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Review Article

A Review of Embryo Transfer Technology in Cattle

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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. It could send female and male genetics worldwide in a cryopreservation tank without the hassle and complications of exporting live animals and the associated risk. The success and economics of cattle embryo transfer programed is dependent on several factors. Using high quality semen with a high percentage of normal, motile cells is a very critical step in any embryo transfer program. The transfer must be to a recipient in the same stage of cycle as the donor. Recipients must have a proven reproductive performance, free of congenital or infectious diseases to obtain high conception rates and have a sturdy body size to avoid problems of dystocia. Embryo transfer techniques can operate in surgical and non-surgical. There are several potential problems which must be overcome in order to make the international movement of embryos commonplace. The risk of transmitting genetic disease via embryo transfer is the same as that involved in natural mating or artificial insemination. It is recommended the process for production, selection of donor and recipients, and transfer procedures of embryos as well as in recipient management will be needed to know before embryo transfer.

Keywords: Embryo transfer, donor, recipients.

INTRODUCTION

In animal husbandry, Application of various biotechnological tools like artificial insemination, estrus synchronization for timed AI, multiple ovulation and embryo transfer, rumen microbial manipulation and modern breeding techniques may be of great use for faster multiplication and propagation of animal species in near future (Mondal *et al.,* 2014). 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 animal numbers which have recently gained considerable popularity with seed stock dairy and beef producers. The history of the embryo transfer procedure goes back considerably farther, but the most modern applicable embryo transfer technology was developed in the 1970s (Steptoe and Edwards, 1978).

Embryo transfer (ET) is a technique by which embryos are collected from a donor female and are transferred to recipient females, which serve as surrogate mothers for the remainder of pregnancy (Curtis, 1991). The first successful transfer of a mammalian embryo occurred in rabbits in 1890 (Heape, 1891). The first embryo transfer in goat was reported by Warwick *et al.*, (1934). There were no further reports of successful ET until the 1920's, and it was not until 1951 that the first calf was born as a result of ET (Willet *et al.*, 1951). In North America, the commercial ET industry began in the 1970's due to the demand for "exotic" breeds of cattle that had been imported from Europe (Hasler, 2003). The primary use of embryo transfer in cattle has been to amplify reproductive rates of valuable females. Ideally, embryo transfer can be used to enhance genetic improvement and to increase marketing opportunities with purebred cattle. Because of their relatively low reproductive rate and long generation interval, embryo transfer is especially useful with cattle (Seidel, 1991). Once transferable embryos are collected from a donor cow, the decision is made as to which of the available recipients should receive embryos to achieve the greatest number of pregnancies (Wright, 1981).

Initially, all collections and transfer were performed surgically through mid-ventral exposure of the uterus and ovaries. However, non-surgical embryo recovery and transfer techniques were developed in the mid-1970's (Hasler, 2003). Advancement in ET technology that occurred in the mid 1970's was the ability to successfully cryopreserve bovine embryos (Wilmut and Rowson, 1973). The ability to freeze embryos for thawing and transfer at a later date eliminated the need for synchronous recipient animals at the time of embryo collection. These important scientific achievements made ET a practical tool for genetic improvement of cattle is a technique of increasing both commercial production and genetic potential in livestock. The ET provides a rapid rate of improvement of the genetic quality offering access to the highest quality genetics at a lower cost than purchasing a live animal (Otter, 1994).

Soon cows were having up to thirty daughters and as many sons from genetically elite cattle whose offspring were worth up to several hundred thousand dollars in the early 1980's, it was profitable to have many offspring. Soon it was possible to flush cows without surgically entering the cow and the recipient. It also became possible to freeze embryos instead of throwing away the ones that there wasn't a recipient set up for. This allowed for international trade in genetics in a much easier way than ever before thought possible. Soon you could send female and male genetics worldwide in a cryopreservation tank without the hassle and complications of exporting live animals and the associated risk (disease and animal loss concerns). Worldwide, close to 500,000 bovine embryos are produced and transferred each year (Hasler, 1991). The transfer of bovine embryos today commonly involves estrus synchronization and superovulation of a donor animal, insemination of the donor animal, and collection of embryos from the donor approximately 7 days after estrus, then transferred to recipients (fresh) or frozen and transferred at a later date. Almost all transfers today are performed non-surgically and most are performed on-farm, rather than in a central clinic (Hasler, 2003).

