

# Embryo Recovery Procedures and Collection Success: Results of 492 Embryo-Flush Attempts

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Embryo-collection success can be maximized by incorporating sound breeding management and flush techniques. Factors that influence embryo-recovery rate are age and fertility of the donor mare, type and quality of the semen used, and number of ovulations. Authors' address: Equine Reproduction Laboratory, 3194 Rampart Road, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado 80523; e-mail: Patrick.McCue@ColoState.edu. © 2010 AAEP.

## 1. Introduction

Embryo transfer is a common reproductive technique in clinical veterinary practice. Advantages of embryo transfer are that valuable mares may have more than one foal per year, older mares can donate embryos to young recipients, subfertile mares can donate embryos to reproductively healthy mares, and mares in athletic competition can donate embryos while remaining in training. The first foal produced by embryo transfer was born in 1974. Over the subsequent 36 yr, specialized equipment has been developed and practical techniques were adopted to enhance embryo-collection success. The goal of this paper is to review embryo-recovery techniques and provide data on embryo-collection success in a clinical embryo-transfer program.

Embryo recovery is usually attempted 7 or 8 days postovulation.<sup>1,2</sup> Collection of small (i.e., <300  $\mu\text{m}$ ) embryos for cryopreservation necessitates flushing at day 6.5 or early on day 7 after ovulation. A sterile silicone catheter, typically of 8.0-mm internal diameter with an inflatable cuff, is used to facilitate transcervical lavage. A 75- $\mu\text{m}$  embryo filter is

either attached to the outflow line or the outflow line is manually regulated at the level of the filter. A sterile Y-tubing set, with clamps to regulate inflow and outflow, is used to connect the catheter, fluid bag, and embryo filter.

The media used for embryo collection can be a commercially prepared complete flush media that contains a buffer system, antibiotic, and surfactant, or it can be as simple as lactated Ringer's solution with the optional addition of either calf serum or purified bovine serum albumen (BSA) as a surfactant to prevent adherence of the embryo to the tubing, filter, or search dish.

The uterus of the donor mare is usually lavaged three to four times in sequence using approximately 1 l of fluid each time. The media may be prewarmed (i.e., 30–35°C) or used at room temperature. The amount of fluid used for each flush is dependent on the size of the uterus and/or parity of the mare. The goal is to expand the uterine lumen enough to allow fluid to effectively reach all parts of the uterus, including the area between or under the endometrial folds. The flush medium is allowed to

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## NOTES

flow back out of the catheter by gravity flow through the embryo filter. The uterus of the mare may be massaged per rectum during the infusion and recovery of the media. Recovery of the uterine lavage fluid may be monitored by collecting the effluent in a graduated cylinder and/or ultrasonographic examination of the uterus at the end of the procedure.

The search for an embryo may begin after each successive lavage or after the final volume of media is recovered. Contents of the filter are poured into a search dish and examined for the presence of an embryo. If an embryo is not recovered, additional media may be infused into the uterus, and the mare is administered 20 units of oxytocin intravenously to stimulate uterine contractions. The media is allowed to stay in the mare for approximately 3 min before being allowed to exit by gravity flow aided by uterine massage per rectum.<sup>3,4</sup> At the conclusion of the embryo-flush procedure, the donor mare is administered prostaglandins to lyse the corpus luteum.

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.<sup>1,2</sup> Embryo-recovery rate is correlated with age and reproductive status of the donor mare. A higher percentage of embryos are recovered from mares <10 yr of age than from mares >15 yr of age. Embryo-recovery rates seem to be ~5–10% below expected pregnancy rates per cycle.

**2. Materials and Methods**

A retrospective analysis of embryo-recovery success in the clinical embryo-transfer program at Colorado State University was performed. A total of 492 embryo-recovery procedures performed on client mares at Colorado State University between 2004 and 2008 were reviewed. Data were included only if the reproductive management of the donor mare was performed on site. A total of 257 embryos were collected; in a limited number of cases, embryo-quality score or developmental stage were not recorded. Accurate age of the embryo relative to collection day was not known in cases of asynchronous double ovulations in which a single embryo was collected. Embryos were evaluated for quality, size, and stage of development as previously described.<sup>5</sup> Comparisons of recovery rates and embryo size between groups were made using statistical analysis software (SAS).<sup>a</sup> All values are presented as the mean ± SEM.

**3. Results**

Overall, the average number of ovulations per cycle and number of embryos recovered per flush were 1.18 ± 0.02 and 0.52 ± 0.03, respectively. Embryo-recovery rates per flush and per ovulation were 48.1% and 45.2%, respectively. A majority of embryos (97.6%) recovered were of excellent (Grade 1) or good (Grade 2) quality (Table 1). Average size (outer diameter) of the embryo approximately dou-

**Table 1. Embryo Quality (Grade) for 251 Embryos Collected From Donor Mares**

Grade	Comment	Embryos	Percentage (%)
1	Excellent	198	78.9
2	Good	47	18.7
3	Poor	6	2.4
4	Degenerate or dead	0	0

bled with each successive collection day (Table 2). Percentage of embryos in various developmental stages (morula, early blastocyst, blastocyst, and expanded blastocyst) is presented in Table 3.

