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Embryo transfer technology in animals: An overview

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Abstract
Early embryos of superior genotype are collected prior to their implantation in uterus, and they are implanted in the uterus of other females of inferior genotype where they complete its actual development which is referred to as embryo transfer. The chief objective of embryo transfer is to achieve greater rate of conception, increase in number of progeny per year from single female animal of superior genotype. Embryo transfer technique helps in increasing commercial production and it can also improve the genetic potential of livestock. The embryo transfer technique provides a rapid rate of improvement of the genetic quality of offspring at relatively lower cost than purchasing a live animal. The present review depicts the basic principles, applications and the different steps in the procedure of embryo transfer in animals.

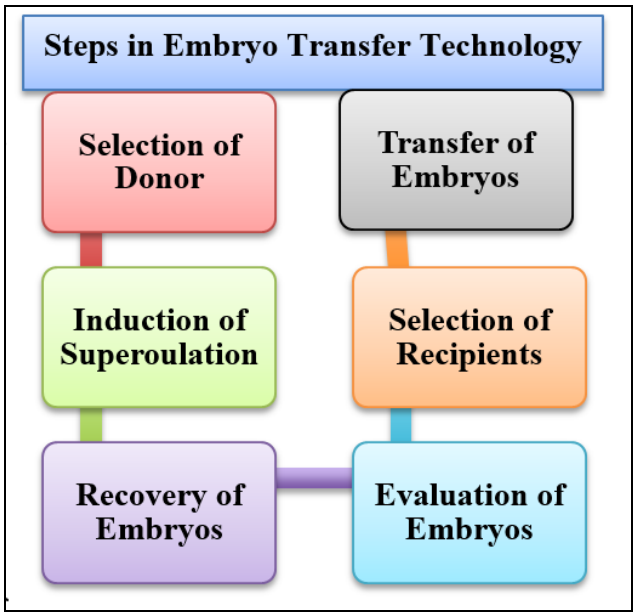
Keywords: Cryopreservation; Donors; Evaluation; Recipients; Superovulation

Introduction
Nowadays, embryo transfer technology is considered to be the principal technique which is very much necessary for achieving success in various assisted reproductive technologies, especially in case of in-vitro fertilization and animal cloning. Apart from natural and artificial insemination, higher pregnancy rates are achieved with the help of embryo transfer techniques [1]. Achieving higher pregnancy rates is mainly dependent on the quality of embryos and reproductive status of the recipient. If a good quality embryo is being used for embryo transfer technique, the success rate will be more. But when poor quality embryos are transferred, it often end up in low pregnancy rates [2]. Upcoming scenario of increased economic values of animals and livestock products, will end up in a drastic use of embryo transfer technology. But achieving a better success rate of embryo transfer still remains as a big challenge. Proper theoratical understanding and technical knowledge is essential to achieve greater success rate in embryo transfer technology [3].

2. Principle applications of embryo transfer technology
The main application is to exploit female reproductive capacity like more offspring from valuable donors (MOET-multiple ovulation embryo transfer technology), offspring from infertile donors. Also to conserve elite genetic materials and to perform gene transfer (Transgeneics) [4]. The technique is also used to produce twins and to manipulate embryos and for developing new breeding concepts like shortening of generation interval, testing of desired gene within a short period of time etc. It is also used to facilitate import and export for valuable genetic materials, to conduct research on embryology, embryo-genomics, early pregnancy etc and to preserve endangered species [3]. Interspecific embryo transfer in the light of lacking of suitable recipients for preservation of endangered species can also be done with the help of this technique.

2.1 Selection of donors
Factors should be considered for selecting a donor animal, the most important ones are genetic superiority of donor, purity of breed under which donor animal being selected, normal physiology and health conditions, normal reproductive status, age and economic value of the potential offspring [5].
For dairy cows, a high cow index value is the best indicator of good genetic potential. The cow index is a measure of genetic transmitting ability for milk, fat, protein and income. Only such

cows having high cow index and popular pedigree with a depth of good breeding and production should be used as a donor [6]. Cows should be thrifty and free of disease, should be in cycling regularly and should have a history of regular calving. As a rule, older cows which have produced well, have had several superior offspring and have classified as high are chosen as donors [6], but dairy producers should not overlook heifers with a high cow index.



Day of cycle	FSH (35-50mg)	eCG (1500-3000 IU)	hMG (1000-1500 IU)
0	Oestrus (spontaneous	or synchronized)	
10	FSH-12h-FSH	PMSG	hMG-Hmg
11	FSH-12h-FSH		hMG-Hmg
12	PG-PG-FSH-12h-FSH	PG-PG	PG-PG-hMG-hMG
13	FSH-12h-FSH		hMG-Hmg
14	Oestrus and insemination	Anti-PMSG	Oestrus and insemination
15	Oestrus and insemination	Oestrus and insemination	Oestrus and insemination
16	Recovery of embryo		

2.2 Induction of superovulation

The principle of susperovulation is to introduce more ovulations than normal rate by giving a gonodotropin stimulus followed by control of luteolysis, synchronous ovulation, high fertilization and early embryonic development rates [2]. The preparations to induce superovulation include mare`s serum gonadotropin (PMSG) sometimes called equine chorionic gonadotropin (eCG)[7], follicle stimulating hormone (FSH) [8] and human menopausual gonadotropin (hMG) [9].

