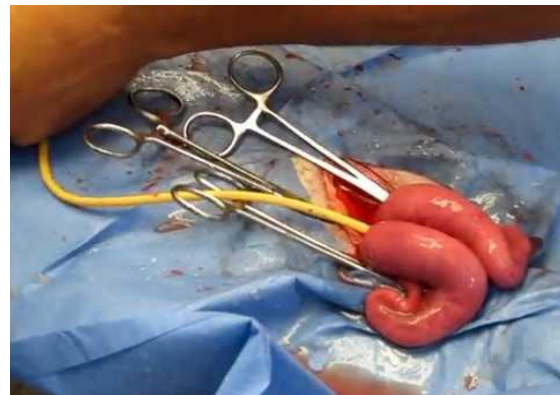
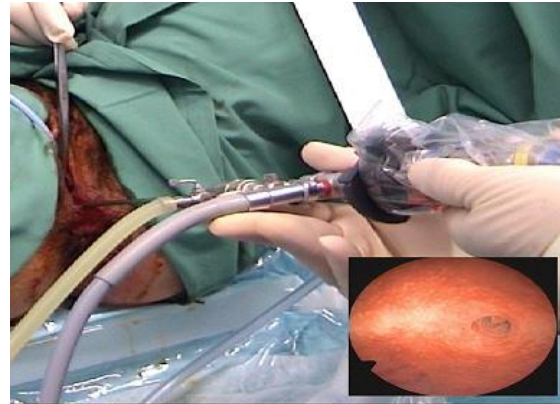
Two-way Foley catheters with and without balloon inflated

Non-Surgical recovery of embryos is relatively simple and can be completed in less than an hour without harm to the cow. Apresterilized stylet is placed in the lumen of the catheter to offer rigidity for passage through the cervix into the body of the

uterus. When the tip of the catheter is in the body of the uterus, the cuff is slowly filled with approximately 2 ml of normal saline. The catheter is then gently pulled so that the cuff is seated into the internal orifice of the cervix. Additional saline is then added to the cuff to completely seal the internal orifice of the cervix. A Y connector with inflow and outflow tubes is attached to the catheter. A pair of forceps is attached to each tube to regulate the flow of flushing fluid. The fluid is sequentially added and removed by gravity. The fluid in the uterus is agitated rectally, especially in the upper one third of the uterine horn. The uterus is finally filled with medium to about the size of a 40day pregnancy. One liter of fluid is used per donor. Each uterine horn is filled and emptied five to ten times with 30 to 200 ml of fluid each time, according to size of the uterus. The embryos are flushed out with this fluid and collected in a filter with the fluid. The pores in the filter are smaller than the embryos, so excess fluid drains out of the filter without losing the embryos. Embryos are separated from the flush media and examined under a microscope to determine their quality and stage of development (George *et al.*, 2008).



ii. **Surgical recovery of embryos**

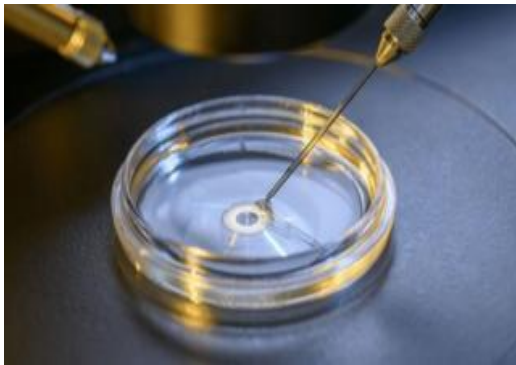


The first successful cattle ET studies obtained from the embryos by a surgical procedure. The donor, which had fasted and been tranquilized, was anaesthetized by intravenous knockdown injection with a halothane/oxygen mixture. This method of recovery is done by performing a Laparotomy (flank or midline abdominal incision) to expose the reproductive tract. A clamp or the thumb and forefinger can be used to block the distal one-third of the uterine horn, so that fluid injected into that segment can be forced through the oviduct with a gentle milking action and collected at the infundibulum. An alternate procedure is to occlude the uterine horn at the body of the uterus. Culture medium is introduced through a puncture at the uterotubal junction or through the oviduct until the uterus is

turgid. The uterus is then punctured with a blunt needle attached to a flexible catheter. The pressure will cause the medium to gush through the catheter, with enough turbulence to carry the embryos into a collection tube. These procedures allow for the recovery of a high percentage of embryos. However, because of the surgical trauma and resulting adhesions they can be repeated only a few times (Peregrino, 2000). 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 (Betteridge, 2003).

