TECHNICAL ASPECTS OF THE RECOVERY, HANDLING AND TRANSFER OF EMBRYOS

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ABSTRACT

This paper discusses the techniques of embryo transplant for ungulates, especially cattle and water buffalo. It outlines techniques in current use, and their success rate. It describes how to recover embryos from donors, by either surgical or non-surgical techniques. Nowadays, non-surgical collection techniques are generally preferred. It describes how to handle the embryos, and how to transfer them. Equipment and procedures are described in detail. Finally, it outlines the factors influencing the success of embryo transfer. Of these, operator skill is one of the most important.

INTRODUCTION

Embryo transfer (ET) techniques are very effective for the propagation of superior genes. This technology has been in use for several years, and allows breeders of livestock to share their most valuable germplasm at an international as well as a domestic level without depleting their genetic base. Embryo movement provides a humane alternative to the stress of transporting live animals. Research to date has shown that this method of moving genetic material can be very safe with regard to preventing the introduction of infectious diseases into previously unaffected populations.

The method of embryo transfer, which is used for the increase of the reproductive ability of female animals, is now a routine practice in cattle breeding in many countries of the world. Its application in buffalo breeding, however, started only about two decades ago, with the birth of the first calf as a result of embryo transfer.

Embryo transfer is a technology that requires expertise in many areas. It includes several consecutive stages such as the selection of donors (genetically superior and with normal reproduction), selection of recipients (of low genetic but high reproductive ability), hormonal treatment of the donors for the induction of superovulation, fertilization of the donors, embryo recovery from the donors, evaluation of embryos recovered and embryo transfer to the recipients by either surgical or non-surgical methods which would complete the pregnancy and give birth to a calf. While each stage is simple and usually a matter of common sense, it must be carried out properly in coordination with all preceding and subsequent steps, otherwise failure will result.

Research with cattle, pig, sheep and goat embryos shows that embryo transfer can be a safe, effective means of preventing the spread of many pathogens, provided that proper sanitary procedures for collection, handling, and transfer are used. The embryo is an unlikely host for many pathogens because of primary resistance provided by the zona pellucida. Secondary resistance factors, such as the early stage of development, small size, and limited mobility, also reduce the potential exposure of the embryo. The facts that many bacterial and viral pathogens are too large to penetrate the intact zona pellucida and that some do not survive well in embryo support media, further reduce the possibility of transmitting an infectious agent via embryo transfer.

Success in ET depends on the ability to perform
a series of technical steps and simultaneously minimize factors known to have a negative effect on the percentage of transfers that result in the birth of healthy offspring. Sound sanitary measures at all stages are vital to ensure success. Poor hygiene will jeopardize the health of donors, reduce pregnancy rates, and increase the risk of transmitting infections when embryos are transferred. This Bulletin addressed some of the technical aspects, with a focus on recovery, handling and transfer of embryos.

**EMBRYO RECOVERY**

Surgical and non-surgical methods are employed in recovering embryos in large animals. Whether the embryos are recovered surgically or non-surgically, cleanliness at all stages is mandatory. For example, it is illogical to pass a sterile catheter into the uterus of a cow past a vulva grossly contaminated with feces. The vulva and tailhead should be scrubbed with soap and water, disinfected, and then adequately rinsed prior to catheterization. Disinfectants must be used with great caution, since many of these chemicals are toxic to embryos.

Restraint of donors should not be overly stressful. However, adequate control is necessary to minimize movement and ensure the safety of animals and personnel. Sedatives or tranquilizers should be used prudently and in accordance with the manufacturer’s instructions. Local, epidural, paravertebral, or general anesthetics should be administered with care. Particular attention should be paid to the site of the injection, the type of drug used, and the concentration of the dose. Animals under general anesthesia should be monitored constantly by a competent person other than the surgeon collecting the embryos. A high level of skill is required for laparoscopic methods of collection and transfer of embryos.