The commercial advantages of embryo transfer in domestic animal include: (i) facilitating genetic improvement in the domestic animal industry by obtaining a large number of desirable progeny from parents of high genetic quality; (ii) enabling embryos to be moved from country to country in the frozen state, thereby reducing the need for long distance domestic animal movement; (iii) permitting high quality breeding stock to be available for sale in much larger number than was previously possible; and (iv) exploiting developments in reproductive technology, such as embryo sexing and embryo splitting. Split or bisected embryos have the ability to develop into identical twins (Lopes *et al.*, 2001). Furthermore, a greater number of pregnancies can be produced from one flush through embryo splitting. The first split embryo twin calves were produced from a day 8 blastocyst-stage embryo (Ozil, 1983).

In the last three decades, embryo transfer has developed into a specific advanced biotechnology which has gone through three major changes, "three generations "the first with embryo derived from donors (in vivo) by superovulation, non-surgical recovery and transfer, especially in cattle embryos, the second with in vitro embryo production by ovum pick

up with in vitro fertilization (OPU-IVF) and the third including further in vitro developed techniques, especially innovated embryo micromanipulation technique, which can promote us to perform embryo cloning involved somatic cells and embryonic stem cells, preimplantation genetic diagnosis (PGD), transgenic animal production etc. At the same time, commercial animal embryo transfer has become a large international business (Betteridge, 2006). The objective of this review is to understand the current status of the embryo transfer technology in cattle.

Current Status of Cattle Embryo Transfer Technology

The embryo transfer technology grew rapidly in the late 1970s, both in terms of the number of practitioners and in the number of donors. These technologies have resulted in new methods for producing embryos, for improving embryo quality, for long-term storage of embryos and oocytes, and for screening of embryos for important genes (Rowson, 1971).

Seidel (1981) reported that more than 17,000 pregnancies resulted from the transfer of bovine embryos in North America in 1979. More recently, Stroud (2012) reported that 572,432 in vivo derived bovine embryos were transferred world-wide in 2011, of which 54% were transferred after freezing and thawing. In addition, 373,836 in vitro produced bovine embryos were transferred. The International Embryo Transfer Society reports that total numbers of collections of transferred cattle embryos have increased which brought the total number of embryos transferred around 590,561.Table 1 shows that more than 104,651 donor cows were flushed 732,227, transferable embryos were attained. In 2011, North America continued to lead in commercial embryo transfer activity with collection of 51,735 donor cows and the transfer of more than 253,671 embryos (42.95% of all embryo transfers).

Table 1: Overall activity of in vivo-derived bovine embryos worldwide

Continent	Flushes	Transferable Embryos	Number of Transferred Embryos			
			Fresh	Frozen	Total	%
Africa	1,515	9,738	4,685	3,730	8,415	1.42
Asia	12,986	131,718	34,148	53,590	87,738	14.86
Europe	17,694	117,813	48,555	60,859	109,414	18.53
N. America	51,735	338,540	106,400	147,271	253,671	42.95
S. America	12,263	77,643	47,353	24,205	71,558	12.12
Oceania	8,458	56,775	21,895	37,870	59,765	10.12
Total	104,651	732,227	263,036	327,525	590,561	100.00

Sources: (IETS, 2011)

The success and economics of a commercial cattle ET programme is dependent on several factors: (i) skill and experience of the ET operator; (ii) selection and management of recipient animals, which must be healthy, cyclic and reproductively normal; (iii) close synchrony of oestrus between donor and recipient; (iv) quality of embryos transferred; and (v) methods used in embryo handling and transfer on the farm. The success of cattle ET is usually measured in terms of the pregnancy rate in recipient animals (Gordon, 2004). Embryo technologies use in cattle breeding has been to increase the number of progeny and top genotypes (Galli *et al.*, 2003). Increase the reproductive rate of the dairy cattle with ET has made it possible to predict accurately the genetic development through higher intensities of selection and shorter generation intervals and more female progenies (Goulding *et al*., 1996). ET has been utilized for enhancement of genetic selection; diagnosis and treatment of infertility, control of infectious disease transmission, screening for genetic defects, propagation of rare and endangered species, and the study of developmental biology (Kraemer, 2005).

Selection, Superovulation and Insemination of the Donor Cow

Selection of Potential Donor cows

The first step in embryo transfer is the selection of the donor cow. According to FAO (2008), there are two broad criteria for selecting donors for most embryo transfer programmes: (1) genetic superiority, those animals that contribute to the genetic objectives of the programmed, and (2) likelihood of producing large numbers of usable embryos. In the

majority of embryo transfer programmes, in both developed and less-developed countries, superiority is determined in practice by market forces.