4. Embryo handling, evaluation and storage

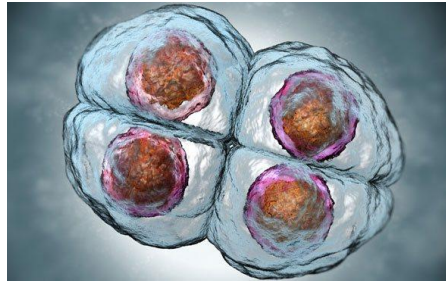
i. Embryo Handling



Careful handling of embryos between collection and transfer is necessary to prevent the transmission of pathogens (Peregrino, 2000). Embryos are located under 10 X magnification with a stereoscopic dissecting microscope after filtering the collection medium through a filter with pores that are approximately 50-70 μm in diameter. Embryos are normally held in the same or a similar medium to that in which they were collected. Media must be buffered to maintain a pH of 7.2 to 7.6 and have an osmolarity around 300 molar osmolarity. Dulbecco's PBS or more complex media with the Hepes buffer and enriched with FCS and antibiotics are normally used in the field. More complex

media with a carbonate buffer generally yield superior results for long term culture of bovine embryos. As indicated earlier, embryo collection holding and freezing media that are free of animal products have recently become available, avoiding the need for refrigeration and increasing biosecurity (IVIS, 2002).

ii. Embryo Evaluation



Embryos are classified and evaluated by morphological examination at 50 to 100 X magnification. The overall diameter of the bovine embryo is 150 to 190 μm , including a zona pellucida thickness of 12 to 15 μm . the quality of an embryo is evaluated and graded as excellent, good, fair and poor depending on several factors discussed below (Dochi *et al.*, 1998):

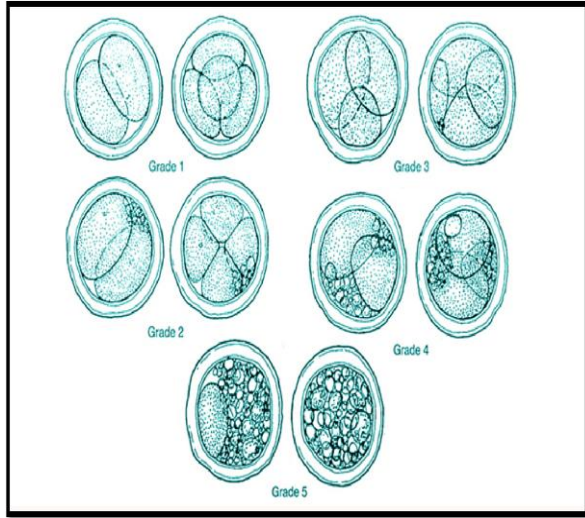
Quality evaluation

Excellent: An ideal embryo, spherical, symmetrical and with cells of uniform size, color and texture.

Good: Small imperfections such as a few extruded blastomeres, irregular shape and a few vesicles.

Fair: Problems that are more definite are seen, including presence of extruded blastomeres, vesiculation, and a few degenerated cells