Equipment that comes in contact with the donor, or is contaminated by her secretions and excretions, must be thoroughly cleaned and disinfected prior to use with another animal. This include items such as squeeze chutes and trolleys as well as surgical instruments. Recovery catheters, tubing, dishes, and other equipment that come into direct contact with uterine fluids or recovery medium should not be used without being resterilized. When it is possible, sterile disposable items should be used. Disposable plastic gloves (or a clean, sterile, rubber sleeve) should be used when embryos are collected non-surgically, and full aseptic procedures are necessary for surgical collections. Embryo recovery media should be of high quality. Biological constituents of media should be specified to be pathogen-free.

Care should be taken during the recovery process to avoid damage to the donor’s reproductive tract. Excessive manipulation of the reproductive tract or collection equipment can cause adhesions of the oviducts or fimbriae and hemorrhage of the uterus. Overinflation of the cuff of the catheter in the case of non-surgical recovery from cattle, or dilating the uterus with an excessive volume of collection medium, can tear the endometrium or rupture the uterus.

The volume of medium infused and recovered should be recorded as a gauge of efficiency of the procedure for recovery of embryos. If direct filtration of collection medium is used in the recovery of embryos, filters should be used with great care according to the manufacturer’s instructions.

**Surgical Collection of Embryos**

Early collection techniques involved either slaughtering the females and excising the oviducts, or surgically removing the oviducts from live females at 72 hours postovulation so that the embryos could be recovered by flushing. 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. 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. Adhesions make it difficult, if not impossible, to expose the reproductive tract repeatedly, and limit surgical interventions to a maximum of around three.

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 way of an initial intravenous knockdown injection, followed by intubation and closed circuit anaesthesia (e.g. with a halothane/oxygen mixture). In the mid-ventral laparotomy procedure, the uterus was exteriorized and a fine cannula introduced into the ovarian end of the oviduct; flushing fluid was then gently forced through from the uterus.

Cattle embryos enter the uterus four days after the end of estrus. The majority of recovery attempts were timed for about seven days after the cow was no longer on heat. In the early days, milking cows were not regarded as good candidates for surgical recovery. There was the danger that they might become ketotic when they fasted prior to surgery, while their recovery from the intervention might be prolonged and occasionally complicated by hypocalcemia.

Before 1976, most bovine embryos were collected via mid-line laparotomy or, less commonly, via a flank incision. The alternative surgical procedure, using the flank incision after employing local anaesthetic and with the donor under sedation, was the preferred option for large animals like cows.

In most cases, embryos are recovered six to eight days after the beginning of estrus (day 0). Embryos can be recovered non-surgically as early as four days after estrus from some cows, but prior to day 6 recovery rates are lower than on days 6 to 8. Embryos can also be recovered on days 9 to 14 after estrus. However, they hatch from the zona pellucida on day 9 to 10, making them more difficult to identify and isolate, and more susceptible to infection. After day 13, embryos elongate dramatically and are sometimes damaged during recovery or become entangled with each other. Procedures for cryopreservation and bisection have been optimized for 6-8 days embryos, which is another reason for choosing this time. A small percentage of embryos remain in the oviduct after day 7. Unfortunately these are not recoverable with current non-surgical procedures.

Several workers during the early years of cattle ET research developed procedures for the non-surgical recovery of embryos. However, it was animal welfare considerations and the problem of adhesions that focused attention in the early 1970s on developing non-surgical procedures. It was clear that non-surgical methods would greatly facilitate the production of cattle ET, especially with certain categories of donors. As already mentioned, high-yielding dairy cows, are likely to have problems if they fast before surgery, while there are often difficulties in exteriorizing the tract.

**Non-Surgical Collection of Embryos**

Non-surgical techniques of recovery have been developed for cows and mares that give results essentially equal to surgical methods. 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. In buffalo, embryos are collected on days 5 to 6 after superovulatory estrus, because the rate of development of buffalo embryo is 24 to 36 hours faster than cattle (Drost and Elsden 1985, Jainudeen 1989, Duran et al. 1998, Misra et al. 1998).

Flushing of superovulated buffalo on day 7 leads to poor embryo recovery, since a sizable proportion of embryos are at the hatched blastocyst stage, which is difficult to identify from debris. Moreover, for micromanipulation/microsurgery of embryos, as well as for movement of embryos from one farm to another, only embryos with intact zona pellucida are required.