Although scarcity and promotion have tended to influence value, true genetic value, the ability to transmit desirable traits, must be the most important long-range consideration. Selection should be based on three criteria: genetic superiority, reproductive ability and market value of the progeny (Mapletoft, 1985). Any cow that is mature and cycling is able to be put through an embryo transfer program. The only problem that could stop the cow or heifer from being flushed is abnormalities of the reproductive tract or with secondary abnormalities due to disease or injury (Hasler, 1992).

Some heifers as young as eight months have been successful in flushing embryos as have cows eighteen years old. Although it is possible for embryos to be flushed from a cow or heifer at these ages the result obtained from the heifer or cow it may not be worth the effort (Wilson, 1992). The first and probably most important step in the process is the selection of the donor cow. A female that is known to be free of reproductive abnormalities or genetic defects can be used in ET. However, this does not necessarily mean she is a deserving donor candidate. Regardless of the selection criteria, the value of the calves from a donor must be high enough to justify the added expense. Selection criteria can be based on actual performance, phenotype, relationship to other outstanding individuals, or some combination of these factors (Grimes, 2008).

Rincker also (2013) stated that the characteristics sought for in a donor cow vary depending on the type of cattle operation. In many cases, objectives measures of genetic superiority can be used, for example milk production, milk composition, growth rates, calving ease and disease resistance. Because phenotypic superiority may not indicate genetic superiority, it is usually desirable to consult someone trained in animal breeding so that the best donors are selected to meet objectives (Hasler, 1991).

Consideration must be given to the marketability of the calves. The purchase of a potential donor female can be an expensive proposition. The breed, selection criteria, and marketing opportunities will eventually determine the value of a donor female. Prices may range from a few thousand dollars to tens of thousands of dollars to an extreme of several hundred thousands of dollars. The individual breeder will have to determine the purchase price that is economically feasible for their operation (Grimes, 2008). Throughout the embryo transfer industry, the current dogma exists that feeding an organic source of mineral prior to superovulation of donors will enhance the total number and quality of transferable embryos (Lamb, 2005).

The potential donor cow should be reproductively sound to produce maximal results. This means that she should have a normal reproductive tract on rectal palpation and have a normal postpartum history, especially with regard to cycle lengths of 18 to 24 days. Both beef and dairy cows should be at least 60 days postpartum before the transfer procedure begins. Selk (2013) has been suggested that prospective donor cows in embryo transfer programs be selected on the following criteria; regular heat cycles commencing at a young age, a history of no more than two breeding per conception, previous calves having been born at approximately 365-day intervals, no parturition difficulties or reproductive irregularities and no conformational or detectable genetic defects.

The donor should be maintained at the level of nutrition appropriate for her size and level of milk production. Both the very obese cow and the thin cow will have reduced fertility, so it is important that the donor cow be in an appropriate body condition score at the time of embryo transfer (Selk, 2013). Lactation in either beef or dairy cows does not decrease response to superovulation provided that cows are cycling and not losing weight. Extremely fat cows make poor donors, both because they do not respond well to superovulation and because their reproductive tracts are more difficult to manipulate. Sick animals usually do not produce many good embryos (Hasler *et al.*, 1987).

Superovulation of the Donor Cow

A cow normally produces only one egg per estrous cycle (which lasts 21 days) and the gestation period is 40 weeks. On average, a cow produces only 2-3 calves in her lifetime. Thus, without intervention, the rate at which a particularly desirable cow can be used to

improve the genetic status of a herd is slow. However, recent advances in techniques for embryo transplantation are revolutionizing the rate of genetic improvement.

Superovulation of the cow is the next step in the embryo transfer process. Superovulation is the release of multiple eggs at a single estrus. Cows or heifers properly treated can release as many as 10 or more viable egg cells at one estrus. Approximately 85% of all normal fertile donors will respond to superovulation treatment with an average of five transferable embryos. Some cows are repeatedly treated at 60-day intervals with a slight decrease in embryo numbers over time (Selk, 2013). In cattle, there are two generally accepted methods of superovulation. One method consists of administering a single IM injection (2,000–2,500 IU) of equine chorionic gonadotropin (eCG), typically on day 10 of the estrous cycle (where day 0 is defined as the day cows are observed in estrus), followed 2–3 days later by two injections of prostaglandin (PG) $_{2\alpha}$ (dinoprost or cloprostenol) 12–24 hour apart. The other commonly used method consists of administering follicle stimulating hormone (FSH). The superovulatory response induced by eCG treatment is often greater than that induced by FSH; however, more embryos of transferable good quality are produced on average after FSH treatment (Selk, 2013).