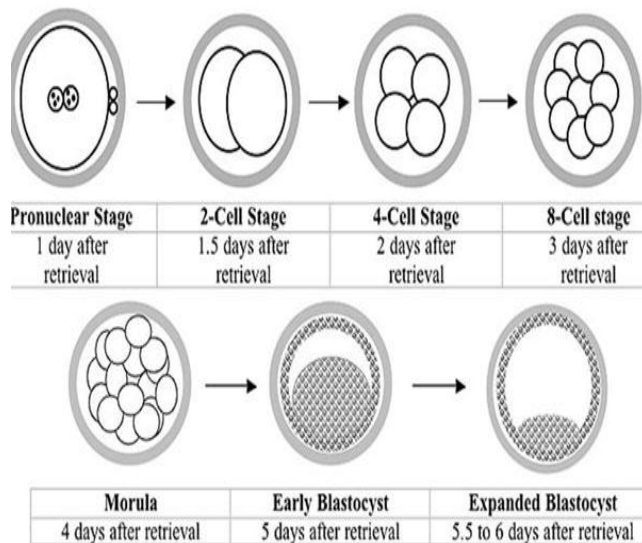
Poor: Severe problems, numerous extruded blastomeres, degenerated cells, cells of varying sizes, large and numerous vesicles but an apparently viable embryo mass. These are generally not of transferable quality (Mapletoft *et al.*, 2002).



Embryo Developmental Stage Evaluation

Embryos also are evaluated for their stage of development without regard to quality. It is advisable to select the stage of embryo development for the synchrony of the recipient (Stroud, 2012):

Embryo Development Prior to Transcervical Embryo Transfer (IVF-ET)



iii. Embryo storage

Donor embryos can be transferred immediately into recipients, or they can be stored for future use, hence it may be

necessary to store embryos until suitable recipients become available for transfer (Sauve, 2002).

a. Short-term storage

Embryos can be stored at room temperature for one day for direct transfer from the donor to the recipients. For periods of 24 to 72 hours, the embryos must be stored at 4°C in PBS, medium 199, or medium L15, each supplemented with 50% FBS. Most media and culture systems are adequate for maintaining the viability of the embryo between donor and recipient (Atsushi *et al.*, 2013).

b. 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 embryos is storage in liquid nitrogen (-196°C) for an indefinite 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 (Atsushi *et al.*, 2013).

5. Selection of recipient, transfer of embryo and post transfer management

i. Selection of recipient females

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 (body condition score 6 for beef cows and dairy body condition score 3 to 4). These cows also must be on a sound herd health program.

ii. Synchronization of the recipients

To maximize embryo survival in the recipient female following transfer, conditions in the recipient reproductive tract should closely resemble those in the donor. This requires synchronization of the estrous cycles between the donor and the recipients, optimally within one day of each other. Synchronization of the recipients can be done in a similar manner and at the same working time as the donor cows (Galina and Orihuela, 2007).

6. Transfer of bovine embryo

i. Surgical Transfer



Embryos can be transferred via mid-line abdominal incision to cows under general anaesthesia, but through flank incision is far more practical. About 60 ml of 2 percent procaine is given along the line of the planned incision. A skin incision about 15 cm long, high on the flank is made just anterior to the hip. The ovary, is located usually about 25 cm posterior to the incision, and CL is visualized or palpated. The uterine horn is exteriorized by grasping. A puncture wound is made with a blunted needle through the wall of the cranial one-third of the exposed uterine horn, because it is very fragile. Using about 0.1 ml of medium in a small glass pipette (<1.5 mm outside diameter), embryo is drawn up from the storage container. The pipette is then inserted into the lumen of the uterus, and the embryo is expelled (Marahall and Minyard, 2002).

ii. Non-Surgical Transfer



First, it is necessary to palpate ovaries accurately in order to select the side of ovulation. The next step is to pass the embryo transfer device through the cervix. The petridish containing the embryo is placed under the stereoscope to load in a

0.25 ml transfer straw. The straw is then loaded into a transfer gun and covered with a transfer sheath. The third step with non-surgical transfer is to be able to insert the tip of the instrument into the desired uterine horn quickly, smoothly and atraumatically. Nonsurgical transfer is preferable, because it is less expensive, it is quicker and does not involve surgical procedures. The most commonly used instrument for non-surgical transfer is the standard Cassou Minseminating gun for French straws, because it is inexpensive and easy to use correctly.

Factors Affecting the Success of Embryo Transfer in Cattle

Many factors may influence the embryo transfer technology that may be beyond the control of practitioners.

