Twin reduction in the mare: current options

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Abstract

Twin pregnancy in the mare causes economic loss. Early transrectal ultrasonographic detection of twins and manual crush of one embryonic vesicle is the method of choice for managing equine twins (90% success rate). Transcutaneous and transvaginal ultrasound-guided twin reduction techniques are described for twin pregnancies that advance beyond 25 days of gestation. Reported success rates for the ultrasound-guided procedures are 50% and 20%, respectively. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

In spite of improved management techniques, twin pregnancy continues to be a source of economic loss in broodmare production. Twinning historically has accounted for 10–30% of abortions in the mare (Roberts, 1986). Recently, Giles et al., (1993) examined causes of abortion, stillbirth and perinatal death in horses between 1986 and 1991. Of 3527 necropsy cases reported, 221 (6%) mares had aborted twins. Early diagnosis and management of twins has reduced the incidence of abortion in mares due to twins; however, twin pregnancies still account for a notable amount of pregnancy wastage in the mare.

2. Management of equine twin pregnancies

Ultrasonographic examination of the reproductive tract early in gestation allows for prompt diagnosis and treatment of twin pregnancies (Chevalier and Palmer, 1982).
Pregnancies are detected as early as Day 9 of gestation with the aid of transrectal ultrasonography (Ginther, 1986). Diagnosis of twin pregnancies is optimally achieved between Days 13 and 15 of gestation to ensure detection of twins arising from asynchronous ovulations (ovulations occurring more than 24 h apart) (Ginther, 1989a). Twin pregnancies detected during the early mobility phase (prior to Day 16 of gestation) are best managed by crushing one embryonic vesicle, with a survival rate for the remaining twin exceeding 90% (Roberts, 1982; Pascoe, 1983; Pascoe et al., 1987).

During the period of embryonic fixation (Days 16–17 of gestation), approximately 70% of twin vesicles will fix in the same horn (unilateral). Of unilaterally fixed twin pregnancies, approximately 85% culminate in natural reduction of one embryo prior to Day 40 of gestation. The remaining embryo develops normally following natural reduction. (Ginther, 1984, 1989b).

Unilateral twins that do not undergo natural reduction to a singleton are challenging to manage. Manual reduction of a unilaterally fixed twin is difficult without damaging the remaining conceptus. Bowman (1987) and McKinnon and Rantanen (1998) reported good success (90–95%) with manual crush of a unilateral twin from Day 17 to 20 if the vesicles could be separated. The technique was less advantageous for inseparable vesicles or unilateral twins ≥ 20 days gestation.

Bilaterally fixed twin pregnancies (one conceptus in each uterine horn) typically do not result in natural reduction to a singleton conceptus during early pregnancy (Ginther, 1984). Instead, both fetuses often survive until later stages of gestation, at which time abortion usually occurs (Jeffcott and Whitwell, 1973). Ginther and Griffen (1994) recently followed bilateral twin pregnancies in 15 pony mares. In two mares, death of one fetus occurred in the first two months of gestation. Eight of fifteen mares (67%) aborted both fetuses by three months of gestation. Four mares aborted one or both fetuses at ≥ 8 months and one mare delivered live twins. The authors noted that early fetal death appeared to be temporally related to the apposition of allantochorions between the fetuses. The actual mechanism causing fetal death is currently unknown.

Manual crush of a bilateral twin results in reduction to a singleton pregnancy in 75% of cases if performed before 30 days of gestation (Pascoe et al., 1987). Delayed attempts to reduce twin pregnancies (i.e., beyond 30 days of gestation) more likely results in abortion of both conceptuses with manual reduction techniques (Bowman, 1987; Pascoe et al., 1987).

