Case Report
Successful reduction of a monozygotic equine twin pregnancy via transabdominal ultrasound-guided cardiac puncture


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Keywords: horse; twin; pregnancy; monozygotic; reduction

Summary
In horses, twin pregnancy is pathological and represents a potentially life-threatening condition to the mare and foal. Twinning occurs in approximately 2% of pregnancies. Of these, the majority of cases are dizygotic twins, resulting from 2 ovulations and monozygotic twins are rare. A 12-year-old Quarter Horse mare was presented for breeding management with shipped cooled semen and embryo transfer. Seven days post ovulation, a single late morula/early blastocyst was recovered and transferred immediately to a recipient mare. Thirty-six days after transfer, transrectal ultrasound examination revealed the presence of 2 embryos. A transcervical reduction of one of the fetuses was performed successfully at 128 days of gestation. This report is the first to describe a successful reduction of monozygotic twins by transcutaneous ultrasound-guided cardiac puncture.

Introduction
In horses, twin pregnancy is a pathological condition, occurring in 1–2% of the equine population and represents 6–30% of all abortions (Ginther 1979; Giles et al. 1993). Untreated twins that do not naturally reduce prior to 30–45 days result in high abortion rates during the second half of pregnancy (Jeffcott and Whitwell 1973; Roberts 1986; Giles et al. 1993). In addition, poor neonatal viability, fetal dysmaturity, dystocia and reduced fertility in subsequent breeding attempts are common sequelae to untreated twinning (Ball 2000).

Case report
A 12-year-old Quarter Horse mare was presented to the NCSU Theriogenology service for breeding management with shipped cooled semen and embryo transfer. Transrectal ultrasound examination was performed daily during oestrus until detection of a 35 mm follicle and...
2000 iu of Human Chorionic Gonadotropin (hCG, Chorulon)\(^1\), was administered i.v. to induce ovulation. The mare was artificially inseminated the following morning with shipped cooled semen and ovulation confirmed 24 h after insemination. Seven days post ovulation the mare’s uterus was lavaged with 4 l of flushing medium (Complete Flush)\(^2\) resulting in the recovery of a single grade I embryo at the late morula/early blastocyst stage. The embryo was transferred transcervically to a 4-year-old Quarter Horse recipient mare housed continuously on the NCSU premises and who had not been exposed to a stallion. The recipient mare was examined via transrectal ultrasonography 7 days after embryo transfer and the presence of one embryonic vesicle was noted. At 25 days gestation, a transrectal ultrasound examination detected one embryo with a heartbeat. A transrectal ultrasound examination detected one embryo with a heartbeat. A transrectal ultrasound evaluation at 36 days of gestation revealed the presence of 2 embryos measuring 3.42 × 1.31 cm and 3.19 × 1.08 cm, respectively. Both embryos were closely apposed and appeared to share an umbilical stalk and chorioallantois (Fig 1). After twin diagnosis, the recipient mare was examined via transrectal ultrasonography weekly to monitor the pregnancy. Due to the stage of pregnancy at the time of diagnosis and proximity of the fetuses, early techniques for twin reduction, including manual crush and transvaginal embryonic aspiration were not deemed safe or efficacious. Although there was an increasing disparity in fetal size during the subsequent examinations, both fetuses maintained normal appearance and heart rates between 35 and 124 days. At 124 days of gestation, transabdominal ultrasound evaluation revealed that the thoracic diameter of one fetus was approximately twice the size of the second one (6.6 × 3.7 cm).