Commercially available preparations of FSH are injected twice daily for four days at the middle or near the end of a normal estrous cycle, while a functional corpus luteum (CL) is on the ovary. A prostaglandin injection is given on the third day of the treatment schedule which will cause CL regression and a heat or estrus to occur approximately 48 to 60 hours later. At this time, the cow will usually produce 7-20 or more viable eggs with an average of 5-15 or more that are transferable (Rincker, 2013).

Many factors may influence how donors respond to superovulation and generate a high number of fertilized good to excellent quality embryos. Outside of genetics, nutrition probably is the single greatest factor that influences the response of donor cows to superovulation. It is important to ensure that cows are maintained on a positive plain of nutrition and are fed a diet that meets maintenance requirements (Lamb, 2011). Hasler (1991) stated that "there have been no significant improvements in techniques for the superovulation of cattle in the last 15 years". Eighteen years later that statement remains largely true. Data provided by both the American and Canadian Embryo Transfer Associations indicate that mean embryo yields per donor are in the range of 5-7 and basically have not changed for many years. The embryo means below are a composite average of individual means of both beef (6.6) and dairy cattle (5.7) flushed by the certified members of AETA (Hasler, 1991). Increased understanding of the processes of oocyte growth and maturation is essential to improving the efficiency of superovulation (Merton, 2003).

Insemination of the Cow

The time when the donor is first observed in standing oestrus is the reference point for insemination treatment. Because of the release of many ova from the multiple follicles on the ovary, there is a greater than normal need to be certain that viable sperm cells reach the oviducts of the superovulated females, the estrus donor is inseminated, usually with at least two straws of semen 12h apart, and 7 days later the uterus is flushed to recover the embryos (Sirard *et al*., 1998; Yoshida *et al*., 1997; Sinclair *et al*., 2000). Using high quality semen with a high percentage of normal, motile cells is a very critical step in any embryo transfer program (Pickett and Olar, 1980). The correct site for semen placement is in the body of the uterus. This is a small target (1/2 to 1 inch) that is just in front of the cervix. There seems to be a tendency for inseminators to pass the rod too deep and deposit the semen into one of the uterine horns, thereby reducing fertility if ovulations are taking place at the opposite ovary (Selk, 2013).

Embryo Collection and Evaluation Procedures

Surgical Embryo Collection Method

Early collection techniques involved either slaughtering the females and excising the oviducts, or surgically removing the oviducts from live females at 72 hours post ovulation so that the embryos could be recovered by flushing (Duran *et al*., 1998). This defeated the primary purpose of superovulation, so other methods were developed. A surgical method was developed first. This 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 (Elsden, 1977). 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. Adhesions make it difficult, if not impossible, to expose the reproductive tract repeatedly, and limit surgical interventions to a maximum of around three (Duran *et al*., 1998).

Non-Surgical Embryo Collection Method

Non-surgical techniques are preferred as they are not damaging to the reproductive tract, are repeatable and can be performed on the farm (Mapletoft, 1985; Mapletoft, 1986). The embryo recovery is unfortunately highly variable, which may limit practical application (Hazeleger *et al*., 1994). The first step in non-surgical recovery is to palpate the ovaries per rectum to estimate the number of corpora lutea. This is very difficult to do accurately if there is a large response to superovulation, although it is not critical to determine how large this response is. Even when only two or three corpora lutea are palpated by skilled personnel, occasionally four or five embryos are recovered. However, it is exceedingly rare to obtain embryos if there are no palpable corpora lutea by day 7 (Macmillan *et al*., 1999). Under most circumstances, cows with no response are not worth flushing, although occasionally an embryo is recovered. It is rare to recover more than one embryo from cows with one palpable corpus luteum. In many situations, donors are palpated the day before recovery or the morning of recovery so that logistical plans can be made, for example, to flush those donors with poor responses first (or last) and cancel those with no response. Ultrasonography (Pierson and Ginther, 1988) provides more accurate information about responses than palpation.

Non-surgical techniques of recovery have been developed for cows and mares that give results essentially equal to surgical methods (Macmillan *et al.,* 1999). They involve the use of a size 18 to 24 French Foley catheter (two-way flow catheter) which allows flushing fluids to pass into the uterus, and then allows fluid to be returned from the uterus to a collecting receptacle. A small balloon near the end of the catheter, which can be inflated just inside the uterine horn to prevent the flushing fluid escaping through the cervix, is also a feature. The Foley catheter is larger in diameter than the normal insemination instrument, and occasionally cannot be passed without first using a cervical dilator on the cervix. With non-surgical collection methods, it is difficult to determine how many ovulation sites are present on the ovary, so it is not possible to determine when all of the embryos have been collected. In controlled experiments, about 50% of embryos resulting from superovulation are recovered whether surgical or non-surgical procedures are used. In cattle, embryos are collected normally on days 6 to 8 (average day 7) after the onset of the estrus induced by Superovulation (Drost and Elsden 1985; Jainudeen 1989; Duran *et al*. 1998; Misra *et al*., 1998).