Buffalo embryos are isolated and evaluated at 75X magnification, and viable embryos are classified and graded on their morphological appearance, as those of cattle (Mapletoft 1986). Excellent and good grade embryos are selected for cryopreservation, while fair and poor quality embryos are transferred to the synchronized recipients. All viable embryos are given 10 serial washings in sterilized holding medium (DPBS supplemented with 0.4% BSA), following the guidelines of the International Embryo Transfer Society.
Estimating Ovulatory Response by Palpation per Rectum

Many workers have now shown that embryo recovery rates comparable to those achieved by surgery can be achieved by non-surgical methods, once the appropriate manipulative experience and skills have been acquired. In contrast to surgical recovery, the precise embryo recovery rate may not always be known accurately when palpation of the corpora lutea per rectum is the method used in estimating the number of ovulations induced. A critical evaluation of ultrasonic monitoring of superovulation in donors treated with PMSG was made by Robertson et al. (1993). They concluded that the presence of luteinized follicles greatly reduced the accuracy of identifying structures in superovulated ovaries.

Catheters and Filters

The non-surgical recovery methods in current use are based on the use of the Foley catheter. Recovery systems may be either two-way or three-way, but an inflatable balloon cuff should always be used. In the two-way system, the flushing medium is introduced and recovered using the same channel. Three-way systems have separate lumens for the introduction and the recovery of the medium from the uterus. Recoveries are generally carried out in any normal animal crush, but in a suitably warm atmosphere. In some crushes, the front is raised about 30 cm above the ground to facilitate the drainage of flushing medium from the donor. During the 1980s, commercially produced filter devices which would retain embryos, while letting much of the cellular detritus pass through, were introduced and found to be very useful. Such filters were especially valuable in speeding up the location and identification of embryos under the microscope.

Various descriptions of equipment designed for embryo collection in cattle have been published. In the former East Germany, Rehbock et al. (1990), provided an illustrated account of the Buhner automatic catheter, designed for transcervical embryo collection. This device gave a recovery rate of flushing medium ranging from 81 to 100%.

Epidural Anesthesia

After the donor is placed in the crush, caudal epidural anesthesia is introduced by injecting 5-10 mL of analgesic (2% procaine or lignocaine hydrochloride) into the space between the first and second coccygeal vertebrae. Care must be taken not to give too large a dose, otherwise the cow may lose control of its hind legs. When the epidural block has become effective, the rectum is completely emptied. The vulva area is washed, first with an antiseptic skin wash and then with surgical spirit, and finally dried.

Flushing Procedures

Consideration in the flushing of donors includes the following:
- Flushing fluid must reach the tip of the uterine horn, since this is where most of the embryos are likely to be one week after estrus.
- All flushing fluid introduced into the uterine horn should be recovered.
- The flushing should always be carried out with a minimum of stress and trauma to the donor.
- The success of a “flush” is directly related to the success of fluid recovery.

An effective flush should return 90-100% of fluid initially introduced. The aim is to recover embryos at the blastocyst stage of development, which normally would be expected seven days after the time of breeding.

Cervical Dilation in Heifers

One problem that can face non-surgical recovery attempts is the passage of catheters through the cervix of the donor animal during the luteal phase of the estrous cycle, particularly in heifers. Simple mechanical dilation with a metal cervical expander may not always provide the solution and there may be a risk of trauma. For this reason, there have been attempts to dilate the cervix by way of special devices (Ushijima et al. 1993) or by applying various agents. In some reports, carbachol has been used. This preparation is reported as being effective, easy to administer and relatively inexpensive (Zraly et al. 1980).

Though surgical methods were employed in recovering embryos in the first successful cattle embryo transfer studies, at present a non-surgical recovery method via the cervix is the technique of choice.

HANDLING OF EMBRYOS

Careful handling of embryos between collection and transfer is necessary to prevent the
transmission of pathogens. The use of aseptic techniques, sterile solutions, and sterile equipment is essential. In dealing with the handling of embryos in laboratories, where inevitably they come into contact with glassware, petri dishes, plastic straws and other equipment, exposure to toxic factors must always be a consideration. It is, for example, essential to provide adequate aeration of straws after their sterilization, in order to remove the powerful antimicrobial, ethylene oxide (Schiewe et al. 1988).

