Management of Embryo Donor Mares With Chronic Infertility

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1. Introduction
Embryo transfer has been used as a management procedure to increase foal production in reproducitively healthy mares, obtain foals from young mares still in sporting competition, and obtain foals from mares unable to carry a foal to term for a variety of reasons such as age, chronic uterine infection, cervical damage, etc. Some uncommon reasons for using embryo transfer to preserve foal production in mares may include chronic laminitis, severe arthritis, chronic, unresolved colic, or dangerous behavioral problems of the donor mare toward handlers, other horses, or her foals. This paper will present selected cases of chronic infertility in which embryo transfer was the treatment of choice to obtain foals.

2. Partial Ovariohysterectomy
A 12-yr-old Standardbred mare was referred for embryo transfer after a unilateral ovariectomy caused by a granulosa cell tumor. On initial palpation and ultrasound examination, the right ovary was absent, and clear fluid was present in the left horn. The cervix was elongated and fibrous. After additional examinations, the right uterine horn was also noted to be “missing.” Only a small 2.5-cm segment (20%) of the right horn was present at the uterine bifurcation. After additional searching to obtain the reproductive history on the mare, it was discovered that the right uterine horn was removed at the time of ovariectomy because of a tumor-like condition involving the right horn.

Over the next 5 consecutive yr, embryo transfer was performed after artificial insemination of the mare. Uterine fluid accumulation was a frequent finding before and after insemination. Initially, endometrial cultures were negative for bacteria and yeast. However, each year that the mare was bred and embryo transfer procedures performed, the incidence of positive cultures and the amount and persistence of uterine fluid increased. Frequently during estrus, the endometrium developed a cobweb, lacy appearance. During years 3 and 4, adhesions of the internal and external cervical os developed. Transluminal uterine adhesions were also suspected, although hysteroscopic examination of the uterus was not performed. Treatment of this mare’s uterine and cervical problems included manual dilation of the cervix, intrauterine antibiotic treatments, therapeutic uterine lavages, and repeated oxytocin treatments before and after ovulation. Therapeutic uterine lavages performed before breeding and ovulation were done using 1 l of lactated Ringers solution 1–2 days before breeding and/or the day after breeding. Oxytocin was ad-
ministered IM before and after breeding when anechoic fluid was present. Oxytocin was given one to three times per day at an interval of 1–2 h using 20 IU. Because of the fibrous nature of the mare's cervix, oxytocin treatments were not always effective in clearing the uterine fluid. The mare was flushed a total of 11 cycles over the 5-yr period. Seven embryos were collected, which resulted in the birth of four live foals.

Mares that are missing a uterine horn because of congenital anomaly, surgical removal, or occluding luminal adhesions of a uterine horn are unable to carry a foal to term or, with the aid of altrenogest or progesterone, may carry a foal to term but deliver a very undersized foal.

3. Repeated Late-Term Abortion
A Standardbred mare delivered a live foal in each of 3 consecutive yr. In the following 4 yr, the mare aborted between 7 and 10 mo of gestation, despite being supplemented with altrenogest for 3 of the 4 yr. On change of ownership, the mare was presented for embryo transfer to obtain live foals.

The mare was put under artificial lighting in December. After the mare began cycling, a reproductive examination was performed. The uterus was astatic, normal in size, and contained no intraluminal fluid. The uterus continued to have an astatic consistency over the next 6 yr. Endometrial culture was negative at first examination. The endometrial biopsy was graded IIb (Kenney-Doig grade) because of the frequency of periglandular fibrosis and cystic glandular distention.1 Cervical function was normal. The mare did not accumulate intruterine fluid after mating.

Embryo transfer was performed for the next 6 consecutive yr, with a live foal being produced each year after embryo recovery attempts on one or two estrous cycles.

The cause of this mare's repeated late pregnancy losses is unknown. However, the mare's initial endometrial biopsy results indicated significant fibrotic and degenerative changes. Mares with an endometrial biopsy grade of IIb may have pregnancy loss rates that approach 50%. Mares with repeated early embryonic death or midterm abortion are very good candidates for embryo transfer because fertilization and embryo development occur normally but fail well after embryo transfer is performed. Embryos from these mares are transferred to young, reproductively healthy mares with a high success rate.