Embryo Handling

Once an embryo is identified in the searching dish, it is immediately transferred to a small Petri dish (35 × 10 mm) containing fresh, filtered (0.22–0.45 μ pore size), sterile medium. As a holding medium, generally phosphate buffered saline (PBS) containing penicillin plus 10–20 percent heat inactivated serum is used (Drost, 1991). Embryos are then serially rinsed through at least three different dishes containing fresh sterile medium using a new sterile pipette for each step. Finally, they are placed in a dish awaiting transfer or cryopreservation. Under certain circumstances, e.g. for export, embryos *must be* rinsed through ten different dishes containing sterile media. All dishes must be kept covered between searches to avoid contamination, and particularly evaporation, when placed in the incubator. Evaporation of the small volume of medium in a flat dish rapidly leads to hypertonic solutions (FAO, 1991). Embryos are located under 10X magnification with a stereoscopic dissecting microscope after filtering the collection medium through a filter

with pores that are approximately 50 - 70 µm in diameter (Mapletoft, 1986).

The temperature of the room should be within the range of 15-30°C. The ideal humidity is 30-70 percent, but this is not a critical factor and need not be taken into account unless, for other reasons, it is desirable to install a special room with controlled environment. Similarly, positive-pressure air filtration reduces the risk of contaminating equipment and containers of embryos; however, unless there are unusual amounts of air-borne microorganisms and dust, such precautions are not required. The presence of insects should be avoided, but special care must be taken to ensure that chemical control measures do not result in aerosols and surface residues that might contaminate equipment or solutions (FAO, 1991).

Although embryos are usually transferred as soon as possible after collection, it is possible to maintain embryos for several hours at room temperature in holding medium (Drost, 1991). It is also possible to cool bovine embryos in holding medium and to maintain them in the refrigerator for 2-3 days. As a final alternative, embryos may be frozen for use at a later date. 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. 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 (FAO, 1991).

Evaluating Embryos

After collection, the embryos are evaluated for quality using a stereoscopic microscope. Shape, color, texture, and size are some factors considered during the evaluation (Elsden, 1977). Embryos are graded on a scale from one (excellent) to four (poor) with no or few extraneous degenerated cells (table 2). Calculation of recovery rate (the number of embryos were recovered/ number of C.L. were Embryo Evaluation: The search for embryos is done detected by ultrasound) were carried out. Excellent and good morula or blastocysts were transferred (one or two embryos) to each recipients buffalo (Drost, 1991).

Fair quality embryos should be transferred fresh, if recipients are available. The International Embryo Transfer Society (IETS) considers the export of poor and fair quality embryos to be improper (IETS, 1998). Currently, however, only microscopic morphology is used for evaluating embryos. Although morphology does not offer predictability on any given embryo, average pregnancy rates relative to embryo quality are highly predictive (Hasler, 2001).

Table 2: Embryo quality grades

Grade	Description
1	Excellent or Good. Symmetrical and spherical embryo mass with individual blastomeres (cells) that are uniform in size, color, and density. This embryo is consistent with its expected stage of development. Structural Irregularities should be relatively minor, and at least 85% of the cellular material should be intact, viable embryonic mass.
2	Fair. Moderate irregularities in overall shape of the embryonic mass or in size, color and density of individual cells. At least 50% of the cellular material should be an intact, viable embryonic mass.
3	Poor. Major irregularities in shape of the embryonic mass or in size, color and density of individual cells. At least 25% of the cellular material should be an intact, viable embryonic mass.
4	Dead or degenerating. Degenerating embryos, oocytes or 1-cell embryos. These embryos are non-viable.

Embryos of appropriate quality (1 or 2 preferably) can be transferred directly to recipient cows or frozen for future use (Drost, 1991).

Classification

Many methods centre round morphological features of the embryo, such as uniformity of cell size, shape of embryo and its color and overall dimensions; various embryo classification schemes have been developed

based on such features. Embryo classification schemes should be based on easily recognizable morphological features and should be backed with firm evidence on the pregnancy rates to be expected with each of the grades. It is likely that techniques for assessing embryos *in vitro*, using objective non-invasive measures will become (Gordon, 2004).