After flushing medium is collected, it should be taken to a separate processing laboratory (or separate work area on the farm) for the recovery, evaluation, washing, manipulation, and freezing of embryos. Embryos should be recovered as soon as possible from the flushed medium. Care should be taken that no embryos are lost. Flushed medium contains a lot of mucus, blood and debris, and these might have detrimental effects on embryos. Discovered embryos should be immediately transferred to fresh storage medium, and washed several times. Throughout this process, the embryos should be kept clean and handled appropriately.

A processing laboratory for embryos should be scrupulously maintained, and separated from animals and other dirty areas. Ideally, it should be built from materials that permit effective cleansing and disinfection. All work surfaces on which embryos are placed must be kept thoroughly clean and dry. In addition to sanitation, there should be strict regulation of temperature, illumination, and movement of personnel.

Conditions on farms vary, so common sense is needed in selecting the best location for handling embryos. It is important to protect the embryo from extremes of temperature. The optimal temperature range for removed embryos prior to freezing or transfer is from 20°C to 30°C. Temperature is of great concern if embryos are to be frozen after they are held for an extended period. Although some investigators feel that embryos should be maintained at refrigerator temperature (4°C) prior to freezing, there have been no definitive experiments to prove this.

Sterility of plasticware and glassware that has been in contact with embryos or media is vitally important for maintaining embryo quality. Embryonic chemicals are also of concern. Known toxins include heavy metals, detergents, disinfectants, gases, and rubber antioxidants on such surfaces as plungers of syringes. To avoid problems or questions related to embryo toxic substances, the usual solution is to implement careful washing of laboratory glassware, using non-toxic detergents that are specially made for use in tissue culture laboratories.

Contamination of the air with dust, engine fumes, aerosol disinfectants, insecticides, tobacco smoke, etc. can be hazardous to embryos. These substances readily accumulate on glass, plastic and bench surfaces, and will also be absorbed by media. All activities associated with the creation of these forms of contamination should be avoided.

Media for collecting, holding, culturing, and freezing embryos should be of high quality, and should be prepared with high-quality water. All holding media should be filtered through a membrane of 0.22 µm pore size prior to use. A separate set of dishes and pipettes should be used to handle embryos from each donor to prevent inadvertent mixing of embryos. Careful labeling of dishes and pipettes will help to assure that this segregation is maintained.

Searching for Embryos

Before embryos are recovered, small dishes which contain 3-5 mL of storage medium (D-PBS + 20% Calf Serum) are prepared and kept warm on a 37°C slide warmer. When embryos are detected, they are aspirated according to the following procedure.

- A mouthpiece is attached to a micropipette. The tip of the pipette is washed by aspirating storage medium 2-3 times. Each aspirate is discarded every time the tip is washed.
- A small amount of storage medium is poured in above the detected embryo, and aspirated with the micropipette. The embryo is then transferred to the storage medium.
- Aspiration of mucus or debris together with the embryo cannot be avoided completely. Thus, the isolated embryo must be washed several times until the medium becomes clean.

Before searching for embryos under the microscope, the volume of recovered medium must be reduced without loss of embryos. For this purpose, the following two methods can be employed.

Cylinder stationary method

- The recovered medium is collected in a 1,000 mL cylinder, and placed in a 37°C water bath or at room temperature.
It is allowed to stand for 30 minutes. During this period, all the embryos will settle to the bottom.

- After 30 minutes, all but 50 mL of medium at the bottom is siphoned out slowly, using a drip tube or a silicone tube with clamp.
- The remaining medium is poured into a dish. The emptied cylinder must be washed with 20-30 mL of medium three times, using a ball pipette. The medium used for rinsing is also poured into a dish.

**Mesh filtration method**

- Using a filter system (“EmCom”: Immuno-system) can speed up the process of isolating embryos. This system filtrates the recovered medium through a 70u meshed filter.
- The bottle is rotated slowly, without making any bubbles. The entire recovered medium is poured into the filter system. As in the first procedure, the emptied bottle should be rinsed thoroughly.
- Throughout the filtrating process, the filter system should never be empty. Some of the remaining medium should always be covering the filter.
- Eventually about 40-50 mL of recovered medium containing embryos will remain in the filter system.
- As embryos are sometimes captured in mucus, all mucus attached to the mesh must be washed out by pipetting with a ball pipette.

