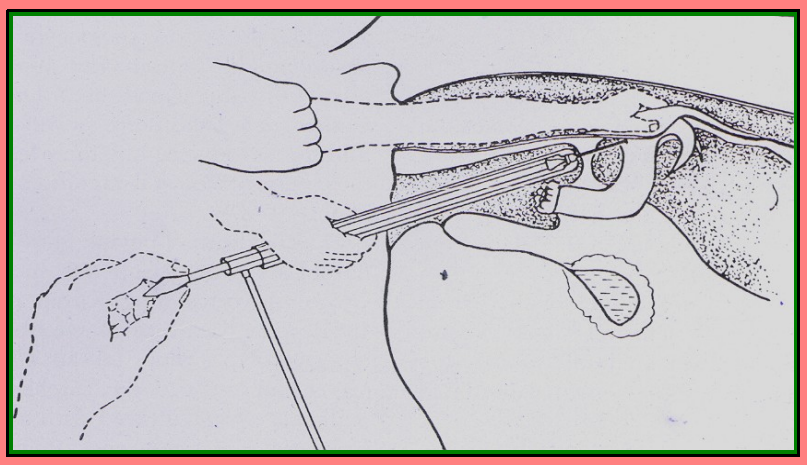
Embryo Transfer



Prepared by :

Dr. P.M. Barua
Professor

Department of Animal Reproduction, Gynaecology & Obstetrics,
CVSc., A.A.U., Khanapara,
Guwahati-781 022

Embryo Transfer

Definition :

Embryo transfer (E.T.) is a technique of transplantation of superior genetic merit embryo to the recipients after collecting from the donors. For successful embryo transfer covering more number of animals within short period the donors are given superovulation treatment for collection more number of ova and subsequently the recipients are treated for synchronization the oestrus. Therefore, the number of offspring that a female can produce in her lifetime can be grately increased by repeatedly allowing her to become temporarily pregnant, recovering the embryos in early pregnancy, and transferring them to the reproductive tracts of other females to complete gestation. The recipient mothers act as foster mother for development of the pedigree calves.

Therefore, it can be defined as a technique in which embryos from a valuable donor mated to a valuable sire are transplanted to non-pedigree recipients that act as foster mothers for development of the pedigree calves.

Historical development of Embryo Transfer and related technique:

First successful embryo transfer was reported by Heape (1890) in rabbits. Later Beidl *et al*. also conducted successful embryo transfer in rabbit in the year 1922. In 1952, Marden and Chang could successfully develop a technique to store the rabbit embryo at 10 degree C. Embryo transfer was successfully conducted in cattle, pig, sheep and goat, and in rat respectively by Willett *et al.* in 1951; Kvnsnickii, in 1951; Warwick and Berry, in 1949 and Nicholas, in 1933. In the year 1971 cattle embryo was first commercially formed by Alberta Livestock Transplants, Ltd. Trial also conducted to produce offsprings from frozen embryos. Later, first mouse offspring was produced from successfully stored frozen embryo by Whittingham *et al.* in 1972. Offspring from frozen cattle embryo was produced in the year 1973 by Wilmut and Rowson. In 1974 first International Embryo Transfer Society was formed. Frozen bovine embryos were first transported successfully from New Zealand to Australia by Bilton and Moor in the year 1977. In 1978 one baby girl was produced using embryo transfer technique by Steptoe and Edwards which opened a new era of Science.

Advantages or applications of Embryo transfer :

1. Embryo transfer can be used to rapidly increase rare blood lines.
2. More number of offspring from valuable females can be obtained by means of embryo transfer and
3. Embryo transfer also helps in rapid genetic make up by facilitating progeny testing of females and reducing the generation interval.

Steps of embryo transfer :

Following steps are necessary to be followed to make embryo transfer successful one.These are :

1. Selection of valuable donor and recipients.
2. Synchronization of oestrous cycles of both donors and recipients.
3. Superovulation of donors.
4. Breeding of donors during oestrus with valuable sire.
5. Recovery of embryos from the donors.
6. Transfer of collected embryos to the recipients at synchronized oestrus.

1. Selection of valuable donor and recipients :

The donors are selected based on good pedigree record like high milk yield, early attainment of puberty and sexual maturity, number of healthy calf born etc. Similarly, healthy with good reproductive organ and young females are selected as recipients.

2. Synchronization of oestrous cycles of both donors and recipients :

In each of group oestrous cycles are synchronized so that the embryos collecting from the donors can be transferred into uterine horn of the recipients in time of the oestrous cycle.

3. Superovulation of donors :

(a) Purpose of technique :

Donors are stimulated for additional follicular growth in ovary so that more number of ova could be released during oestrus.

(b) Procedure :

Usually, pregnant mare serum gonadotropin (PMSG) or follicle stimulating hormone (FSH) are injected subcutaneously or intramuscularly. This treatment is followed by intravenous administration of luteinizing hormone (LH) or human chorionic gonadotropin (hCG) several days later to induce ovulation of the follicles. In adult cow, sheep and goat exogenous LH or hCG is not required because sufficient endogenous LH is usully released following follicular growth.

(c) Time of treatment :

Generally 15 to 16 days of the oestrous cycle is recommended for better result. Of course, in ewe and lamb superovulation treatment is necessary to be conducted 12 to 14 days of the oestrous cycle.