Embryos are also classified on their stages of development. Embryos are collected on day six to eight after breeding and are usually in the morula through blastocyst stage (Robertson and Nelson, 1998). It should be noted that the visual evaluation of embryos is a subjective evaluation and is not an exact science. The following standardized coding system (table 3) is recognized by the International ET Society. Obviously, the higher the stage, the more developed the embryo (Seidel, 1991).

Table 3: Stages of normal embryonic development

Stage	Status	Days after Estrus
1	Unfertilized	0-2
2	2-12 Cells	1-5
3	Early Morula	5-6
4	Morula	5-7
5	Early blastocyst	7-8
6	Blastocyst	7-9
7	Expanded blastocyst	8-10
8	Hatched blastocyst	9-11
9	Expanding hatched blastocyst	11-12

Sources: (Seidel, 1991)

Storage and Freezing of Cattle Embryos

The freezing of embryos is already developed but not a real routine in most countries and often pregnancy rates have been substantially lower than with fresh embryos. However, quite advanced yet simple technologies are developed for freezing and are already successful in research (Niemann, 1992).

Freeze-thawing of Embryo

The purpose of freezing embryos is to hold the embryo in a state of suspended animation. When the embryo is thawed for transfer at a later date or place, normal biochemical processes in the embryo may be resumed and the embryo can then go on to develop normally. Embryos of good to excellent quality can be frozen and thawed with a slight reduction (10-20%) in the pregnancy rate normally expected with fresh embryos (McOnie, 2013).

They are two types of procedures to freeze and thaw embryos for transfer: 1) Thawing and transferring or 2) direct transfer. Freezing needs to be done in a lab environment and requires some specialized equipment. Traditionally, embryos are frozen with a cryoprotectant called glycerol. Even though glycerol protects the embryo during the freezing process, it is lethal to the embryo if not removed before transfer. Once the embryos are frozen the thawing procedure requires 3 or 4 steps of rehydration (washing) to remove the glycerol before transferring. This procedure can take 15 to 20 minutes (Rozeboom, n.d). The widespread adoption of freezing embryos in ethylene glycol for direct transfer after thawing (Voeklel and Hu, 1992) has made the transfer of frozen-thawed embryos more practical under a wide variety of conditions in the field. Frozen embryos are thawed in a manner quite similar to frozen semen, and should be handled under carefully controlled conditions of time and temperature. Improper handling and partial thawing and re-freezing are a potential cause of poor pregnancy rates with frozen embryos. They must remain immersed in liquid nitrogen in a storage tank (McOnie, 2013). Successful embryo freezing has many applications in embryo transfer programmes. Firstly, recipient management is improved and made more cost-effective. In addition, season of parturition can be controlled, even though embryo collection and freezing may take place year around. Finally, Embryo freezing is necessary for international movement of embryos because it eliminates critical timing and allows disease testing while the embryos are held in quarantine (Mapletoft, 1985).

Recipient Selection and Management

Recipients must have a proven reproductive performance, free of congenital or infectious diseases to obtain high conception rates and have a sturdy body size to avoid problems of dystocia (Larson, *et al.*, 2010). A variety of factors should be considered when identifying females suitable for potential use as recipients. Firstly, females should be in the bottom half of the herd from a genetic standpoint. Given the overall goal of embryo transfer is to facilitate genetic improvement, it seems unwise to use genetically above average females as recipients.

Instead, use the genetically below average females as "incubators" for embryos possessing high genetic merit. Secondly, females should be reproductively healthy and assured of not being pregnant (Rayos, 1989).

The selection of recipient cows is very important but is sometimes underemphasized. Even though the surrogate contributes no genetic material to the offspring, she does exert important maternal environmental influences which affect the physical makeup (phenotype) of the calf. For example, her milk production will affect calf weaning weight. There is also a maternal environmental effect on calf birth weight. Cows with reproductive problems or low calf weaning weights should not be used as recipient cows (Marahall and Minyard, 2002).

The main criteria for selecting recipient cows were animals not pregnant and with more than 90 days postpartum, fewer than five calving, and without any gross pathological features in genital tract. Only animals with a CL detected by rectal palpation and a body condition score of 2.5-3.5 in the scale 1-5 proposed by Pullan (1978) were selected. Once elected, the animals were separated from the rest of the herd to guarantee they were away from the bulls. The animals were estrous synchronized 20 days later and an ultrasound examination was performed to reassure that the animals were not pregnant. The selection of cows and heifers suitable for use as recipient females is a two-stage process. The initial selection of recipients should be done at the same time or earlier than selection of donor females, but the final selection of recipients should not be done until the day of embryo transfer (Barr, 1986).