Donor mare age affected embryo-recovery rate, with one or more embryos recovered from 144 of 252 flushes (57.1%) from mares ≤15 yr of age and 93 of 236 flushes (39.4%) from mares >15 yr of age. Embryos collected from mares ≤5 yr of age tended (p < 0.1) to be larger on a given flush day than embryos collected from mares >5 yr of age. There was no difference in size of embryos collected from mares ≤15 or >15 yr of age.

Mares inseminated with fresh or cooled-transported semen yielded one or more embryos on 51.9% (27/52) and 51.6% (182/353) of cycles, respectively. In contrast, mares bred with frozen semen donated one or more embryos on 33.3% of cycles (26/78). Diameter of embryos recovered on day 7 (n = 114) from mares bred with cooled semen (401.9 ± 19.6 μm) were larger (p < 0.05) than embryos recovered on day 7 from mares (n = 11) bred with frozen semen (258.2 ± 33.3 μm) (Fig. 1). Embryos (n = 24) collected on day 8 from mares bred with cooled semen tended (p = 0.0553) to be larger (716.9 ± 104.9 μm) than embryos (n = 10) collected on day 8 from mares bred with frozen semen (383.5 ± 54.9 μm).

One or more embryos were recovered from 43 of 72 cycles (59.7%) with spontaneous double ovulations and 190 of 406 cycles (46.8%) with single ovulations (p < 0.05). Embryo-recovery rate per ovulation was 46.8% for cycles with single ovulations and 41.1% for cycles with double ovulations. The number of embryos recovered from 34 mares with unilateral double ovulations (0.9 ± 0.8 embryos per flush) was not significantly different (p > 0.05) than the number of embryos recovered from 34 mares with bilateral double ovulations (0.8 ± 0.8 embryos per flush).

Mares with no or minimal fluid in their uterus after breeding yielded an embryo on 158 of 307

**Table 2. Diameter (in Micrometers) of 238 Embryos Relative to Day of the Collection Procedure**

Collection Day	Embryos	Mean ± SD (μm)	Range (μm)
6	20	191.8 ± 13.2	150–325
7	183	354.0 ± 13.9	150–900
8	35	623.9 ± 72.9	150–2,500

**Table 3. Stage of Embryonic Development for 256 Embryos Collected From Donor Mares**

Stage	Embryos	Percentage	Mean ± SD (μm)	Range (μm)
Morula	31	12.2	156.7 ± 3.0	125–200
Early blastocyst	66	25.9	188.5 ± 3.9	150–225
Blastocyst	44	17.3	295.9 ± 6.3	200–400
Expanded blastocyst	115	45.1	598.0 ± 22.5	250–2,500

flushes (51.5%), whereas embryos were recovered on 78 of 176 cycles (44.3%) in which mild to moderate fluid was present after breeding ( $p = 0.10$ ). Mares in the latter category were typically managed by a combination of uterine lavage and ecbolic agents (i.e., oxytocin or prostaglandins). Minimal fluid was defined as <1 cm in depth. Moderate fluid was defined as >1 cm in depth.

The quality or character of the media recovered was associated with success of embryo recovery. Mares with minimal debris in the uterine effluent yielded an embryo on 235 of 462 flushes (50.9%), whereas embryos were only recovered from 2 of 21 flushes (9.5%) when the media recovered were cloudy or contained an abnormally large amount of debris ( $p < 0.001$ ).

In 31 instances, a donor mare was flushed again the day after a negative embryo-recovery procedure. Two liters of flush media were infused into the uterus and the mare was administered 20 units of oxytocin. The fluid was allowed to remain in the uterus for 3 minutes before being evacuated through an embryo filter. Embryos were recovered on 3 of 31 attempts (9.7%). An increase in cloudiness or debris in the recovered media was noted in a majority of the next day reflushes.

Mares were commonly flushed more than one time per season. The percentage of embryo-recovery attempts yielding one or more embryos tended to be higher ( $p = 0.1009$ ) for mares flushed from one to four times per season (217/439; 49.4%) than for

mares flushed five or more times per season (20/53; 37.7%) (Table 4).

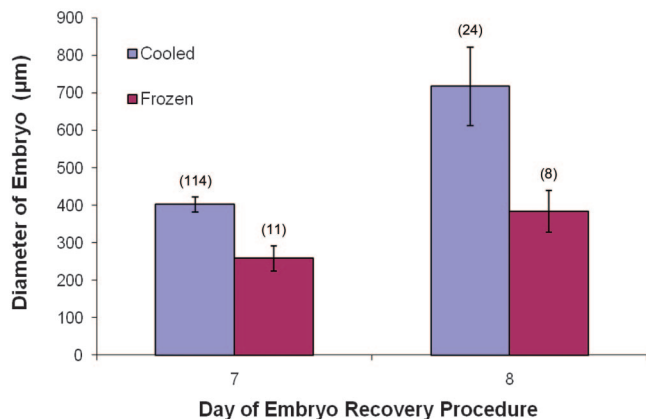
The presence of an unfertilized oocyte (UFO) in the recovered media was recorded in 3 of 495 flush procedures. An embryo was recovered concurrently with the UFO during one of three flushes. In some cases, recovery of an UFO may not have been recorded.