The remaining medium and rinsed medium are poured into one or two examination dishes. Square grid lines of 10-15 cm should have been drawn on bottom of the dishes. These dishes are examined for embryos under a stereo microscope, at 10-15 x magnification.

A sterilized glass stick, one end of which is stretched to a fine point, is useful in searching for embryos in the mucus.

Throughout the whole process of embryo handling, the greatest care should be taken not to damage the embryos before transfer or freezing. In this regard, the following hygiene precautions are important:

**Hygiene Precautions**

**Sterile Technique**

If a clean booth is available, it is convenient to use this for embryo handling, to reduce the chance of infection with bacteria. If no such booth is available, the micropipette should be put in a test tube laid slant-wise in a tube rack, in order to keep it clean.

**Contamination**

All instruments and media that come into contact with the embryos should be kept free of any contamination by chemicals, mineral deposits on glassware, lubricants used in disposal syringes etc.

**Temperature**

After the recovered embryos are moved to fresh sterile medium, they should be kept at 37°C on a slide warmer or at room temperature (20-30°C). Sudden changes of temperature should be avoided. High temperatures (of 40°C or more) can easily kill the embryos.

**Ultraviolet Rays**

Ultraviolet rays are known to have a detrimental effect on embryos. Therefore, direct irradiation of sunlight on embryos should be avoided.

**Changes in the Medium**

A small volume of medium in an uncovered dish may evaporate, so there is a change in concentration. The dish should always be kept covered, as much as possible. This is also important for preventing contamination.

**TRANSFER OF EMBRYOS**

Various methods of transferring embryos have been developed. The efficiency of transfer depends on many factors, but the most important are the experience and skill of the person(s) doing the transfer.

There are two methods of transferring embryos; surgical and non-surgical. The surgical methods have in the past given higher and more consistent pregnancy rates. However, skillful technicians can now effect similar high pregnancy rates with non-surgical (through the cervix) methods.
Surgical Transfer

The work of Rowson et al. (1969) marked an important turning-point in cattle ET prospects, by showing that an acceptable pregnancy could be achieved, albeit by a surgical technique. The mid-ventral laparotomy technique employed in these Cambridge studies involved general anesthesia, with surgical preparation of the midline just anterior to the mammary gland of the recipient. After the uterus had been brought to the site of the incision and the location of the corpus luteum confirmed, a small puncture was made in the uterine wall to provide access to the lumen of the uterine horn ipsilateral to the corpus luteum. The transfer pipette was introduced through the puncture, and the embryo deposited. Although the mid-ventral procedure could be carried out quickly on heifers, it was clearly not a procedure for use on farms. As well as being both labor and capital intensive, the technique was not at all suitable for milking cows.

When surgical transfers are used, most commercial ET operators have adopted the flank surgical approach. The recipient stands sedated and under local anesthesia (paravertebral block). The site of the incision is the sublumbar fossa, and the embryo is transferred into the upper third of the uterine horn. Prior to surgery, the location of the corpus luteum is established to show the side on which the transfer should be made. In comparison with the mid-ventral procedure, the flank approach is widely recognized as being a highly effective, practical and rapid means of performing surgical transfers with a minimum of equipment, facilities and labor.

As to the reasons for using the surgical approach, this is likely to be in an effort to give the recipient the greatest opportunity of becoming pregnant with the embryos that are available. According to Coultard (1991), if the embryo has been frozen and thawed or is second grade, a 10% or so improvement in pregnancy rate may be achieved with the flank surgical approach, in comparison with non-surgical methods.

In experiments with water buffaloes, the surgical method of embryo transfer has been used to only a limited extent. Bhattacharya et al. (1998) transferred by this method 9-day old fresh buffalo embryos and registered pregnancy in a buffalo cow, which, unfortunately, aborted. In Bulgaria, two transfers were performed but no pregnancy was registered. A success was achieved only in 1987, when Misra and associates in India obtained the first buffalo calf born by surgical embryo transfer (Kurup 1988, Bhattacharya et al. 1988). Subsequently, a few more calves were born in India in this way. The surgical method, however, was abandoned as impractical. In all later experiments, non-surgical methods of embryo transfer were used.