Suitable recipient females should be free of reproductive anatomical abnormalities (e.g., they should have two functional ovaries plus normal oviducts, uterus and cervix), free of reproductive tract adhesions, and free of ovarian follicular cysts. Females should be consistently exhibiting estrous cycles of normal length (Youngs, 2007). The success of an embryo transfer program is primarily dependent on the quality and reproductive capabilities of the recipient females. Many commercial and research transfer programs fail because insufficient attention is given to the logistics of maintaining a good pool of recipients (Church, 1974). Since pregnant recipients are carrying valuable calves, they should receive better than average care. Nutrition is clearly important as well as prevention of abortion. The most critical time is at parturition. It is easy to lose 10 percent of calves at and within a few days of birth (King *et al.*, 1985); most of these losses are due to poor management.

Donor-Recipient Synchronization

Acceptable pregnancy rates in embryo transfer are partially dependent upon the onset of estrus in the recipient being within 24 hours of synchrony with that of the embryo donor (Hasler *et al*., 1987). Recipients can be selected for an embryo transfer program by detection of natural estrus in untreated animals or by detection after drug-induced estrus synchronization. Regardless of the method of synchronization used, timing and critical attention to estrus detection are important. Recipients synchronized with PGF must be treated 12 to 24 hours before donor cows because PGF-induced estrus will occur in recipients in 60 to 72 hours (Kastelic, *et al*., 1990) and in superovulated donors in 36 to 48 hours (Baruselli and Moreno, 2002).

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 (Gordon, 2004). In Arkansas, for example, the use of an electronic oestrus detection system to continuously monitor cattle permitted more precise timing of ET and resulted in improved pregnancy rates; Rorie *et al*. (2002) reported data suggesting that continuous monitoring of embryo donors and recipients and selection of recipients with synchrony of ±12 h could improve pregnancy rates.

If embryo transfer work is done on an essentially daily basis, with an average of two or more donors per day, most of the recipients coming into oestrus will be required as recipients, and oestrus synchronization will not be advantageous on most days. On the other hand, if embryo transfer is scheduled less than three or four times per week, oestrus

synchronization will be very useful. There is some evidence that oestrus synchronization with prostaglandins may result in higher pregnancy rates than natural oestrus (Hasler *et al.*, 1987).

Preparing Embryos for Transfer

Surgical and Non-Surgical Embryo Transfer

The successful utilization of both surgical and non-surgical embryo transfer in animal breeding programs represents a significant new approach for animal breeders, since it permits the production of increased numbers of offspring from mating of genetically superior parents (Baruselli *et al.*, 2006).

The transfer must be to a recipient in the same stage of cycle as the donor. For non-surgical transfer, the deposition of the embryos in the uterus horn with corpus luteum is performed under rectal control with a transfer catheter similar to the technique of flushing for embryo collection. Care must be taken especially with young animals to avoid any injuries in the still narrow uterus (Small *et al*., 2004). Surgical transfer is not more difficult nor takes it longer than non-surgical transfer. It needs local anesthesia at the standing animal and incision from the flank and even better results have been reported for the surgical transfer (Holtz, 1994).

Success rates with embryo transfer in many commercial situations are consistently high, often exceeding 70% pregnancy rates. In fact, when high quality fresh embryos are transferred into suitable recipients, pregnancy rates can average nearly 80% (Hasler, 2001). Recent reports in cattle have indicated that such differences in synchronous transfer can be tolerated without a significant reduction in the pregnancy rate (Seidel, 1981).

Non-surgical embryo transfer techniques utilized today involve the use of an artificial insemination pipette and more recently, specialized embryo transfer pipettes. After confirming synchrony of estrus, the recipient is restrained and the rectum is evacuated of feces. At the same time, the presence and side of a functional CL is confirmed. The big problem with non-surgical transfer is the difficulty in becoming proficient in this technique. First, it is necessary to be able to palpate ovaries accurately in order to select the side of ovulation. Pregnancy rates are markedly lowered if embryos are transferred to the uterine horn contra lateral to the corpus luteum (Seidel, 1981). Also, recipients should be rejected if no corpus luteum is present or pathology of the reproductive tract is noted. Even very experienced palpators make some errors in palpating corpora lutea.

Care is taken to prevent ballooning of the rectum with air. An epidural anesthetic is administered and the vulva is washed with water (no soap) and dried with a paper towel (Selk, 2013). The embryo is loaded in 0.25 ml straw between at least two air bubbles and the straw is loaded in the embryo transfer pipette. Care must be taken to insure that the straw engages the sheath tightly so as to avoid leakage. The embryo is placed in the uterine horn adjacent to the ovary bearing the CL by passing the pipette through the cervix, very similar to artificial insemination (Melissa, 2009). However, an attempt is usually made to pass the transfer pipette at least half-way down the uterine horn. The uterine lumen should be lined-up prior to transfer so as to prevent trauma to the endometrium during passage. The embryo is deposit slowly and firmly while slightly withdrawing the tip of the transfer pipette. Great care must be taken to not cause damage to the lining of the uterus (Melissa, 2009).