Management of equine twin pregnancies after 30 days gestation is further complicated by the formation of endometrial cups at approximately Day 36 of gestation. The endometrial cup cells secrete the hormone equine chorionic gonadotropin (eCG) (Allen and Moor, 1972). This hormone is reported to stimulate the formation of secondary corpora lutea thereby leading to increased progesterone production for pregnancy maintenance (Cole et al., 1946; Amoroso et al., 1948). Endometrial cups remain functional until approximately Day 80–120 of gestation in either the presence or absence of a viable fetus (Clegg et al., 1954; Mitchell, 1971). Thompson et al. (1982) demonstrated that irregular estrous cycles and impaired fertility occurred with pregnancy loss in the presence of endometrial cups. Consequently, if twin pregnancies are not successfully managed prior to cup formation and both pregnancies are lost after Day 35, the mare may not return to fertile estrus for a prolonged time. Methods for managing
equine twins beyond Day 30 of gestation have resulted in inconsistent outcomes. Methods include dietary energy restriction (Merkt et al., 1982), needle aspiration of one embryonic vesicle per vagina (Pascoe, 1979), surgical removal of one vesicle (Pascoe and Stover, 1989) and transvaginal and transcutaneous ultrasound-guided fetal reduction techniques (Rantanen and Kincaid, 1988; Bracher et al., 1993; Jonker et al., 1995). Currently, ultrasound-guided techniques are commonly used methods for reduction of fetal twins.

3. Transvaginal ultrasound-guided pregnancy reduction in mares

Selective reduction of pregnancy using transvaginal ultrasonography has been examined in mares having both singleton (Squires and Tarr, 1994; Macpherson et al., 1995) and twin pregnancies (Bracher et al., 1993; Jonker et al., 1995). The technique involves a 5- or 7.5-MHz transvaginal ultrasound transducer designed for use in large animals. Typically, the transducer and casing are cold-disinfected or sterilized prior to placement in the mare. Some individuals cover the transducer with a sterile latex cover (latex ultrasound transducer cover, Civco Medical Instruments, Kalona, IA) or sterile sleeve filled with sterile lubricating jelly. The mare’s tail is wrapped and the perineal area cleansed. Methods of restraint include epidural anesthesia (Macpherson et al., 1995), tranquilization (Bracher et al., 1993; Macpherson et al., 1995) and infusion of 2%
lidocaine in the rectum (Bracher et al., 1993; Jonker et al., 1995). Bracher et al. (1993) tranquilized one mare with detomidine hydrochloride prior to twin reduction and noted substantial uterine relaxation after the tranquilizer was administered. They found it difficult to localize the pregnancy for visualization after sedation. The group elected to use lidocaine in the rectum rather than tranquilization to restrain mares for future procedures. Macpherson (unpublished results) has also experienced uterine relaxation when administering detomidine hydrochloride prior to fetal reduction and has adopted the practice of rectal infusion of lidocaine for local anesthetic effect.

Prior to performing the procedure, the mare often receives 350–500 mg flunixin meglumine, IV, to counteract prostaglandin release during uterine manipulation. Wearing a sterile obstetrical sleeve, an operator carries the transducer into the anterior vagina. The operator’s arm is then removed from the vagina and placed in the rectum for manipulation of the reproductive tract. The operator manually secures the pregnancy transrectally, and the transducer is manipulated transvaginally until the pregnancy is imaged on the ultrasound screen. The fetal position is clearly identified. A puncture guide on the ultrasound screen is used to select a path for needle placement in the yolk or allantoic sac (Fig. 1). An assistant passes a sterile, 16- to 18-gauge, 60 cm needle with an echogenic tip (Echogenic tip spinal needle, Cook Ob/Gyn®, Spencer, IN) through a needle channel in the transducer casing. A sharp jab of the needle is made for passage of the needle through the vaginal and uterine walls into the yolk or allantoic

![Image](image.jpg)

Fig. 2. Needle placement within allantoic sac.
space. After ultrasonographic identification of the echogenic needle tip in the yolk or allantoic space (Fig. 2), a 60-ml luer-tip syringe or suction pump is connected to the needle and fluid is aspirated (Fig. 3). To facilitate complete fluid aspiration, the needle can be moved within the sac into areas of detectable fluid. The orientation of the twins (unilateral vs. bilateral) influences when aspiration is discontinued. For unilateral twins, aspiration is discontinued when there is danger of aspirating the fetal membranes between the twins, the conceptus can no longer be visualized because of fluid removal, or it is no longer possible to obtain fluid (Fig. 4). When performing the procedure on a bilateral twin, complete fluid evacuation is ideal. Trauma to the treated fetus is not a concern with bilateral twins, and may actually be advantageous.

