

## Commercial Embryo Transfer in the United States

Margo L. Macpherson, DVM, MS, DACT<sup>1</sup>

Mats H.T. Troedsson, DVM, PhD, DACT, DECAR<sup>2</sup>

Patrick M McCue, DVM, PhD, DACT<sup>3</sup>

<sup>1</sup>Dept of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, FL 32610

<sup>2</sup>Dept of Veterinary Science, University of Kentucky, Lexington, KY 40506

<sup>3</sup>Dept of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences,  
Colorado State University, Ft. Collins, CO 80523

### Introduction

The use of embryo transfer (ET) in the equine breeding industry provides a unique opportunity to obtain foals from mares that are in competition, have suffered debilitating injuries or who have reproductive abnormalities. In the United States, the use of ET increased dramatically in 2003 due to changes in breed registry regulations. Currently, Thoroughbreds are one of the few registries that will not accept foals obtained through embryo transfer. Additionally, with more veterinary clinics providing ET services, as well as increasing numbers of ET 'recipient stations' throughout the country, ET has become a readily available reproductive tool. Limitations to the procedure include cost (\$3,000 to \$6,000 per pregnancy) and more intensive management of mares. The horse owner should consider the cost of the procedure relative to the potential profit from the foal as well as time lost from the mare due to pregnancy.

### Embryo Recovery Procedure

Embryo recovery is usually attempted 7 or 8 days post ovulation. Embryos recovered at this time are generally expanded blastocysts which are easily observed under the microscope. Embryos recovered later (i.e. day 9) are often too large to be handled without damaging the embryo.

Techniques for embryo recovery have not changed significantly in recent years. A sterile silicone catheter, with an inflatable cuff, is used to facilitate transcervical lavage of the uterus. The catheter and flush media are attached to a closed system of tubes containing a "y" junction. An in-line filter is either attached to the outflow line or the outflow line is manually regulated at the level of the filter (Figure 1). The uterus of the donor mare is lavaged three to four times with 1-2 liters aliquots of prewarmed (30-35° C) embryo flush medium each time. The amount of fluid used for each flush is dependent on the size of the uterus. The goal is to expand the uterus enough to allow fluid to effectively reach all parts of the uterus, including the area between the uterine folds. The mare often becomes mildly uncomfortable upon stretching of the uterus and associated structures and this can be used to indicate that sufficient infusion of fluid into the uterus has occurred. The flush medium is then allowed to flow back out the catheter by gravity flow through the embryo filter. The uterus of the mare is usually massaged *per rectum* during the infusion and recovery of the final liter of media. Some practitioners believe it is advantageous to leave the final liter of media within the uterine lumen for 3-5 minutes prior to recovering the efflux. Complete recovery of the uterine lavage fluid is monitored by collecting the fluid in a graduated cylinder, and/or ultrasonographic examination of the uterus at the end of the procedure. Additionally, 10-20 units of oxytocin can be administered, IV, prior to recovery of the final liter of fluid to facilitate uterine contraction and emptying.

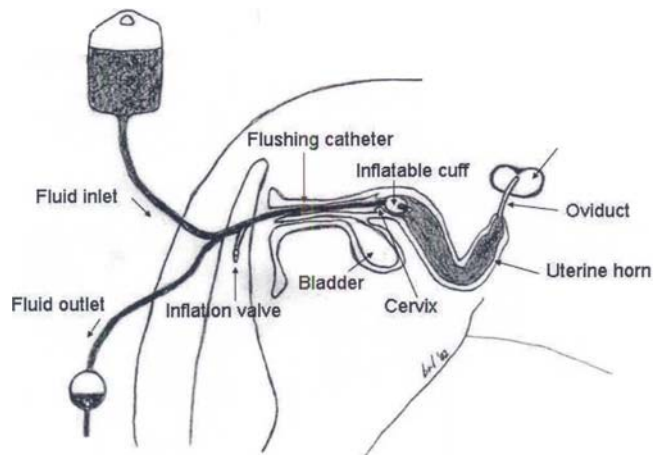


Fig 1: Non-surgical embryo collection procedure. (Illustrated by B. Lindsey).

The search for an embryo may begin after each successive lavage or after the final liter of media is recovered. Filter contents are poured into search dishes and filters are rinsed with flush media. Dishes are then examined for the presence of an embryo using a dissecting microscope. Recovered embryos are 'washed' to remove bacteria and other impurities by transferring them sequentially through several drops of holding medium. The embryo is graded for quality (grade 1-4, excellent to degenerate), and held in this medium until transferred. If an embryo is not recovered following the initial series of lavages, additional media can be infused. Further, if an unfertilized oocyte (UFO) is identified in the flush media (flat, football shape) additional flushes or searching should be performed as UFO's are typically released from the oviduct through signals transmitted by the traveling embryo.

Embryo recovery rate is influenced by many factors, such as age and fertility of the donor mare, quality of the sire's semen, day of recovery, number of ovulations and clinical expertise. A higher percentage of embryos are recovered from mares less than 10 years of age compared to mares less than 15 years of age. Embryo recovery rates appear to be approximately 5-10% below expected pregnancy rates per cycle (Morris and Allen, 2001).

