Management of twins
K.E. Wolfsdorf,a M.L. Macphersonb
aHagyard Equine Medical Institute, Lexington, KY; College of Veterinary Medicine, University of Florida, Gainesville FL

Introduction
In spite of improved management techniques, twin pregnancy continues to be a source of economic loss in broodmare production. The introduction of transrectal ultrasound for pregnancy evaluation has revolutionized the identification and management of twin pregnancies in mares. As such, the rate of abortion due to twin pregnancies has dropped from an average of 20%1 to 6%.2 While improvements in management of twins are certainly notable, pregnancy loss as a result of missed twins is costly. Not only does the mare owner suffer lost income from the foal(s), the mare is barren for the season while incurring expenses. As a consequence, management of twin pregnancies to ensure delivery of a viable singleton is essential.

Keywords: Twins, pregnancy, ultrasound

Management of equine twin pregnancies
Ultrasonographic examination of the reproductive tract early in gestation allows for prompt diagnosis and treatment of twin pregnancies.3 Pregnancies are detected as early as Day 9 of gestation with the aid of transrectal ultrasonography.4 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 hours apart).5

During the period of embryonic fixation (Day 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.6,7

Bilaterally fixed twin pregnancies (one conceptus in each uterine horn) typically do not result in natural reduction to a singleton conceptus during early pregnancy.7 Instead, both fetuses often survive until later stages of gestation, at which time abortion usually occurs.9 Ginther and Griffen9 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.

Management of equine twin pregnancies after 30 days gestation is complicated by the formation of endometrial cups (Day 36 of gestation). The endometrial cup cells secrete equine chorionic gonadotropin (eCG).10 This hormone is reported to stimulate the formation of secondary corpora lutea thereby leading to increased progesterone production for pregnancy maintenance.11,12 Endometrial cups remain functional until approximately Day 80-120 of gestation in either the presence or absence of a viable fetus.13,14 Thompson, et al.15 demonstrated that irregular estrous cycles and impaired fertility occurred with pregnancy loss in the presence of endometrial cups. Consequently, if both twin pregnancies are lost after Day 35, the mare may not return to fertile estrus for a prolonged time. However, methods for managing equine twins beyond Day 30 of gestation have resulted in inconsistent outcomes. Dietary energy restriction,16 needle aspiration of one embryonic vesicle per vagina,17 surgical removal of one vesicle,18,19 cranio-cervical dislocation,20 and transvaginal21-23 and transcutaneous ultrasound-guided23-25 fetal reduction techniques are methods that have been investigated for twin reduction. This paper will review commonly used techniques to manage twin pregnancies in a clinical setting.

Manual crushing during mobility phase
Although pregnancies can be diagnosed with ultrasound as early as 9 days after ovulation, twin pregnancies are optimally detected between Days 13 and 15 of gestation. During this time period, the embryonic vesicles are mobile within the uterus, and both embryonic vesicles are reliably identified (even after asynchronous ovulation).4,5,26 Twin pregnancies that are detected during the mobility phase (up to Day 16 or 17) are best managed by manually crushing one embryonic vesicle.27 Using this procedure, one embryonic vesicle is manually moved to the tip of a uterine horn and crushed under pressure. Survival rates of the remaining vesicle after crushing exceed 90%.28,29

The location of the embryonic vesicles is determined with transrectal ultrasound evaluation of the reproductive tract. Early detection of embryonic vesicles may reveal vesicles that are too small to crush. Vesicles
smaller than 10-12 mm are challenging to crush. Further, if the vesicles are located in the uterine body it may be
difficult to manipulate them and/or perform an embryonic crush. Re-evaluation the following day(s) may be
necessary. Embryos located in different uterine horns are easy to manipulate by milking one vesicle into the tip of a
uterine horn for a crush. Embryos that are next to one another or superimposed upon each other may be more
challenging to manage. Some recommend re-evaluation of the mare later in the day or the next day to determine if
embryo position has changed. When both embryonic vesicles continue to be in close proximity to one another,
movement of the vesicle closest to the tip of a horn can be facilitated with manual manipulation. Alternatively, the
ultrasound probe can be placed between the vesicles and pressure is used to move one vesicle up the uterine horn for
crushing. Using the ultrasound probe to move a vesicle allows for continuous visualization of the vesicle during the
procedure. Additionally, the pressure of the probe during movement of the vesicle frequently results in rupture of
that vesicle. If the chosen vesicle is still intact once it reaches the tip of the uterine horn, continued pressure can be
applied to the vesicle with the fingers or the ultrasound probe. The advantages of performing a crush at the tip of the
horn include easy isolation of the vesicle in a smaller diameter uterine lumen and fluid release after rupture that is
far removed from the remaining vesicle.

