

Nonsurgical Embryo Transfer in a Private Practice (1998)

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Nonsurgical transfer of equine embryos can achieve success rates similar to surgical transfer. Preparation of recipients that includes medication to decrease uterine motility may be beneficial. Author's address: Equine Medical Services, Inc., 5851 Deer Park Rd., Columbia, MO 65201. © 1999 AAEP.

1. Introduction

Embryo transfer has become an important reproductive tool in many equine breeding programs throughout the world today. It is commonly utilized to produce multiple foals in a season from a particular mare, to produce foals from mares involved in some other activity such as showing or racing, and to produce foals from mares otherwise unable to successfully carry a foal to term. Surgical embryo transfer via flank laparotomy has been accepted as providing higher pregnancy rates than other methods, with an anticipated 75–80% of transfers resulting in pregnancies.¹ The time, expense, and personnel necessary for surgical transfer have led to increasing interest in nonsurgical transcervical embryo transfer in recent years. Improvements in technique combined with operator experience have yielded sometimes inconsistent results, with pregnancy rates of 50–75%, approaching those of surgical transfer.² This paper will report the results of a nonsurgical technique used in a commercial embryo-transfer program during the 1998 breeding season.

2. Materials and Methods

Fifty-five mares of various breeds were presented by clients for embryo recovery and transfer. Fifteen of

these mares had no history of reproductive problems and were presented simply to produce multiple pregnancies; 10 show mares were presented to produce foals while still showing; and 30 mares were admitted with a history of infertility. All mares were bred via artificial insemination, 5 with fresh semen, and the remainder with transported fresh-cooled or frozen semen. Embryo-collection flushes were performed 7 days after ovulation with a 30F silicone balloon catheter. The uterus was flushed with three successive liters of Dulbecco's phosphate-buffered saline (PBS) with 1% fetal calf serum containing 100 U/ml of sodium penicillin and 100 µg/ml of streptomycin sulfate. After initial infusion, each liter was drained from the uterus through a 75-µm embryo-recovery filter.^a The uterus was manipulated per rectum to maximize fluid contact with the endometrium and fluid recovery. Larger volumes of PBS (1.5–2 liters per infusion) were utilized in mares that had particularly large uteri. Each mare was administered a luteolytic dose of prostaglandin at the completion of the flushing procedure. The contents of the filter were emptied into a gridded petri dish, and the filter rinsed with 35 ml of fluid with the same composition as the PBS

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mixture used for embryo recovery. The petri dish was searched for embryos under 10–40× magnification using a dissecting stereomicroscope. Recovered embryos were rinsed in PBS containing 10% fetal calf serum with 1000 U/ml sodium penicillin and 1000 µg/ml streptomycin sulfate; they were then loaded into a 0.5-ml wick and powder semen straw, with the embryo surrounded by fluid and air dams. The straw was loaded into a 21- or 25-inch disposable cassou-type embryo-transfer gun^b for transfer to a recipient mare.

The recipient herd consisted of mares from three to ten years of age representing many breeds, but predominantly of quarter horse type. The recipients were selected for inclusion in the program on the basis of age, size, manageability, and continuing normal results of daily ultrasound examinations. Uterine cultures and biopsies were performed only if findings of history or ultrasound examination were abnormal. A recipient mare that ovulated after a normal cycle from 1 day before the donor (+1 degree of synchrony) to 5 days after (–5 degrees of synchrony) the donor would be selected for transfer. At the time of transfer, recipient mares were restrained in stocks, tranquilized with 300 mg xylazine, and given 15 mg of terbutaline sulfate, 500 mg of flunixin meglumine, 40 mg of dexamethasone sodium phosphate, and 1 g of gentamicin sulfate IV. Ten million units of procaine penicillin G were administered IM. The tail was restrained and the perineum scrubbed with a solution of 0.4% chlorhexidine and 1% isopropyl alcohol. The embryo-transfer gun was guarded with the sleeved hand (no sterile chemise was used) and passed into the anterior vaginal vault. The cervix was not dilated with the operators finger, but was aligned axially with the mare by grasping it with the second, third, and fourth fingers. The embryo-transfer gun was advanced carefully while the cervix was gently manipulated to facilitate advance of the gun. When the gun could be advanced no farther, the operator's sleeved hand was removed from the vagina, and the uterus was gently manipulated rectally to allow the gun to be advanced well into either uterine horn. After transfer, the recipient mares were examined with ultrasound for pregnancy on day 12 (5 days after transfer), day 14, day 30, and immediately before being shipped to the client.

In addition, 25 cooled embryos collected by referring veterinarians were shipped to the facility for transfer. Embryos were shipped cooled via air courier in Ham's F-10 gassed with 5% carbon dioxide and supplemented with 10% fetal calf serum with 1000 U/ml sodium penicillin and 1000 µg/ml streptomycin sulfate. All embryos were transferred less than 18 h after recovery. Recipient selection criteria and embryo-transfer techniques were unchanged.

