

Transcervical embryo transfer in performance mares

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Summary: Pregnancy was established by transcervical transfer of embryos from performance mares into recipient mares. Estrus was synchronized between donor (n = 17) and recipient (n = 4.3) mares. After a ≥ 25 -mm follicle was detected, donor mares were bred artificially daily until ovulation. Day of ovulation was recorded. Uterine flushes (n = 111) were performed on donor mares 7 days after ovulation, and recovered embryos were transferred transcervically to recipient mares within 2 hours. Embryos were recovered from 40.5% of uterine flushes. Of transferred single embryos, 65.7% resulted in pregnancy, detectable by ultrasonographic examination 23 days after transfer. Only 35.3% of twin embryos resulted in pregnancy. Results over a 4-year period were as follows: uteri were flushed on 14, 44, 31 and 22 occasions, and 8, 21, 15, and 11 embryos were recovered (1 embryo was not transferred), with 6, 11, 4, and 6 resulting in 30-day pregnancy in years 1 to 4, respectively.

Equine embryos can be transferred transcervically into synchronized recipient mares. Studies from research facilities¹ and embryo transfer laboratories² report success, for example, 67%.² Success rate depends on factors such as reproductive status of donor and recipient mares (e.g., maiden vs. postpartum),² site of embryo deposition,³ skill of a technician, time of year, age of embryo, and degree of synchrony of donor and recipient mares.⁴

Horses are seasonally polyestrous breeders for which the optimal natural breeding season in the northern hemisphere is April through August. Unfortunately, horses involved in competition may not be able to reside at a breeding farm during the natural breeding season. Show and training schedules may restrict equine participants of an embryo transfer program to irregular visits to a fertility clinic. The purpose of the study reported here was to describe an embryo transfer program conducted at a fertility clinic that deals with a wide range of reproduction activities involving actively competing horses.

Materials and methods

Mares—Donor (n = 17) and prospective recipient (n = 43) mares were examined for breeding soundness. External genitalia were examined visually, and internal genitalia were palpated per rectum. A swab sample of the endometrium was collected and cultured aerobically. The cervix was evaluated digitally, and an endometrial biopsy specimen was obtained and evaluated.⁵ Before they were bred, 3 donor mares with endometritis were treated with antimicrobial uterine injections appropriate for the organisms recovered on culture.

Donor mares were nulliparous, 2 to 16 years old, weighed 500 to 800 kg. And were of Thoroughbred or European warmblood breeding. Most mares participated in horse shows on a regular basis, which entailed daily training and frequent transport. While at the breeding center, donor mares were stabled indoors with 1 to 2 hours' daily paddock exercise and were given sweet feed and mixed grass hay.

Recipient mares (n = 43) were 2 to 10 years old (mean, 5.3 years), weighed 450 to 550 kg., and were Standardbred, Thoroughbred, Quarter Horse, or crossbred. These mares had no history of reproductive problems or were maiden mares (n = 35). Maiden mares were preferred because they generally had a long, right, functionally competent cervix. Examination of biopsy specimens revealed that all recipient mares had a category I or I-to-II endometrium.⁵ Recipient mares were easy to handle and fared well in a herd situation. Recipient mares were maintained on pasture at the breeding center and were given sweet feed and mixed grass hay.

Synchronization of ovulation and breeding—All procedures were performed between Apr. 1 and Dec. 1 over a 4-year period. Years 1 to 4 involved 6 mares and 15 flushes, 8 mares and 43 flushes, 9 mares and 31 flushes, and 11 mares and 22 flushes, respectively. Ovulation was synchronized between donor and recipient mares by daily IM injection of 150 mg. of progesterone and 10 mg. of estradiol for 10 days. In addition, 10 to 15 mg. of prostaglandin (PG)F_{2a} was administered IM on day 10.⁶ Ovulation in the recipient mare was usually synchronized to occur 2 days after ovulation in the donor mare. Prostaglandin F_{2a} or human chorionic gonadotropin (3,000 IU) was also administered IM to hasten time to ovulation if needed. Ovulation was scheduled so that the stallion and mare would be available for breeding and/or subsequent uterine flush. Because of show conflicts, stallions sometimes were not available at the optimal time for breeding.

Donor mares were brought to the same location as the recipient mares at various times depending on the show schedule and were teased daily in their box stalls. Recipient mares also were teased daily as a group with a teaser stallion across a fence. Ovaries, cervix, and uterus of both groups of mares were palpated and examined by ultrasonography per rectum daily during estrus (2 to 8 days: Fig 1). After a follicle ≥ 2.5 mm was detected, each donor mare was bred artificially daily until ovulation.

Stallions (n - 4) included 3 Dutch warmbloods and 1 Thoroughbred, ranged in age from 4 to 10 years, and weighed 500 to 600 kg. Except for stallion 4 that had low fertility, stallions were managed routinely.⁸ Semen was extended with a nonfat dry, skim-milk, glucose extender containing penicillin and streptomycin or gentamicin.⁸ Mares were inseminated within 1 hour of semen collection.