Doses of Gonadotropins for superovulation are shown below the table :

Animal	Day of estrous cycle	Gonadotrophin for Follicular Growth			Gonadotrophin for Ovulation	
		PMSG[b] (IU)	or	FSH[c] (mg)	hCG[d] (IU)	LH[d] (mg)
Cow	15 to 16[a]	1500 to 3000		20 to 50	1500 to 2000	75 to 100
Calf	—	1000 to 2000		20 to 50	1000 to 1500	50 to 75
Goat	16 to 17[a]	1000 to 1500		12 to 20	1000 to 1500	50 to 75
Kid	—	1000 to 1200		10 to 15	1000 to 1500	50 to 75
Ewe	12 to 14[a]	1000 to 2000		12 to 20	1000 to 1500	50 to 75
Lamb	—	1000 to 1200		10 to 15	1000 to 1500	50 to 75
Pig	15 to 16	750 to 1500		10 to 20	500 to 1000	25 to 50
Rabbit	—	25 to 75		2 to 3	25 to 75	2 to 3

[a]Alternatively, treatment may be initiated on days 6 to 15 of the cycle in the goat and cow and days 6 to 13 in the ewe if followed with a luteolytic dose of prostaglandin $F_{2\alpha}$ 2 to 3 days later with the cow and 1 day later with the ewe and goat.

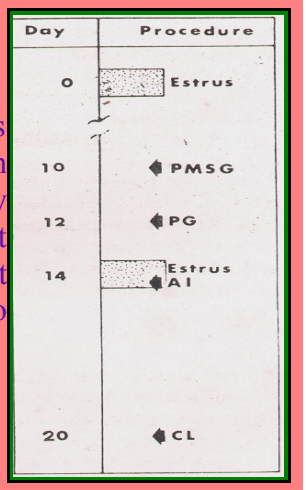
[b]PMSG is given as a single dose intramuscularly or subcutaneously.

[c]FSH (NIH-FSH-S1 equivalents) is divided among daily or twice daily subcutaneous injections for 4 to 5 days in the bovine and 3 days in the other species.

[d]LH (NIH-LH-S1 equivalents) or hCG is given intravenously 5 days after initiation of superovulation in the calf; 3 days after initiation in the rabbit, and at the beginning of estrus in the other species. The gonadotrophin injection for ovulation in the cow, ewe and goat may be omitted because sufficient endogenous LH is usually released following follicular growth.

(d) An alternate treatment using prostaglandin :

Use of prostaglandin F2 alpha or PGF2 alpha analogues such as chloprostenol has a greatest advantage over superovulation treatment with PMSG. Because superovulatory treatment can be initiated any time between day 6 of the oestrous cycle and natural corpus luteum regression. In cattle, treatment between 8 to 12 day of the oestrous cycle is found to have better result producing more number of normal embryos. Most of the donors come into oestrus within 2 to 3 days of prostaglandin treatment.



Superovulation with prostaglanins

4. Breeding of donors with valuable sires :

The sires are selected based on its pedigree record before breeding with the donors during oestrus.

5. Recovery of embryos from the donors :

After breeding is over embryos are recovered from the donors by two different techniques . These are :

a) Surgical method

b) Non-surgical method

(a) Surgical method :

Embryos are collected from the donors either slaughtering the animal or excising the reproductive tract. Then embryos are collected from the reproductive tract by flushing the organ with media. The flushing design for embryo collection varies based on different species.

i) In cattle, sheep, goat and rabbit : Flushing with 2 to 20 ml medium through the oviduct from the upper part of the uterine horn towards the fimbria using a syringe and blunt needle. The flushings are then to be collected through a small glass tube inserted into the infundibulum.

ii) In pig and mare : Flushing with 15 to 20 ml medium through the oviduct from the infundibulum, through the utero-tubal junction into the upper part of the uterine horn using a small glass tube attached to a syringe. Then collection of flushings through a blunt needle or fine glass tube inserting into the uterine lumen through a punctured wound.

iii) Applicable for all species : Flushing the uterus from the base of the uterine horn towards the utero-tubal junction or in the reverse direction using 10 to 100 ml of medium depending on the size of the uterus. Then collection of flushings through a blunt needle or small glass tube inserting into the uterine lumen through a punctured wound.

Flushing of oviduct 1 to 3 days after oestrus yields more ova than flushing the uterus 5 or more days after oestrus.

(b) Non-surgical mehod :

Embryos are collected non-surgically using two types of Foley catheter. These are :

a) Two-way flushing system and

b) Three-way circulation system

The cervix is dilated with cervix dilator or expander. Then, Foley catheter is inserted into the uterine horn by manual guidance per rectum and inflate the balloon in the cervix with air. Uterine horn is then irrigate using 100 to 800 ml of flushing medium. Thus, both the uterine horns are flushed simultaneously.

Flushing is done for collection of embryos within 6 to 8 days of artificial insemination or breeding. This non-surgical method of collection of embryos are commonly applied in cattle and horse.

6. Transfer of collected embryos to recipients :

The embryos after collection are selected based on certain criteria prior transfering to the recipients. Embryos are categorized into defective based on the following points :

* Nonuniform size of blastomeres.
* Presence of cellular debris in morulla
* Collapsed blastocyst with oblong-shaped zona pellucida.
* Disintigrating mitotic figure.
* Indistict foamy blastomeres
* Fragmentation of cytoplasmic and nuclear material.

* Amnormal shape of morulla or blastocyst.

The quality embryos are transfered to the recipients by means of two different ways. These are :

(a) Nonsurgical transfer

(b) Surgical transfer

In nonsurgical method ova is deposited in the uterus either bypass the cervix or through the cervix with an artificial insemination straw gun 6 to 12 days after oestrus.

Embryos are transfered surgically commonly by Midventral laparotomy under general anesthesia, although flank and lumber approaches are often practiced in cattle.

After successfully transfer the embryos pregnancy is necessary to confirm in the recipients.

Suggested reading :

Reproduction in Farm Animals. 5th Edition. E.S.E. Hafez.