4. Transvaginal ultrasound-guided twin reduction in the mare

Bracher et al. (1993) at the University of Utrecht were the first to report using a transvaginal ultrasound-guided technique to reduce equine twin pregnancies to a singleton. Aspiration of the yolk sac or allantois was attempted in 13 Dutch Warmblood mares with twin pregnancies ranging from 20 to 45 days of gestation. Nine of 13 mares in the study had unilateral twin pregnancies and 4 of 13 mares had bilateral twin pregnancies. Prior to twin reduction, mares received 350 mg flunixin meglumine, IV, and 50 ml of 2% lidocaine was instilled in the rectum. A 5- or 7.5-MHz transvaginal transducer was
used for the procedure. The conceptus was penetrated with an 18-gauge needle and fluid was aspirated using a syringe. Mares did not receive additional treatment after the procedure. Mares were examined for a viable conceptus using transrectal ultrasound 3 and 10 days after the procedure. Success of the procedure was defined as a viable, singleton conceptus 10 days following the procedure. Six of 13 (46%) mares had a viable conceptus 10 days after the procedure. The procedure was considered successful in 3/9 (33%) mares with unilateral twins and 3/4 (75%) mares with bilateral twins. When the procedure was performed at ≤ 35 days on unilateral twins, 2/5 (40%) mares had a viable singleton 10 days later. Only 1/4 (25%) mares ≥ Day 36 had a viable singleton at the 10 day examination. All bilateral twin reductions in this study were performed at ≤ 35 days and three of four mares (75%) had viable singletons 10 days later. Live foal data was not available from this study.

Workers at the University of Utrecht (Jonker et al., 1995) reported additional results for transvaginal ultrasound-guided twin reductions. The group at Utrecht implemented a similar technique as Bracher et al. (1993), with the exception of using a suction pump to aspirate fluid. Aspiration of the yolk sac or allantois was performed on 16 mares, all of which had unilateral twins, ranging from 26 to 71 days of gestation. Mares received flunixin meglumine 30 min prior to the procedure, and therapy with altrenogest was begun on the day of the procedure. Nine of 15 mares (56%) had a viable singleton remaining 7 days after the reduction. Five of 16 mares (31%) delivered live foals, and
all five mares had the procedure performed at 35 days or less (Jonker et al., 1995; Jonathan Pycock, personal communication). Pycock (personal communication) concluded from this work that transvaginal ultrasound-guided twin reduction was best performed before Day 35 of gestation.

Transvaginal ultrasound-guided twin reductions performed by Macpherson have had variable results. The procedure was used in 19 mares with twin pregnancies (11 unilateral twins, eight bilateral twins). All mares were treated with 500 mg flunixin meglumine 30 min prior to the procedure and 1 to 2 days after the procedure. Mares were also treated with exogenous progestins starting the day prior to the procedure until documentation of fetal death or at the discretion of the referring veterinarian. Two of eight (25%) mares with bilateral twin pregnancies have either an ongoing pregnancy or a live foal. The length of gestation in these mares was 50 and 43 days at the time of the procedure. For unilateral twin pregnancies, 1/11 mares (9%) delivered a live foal following twin reduction performed at 25 days gestation. The gestational length at the time of the reduction in 7 of 10 mares with unilateral twins was >35 days and all failed. One mare with unilateral twin vesicles that were separated by a few centimeters carried a viable singleton for 90 days after the procedure (performed at Day 25) and aborted. In most cases, fetal death of the remaining twin was noted 10–14 days after the procedure; however some mares carried viable singletons 3–9 months after the procedure and then aborted.

