

How to Increase Embryo Recovery Rates and Transfer Success

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1. Introduction

Changes in breed registry rules have resulted in an increase in the number of embryo transfers performed in the United States in the past 2 yr. The basic technique used by practitioners to recover embryos from donor mares has remained the same for many years. The standard method of embryo collection in the mare is a nonsurgical transcervical uterine lavage.^{1,2} A sterile catheter with an inflatable cuff is inserted through the cervix, and the cuff is inflated with 60–80 ml of air. The uterus is lavaged three times with 1–1.5 l of prewarmed (30–35°C) embryo flush medium each time. The flush medium is allowed to flow back out the catheter by gravity flow and is passed through an embryo filter. Recovery of the flush medium may be aided by massage of the uterus per rectum, and occasionally, administration of oxytocin (i.e., 20 IU, IV). Recovery of the uterine lavage fluid is monitored by collection of the effluent fluid in graduated cylinders. Contents of the filter are poured into a search dish and examined for the presence of an embryo.

Transfer of equine embryos is generally performed using a nonsurgical transcervical approach. The goals of this paper are to (1) present a modified embryo flush technique³ used in our commercial practice to enhance embryo recovery rates and (2)

confirm a previous report that some equine embryos may fail to leave the tip of the Cassou gun during nonsurgical transfer.

2. Materials and Methods

Embryo recovery attempts were performed on day 7 or the morning of day 8 after ovulation. The materials used to perform an embryo flush included a complete flush media,^a 80 cm of silicone catheter with an inside diameter of 8.0 mm and an inflatable cuff,^b Y-junction tubing,^c and a 75- μ m in-line embryo filter.^d

An initial embryo recovery attempt was performed using standard procedures. Oxytocin was administered only if fluid recovery was inadequate. After a series of three lavages, the filter was searched for the presence of an embryo. The catheter remained in the mare during the search process. If an embryo was recovered, the cuff was deflated, the catheter was removed, and the mare was administered prostaglandins to lyse her corpus luteum and bring her back into heat. However, if an embryo was not recovered after the initial series of three lavages, an additional 1–2 l of media was infused into the uterus, and the mare was administered 20 units of oxytocin IV. The media were allowed to stay in the mare for ~3 min before being

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allowed to exit by gravity flow aided by uterine massage per rectum. A search for the embryo was again performed after the "extra" flush. The embryo collection procedure was usually terminated after one "extra" flush was performed, whether or not an embryo is recovered.

In our practice, embryos <1000 μm are transferred using a 0.25-ml straw inserted into a Cassou gun that is placed within a sterile outer sheath.^e Embryos >1000 μm are transferred in a standard 21-in artificial insemination pipette.^f After transfer, the tip of the sheath or the insemination pipette is always rinsed with flush media, and the media are examined for the presence of the embryo. Recipient mares are administered flunixin meglumine (500 mg IV) and either acepromazine (20 mg IV) or xylazine (150 mg IV) before transfer.

3. Results

In 2002 and 2003, a total of 209 embryo recovery attempts was performed at Colorado State University on client-owned mares. Embryos were recovered on the first set of flushes (three rounds of infusion using a total of 4 l of media) on 31.6% of attempts. A total of 32 embryos was subsequently recovered during the "extra" flush attempt when no embryo was recovered during the first series of flushes. Therefore, the final embryo recovery rate (91/209) was 43.5%.

On 3 of 81 transfer attempts (3.7%) made using the Cassou gun system, the embryo was recovered from the tip of the sheath after transfer. On each occasion, the embryo was reloaded into another straw and transferred back into the same recipient. Two of the three recipient mares became pregnant after the "double transfer."

4. Discussion

The possibility that an embryo may still be present in the uterus after an initial embryo collection attempt is not new. Betteridge et al⁴ noted that pregnancies were established in four mares from which no embryos were recovered during a uterine flush initially performed on days 6.5–9.5 after ovulation. Similarly, McKinnon et al⁵ noted that 9 of 27 mares were determined to be pregnant after a failed embryo recovery attempt 6.5 days after ovulation.

Squires et al⁶ demonstrated that additional flushes per collection attempt increased the probability of recovering an embryo. Extra embryos were collected from several superovulated mares after uterine lavage with an additional 3 l of media (6 l total). In a second experiment, embryos were recovered from 2 of 21 mares (9.5%) from which no embryos were recovered during an initial flush attempt after uterine lavage with an addi-

tional 3 l of Dulbecco's phosphate buffered saline (PBS).⁶

Hinrichs⁷ recorded embryo recovery success after three successive uterine lavages with 1 l of modified Dulbecco's PBS. Of the 21 embryos recovered, 12 (57.1%) were noted after the first flush, 5 (23.8%) were found after the second flush, and 4 (19.0%) were detected after the third flush. It was also reported that embryo collection rates seemed to be enhanced if the flush medium was allowed to remain in the uterus for 3 min before recovery.

Embryo recovery rate is influenced by many other 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.²

The increase in embryo recovery rate provided by the modified recovery procedure is economically very significant in a clinical embryo transfer program. The cost of the additional media, oxytocin, and clinician time is usually minimal compared with the potential value of a recovered embryo.

It is recognized that there are differences in technique during the "extra" flush that were not routinely used in the initial flush procedure. Specifically, during the "extra" flush, the medium was allowed to remain in the uterus for several minutes before being recovered, and oxytocin was always used before the "extra" flush to stimulate uterine contractions, even if fluid recovery during the initial flush attempts was adequate. Additional studies are needed to determine if the increased embryo recovery rate is because of the additional flush, the 3-min waiting period, or the oxytocin.

A recent study compared pregnancy rates after nonsurgical transfer of equine embryos using various transfer devices.⁸ It was noted that equine embryos would occasionally be retained in the tip of the side delivery embryo transfer (ET) sheath. Our observations corroborate those of the earlier report. Consequently, it is suggested that the tip of the transfer sheath be rinsed after transfer to confirm that the embryo was not retained in the device.

In summary, embryo recovery can be enhanced by slight modifications of the standard flush technique, and transfer success can be improved by verifying that the embryo was not retained in the tip of the ET sheath.

References and Footnotes

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