Identification, Certification and Registration of Offspring

Records for the accurate identification of parentage and of embryo transfer offspring is of vital importance for both domestic and international application of embryo transfer technology. The International Embryo Transfer Society (IETS) has developed three forms for certification of embryo recovery, freezing and transfer, respectively. In addition, a fourth form is recommended for use in embryo exports (IETS, 1998). The IETS also allocates embryo-freezing codes that must appear on all embryo containers and all documentation so that the organization freezing embryos can be identified. Finally, standard procedures for labeling embryo freezing containers are also recommended e.g., embryos frozen for Direct Transfer are to be frozen in yellow straws and placed in yellow goblets. Examples of the above forms and specific instructions on their use, the labeling of embryo freezing containers and the identification of embryo developmental stages

and quality grades are available in the Manual of the IETS (IETS, 1998).

International Trade In Frozen Cattle Embryos

Commercial ET in cattle started in North America in the early 1970s, primarily as a result of the high prices being paid for various breeds of so called "exotic" beef cattle that had been imported in small numbers from Europe. Initially, all embryo recoveries and transfers were performed surgically. Although early investigators described nonsurgical embryo recovery techniques (Dziuk *et al.*, 1958), these worked rather poorly and few embryos were recovered.

The intercontinental transport of a live animal may cost $1,000 or more; where as an entire herd can be transported, in the form of frozen embryos, for less than the price of a single plane fare. This may be the single most important potential application of embryo transfer. Additional benefits of the export of embryos over that of live animals include a wider genetic base from which to select, the retention of genetics within the exporting country and adaptation. This is particularly true of tropical and subtropical climates where the embryo would have the opportunity to adapt both in the uterus and then suckling a recipient indigenous to the area (Mapletoft, 1985). If cows are imported, the genetic influence on the general population is limited until their bull calves reach breeding age. While the genetic influence of imported semen can be distributed over a larger portion of the herd, offspring have only 50 percent of the new genes and will not become producing members of the herd for two to three years. With imported embryos, the resulting offspring have 100 percent of the desired genes (FAO, 2008).

There are several potential problems which must be overcome in order to make the international movement of embryos commonplace. Firstly, this use is dependent on the successful freezing of embryos. Secondly, the inadvertent introduction of disease into a herd and/or country with or within the embryo presents some very difficult regulatory problems. Well defined methods of collection, handling and washing embryos must be followed to ensure that disease transmission is avoided. Finally, the international movement of embryos is heavily dependent on technology transfer as personnel within the importing country must be able to successfully thaw and transfer embryos (IETS, 2011).

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. For example, selection of specific donor cows and service sires are ultimately chosen by the owner. 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).

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). Many factors go into cattle management and there are sometimes opportunities for ET practitioners to make a meaningful contribution to improving or changing certain management programs. In some cases, however, change either is not welcome or not possible and then the practitioner must make the best of the situation at hand. 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, no matter how cattle are propagated and there is no increased incidence of abnormal offspring due to these procedures (King *et al.*, 1985).

CONCLUSIONS AND RECOMMENDATIONS

Embryo transfer in cattle has grown into a mature international business with high success rates and it becomes a well-established industry. Its impact is large because of the quality on animals being produced. Embryo transfer is now being used for real genetic gain, especially in the dairy industry, and most semen used today comes from bulls that have been produced by embryo transfer. Superovulation and embryo transfer are used to increase the number of offspring from genetically outstanding females as well as superior sires. The superovulation

procedure causes more oocytes to be ovulated than is normally the case and these oocytes are fertilized using AI.

An even greater benefit of bovine embryo transfer may be that in vivo derived embryos can be made specified pathogen free by washing procedures, making this an idea process for disease control programs or in the international movement of cattle genetics. It can be stated that embryo transfer is the method of choice for such movements because it is the safest means to do so.

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. Because international movement of embryos is heavily dependent on technology transfer as personnel within the importing country must be able to successfully thaw and transfer embryos. A generally successful approach is to build embryo transfer on a programme that has been successful for artificial insemination. Facilities and logistics of handling animals are similar for both techniques. Also, the skills of good oestrus detection and passing catheters through the cervix are an excellent foundation for embryo transfer.

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