At Day 128 of gestation, a transabdominal ultrasound-guided fetal cardiac puncture was performed to reduce the smaller twin. The mare was properly restrained in stocks and sedated with 100 mg/animal xylazine (AnaSed)\(^3\), 2.5 mg/animal detomidine hydrochloride (Dormosedan)\(^4\) and 2.5 mg/animal butorphanol tartrate (Torbugesic)\(^5\). The abdominal area ventral to the location of the smaller twin was clipped and scrubbed. Using a 3.5 mHz convex ultrasound probe (Aloka SSD 900)\(^6\), the fetal thorax was located and 2 ml lidocaine administered subcutaneously at the predicted puncture site to desensitise the area. The probe was placed in a sterile sleeve and the fetal thorax relocated. The trocar from a 14 gauge, 5.25 inch, 2.1 x 133 mm i.v. catheter was advanced through the skin and ventral abdominal wall and guided with the aid of ultrasound through the uterine wall and fetal thorax into the heart of the smaller fetus. Ten ml of 149 mg/mL potassium chloride\(^7\) were injected directly into the fetal heart. The mare was administered sulphonamethoxazole-trimethoprim USP\(^8\) (30 mg/kg bw per os q. 12 h) for 5 days and flunixin meglumine (Flumeglumine)\(^9\) (1.1 mg/kg bw i.v. q. 24 h) for 4 days. She was also administered 0.088 mg/kg altrenogest (Regu-Mate)\(^1\) per os q. 24 h, for 5 days, then 0.044 mg/kg bw per os q. 24 h for 5 days. On the day following the procedure, the reduced fetus had no evidence of a heartbeat. Post reduction, the mare was examined via transrectal and transabdominal ultrasonography every 2 days for 2 weeks. After this period, the recipient mare was transported to the foal owner’s farm as part of a lease contract. Transrectal and transabdominal ultrasound examinations were performed once a month at the foal owner’s farm and no abnormalities were noted during any of these examinations. The reduced fetus failed to increase in size and was last detected via transabdominal ultrasonography at 173 days gestation. At this time, the thoracic diameter of the reduced fetus measured 3.1 cm, while the viable foal had a thoracic diameter of 13.6 cm.

The mare foaled at 340 days of gestation with no complications and produced a healthy filly weighing 40.8 kg. The fetal membranes weighed 7.2 kg. Gross evaluation of the placenta revealed the presence of 2 sets of fetal membranes (dichorionic diamniotic) and one small mummified fetus. The chorionic surfaces of the placenta were intimately adhered to each other and a small area of vascular anastomosis was seen (Fig 2 and 3). Tissue from the mummy and hair follicles from the viable foal were submitted for genetic comparison (UC Davis, Veterinary Genetics Laboratory). Fourteen microsatellites were matched between the 2 samples, confirming monozygosity of the twins.

Discussion

To the knowledge of the authors, this is the first reported case of successful reduction of monozygotic twins.
resulting in a viable foal. Previous reports of monozygotic twinning in the mare have described this condition after natural breeding as well as embryo transfer; however, all cases resulted in abortion (Rooney 1970; Meadows et al. 1995; McCue et al. 1998; Govaere et al. 2009). The specific cause and time of embryo division, as well as the frequency of occurrence are unknown in the horse. In man the occurrence of monozygotic twins following nonstimulated in vivo conception is about 0.4–0.45% (MacGillivray 1986; Derom et al. 1987). There has been a marked increase in monozygotic embryonic twinning rates in women since the advent of assisted reproductive technologies (ART). The monozygotic twinning rates in women following ART procedures are between 2 and 12 times higher than that natural occurrence (Edwards et al. 1986; Blickstein et al. 1999, 2003; Sills et al. 2000; Alikani et al. 2003). Many different theories for an increased incidence of monozygotic twins after ART have been discussed, but none have been clearly elucidated. Some theories include: 1) insult to the morula resulting in disruption of communication between inner blastomeres and the independent formation of 2 separate internal cell masses (ICM) within the same blastocyst. 2) Blastocyst collapse, with adhesion of ICM cells to another point within the trophectoderm and the formation and growth of a second ICM. 3) Breach in, or hardening of, the zona resulting in abnormal blastocyst hatching with adhesion of ICM cells to another point within the trophectoderm and the formation and growth of a second ICM (Aston et al. 2008). Mares undergoing ART are also exposed to many of these factors and the authors speculate that an increased incidence of monozygotic equine twinning may exist; however, there are no conclusive data to support it. With regard to previously reported monozygotic twins, 3 cases reported by McCue and coworkers underwent embryo transfer. The authors speculate that it is possible that mares undergoing embryo transfer are at greater risk for monozygotic twinning (McCue et al. 1998). Monochorionic twins are believed to arise from a single monozygotic embryonic vesicle whereas dichorionic twins arise from 2 separate monozygotic or dizygotic embryos (Hall 2003). The presence of 2 separate chorions (dichorionic) in this case of monozygotic twins indicates embryo splitting occurred at some point during early embryo development. Prior to transfer, the authors were unable to identify any degree of separation or irregularity within the embryo. Therefore, it is most likely that the embryonic separation occurred during or after embryo transfer in this case.