**4. Discussion**

A majority of embryos collected from mares are good to excellent in quality as a result of selective transport of viable embryos through the oviduct facilitated by secretion of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) by the embryo.<sup>6</sup> Poor-quality embryos, dead embryos, and unfertilized oocytes are likely retained in the oviduct.

Age and reproductive health of the donor mare have a significant influence on success of embryo recovery. Embryo-collection rates were highest from mares <15 yr of age, mares that did not accumulate fluid in their uterus after insemination, and cycles in which there was limited or no debris in the uterine flush. We had anticipated that there would be a relationship between mare age and embryo size, with older mares yielding smaller embryos than younger mares. However, there was no significant difference in diameter of embryos collected on either day 7 or day 8 from mares <15 yr of age and mares >15 yr of age.

Semen type influenced embryo-recovery rate and the average diameter of embryos collected on a given flush day. Embryo-recovery rates for mares bred with frozen semen were lower than rates for mares inseminated with fresh or cooled-transported semen. Mares inseminated with frozen semen yielded smaller embryos on each collection day versus mares inseminated with fresh or cooled-trans-



**Fig. 1.** Average diameter of embryos collected on day 7 or 8 after ovulation from mares inseminated with cooled-transported semen or frozen semen.

**Table 4. Relationship Between the Number of Embryo-Flush Attempts per Year and the Percentage of Flushes in Which One or More Embryos Were Recovered**

Number of Flushes per Year	Total Number of Positive Flushes	Total Number of Cycles	Percentage of Positive Flushes
1	83	174	47.7
2	64	132	48.5
3	47	89	52.8
4	23	44	52.3
5	9	24	37.5
6	4	13	30.8
≥7	7	16	43.8

ported semen. It is hypothesized that this may be because of a delay in fertilization or a delay in early embryonic development associated with the use of frozen semen.

An increase in ovulation rate is generally associated with an increase in embryo-recovery rate,<sup>7,8</sup> and this was observed in our results as well. In the current study, there was no difference in embryo-recovery rate for mares with unilateral or bilateral double ovulations. This is in contrast to a larger study in Argentina in which embryo-recovery rates were higher in mares with bilateral double ovulations than mares with unilateral double ovulations.<sup>9</sup>

Practitioners should understand that an embryo may still be present in the uterus or oviduct after a negative flush attempt. Betteridge et al.<sup>10</sup> reported 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 postovulation. Similarly, McKinnon et al.<sup>11</sup> noted that 9 of 27 mares were determined to be pregnant after a failed embryo-recovery attempt 6.5 days after ovulation. Consequently, it is recommended that prostaglandins be administered at the conclusion of an embryo-recovery procedure to cause regression of the corpus luteum, allow the mare to come back into heat, and insure that she does not remain pregnant in the event that an embryo is not recovered.

In our clinical practice, if an embryo is not recovered after an initial series of uterine lavages, an additional flush procedure is immediately performed. The extra flush procedure has markedly increased our overall embryo-recovery rate.<sup>4</sup> It is not known if the primary reason for recovery of an embryo during the extra flush is the oxytocin administered, the 3 min of uterine dilation with flush medium, or simply, one more round of fluid infusion and recovery. It is not typical for our program to reflush a mare the day after a negative embryo-recovery attempt. However, we have performed multiple procedures over the years in a final desperate attempt to recover an embryo. Interestingly, we were successful in recovering an embryo on 9.7% of such attempts. It was common for the media to be mildly cloudy or contain cellular debris on the next day reflush.

The percentage of positive flushes was higher during the first four attempts of a breeding season than on subsequent attempts. This may be related to the fact that some mares in our program were considered subfertile, and it required more estrous cycles to recover an embryo. However, it may also be because of changes in uterine health after multiple cycles of insemination and flushing. A previous study reported that mares subjected to repeated cycles of insemination and embryo collection have chronic inflammatory changes present in their uterus.<sup>12</sup>

A UFO is generally retained within the oviduct near the ampulla-isthmus junction.<sup>6,13,14</sup> Recovery of an UFO during an embryo-flush procedure usu-

ally only occurs if the UFO transported through the utero-tubular junction along with a viable embryo. If an UFO is recovered in the absence of an embryo, an additional flush procedure should be performed in an attempt to recover an embryo.<sup>5</sup>

In summary, embryo-collection rates were highest in young mares and mares that did not accumulate uterine fluid after insemination. An increase in ovulation rate was associated with an increase in embryo recovery, but the site of the multiple ovulations (unilateral versus bilateral) did not affect embryo recovery. Additional embryos were recovered when another flush procedure was performed immediately after a negative flush or the next day.

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