### Recipient Management and Selection

Mares intended to be embryo recipients are usually purchased in the late fall, winter or early spring. A common trend now is to lease recipient mares from a horse broker. Mares not used or not pregnant at the end of the breeding season are returned. Recipient mares range in age from 3 to 10 years. Since owners of donor mares often wish to begin embryo transfers in February or March, ET recipient mares are commonly placed under lights in early December to advance the onset of the first ovulation of the year. A donor embryo can be successfully transferred into a recipient mare that ovulates the day prior to (+ 1), the same day (0) or up to 3 days (-3) after the donor mare. If a limited number of recipients are available, an individual recipient must be synchronized with each donor mare. Most big embryo transfer programs maintain large numbers of recipients and consequently have several mares ovulating on any given day. Synchronization of an individual donor and recipient mare is often requested by owners, but is discouraged. If necessary, it is recommended that 2 to 3 recipients be synchronized along with the donor in order to have at least one recipient mare ovulating during the critical time window. Ideally, the recipient mare would have ovulated 1-2 days after the donor mare.

Success of an embryo transfer program is highly dependent on the quality of recipient mares. Potential recipients are evaluated 5 days after ovulation to determine if they qualify to receive an embryo that cycle. Criteria used for evaluation include palpation and ultrasonography of the reproductive tract *per rectum* and occasionally analysis of blood progesterone concentrations. Mares are examined for tone in the uterus and cervix, presence of a corpus luteum on ultrasonography and an absence of endometrial folds (edema) or free fluid within the uterine lumen. Mares that 'pass' this examination are available for use as recipients for the next 2-3 days. Mares are rejected as potential recipients if poor uterine or cervical tone, a small (or absent) corpus luteum, endometrial edema or uterine fluid are detected.

### Transfer Procedures

Transfer of embryos into synchronized recipient mares can be performed surgically or non surgically. Non-surgical transfer is preferred at most embryo transfer centers. Most clinicians use similar techniques and premedications (if any). Recipient mares are usually administered flunixin meglumine (Banamine, 500 mg or 10 ml) intravenously 5-10 minutes prior to nonsurgical transfer. Additional medications such as antibiotics, and other drugs are administered at some facilities (Foss *et al.*, 1999). Administration of exogenous progesterone to recipient mares following ET is routinely performed at some facilities and not at all at others.

Embryos are typically transferred using a 0.25 to 0.5 ml straw, disposable sterile sheath and embryo transfer gun. Factors affecting transfer success include embryo quality, age of the donor mare, transfer technique, recipient quality and synchrony of the recipient (Squires *et al.*, 1999). As an example, pregnancy rate for Grade 1 embryos transferred surgically at CSU in 1999 was 71.8 %. Pregnancy rate at day 16 for grade 1 or 2 embryos transferred nonsurgically between 2000 and 2003 was 69.8 %.

### **Transported Embryos**

The use of embryo transfer in the US has become more popular as technology that allows for short-term storage and transportation of equine embryos has become available (Carnevale *et al.*, 1987). Cooled-transported embryo programs have made embryo transfer available to horse owners that do not want to ship a donor mare to a referral center. If an embryo is recovered it is packaged in a small (5 ml) plastic tube filled with holding media. The small tube containing the embryo is placed within a larger (50 ml) conical tube filled with either flush or holding media. Both tubes are sealed with parafilm strips and placed into a passive cooling system (Equitainer®, Hamilton-Thome Biosciences, Beverly, MA). In most instances, embryos maintained in holding solutions are shipped by counter-to-counter airline service and the embryo is transferred into a synchronized recipient within 12 to 16 hours of recovery. Alternatively, embryos can be retrieved late in the day and sent overnight using a courier such as FedEx or UPS.

### **Selected References**

Carnevale, E.M, Squires, E.L., McKinnon, A.a. (1987) Comparison of Ham's F-10 with CO<sub>2</sub> or Repes buffer for the 24-hour storage of equine embryos at 5° C. J Anim Sci 65, 1775-1781.

Foss, R., Wirth, N, Schiltz, P., Jones, N (1999) Nonsurgical embryo transfer in a private practice (1998). Am Assoc Equine Pract 45,210-212.

McCue, P.M, Scoggin, CF., Meira, C, Squires, E.L. (2000) Pregnancy rates for equine embryos cooled for 24 hours in Ham's F-10 vs. emcare™ embryo holding solution. Proc Ann Conf Soc for Theriogenology p. 147.

McKinnon, A.G., Squires, E.L., Carnevale, E.M, Hermet, MJ. (1988) Ovariectomized, steroid-treated mares as embryo recipients and as a model to study the role of progestins in pregnancy maintenance. Theriogenology 29, 1055-1063.

Morris, L.H.A., Allen, WR. (2001) Reproductive efficiency of intensively managed Thoroughbred mares in Newmarket. Pferdeheilkunde 17, 548-556.

Moussa, M, Duchamp, G., Mahla, R., Bruyas, J.-F., Daels, P.F. (2002) Comparison of pregnancy rates for equine embryos cooled for 24 h in Ham's F-10 and emcare holding solutions. Theriogenology 58, 755-757.

Squires, E.L, Carnevale, E.M, McCue, P.M, Bruemmer, J.E. (2003). Embryo technologies in the horse. Theriogenology 59, 151-170.

Squires, EL, McCu,e P.M, Vanderwall, D. (1999) The current status of equine embryo transfer. Theriogenology 51,91-104.