Regardless of embryonic origins, twin pregnancy carries a high risk of pregnancy loss and maternal disease in the horse. It is considered a pathological condition and several methods have been described for twin reduction. Manual crushing of one vesicle between 14 and 18 days post ovulation is the preferred technique, with success rates of greater than 90% prior to embryonic fixation (Roberts 1982; Pascoe 1983; Pascoe et al. 1987). Different techniques have been described to reduce twin pregnancies post fixation, with variable outcomes. Techniques are listed here in ascending order according to the gestational age performed. Merkt and colleagues described twin reduction by dietary deprivation, where food intake in mares carrying twins is reduced in order to cause death of one conceptus. Forty-one Thoroughbred mares carrying twins between 21–49 days were treated by complete removal of oats, concentrates or alfalfa and fed free choice grass. In this study, 63% of the mares delivered a single foal (Merkt et al. 1982). No comparison was made with nontreated mares, making it difficult to attribute the reduction to dietary deprivation solely. Transvaginal ultrasound-guided twin reduction is performed by aspiration of yolk sac or allantoic fluid with a syringe or pump and depositing of procaine-penicillin.
into the fetal membranes, abdomen or heart (Bracher et al. 1993; Jonker et al. 1995; Macpherson et al. 1995; Macpherson and Reimer 2000; Klewitz et al. 2010). Success for this procedure varied between 33 and 75% when performed between Days 20 and 71, where bilateral twins had higher positive outcomes. More recently, craniocervical dislocation of one fetus (Wolfsdorf et al. 2005) was described between Days 60 and 110 of gestation. Dislocation of the first cervical vertebrae from the cranial transects the spinal cord, resulting in demise of one fetus over 2–10 weeks. Success rates for this procedure were reported to be around 60%; however, limited numbers of procedures had been performed at the time of publication. Transcutaneous ultrasound-guided twin reduction can be performed between 66 and 168 days of gestation. A needle is introduced in the fetal thorax via a transabdominal approach, under ultrasonographic guidance. Potassium chloride or procaine penicillin have been used for injection into the fetal heart, thorax or abdomen. Success rates for this procedure are reported between 38–60% (Rantanen and Kincaid 1988; Rantanen 1990; McKinnon and Rantanen 1998).

In this case, the late detection (36 days) of the second embryo, the intimate contact between conceptuses (unilateral) and the potential that they could be monoamniotic were each sources of concern. The mare was monitored for 10–14 days, with the hope that one fetus would be reduced naturally prior to 45 days, as described for unicarnual twins (Ginther 1984, 1989). After this time, craniocervical dislocation and transcutaneous reduction were considered. Transcutaneous reduction was elected over craniocervical dislocation based on the potential complications related to craniocervical dislocation (rectal tear via transrectal approach and surgery complications via flank incision) and the close proximity of the 2 fetuses and the relative success rates of the available techniques (Rantanen and Kincaid 1988; Rantanen 1990; McKinnon and Rantanen 1998; Macpherson and Reimer 2000; Wolfsdorf et al. 2005).

In conclusion, twins in the horse most commonly arise from multiple ovaations. However, mares may be at increased risk for monozygotic twins after advanced reproductive techniques, such as embryo transfer, oocyte transfer and intracytoplasmic sperm injection. With the increasing application of these techniques in the equine industry, it is critical for equine practitioners to be aware of the potential for twins when performing pregnancy diagnosis, even in recipient mares receiving a single oocyte or embryo. In these cases, twins may not be detectable at 14 days, when ultrasounds are routinely performed and each subsequent pregnancy diagnosis should be used to confirm a singleton pregnancy. Furthermore, the current case demonstrates that late term twin reduction can be performed successfully in a case of monozygotic twinning and despite the presence of vascular anastomosis.

Manufacturers’ addresses

1Intervet, Millsboro, Delaware, USA.
2Vigro from Bioniche Animal Health USA, Inc, Pullman, Washington, USA.
3Lloyd Laboratories, Shenandoah, Iowa, USA.
4Pfizer Animal Health, Exton, Pennsylvania, USA.
5Fort Dodge Animal Health, Iowa, USA.
6Aloka, Wallingford, Connecticut, USA.
7Hospira, Inc., Lake Forest, Illinois, USA.
8Amneal Pharmaceuticals, Glasgow, Kentucky, USA.
9Phoenix Pharmaceutical, Inc, Joseph, Missouri, USA.