The day that ovulation was first detected was designated day 0. In mares that ovulated 2 follicles 1 day apart, the day of the earlier ovulation was designated day 0. If the second ovulation was detected 2 days after the first, day 0 was considered to be the day between the 2 ovulations. The goal was to flush the uterus to obtain 6- to 8-day embryos.

Embryo recovery—On day 7, the donor mare was restrained in stocks, and aseptic techniques and nonspERMICIDAL, water-soluble lubricant were used for flushing the uterus of the donor and for embryo transfer.

A 30-F extended Foley or a modified^a uterine catheter was placed into the uterine body and its cuff was inflated with 30 ml of air. Phosphate-buffered saline solution (warmed to 37C) was used as the flush medium and was prepared using double-distilled and deionized water with 1% newborn calf serum, 60 IU of crystalline penicillin, and 60 ug of crystalline dihydrostreptomycin/ml. Each lavage involved infusing 1 L of flush medium into the uterus by gravity flow and by collecting the fluid into a 1-L graduated cylinder by gravity flow. The operator's hand remained in the vagina, and the uterus was not massaged per rectum. Lavage was performed 3 times. If most of the lavage fluid was not recovered, 20 IU of oxytocin was administered IV, resulting in expulsion of remaining fluid in 1 to 5 minutes. After the third lavage, the donor mare was given PGF_{2a}(0.022 mg/kg of body weight, IM).

The graduated cylinder containing the flush medium was maintained at 23 C for 20 to 30 minutes to permit the embryo to sink to the bottom. The top portion of the flush medium was siphoned through an embryo-collecting filter,^b leaving 50 ml of fluid in the cylinder. This 50 ml was poured into a sterile disposable 100-mm x 15-mm petri dish. The cylinder was rinsed with an additional 50 ml of flush medium that also was placed in a petri dish. The fluid was inspected under a dissecting microscope for the presence of an embryo. All petri dishes were examined twice regardless of whether an embryo had already been located. After the embryo was located, it was washed by successively transferring it into 3 petri dishes of microfiltered^c (0.2um) flush medium modified to contain 20% heat-inactivated newborn calf serum.

Embryo transfer—The embryo was transferred into a recipient mare that had ovulated 3 to 10 days before the day of transfer. A 53-mm bovine infusion pipette^d and 10-ml syringe^e were used to transfer the embryo. Syringes^f (3 ml) were used for the first 8 transfers. Using aseptic conditions, the pipette was loaded in the following sequence⁷: 0.2 ml of penicillin (60 U/ml) and streptomycin (60 ug/ml), 0.2 ml of air, 0.2 ml of medium, 0.2 ml of air, 0.2 ml of medium containing the embryo, 0.2 ml of air, and 0.2 ml of medium. Antimicrobial drugs were omitted from the medium in the last 26 transfers because effects on sperm motility, using these antimicrobial agents in proper concentration in semen extender, had been detrimental. The pipette was passed alongside the index finger in the cervix and its tip was placed into the uterine body. The index finger was withdrawn from the cervix and was used to hold the pipette in the uterine body position. Contents of the pipette were injected into the uterus, and the pipette was withdrawn while the operator maintained pressure on the syringe plunger. Each recipient was examined ultrasonographically for an embryonic vesicle 6 days after transfer and after 3 to 4 weeks.

Results

Only 1 of 8 mares bred to stallion 4 produced an embryo at the time of uterine lavage during the first year. Because of low numbers of progressively motile, morphologically normal sperm, stallion 4 was limited to breeding no more than 2 mares/synchronized ovulation. Semen was collected from stallion 4 daily for several days before mares were bred to ensure he was not ejaculating stored sperm. To determine the best extender regimen for this stallion, several semen extenders of various sperm-to-extender ratios were evaluated. Gentamicin and nonfat, dry, skim-milk glucose extender (50×10^6 sperm/ml dilution) were used for insemination.

Double ovulations were detected in 40 to 111 estrous cycles of donor mares. In 3 of 111 cycles, triple ovulations were detected. Of 111 uterine flushes, 45 yielded embryos (40.5% successful flush rate). Of 45 successful flushes, 35 yielded single embryos and 10 yielded twin embryos. All twins were from detected double or triple ovulations. Seven flushes yielded unfertilized oocytes, 3 of which were detected with an embryo. Of the embryos, 44% were located in the first flush and 27 and 20% were located, respectively, in the second and third flushes. Over the 4-year period, the uteri of 5 mares were classified as infected when the uterine flush yielded a cloudy fluid. Infection was detected in 4 mares on 2 occasions each and 1 mare on 5 occasions. The last mare, although infected 5 of 22 cycles bred, still produced 8 embryos, of which 2 resulted in live foals. Swab samples from the uteri of these 5 mares revealed a positive bacteriologic culture. The overall treatment approach to infected mares was conservative, with treatment consisting of 3 days of uterine infusion with an appropriate antimicrobial drug and/or shortening of the estrous cycle with PGF_{2a} administration.