Non-Surgical Transfer

Although numerous instruments designed specifically for the non-surgical transfer of cattle embryos were described in the literature during the 1960s and early 1970s (Gordon 1983), it was the successful application of the Cassou AI gun which eventually proved to the answer to this particular problem. Since the mid-1970s, several variants of the standard inseminating gun have been marketed for cattle ET. Coultard (1991) describes his transfer procedure in the United Kingdom, using a modified insemination gun. This gun has a special sheath with a metal tip and two side outlets. In carrying out the transfer, the recipient is given epidural anesthetic and the sheathed, loaded gun is inserted into a sterile, loose plastic outer sheath; this is passed up into the cervix and the gun forced through the outer sheath.

ET of buffalo is of recent origin. The pioneering work of Drost and associates (1983) in Florida resulted in the birth of first live buffalo calf through non-surgical embryo transfer. This work aroused considerable interest in several buffalo rearing countries. Subsequent work was carried out in India, Bulgaria, Thailand, Pakistan, Malaysia (for review see Misra 1993), Italy (Schallenberger et al. 1990), Vietnam (Uoc et al. 1992a,b), Philippines (Cruz et al. 1991), Japan (Ocampo et al. 1988), Egypt (Ismail et al. 1993) and China (Wang et al. 1994).

The first non-surgical embryo transfer in buffaloes was performed by a research team at the University of Florida, Gainsville, USA. The team transferred a seven-day buffalo embryo to a recipient, resulting in the first live-born buffalo calf in the world by this method in 1983 (Drost et al. 1983). Drost is also the pioneer in trials on between-species transfer. Under his management, a team transferred a buffalo embryo to a recipient cow, which, however, aborted at 2-3 months of pregnancy (Drost et al. 1986). This between-species experiment was later repeated in India, where Misra et al., Kurup (1988), transferred a cow embryo to a buffalo cow, which, however, aborted after 82 days of pregnancy.

The first non-surgical embryo transfer in
Bulgaria was performed at the end of 1984 and resulted in pregnancy, but the recipient buffalo cow aborted in the third month of pregnancy. In April, 1986, at the Buffalo Research Institute in Shumen, Bulgaria, a large scale Bulgarian-American experiment on embryo transfer in buffaloes was carried out. As a result of this experiment, for the second time in the world and for the first time in Europe, four live born buffalo calves were obtained at the beginning of 1987. In February, 1988, the Bulgarian team obtained three more live born buffalo calves (Alexiev et al. 1988).

The very first experiments showed that the embryo transfer technology applied to cows cannot produce the same results in buffaloes, due to the reproductive peculiarities of the latter species. Following a number of experiments, the Bulgarian and American scientists established that buffalo embryos develop more intensively during the first four days than cattle. Hence, the optimal time for their recovery is between the 5th and the 6th day from the beginning of the true estrus of the donor buffalo cows. Transfer at this time contributes greatly to its success.

Non-surgical transfers are made through the cervix into the uterine horn, using a miniature ET gun. Synchronized recipients are checked for the presence of an active corpus luteum (CL). Epidural anesthesia is induced to minimize straining. The embryo is aspirated into a 0.25 mL French straw in a central column of 20 mm holding medium, between two air pockets (of about 30 and 20 mm). The straw is loaded into the ET gun, and a sheath with a metal tip is fitted over the top. One sanitary sheath is then rolled on top to avoid any contamination from the vaginal microflora. The ET gun is now passed through the vagina to reach the external os uteri. The sanitary sheath is then perforated, and the gun is gently guided through the cervix and uterine body to reach the upper one-third of the uterine horn, ipsilateral to the ovary bearing the CL. The piston of the gun is then pushed gently to deposit the embryo into the uterine horn and the gun is gently withdrawn. In recipients where cranial placement of ET gun is difficult, a rapid placement of the embryo in a more caudal position is preferred.

Factors Influencing Success of Non-Surgical Transfers

There are a number of embryonic, maternal and environmental factors which may affect the pregnancy rates established in recipient cattle after non-surgical transfer (Sreenan and Diskin 1987, Hasler 1992). With heifers, for example, there may be about 10% of potential recipients for which it is difficult, if not impossible, to carry out ET via the cervix (Coultard 1991). The embryo should always be transferred to the uterine horn associated with the ovulating ovary (ipsilateral horn). This is because pregnancy rates are higher when single transfers are made to the ipsilateral rather than the contralateral horn.