Authors’ declaration of interests

No conflicts of interest have been declared.

References

PER ORAL IN HORESES ONLY
For the treatment of equine protozoal myeloencephalitis (EPM) caused by Sarcocystis neurona.

CAUTION
Do not use in horses intended for breeding. Do not use in horses with known hypersensitivity to diclazuril. Do not use in pregnant mares. Federal (U.S.A.) law restricts this drug to use by or on the order of a licensed veterinarian.

INDICATIONS: PROTAZIL® (1.56% diclazuril) Antiprotozoal Pellets are indicated for the treatment of equine protozoal myeloencephalitis (EPM) caused by Sarcocystis neurona in adult horses.

DOSAGE AND ADMINISTRATION
DIAGNOSE: PROTAZIL® (1.56% diclazuril) is administered as a top-dress on the feed. One 2-lb bucket of PROTAZIL® will treat one 1100-lb horse for 28 days. One 10-lb bucket of PROTAZIL® will treat five 1100-lb horses for 28 days.

ADMINISTRATION: One 2-lb bucket of PROTAZIL® will treat two 1100-lb horses for 28 days. One 10-lb bucket of PROTAZIL® will treat 1100-lb horses for 28 days.

CONTRAINDICATIONS: Use with caution in horses with known hypersensitivity to diclazuril.


PRESERVATIVES: The enteric coating of PROTAZIL® (1.56% diclazuril) Antiprotozoal Pellets in horses used for breeding purposes, during pregnancy, or in lactating mares has not been evaluated.

ADVERSE REACTIONS: No adverse effects related to the field study which could be attributed to drug administration were observed. No reported adverse reactions, to initiate a MEDS, or for technical assistance call 1-800-224-5318.

CLINICAL PHARMACOLOGY: The effectiveness of PROTAZIL® (1.56% diclazuril) in reducing the number of Sarcocystis neurona tachyzoites in the brain and spinal cord was studied by Givens and Sykes (2005). Diclazuril inhibited intracellular parasite multiplication by more than 60% in cultures of Sarcocystis neurona. Diclazuril reduces intracellular parasite load by more than 60%. Intracellular parasite load of Sarcocystis neurona was observed when the fellulae were treated with 0.5 mg/kg (oral) dose. The clinical relevance of the intracellular parasite load was not determined.

PHARMACOGENETICS: The oral bioavailability of diclazuril from the PROTAZIL® (1.56% diclazuril) Antiprotozoal Pellets is 61% compared with 60% oral bioavailability from the enteric coated oral formulation. The safety of top-dress administered to horses at 0.1 mg/kg dose cannot be determined based on the clinical study data.

EFFECTIVENESS: Ten horses (5 males and 5 females, ranging from 9 to 9 months of age) were treated with PROTAZIL® (1.56% diclazuril) Antiprotozoal Pellets at the rate of 0.5 mg/kg dose per day. Clinical and hematologic response was observed as a reduction in clinical signs. There were no test article-related findings during the study. The clinical and hematologic response was compared to the pre-treatment values. The safety of diclazuril top-dress administered to horses at 0.1 mg/kg dose cannot be determined based on the clinical study data. Because of the lack of an adequate control group (control horses tested positive for the test article in plasma and CSF), however, possible associations were not evaluated because of the lack of an adequate control group (control horses tested negative for the test article in plasma and CSF). Therefore, possible associations should be monitored in clinical practice.

ANIMAL SAFETY: PROTAZIL® (1.56% diclazuril) Antiprotozoal Pellets were administered to 30 horses (15 males and 15 females, ranging from 9 to 9 months of age) in a target animal safety study. The study involved 6 horses in each of the 3 groups (15 males and 15 females). The studies were conducted at 0, 1, and 5 mg/kg doses. The study was conducted at 28 days.

STORAGE INSTRUCTIONS: Store between 10 °C to 30 °C (50°F to 86°F).

HOW SUPPLIED: PROTAZIL® (1.56% diclazuril) Antiprotozoal Pellets are supplied in 2- to 2.5-lb (900- to 1135-g) bags and 5-lb (2.25-kg) bags.


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