Recipient mares ovulated 3 to 10 days (average, 6 days) before the day of transfer. Pregnancy rates, according to transfer on days after ovulation, were as follows: day 3, 100%; day 4, 50%; day 5, 71%; day 6, 67%; and day 7, 40%. Of 7 mares given embryos \geq 8 days after ovulation, only 1 produced a live foal. Of 35 single embryos transferred, 23 (65.7%) were detected on ultrasonographic examination before 23 days of gestation. One single embryo was resorbed between 23 and 28 days. This embryo had been recovered from a mare with a B-hemolytic streptococcal uterine infection and had been classified as abnormal at the time of transfer. The embryo's zona pellucida was irregular, blastomeres were shrunken away from the zona, and debris was in the perivitelline space.

The progesterone estradiol synchronization regimen was used to synchronize 123 estrous cycles, of which 105 (85%) resulted in ovulation 9 to 13 days after the PGF_{2a} injection. Of synchronized mares, 65% ovulated 10 to 12 days after treatment with PGF_{2a}. Only 6 (5%) mares failed to ovulate in response to treatment.

Of 20 twin embryos recovered, 17 were transferred individually. Of the 17, 6 (35.3%) were seen as vesicles on ultrasonographic examination before 30 days of gestation. Two of these vesicles disappeared, one between days 15 and 27 and one between days 25 and 33. One embryo of a set of twin embryos could not be transferred because a suitable recipient was not available. One pair of twin embryos was transferred simultaneously into one mare because a second recipient was not available. This twin transfer resulted in a single pregnancy.

Length of gestation, defined as the time between ovulation and parturition, ranged from 316 to 354 days. Gestation was < 325 days for 10 of 27 foals born. Of these 10 foals, 9 were born after May 1. Despite efforts at resuscitation, one foal died at birth after 322 days' gestation. One mare aborted at 218 days.

Discussion

The overall embryo recovery rate of 49.5% for all flushes was lower than that reported by others.^{1,7} The recovery rate for healthy mares was 52.1%, whereas that for mares with endometritis was 35.3%. Recovery rate from mares with reproductive problems has been lower than that of healthy mares when embryos were collected 7 to 10 days after ovulation.^{1,7,9} The principal stallions 1 to 3 resulted in 42.6, 31.6, and 43.5% of the embryos. Stallion 4 with low fertility, when managed intensively, achieved an embryo recovery rate in mares he bred comparable with that of the 3 other stallions.

Multiple ovulations are more frequent in Thoroughbreds (19%) than in Quarter Horses (9%) or Appaloosas (8%).¹⁰ Maiden Standardbred and Thoroughbred mares had a 22.4% prevalence of multiple ovulations.¹¹ European warmbloods had a much higher percentage of multiple ovulations (55.9%) than did traditional American breeds.¹² The donor mare group was predominantly European warmbloods with a few Thoroughbreds, and multiple ovulations were detected in 43 of the 111 (38.7%) donor mare cycles. Many ovulations took place after human chorionic gonadotropin stimulation, and this treatment may have had an effect on ovulation rate.⁶ Ultrasonographic examination for the detection of ovulations permits greater accuracy in detecting adjacent follicles than does manual palpation alone.

The incidence of twins recovered was 10 of 111 flushes (9%). Ultrasonographic examination 12 to 20 days after breeding revealed twin vesicles in 13.9% of Thoroughbred and 5.2% of Standardbred mares.¹³ These values are higher than twin foaling and twin abortion rates, probably as a result of the embryonic loss associated with twin pregnancies. All twin embryos recovered were from the mares with detected multiple ovulations, thereby providing supportive evidence that equine twins are usually dizygotic.¹⁴

The present study finding that twin embryos did not develop into 2 live foals is in accordance with the observations that the pregnancy rate per embryo for embryos collected from multiple ovulating mares was less than that for single-ovulating mares.^{4,15} One twin embryo in each of 4 of 5 sets was abnormal.¹⁶ However, pregnancy rates from single or multiple embryos transferred individually into recipients were not different.¹²

In this and another study,¹⁷ most embryos were recovered from the first liter of flush medium, with progressively fewer embryos recovered from second and third flushes. However, a fourth flush may still be justifiable, because 20% of the embryos were recovered from third flushes.

Recipient mares that ovulated 2 days after the donor and that received an embryo 5 days after their own ovulation were most successful in developing pregnancy. Pregnancy rates decreased if donor mare and recipient mare did not ovulate synchronously.

Embryo transfer foals may have a shorter than normal gestation length. Although many foals were born at the shorter end of the gestation range, they were still considered normal for the month of

parturition.¹⁸ The relative sizes of the recipient and donor mares may have a role in influencing gestation length. Genetic parents of the embryos were larger than those of recipients. The uterine capacity for fetal support may have been exceeded in the recipients, resulting in slightly earlier delivery.