The PMSG is a glycoprotein in that produces both FSH and LH biological effects [8]. Due to its carbohydrate side chains and sialic acid, PMSG has a very long half-life of about 5 days. A luteolytic dose of PG is administered 2 to 3 days after PMSG treatment and donor is expected to show heat signs 2 days after PG injection [7]. The advantage of using PMSG is its availability in large quantities at low cost and single dose is sufficient for ovulation. The main disadvantage of PMSG treatment is long half-life of PMSG inducing extra follicular growth with a resultant increased estradiol secretion that persists during post ovulatory period affecting early embryonic development [10]. FSH treatment shows higher and more consistent ovulatory response [8]. The half-life of FSH is approximately 5 hours. To get optimum response, FSH to LH ratio 5:1 should be administered [9]. The hMG is a protein purified from urine collected from menopausal women possessing both FSH and LH activities. Superovulation can be adequately induced using only one injection of 450 to 600 IU of hMG on polyvinylpyrolidone [11].

2.3 Recovery of embryo

In bovine including cattle and buffalo, embryos are recovered by nonsurgical methods, where a special type of catheter is introduced via cervix into uterine cornea and embryos are flushed with 250-300 ml flushing medium [3]. But, the recovery rate depends on the expertise of the person who is performing flushing, position of the embryos in the uterus, time of recovery, superovulatory response and embryo viability etc. From one flushing an average of 4-6 transferable embryos are recovered [12]. Flushing just beyond uterine bifurcation gave improved embryo recovery as compared to flushing at the tip of the uterus. In sheep, goat and pig, embryo recovery is performed by surgical methods. The abdomen of donors is opened by a midline incision and embryos are recovered by flushing the oviduct and/or the uterine horns [13].

2.3.1 Step-wise procedure for embryo collection

A local anesthetic is injected in between the vertebrae on the rump of the cow to reduce rectal contractions. The vulva of the donor is thoroughly cleansed so as to reduce contamination of the embryos. The Foley catheter is passed through the cevix. When the catheter is in place, the balloon is inflated so that it closes the lumen of the appropriate uterine horn slightly passed the bifurcation of the uterine horns. The collection container and tube is filled with the fluid at body temperature and about 15 to 20 ml of fluid are infused to the prime tube. Then additional medium is infused into the uterine horn until it becomes rigid as determined by palpation per rectum. The delivery tube clamp is released and the fluid is drained into the collection container. The process is repeated several times, with uterus massaged per rectum. Upto 500 ml of fluid may be used for each flushing so as to recover as many embryos as possible. Following the flushing, the balloon is deflated and the catheter is removed. Then, some antiseptic solution should be infused into the uterus to reduce the irritation of cervix developed from introduction of catheter. The flushed media is allowed to stand for several minutes to let the embryos settle to the bottom. The supernatant is removed and the bottom portion is placed in a petridish and see under microscope at a magnification of 10X to 60X. the embryos selected for transfer or storage should be round and full with un-interrupted zona pellucida and normal cells development with respect to time of recovery. The embryos considered normal will generally have 2 to 64 cells with number of cells varying with the time flushing [14]. The recovered embryo selected for use should be maintained at a temperature of 37 ^{0}C and kept sealed in the collection vessel until transfer or frozen. After selection, embryos should be transferred to the recipient animal as soon as possible to ensure high viability [1].

Embryo recovery is performed at morula stage and it is varied from species to species.

Cattle	:	6-8 days
Sheep and goat	:	4-5 days
Pig	:	3-5 days
Buffalo	:	150 hour post estrous (day 6 of the cycle)

2.4 Evaluation of embryo

Embryo viability can be evaluated by several methods. The two principle methods which are used to evaluate embryo viability are morphological method and staining method

2.4.1 Morphological method

Morphological evaluation is done at various microscopical magnifications to see the developmental stages. The criteria in embryo evaluation includes shape, colour, number, compactness of cells, size of perivitelline space, number and size of vesicles and status of zona pellucida [13].

In morula stage, individual blastomeres are difficult to discern from one another and the cellular mass of the embryo occupies most of the perivitelline space. In compact morula stage, individual blastomeres have coalesced, forming a compact mass and the embryo mass occupies 60-70% of the perivitelline space [3]. In early blastocyst stage, an embryo form a fluid-filled cavity or bastocoel and has a general appearance of a signet ring and embryo occupies 70-80% of the perivitelline space maintaining differentiation between trophoblast and inner cell mass. At mid blastocyst stage, pronounced differentiation of the outer trophoblast layer and the more compact inner cell mass is found. The blastocoel is highly prominent with embryo occupying most of the perivitelline space. At expanded blastocyst stage, overall diameter of the embryo dramatically is increased to 1.2-1.5X with a concurrent thinning of the zona pellucida to approximately one-third of its original thickness. A hatched blastocyst stage, embryo completely shed zona pellucida [13].