A variety of factors figure into the success of the transvaginal ultrasound-guided twin reduction procedure. Day of gestation at the time of reduction appears to impact pregnancy outcome following the procedure. When examining success rates across the aforementioned studies (Bracher et al., 1993; Jonker et al., 1995; Macpherson), there appears to be an advantage to performing the procedure before 36 days of gestation, particularly in the case of unilateral twins. One could argue that unilateral twins prior to Day 40 might reduce naturally and intervention is not necessary (Ginther, 1989c). However, by Day 25–30, a size discrepancy is often noted in unilateral twins that are in the process of natural reduction (Ginther, 1984). For those mares having unilateral twins with a distinct size discrepancy prior to Day 40, twin reduction should be unnecessary. When twin embryos are similar in size between 25 and 35 days gestation, the transvaginal reduction procedure may be the best option.

The application of transvaginal ultrasound-guided twin reduction to unilateral twins has limitations due to the close proximity of the embryos/fetuses and associated membranes. Extreme care must be taken when advancing the needle into the allantoic sac of a unilateral twin as it may be difficult to visualize the location of all of the placental membranes. One may inadvertently penetrate the adjacent vesicle, and possibly the embryo or fetus, if the placental membranes are not seen in the imaging plane. When aspirating placental fluids for termination of a unilateral twin one can easily aspirate placental membranes into the needle tip causing damage to the remaining fetus. Additionally, when fluid is withdrawn from a unilateral twin vesicle, the adjacent vesicle tends to pull from the endometrium and “fall” into the evacuated space (Macpherson, unpublished data). Fluid may also leak from the incompletely evacuated vesicle causing the placental membranes to separate from the endometrium (Macpherson et. al, 1995). Treating the mare with exogenous progestins to enhance uterine tone might prevent
separation of the membranes from the endometrium. Alternatively, injection of a toxic substance into the vesicle without removal of fluid would terminate the embryo/fetus without altering the shape of the vesicle.

The data for transvaginal ultrasound-guided reduction of bilateral twins is far less plentiful, thus strong conclusions about success and the best time to perform the procedure cannot be made. Theoretically, the transvaginal ultrasound-guided twin reduction procedure should be most useful for bilateral twins. With bilateral twin pregnancies, one has far more flexibility with regard to penetration of the conceptus and surrounding membranes because there is less likelihood of damaging the other conceptus. Limiting factors in reduction of bilateral twins using the transvaginal ultrasound-guided method include age of the mare, parity, size of the mare, position of the uterus, tone of the uterus and experience of the personnel. In the author’s experience (Macpherson), the procedure is significantly more difficult if the pregnant uterus is pendulous within the abdomen as occurs in aged mares or advanced pregnancy. The pregnant uterine horn must be stabilized adequately, per rectum, to allow visualization and penetration of a maximal area of fluid. When this is not possible, it is difficult to determine the optimal area for needle penetration, and it is more difficult to fully aspirate the allantoic fluid. Thus, while adhering to a time frame prior to 36 days of gestation should not be necessary for transvaginal ultrasound-guided reduction of bilateral twin pregnancies, the procedure is more challenging to perform beyond 45 or 50 days in older, multiparous mares. From the known data, it would appear that performing transvaginal ultrasound-guided twin reduction as early as possible in both unilateral and bilateral twin pregnancies is prudent.