4. Chronic Bacterial and Yeast Infection
A 1993 Hanoverian mare delivered two live foals in Germany before being exported to the United States. The horse was used in riding competition for 3 yr. In 2002, the mare was bred using frozen semen. After being given human chorionic gonadotropin (HCG), the mare suffered an anaphylactic reaction resulting in difficult breathing and hives. During the second estrous cycle, the mare became pregnant but lost the pregnancy. The mare was diagnosed with Cushing's disease and put on medication. The type of medication was not known. The mare was bred in 2003 but did not become pregnant. In 2004, the mare was diagnosed with a mixed bacterial uterine infection that included yeast, E. coli, beta hemolytic streptococcus, and non-hemolytic streptococcus. The mare had normal perineal conformation and did not have evidence of uterine fluid accumulation or externally visible vulvar discharge. The mare was treated with chlorhexidine (concentration of the chlorhexidine was not known) because of the broad array of microorganisms recovered from the uterus. No bacteria were isolated from culture swabs after uterine treatment, and the mare was bred. The mare became pregnant, but the pregnancy was lost by day 60 of gestation. The mare was referred to Nandi Veterinary Clinic for breeding.

On initial evaluation of the mare's reproductive tract, large quantities of intruterine fluid were visualized on ultrasonography. A diagnosis of pyometra (hyperechoic cloudy fluid) was made. Manual examination of the vagina and cervix showed cervical adhesions and scarring. Endometrial swab culture resulted in growth of yeast. Evaluation of an endometrial biopsy showed acute endometritis with infiltration of eosinophils, periglandular fibrosis of many endometrial glands, and moderately frequent cystic glandular distention. The mare was treated by manually dilating the cervix, draining the uterine fluid, uterine lavage, infusion of clotrimazole into the uterus (500 mg every other day for three treatments), and oxytocin to help empty the uterus before treatments (20 IU). The mare was not bred and was sent home with a poor prognosis for future breeding potential.

The mare returned the subsequent year for breeding. Reproductive findings were similar to those identified in the previous year, i.e., uterine fluid, cervical adhesions and fibrosis, and isolation of Candida parapsilosis. The mare was given a poor prognosis for fertility and was not bred.

Two years later, the mare returned to the clinic with the hope that a foal could be obtained from the mare using embryo transfer technology. Reproductive evaluation of the mare showed the same problems as previously diagnosed except the culture of uterine fluid was negative for growth of bacteria or yeast. Initial treatment consisted of manual cervical dilation, evacuation of uterine fluid, and administration of oxytocin (20 IU).

The mare was bred during two estrous cycles using fresh cooled semen. HCG was used to induce ovulation. Numerous treatments were performed during estrus. Treatments included uterine lavage with lactated Ringers solution, oxytocin, manual cervical dilation, post-breeding antibiotics, and uterine lavage. Embryo transfer was performed on days 7 and 8 after asynchronous double ovulation. Two poor-quality embryos were recovered during
the first cycle but did not result in a pregnant recipient. After breeding on the second estrous cycle, a day 8, grade 1 embryo was transferred to a recipient that became pregnant.

5. Irregular Cyclicity in an Older Mare
A 19-yr-old barren Standardbred mare was purchased at public auction. The mare had 12 foals in her lifetime. Although the mare cycled sporadically the subsequent year, the mare became pregnant and delivered a foal. Her cyclic pattern continued to be erratic for the next two seasons, and she ovulated only once at age 22. The mare was referred to the clinic for embryo transfer at age 23.

On presentation, the mare had very small, inactive ovaries (2 × 1 × 1 cm), an atonic uterus, and a flaccid cervix despite examination during the breeding season and having been under artificial lighting since December 1. Based on the erratic cycling pattern during the prior years and the current physical examination, a diagnosis of senile ovarian atrophy was made. A final attempt was made to stimulate follicular activity using equine follicle stimulating hormone (eFSH)\textsuperscript{a2} The mare was given twice daily injections of 8 mg eFSH. After 12 days of treatment, significant follicular activity did not occur. The mare was retired. Treatment of other cases of senile ovarian atrophy have also been unsuccessful using eFSH. Other treatments to induce cyclicity in these older mares, such as domperidone, sulpiride, prostaglandin, and artificial lighting, have also been routinely unsuccessful.