3. Results

Overall embryo recovery rate was 44.5%, or 98 embryos recovered from 220 flushes. Show mares

had the highest recovery rate, while mares presented for infertility had the lowest rate (Table 1). Pregnancies were established from 82.9% of the transferred embryos (102/123). No significant ($p \geq 0.05$) difference was observed in pregnancy rate for transported embryos versus embryos harvested on site. Grade 1 or 2 embryos had significantly ($p \leq 0.05$) higher pregnancy rates than grade 3 embryos (Table 2). Neither degree of recipient mare synchrony (Table 3) nor embryo morphological stage (Table 4) was significantly associated with pregnancy rates ($p \geq 0.05$). Total pregnancy loss was 12.7%, with the greatest losses occurring before 30 days of gestation. Pregnancy loss was more common in grade 3 embryos (Table 5).

Several adverse effects of the use of IV terbutaline were noted. Heart rate quickly increased to approximately 90 beats/min in treated recipients. Most mares exhibited a dry cough and sweating was increased over that normally seen in xylazine tranquilized mares. Adverse effects lasted 20–30 min and did not require additional treatment.

4. Discussion

Several reasons for decreased pregnancy rates frequently seen in nonsurgical versus surgical transfer have been suggested. These include local inflammatory response, embryo expulsion, pipette-induced trauma, site of deposition, and embryo damage during transfer.³ Distinct operator-related differences have been noted as well as the benefit of operator experience.⁴ The pregnancy rates reported in this clinical study suggest that nonsurgical

Table 1. Embryo Recovery Rate

	Embryos/Attempt	% Recovery
Mares presented for		
Show	16/19	84.2 ^a
Multiple Foals	43/72	59.7 ^{a,b}
Infertility	39/130	30 ^b
Overall recovery	98/220	44.5 ^{a,b}

^{a,b}Recovery rates designated by different superscripts differ significantly ($p \leq 0.05$).

Table 2. Transfer Success Rates

	Pregnancies/ Transfers	Percentage Pregnant
In-clinic		
Grades 1 & 2	77/89	86.5 ^a
Grade 3	4/9	44.4 ^b
Total in-clinic	81/98	82.6 ^{a,b}
Transported Embryos		
Grades 1 & 2	19/22	86.3 ^c
Grade 3	2/3	66.7 ^c
Total, Grades 1 & 2	96/111	86.5 ^d
Total, Grade 3	6/12	50 ^e
Overall Total	102/123	82.9 ^{d,e}

^{a-e}Transfer success rates designated by different superscripts differ significantly ($p \leq 0.05$).

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Table 3. Effect of Synchrony, Grade 1 & 2 Embryos

Degree of Synchrony	Pregnancies/Transfers	Percentage Pregnant
+1	13/15	86.6
0	26/30	86.6
-1	12/14	85.7
-2	18/19	94.7
-3	17/18	94.4
-4	5/9	55.6
-5	5/6	83.3

Table 4. Morphology of Grade 1 and 2 Embryos

Morphology	Pregnancies/Transfers	Percentage Pregnant
Morula	8/10	80
Early blastocyst	17/18	94.4
Expanded blastocyst	61/72	84.7
Large expanded blastocyst	10/11	90.9

Table 5. Pregnancy Losses

Gestational Age	Embryo Grade	Losses/Pregnancies	Percentage Lost
<30 days	1 & 2	7/96	7.29 ^a
	3	3/6	50 ^b
>30 days	1 & 2	3/89	3.4
	3	0	0
Total	1 & 2	10/96	10.4 ^c
	3	3/6	50 ^d
All embryos	1, 2, & 3	13/102	12.7

^{a-d}Pregnancy loss rates designated by different superscripts differ significantly ($p \leq 0.05$).

embryo transfer can result in pregnancy rates comparable or superior to surgical transfer. Increased uterine contraction rates in women receiving embryos have been linked to decreased pregnancy rates when compared with women with fewer uterine

contractions.⁵ The successful use of the tocolytic agent terbutaline suggests stimulation of uterine contractions may be detrimental to retention of the embryo in the uterus. Initiation of contractions by manipulating the cervix of the recipient mare and stimulation of the uterus itself with the embryo-transfer gun may be responsible for the active expulsion of the transferred embryo. Further research will be necessary to determine the true effect, if any, of uterine contractions in embryo transfer, as well as the effect of tocolytic agents such as terbutaline.

A wider range of degree of synchrony between donor and recipient ovulation days was utilized in this protocol than most programs.⁶ Synchrony of +1 to -3 is most typically used, but in this group synchrony up to -5 provided acceptable rates. The flexibility provided by this larger window of synchrony made selection of an appropriate recipient easier.

References and Footnotes

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^aLow Volume ET Filter, Veterinary Concepts, 303 South McKay Ave., Spring Valley, WI 54767.

^bDisposable Implant Gun, Veterinary Concepts, 303 South McKay Ave., Spring Valley, WI 54767.