Operator experience and uterine trauma also impacts success of the procedure. One must learn to pass the needle into the uterus while minimizing the insult to the mare’s uterus. The needle is advanced with a quick, controlled thrust to allow proper placement but avoid penetration through the uterus. In some cases (Macpherson, unpublished results) more than one needle puncture into the uterus was necessary for aspiration. Repeated insult to the uterus likely stimulated prostaglandin release and subsequent pregnancy loss. Twelve of 14 mares in a Colorado study (Squires and Tarr, 1994) developed intra luminal fluid from 2 to 10 days after transvaginal ultrasound-guided reduction of singleton pregnancies. The authors speculated that the presence of uterine fluid indicated uterine irritation resulting from the transvaginal procedures. They suggested that progestin therapy might be beneficial in enhancing uterine and cervical tone which would reduce intraluminal accumulation of fluid. Macpherson et al. (1995), in a similar study involving singleton pregnancies, treated mares with altrenogest (Regumate™, Hoechst-Roussel Agri-Vet, Somerville, NJ) for 30 days after performing transvaginal ultrasound-guided pregnancy reduction. Mares did not develop profound intra-luminal uterine fluid after the procedure as described in the Colorado study. Instead, placental membranes separated from the endometrium in terminated pregnancies, and the overall fluid volume within the uterus decreased rather than increased. The effect of exogenous progestin therapy when performing a twin reduction could be two-fold: (1) to prevent endogenous prostaglandin release resulting in subsequent luteolysis and increased uterine contractility, (2) to enhance uterine tone and optimize contact between the endometrium and the remaining conceptus.
5. Transcutaneous ultrasound-guided twin reduction in mares

The use of transcutaneous ultrasonography to aid in twin reduction in the mare was pioneered by Rantanen and Kincaid (1988). In the initial report, the procedure was attempted in 19 mares at 66 to 168 days of gestation. Mares were examined using transcutaneous ultrasound while standing and tranquilized with a combination of acepromazine, xylazine and butorphanol. A 3-MHz transducer fitted with a biopsy guide was used. Fetal location and differences in fetal size were determined so that the smallest and/or most easily accessible fetus could be selected for reduction. Fetal accessibility was considered a priority for the procedure unless one fetus was substantially smaller than the other fetus. The mare’s abdominal area adjacent to the fetuses was clipped and surgically prepared prior to the procedure. The transducer was placed in a sterile obstetrical sleeve which contained a small amount of scan gel in the thumb of the sleeve. Sterile gel was used on the abdomen. Once the fetuses were located, the depth from the abdominal wall to the fetal heart of the selected fetus was determined via ultrasound. If the heart was 5–6 cm from the skin surface, a 3.5-in., 20-gauge spinal needle was used. If the heart was located 10–12 cm from the skin surface, a 6-in., 18-gauge spinal needle was used. The area of the mare’s abdomen directly over the fetal heart was identified and infiltrated with 2% lidocaine. Using the biopsy guide apparatus attached to the transducer, the needle was passed through the skin and abdomen in one motion. The needle tip was located on the ultrasound image, advanced through the skin and abdomen in a quick, thrusting motion. Free flow of blood from the needle indicated needle placement within the fetal heart. If blood flow was not evident, the needle was withdrawn within the thorax and replaced at the area of the fetal heart. Potassium chloride (KCl, 2 mEq/ml) was injected into the heart in 1 ml aliquots until cessation of cardiac activity. Up to 32 mEq KCL was injected into the fetal heart without affecting the remaining fetus (N. Rantanen, personal communication). Cardiac activity in the remaining fetus was monitored. Mares were treated with 500 mg flunixin meglumine on the day of the procedure and for 3 additional days. Most mares were also treated with exogenous progesterone. Seven of 18 (40%) mares in the original study (Rantanen and Kincaid, 1988) delivered live foals (one mare was lost to follow-up examination). From these and subsequent twin reductions, Rantanen (1990) has concluded that the procedure is best performed between 115 and 130 days of gestation. Reporting on 59 mares with twins greater than 115 days gestation, 29 (49%) delivered single, live foals (McKinnon and Rantanen, 1998).