6. Additional Comments
Management and treatment of a mare with chronic uterine infection must be tailored specifically for the individual because there is no single protocol that is appropriate for all mares. The treatment plan may change daily and is based on reproductive examination on a specific day and response to treatment. In the fourth case presented here, the cervical fibrosis and adhesions may have resulted from chlorhexidine intrauterine treatment that was performed 3 yr earlier. If a uterine culture is obtained routinely from mares before breeding and organisms are recovered, the clinician should make every effort to correlate these culture results with inflammation of the reproductive tract, especially if more than two organisms are recovered from the swab. Reproductive tract inflammation is more frequently observed in mares if the mare has abnormal perineal conformation, a fistula in the caslick suture line, a positive uterine cytology, endometritis based on endometrial biopsy, a cervical laceration, or presence of uterine fluid before or after mating.\textsuperscript{5}

It is possible to recover embryos and produce viable pregnancies from mares with chronic uterine inflammation and infection during the mated cycle. Mares with anatomical defects that interfere with uterine drainage, such as cervical adhesions or fibrosis, may need to be bred even though bacteria or yeast are recovered from the uterus because it is unlikely that the infection will ever be completely cleared. Mares are treated aggressively before and after breeding. The goal of treatment is directed at improving uterine health for a short period of time, i.e., days 5–8. If an embryo is recovered, the embryo needs to be washed numerous times (8–10) to minimize the chance that a bacterial or yeast infection will be carried to the recipient. The most common reproductive tract problem noted in mares with chronic uterine infections is abnormal cervical function. Mares with long-standing uterine infections may be good candidates for ova transfer as a method of obtaining foals.\textsuperscript{4}

When presented with mares with chronic infertility in an embryo transfer program, it is important to prepare the mare for the breeding season. The mare’s specific health needs, such as chronic arthritis and poor body condition, should be addressed and corrected. It is advantageous to have the donor mare exposed to increased lighting in early December so the mare begins cycling in February even if breeding is planned for later in the spring. This practice will allow evaluation and treatments of reproductive tract problems while the mare is cyclic. Remember, if the donor is exposed to artificial lighting so that breeding can start in February or March, recipient mare candidates should also be included in the artificial lighting program.

The procedure for flushing a mare for embryo recovery has been described many times and in numerous textbooks. Most of the flushing methods recommend the use of a large diameter catheter (34-F), 3–6 l of flush fluid, and sealing the internal cervical os by dilating the catheter balloon with 60–100 ml of air or saline.\textsuperscript{5} Each practitioner tends to develop his or her own technique for the embryo transfer flush procedure. Many mares with chronic uterine pathology or cervical scarring or lacerations and aged mares will have a pendulous uterus and unilateral or bilateral sacculations of the uterus. When the flush fluid is allowed to enter the uterus by gravity flow, an uneven distribution of fluid frequently occurs. This disparity of fluid distribution becomes exaggerated with each aliquot of fluid infused and retrieved from the uterus. Recovery of 100% of the flush fluid is difficult. In my opinion, this may account for poor embryo recovery efficiency.

I would suggest a modification of the usual flushing method for the above described mares. A total of 2 l of flush fluid is used. The flush fluid should contain a surfactant such as polyvinyl alcohol to minimize foaming of the return flush fluid and minimize the chance of the embryo sticking to surfaces. The 2 l of flush fluid is divided into three or four aliquots of 500–700 ml of fluid. After the catheter is passed into the uterus, the balloon is seated at the internal cervical os and distended with 60 ml of fluid or air. A 500- to 700-ml aliquot of fluid is allowed to flow into the uterus. While fluid is being added to
the uterus, the fluid is manually pushed or massaged to achieve uniform distribution throughout the uterus. When the return fluid port is opened, a steady stream of fluid should flow through the filter dish. If fluid tends to remain in dependent portions of the uterus, the catheter is passed forward into the fluid. While the catheter is in the forward position, the practitioner should continue to massage the uterus to keep the fluid “in motion.” This process is repeated three times. A serious attempt should be made to retrieve the entire 500- to 700-ml aliquot before infusion of the next aliquot. The loss of fluid through the cervix using this method is very unusual. Fluid loss around the catheter and through the cervix usually occurs when the practitioner attempts to “pull” the fluid back into the uterine body with the catheter balloon seated at the internal os. If the technician is unable to retrieve all the fluid from the uterus, the uterus should be scanned with an ultrasound examination. When the pocket of fluid is located, the catheter can be advanced to the fluid for retrieval.

Another practice tip that may be helpful in these difficult mares is to pre-medicate the mare using 10–15 mg acepromazine and 10–15 mg N-butylscopolammonium bromide IV just before the embryo flushing procedure. Acepromazine allows a “relaxation” of the uterus so that the instilled fluid can be manually distributed throughout the uterus. Buscopan minimizes rectal pressure and peristalsis, which allows easier manipulation of the uterus per rectum.6

References and Footnotes

*bBuscopan, Boehringer Ingleheim, Vetmedica, Inc., St. Joseph, MO 64506.AQ1: Please provide take home message.
AQ2: Please spell out eFSH.
AQ3: Please provide footnote for Buscopan with manufacturer and location of manufacturer.