This is presumably because maternal recognition of the embryo is more positive when it is in the ipsilateral horn. In Japan, Cerbito et al. (1994) found evidence that the level of progesterone and its distribution in the uterus are dependent on luteal function and corpus luteum location. This may be a factor influencing the survival of the embryo in early pregnancy. In France, seasonal effects were recorded by Lonergan et al. (1995), who found a significant difference in the pregnancy rates from summer transfers (52%) and those carried out in winter (21%).

Importance of embryo quality

The single most important factor affecting the success of transfers is embryo quality, according to Janowitz (1994). He carried out an analysis of 2,478 transfers of fresh or frozen embryos in Germany. Other work in the same country by Piturru (1994) recorded a pregnancy rate of 59% after the transfer of 292 fresh embryos in Piedmont cattle.

Operator skill

One important factor influencing the pregnancy rate is likely to be the skill and experience of the transfer operator, especially in the matter of avoiding trauma to the endometrium. A six-year study by Park et al. (1991) showed that an ET program can be conducted by herdsmen, but only after an appropriate training period. In this regard, it should be remembered that transfer is being carried out at a time when the uterine environment is markedly different from that of a cow in estrus, in terms of its susceptibility to infection and injury. In certain species (e.g. hamsters) it is known that low pregnancy rates after ET are mainly due to trauma of the endometrium and to prostaglandin release (Jarosz and Dukelow 1990).

For cattle ET, the importance of avoiding damage to the endometrium or other trauma to the uterus during the transfer process cannot be overemphasized. According to some reports, embryo survival is significantly higher for transfers into the
middle third of the uterine horn than for transfers to the apical and basal thirds of the horn (Kurykin 1992). Introducing the transfer instrument for some distance up the uterine horn, however, clearly increases the likelihood of such trauma unless the operator takes appropriate care.

The manipulations involved in ET, if not carefully conducted, may create an endometrial inflammatory response, which would imply the migration of macrophages and immunocompetent cells to the inflammatory site. This could result in an environment hostile to the embryo. There have, however, been studies in Sweden which have shown that mechanical manipulation of the cow’s tract during non-surgical ET does not increase plasma prostaglandin level during the hour after transfer (Odensvik et al. 1993). According to Thibier and Nibart (1992), high pregnancy rates occur when transfers take place quickly and smoothly. Trainees may be too slow, as well as being less careful than skilled operators.

It is also important to restrain the recipient animal during the transfer procedure, to avoid any unexpected movements. The recipient is examined for the location of the corpus luteum, and an epidural anesthetic given to eliminate rectal contractions. According to Broadbent et al. (1991), the use of a sedative and epidural anesthesia may not be essential in recipient management, but seems advisable and necessary for the welfare of the animal and for operator comfort. It may also contribute to good pregnancy rates.

Maintaining sterility

The success of non-surgical transfer depends on maintaining adequate sterility during the deposition of the embryo in the uterus. The uterus is much more susceptible to low-grade infection a week after estrus than at the time of heat. More than 40 years ago, Cambridge workers drew attention to the fact that uterine infections could be readily established during the luteal phase of the cow’s estrous cycle, but that they could be controlled using appropriate antibiotic cover. In practicing ET, this means employing trace amounts of certain antibiotics (penicillin/streptomycin) in the transfer medium.

The main factors affecting the success of embryo transfer are listed below:
- Preventing infection;
- Site of transfer;
- Non-surgical technique and skilled operators;
- Quality of heifer or cow recipient and
- Nutritional status of recipient.

**INSERTION TECHNIQUES**

In both recovery and transfer of embryos, the most important step is inserting a device such as a balloon catheter, and transferring the gun to the cervix and uterine horn. Careless use of such devices always injures the cervix and endometrium, and causes bleeding. My final advice to ET techniciens is:
- Don’t move the devices unless you know their location.
- Don’t think you have to insert them into the tract. The tract should be manipulated and adjusted to the devices by your hand in the rectum.

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