Johanna Reimer used a slightly modified technique for transcutaneous ultrasound-guided twin reduction. The procedure was performed in the standing, heavily sedated mare to promote movement of the fetuses into the cranial abdomen for easier accessibility and to minimize fetal movement during the procedure. The smaller fetus was preferentially selected for reduction. Using a 5-MHz transducer fitted with a biopsy guide, Reimer located the fetal heart and passed a 6-in., 18-gauge spinal needle into the thorax. Once needle placement within the heart was confirmed, up to 12 mEq KCL were injected into the heart. Mares were treated with flunixin meglumine immediately after the procedure and for 4 additional days. Progestin therapy was implemented after the procedure and continued for 2 months or at the referring veterinarian’s discretion.
Reimer performed the procedure in 24 mares with twins at gestation \( \geq 120 \) days. Normal, live foals were delivered from 9 of 24 mares (38%) following the procedure. Three additional foals were born live (3/24, 12%) but were weak and small at birth. One foal survived but was unthrifty, one foal died and one foal was euthanized. The remaining mares aborted after the procedure. Most mares aborted 1 to 2 months after the procedure. One mare carried a viable singleton fetus to 9 months gestation and then she aborted.

McKinnon and Rantanen (1998) used a different technique from Rantanen and Reimer for transcutaneous ultrasound-guided twin reduction. McKinnon initially tranquilized the mare with detomidine hydrochloride. Using a 6–10-in., 18- or 16-gauge needle with a stylet and echogenic tip (Cook Veterinary Products, Brisbane Australia), the needle was passed through a biopsy guide into the fetal heart, thorax or abdomen. Ten to 20 ml of procaine penicillin were injected into the area and the fetus monitored for 5–10 min. McKinnon reported that fetal death took slightly longer when injecting penicillin into the thorax or abdomen than directly into the heart. The reported advantages of using procaine penicillin vs. KCl are (1) reducing the possible risk of iatrogenic infection, (2) better visualization of the agent as it is injected, and (3) fetal death even in the absence of cardiac placement. Using this technique, McKinnon reported the birth of eight live, singleton foals from 13 mares (8/13; 62%) with twin pregnancies.

6. Conclusions

Diagnosis and management of twin pregnancies in the mare are an ongoing challenge for the equine veterinarian. Early detection of twins using transrectal ultrasonography and manual crush of one embryonic vesicle are the two most important tools an equine veterinarian can implement for management of twins. Procedures such as transvaginal and transcutaneous ultrasound-guided fetal reduction should be reserved for those cases in which the window for early crush of a twin vesicle has been inadvertently missed. In skilled hands, the reported rate for delivery of a singleton foal following the transcutaneous procedure is 40–60% (Rantanen, 1990; Reimer; McKinnon and Rantanen, 1998). In the 10 years that transcutaneous ultrasound-guided fetal reduction has been used the success rate for the procedure has improved 20% for some individuals. Currently, success using the transvaginal ultrasound-guided reduction lags behind that seen with the transcutaneous approach. With transvaginal ultrasound technology one has the advantage of performing twin reduction earlier in gestation. However, the conceptus at 30 to 50 days gestation may suffer more profound effects from the slightest disruption. Furthermore, direct penetration of the fetal heart is nearly impossible with a highly mobile fetus in a large, fluid-filled sac. Efforts at improving the transvaginal technique should be geared toward: (1) determining the optimal time for performing the procedure in both unilateral and bilateral twins (as has been achieved by Rantanen using the transcutaneous twin reduction technique), (2) developing a minimally invasive approach which causes the least adverse reaction in the uterus after the procedure (IE, aspiration of fluid vs. injection of a toxic substance), and/or (3) identifying a substance that is
both toxic to the embryo/fetus of choice but does not readily cross fetal membranes in the case of unilateral twins or does not cause uterine irritation should leakage from the puncture site occur. With continued refinement of ultrasound-guided twin reduction techniques, one will have options other than pregnancy termination in mares with twins that are undetected in the first 30 days of gestation.

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