1. Long term weather problems or storms during the superovulation/recipient synchronization process are beyond the control of anyone and can wreak havoc with ET success (Armstrong and Evans, 1983).
2. Travel problems sometimes means traveling to a farm a day or two late, which mandates working with older embryos than planned. Probably the single most important variable affecting success in ET is the level of donor and recipient management (Schneider *et al.*, 1980).
3. The risk of transmitting *genetic disease* via embryo transfer is the same as that involved in natural mating or artificial insemination; wise selection of dams and sires is mandatory, (King *et al.*, 1985).

Advantages of embryo transfer

1. Increased reproductive potential: This should be considered in relation to the female animal. The sperm that can be

obtained from a genetically superior bull and be utilized widely through artificial insemination.

2. Naturally, the genetically superior cow can produce approximately 10 calves in its lifespan but through adoption of ET, there is a notable increase in the number of offspring that a genetically superior cow can produce through surrogates.

3. Relatively faster genetic improvement: There is a noticeable positive upward trend based on the different techniques adopted to improve a herd's genetic base. For example, when using natural breeding, it could take up to 20 years while when using AI, the time span is reduced almost by half. The use of ET reduces this time further to approximately four to five years.

4. Outsmarts natural catastrophes: At times we may have a very good and genetically superior cow that has suffered disease bouts, injuries or has grown relatively old and become moribund. This animal is often considered infertile but through embryo transfer technology, the animal can be super ovulated to harvest its ova and fertilise them in-vitro.

5. Environmental advantage: In ET, passive immunity is passed on by the native dam, giving the offspring a better chance of survival despite the embryo being 100 per cent genetically different from the surrogate mother.

6. Financial benefits: Once the desired genetic impact is realised, the production of milk and beef will improve culminating to increased incomes. In well-established enterprises, one can also undertake selling of the embryos as additional income.

7. Embryos can also be stored indefinitely through freezing, thus making the highly superior genetic material available for future use. This technology is also considered cheaper than exporting/importing live animals.

Disadvantages of Embryo Transfer

1. Technical specificity: The technology requires trained technical personnel and a high level of management. It needs technical knowledge especially in relation to flushing the embryos and observation of oestrus in the recipient.
2. High cost: The high cost can be attributed to the necessary equipment that must be put in place and the maintenance of recipient animals, among other reasons. However, governments can establish these stations where the service can be accessed when necessary.
3. Reduced genetic variability: The relatively small gene pool sourced from the donor cows may have a negative impact in future especially in reference to the rare breeds.
4. Low success rate: The success rate of embryo transfer is reportedly lower than use of artificial insemination. This may be attributed to the numerous processes that have to be undertaken before the embryo is successfully implanted.
5. This being a labour intensive and time consuming technology, it may be prudent that farmers weigh their options and understand their abilities and goals before embracing embryo transfer technology.

Costs of embryo transfer

Costs of embryo transfer vary greatly from country to country, and within countries, depending on a variety of factors. No matter how it is done, embryo transfer programs are relatively expensive. The costs of the actual embryo transfer services or technology may be quite low; however, labour and feed costs averaged over the number of calves produced are high since normal, healthy cows or heifers are kept out of production in order to be available as recipients. Although embryo transfer is generally costly, it is

frequently still profitable. Obviously, one must analyse the costs and benefits. When the benefits exceed the costs, this technology would be used by all means. However, if costs are not justified by benefits, embryo transfer programs should not be initiated in most cases.

In conclusion, commercial embryo transfer in cattle has become a well established industry throughout the world. Although a very small number of offspring are produced on an annual basis, its impact is large because of the quality of animals being produced. Embryo transfer is now being used for real genetic improvement, especially in the dairy industry, and most semen used today comes from bulls produced by embryo transfer. An even greater benefit to embryo transfer is that in vivo-produced bovine embryos can be made specified pathogen-free by washing procedures, making this an ideal procedure for disease control programs or in the international movement of animal genetics. Techniques have improved over the past 60 years so that frozen-thawed embryos can be transferred to suitable recipients as easily and simply as artificial insemination. In vitro embryo production and embryo and semen sexing are also successful, but time and cost limit their widespread use. A combination of embryo transfer using proven cows inseminated with semen from proven bulls, followed by industry-wide artificial insemination appears to be the most common use of bovine embryo transfer in the near future.

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