According to the morphological appearance embyos classified in to four groups which includes excellent embryo (perfect morphology), good embryo (slight morphological deviation), degenerated or retarded embryo and unfertilized ova [10].

2.4.2 Staining method

Evaluated by various vital staining methods and fluorescence techniques.

2.5 Selection of recipients

The selection of suitable recipient is out-most important for successful embryo transfer programme [3]. The criteria for selecting recipients are normal physiological and health status of the animal, good reproductive condition, lack of any reproductive disorders, compatibility to the donor with respect to the size of the foetus and easy to synchronise the oestrus [15].

The oestrus of donors and recipients should be synchronised within 24 hours, otherwise pregnancy rates will be considerably lower because highest conception is achieved when an embryo is transplanted to a uterine environment that most closely resembles the environment that embryo originated from [16]. For synchronisation of oestrus, implantation under the skin of the ear and 2 ml injection of norgestomet/estradiol valerate and removal of implants on day 10 will show oestrus 36 hours after removal of implant [10].

PGF2α treatment can bring out oestrus synchronization and it can be done by three ways which includes PGF2α single injection with implantation to know the status of ovaries stage i.e. days 6-16, PGF2α single injection without palpation may cause oestrus in approximately 45% of animals and PGF2α double injection at 11 days interval may bring 90% animals into heat [8].

Minimizing stress in recipient animals is very much essential for proper development and delivery of the foetus. Changes of feeding regime should be prohibited for 3-4 weeks before and after transfer. Recipients should be provided adequate space so that they can be easily and quietly handled on the day of transfer [3].

2.6 Transfer of Embryos

Special type of catheters are used to perform nonsurgical embryo transfer in cattle. It is important to place embryo into the tip of uterine horn without damaging endometrium. The pregnancy rate in non-surgical method is similar to surgical method. For getting success in embryo transfer [17], a number of factors are important which includes embryo should be of good quality, emvryo should be transferred into uterine horn bearing corpus luteum, recipients should be healthy and free from any reproductive disorder and expertise in handling and transfer of embryo [3].

In sheep, goat and pig, surgical method is applied, abdomen is opened and embryo is placed into the tip of the uterine horn [18]. Recently laproscopic method is followed to transfer the embryo. In pig, approximately 16-20 embryos are transferred to achieve a normal litter size.

2.6.1 Non-Surgical method of embryo transfer

The recipient animal is given a light epidural injection of anesthesis between the vertebrae of the rump to reduce rectal contractions. The embryo to be transferred is taken into a 0.25 ml straw and then placed into the AI gun. Place the left hand into the rectum and feel the cervix and hold it. Thee insemination gun is carefully passed through the cervix and into the uterus corresponding to the ovary that has a corpus luteum. The embryo should be disposed as deep into the uterine horn as feasible without using force. If twins are required, an embryo should be placed in both horns of the uterus of the recipient with presence of corpus luteum [18].

2.6.2 Surgical method of embryo transfer

The recipient is prepared for surgery by shaving an area about 6 inches square located some 6 inches in front of the hip joint. The area prepared should be on the side where the corpus luteum is present. A local anaesthetic is injected at the shaved area. The area is scrubbed with alcohol and a 2 inch incision is made with a scalpel. The uterus and the ovaries are brought near the opening of incision by grasping the uterus with the fingers of a hand. A small incision is made in the exposed uterine horn with a blunt needle. The embryo is drawn into a 0.25 ml straw attached to a small syringe and deposited into the uterus. The incision is closed with a few stitches and antibiotic solution is applied into the stitch area to remove infection [18].

3. Cryopreservation of embryo

It is an essential part of embryo transfer programmes. Blastocysts with a multi-layered trophoblast are best suited for rapid freezing and thawing method [4]. Reliable freezing methods have been developed for bovine and sheep embryo. This method includes a one-step addition of 1.4 M glycerol as cryoprotectant, a 20 minute equilibration period, packaging

into 0.25 ml straw, slow cooling (0.3 to 0.1 ^{0}C/min) down to -35 ^{0}C and subsequent plunging into liquid nitrogen (-196 ^{0}C) [19]. The frozen embryo can be used as and when required. Embryos are thawed by placing the straws directly into warm water. Glycerol is removed using sucrose [20].

4. Conclusion

Embryo Transfer Technology can been used as a principle technique for most of the assisted reproductive technologies, because of its impressive and successful history. It has gained more importance and attention and lot of scientific research activities are going on related to embryo transfer. Because of various innovations and development in the field of embryo transfer, various advanced assisted reproductive technologies are gaining importance which includes mainly in-vitro fertilization and animal cloning. Multiple Ovulation Embryo Transfer (MOET) technique, which is an essential component in the embryo transfer practices will be of greater importance and it will exist for years to come. The major disadvantage associated with embryo transfer technique is that proper training should be given to field veterinarians, otherwise the success rate will be very less. Hence, proper training should be given to the practitioners and researchers in order to achieve the greater success rate.

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