Historically, it is recommended to crush the smaller vesicle or the vesicle that needs the least amount of
uterine manipulation. While it is disconcerting to crush the larger of the two vesicles, it might be the wisest choice
if the larger vesicle is easily accessed for the operator. Minimizing uterine manipulation is a priority when
performing a manual embryonic crush. Further support during the procedure can be provided pharmacologically.
To promote uterine or rectal relaxation during the procedure, the mare can be administered N-
butylscopolammonium bromide (Buscopen™, Boehringer Ingelheim, St. Joseph, MO; 0.3mg/kg, IV) or
propantheline bromide (15mg , IV) can be administered. Treatment during and after the procedure is not consistent
between clinicians. Flunixin meglumine is often administered (1 mg/kg, IV) at the time of the procedure to
circumvent prostaglandin release during uterine manipulation. Additional administration of flunixin meglumine for
the subsequent few days may provide anti-inflammatory support. Exogenous progestins, most frequently altrenogest
(Regumate™, Intervet-Schering Plough Animal Health, Millsboro, DE, 0.044 to 0.088 mg/kg, PO, once daily), is
administered for enhanced uterine tone and progestin support in the face of luteolysis. Re-evaluation for continued
growth and presence of the remaining vesicle should be performed 2-3 days post crushing and every few weeks until
placentation occurs.

Manual crush of a twin embryonic vesicle is the preferred method for twin reduction. The procedure is
minimally invasive, inexpensive, easy to perform and highly reliable. Additionally, if both pregnancies are lost
prior to endometrial cup formation, the mare will cycle back for breeding. The sole reason for not performing a
manual crush during the mobility phase would be having missed twins.

Manual reduction after fixation

Manual reduction is preferentially, and most successfully, performed before fixation of the embryonic
vesicles. If twin conceptuses are observed after fixation (Day 16-17), manual reduction can be attempted. Manual
reduction of unilaterally fixed twins is difficult without damaging both conceptuses. If the vesicles can be separated,
90% of unilateral twins can be manually crushed between days 17-20. An attempt at manual reduction of
bilateral twins between Days 16 and 40 is a necessity if abortion at a later stage of gestation is to be avoided.
Seventy-five percent of bilateral twins may be successfully reduced to a singleton pregnancy by crushing one vesicle
before 30 days of gestation. However, with bilateral twins of gestational age >35 days, there is a greater risk of
abortion at a later stage if a vesicle is crushed potentially because fluid released from the crushed vesicle gets
between the chorioallantois and endometrium and causes a loss of contact.

Transvaginal, ultrasound-guided twin reduction

Selective reduction of pregnancy using transvaginal ultrasonography has been examined in mares having
both singleton and twin pregnancies. The technique involves a 5 or 7.5 MHz transvaginal ultrasound
transducer designed for use in large animals. Prior to the procedure, mares are administered broad spectrum
antibiotics, flunixin meglumin and altrenogest. 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. Ideally, chemical restraint is not use to perform the
procedure because of the uterine relaxation induced by some agents (alpha agonists such as xylazine and detomidine
hydrochloride). Local anesthetics (2 % lidocaine) can be infused directly into the rectum or mixed with lubricant
and carried into the rectum at the time of the procedure. Prior to performing the procedure, the mare often receives
flunixin meglumine (1 mg/kg, 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. 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 space. After ultrasonographic identification of the echogenic needle tip in the yolk or allantoic space, a 60-ml Luer-tip syringe or suction pump is connected to the needle and fluid is aspirated. 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. When performing the procedure on a unilateral twin, complete fluid evacuation is ideal. Trauma to the treated fetus is not a concern with bilateral twins, and may actually be advantageous.

The success rate of transvaginal ultrasound-guided twin reduction is highly variable and dependent on many factors. In experienced hands, the live foal delivery rate can approach 50% for mares with unilateral twins at Day 35 or less, or mares with bilateral twins up to Day 55 (Jonathan Pycock, personal communication). Day of gestation at the time of reduction appears to impact pregnancy outcome following the procedure. When examining success rates, 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. However, by Day 25-30 a size discrepancy is often noted in unilateral twins that are in the process of natural reduction. When twin embryos are similar in size between 25 and 35 days gestation, aggressive management of twins is probably the best option. However, performing the transvaginal ultrasound-guided procedure in mares with unilateral twins has significant limitations due to the close proximity of the embryos/fetuses and associated 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. Fluid may leak from the incompletely evacuated vesicle causing the placental membranes to separate from the endometrium. Direct embryonic/fetal damage, without aspiration, may be advantageous when performing this procedure in unilateral twins. This prevents partial collapse of the vesicle and possible fluid leakage into the endometrium. Treating the mare with exogenous progestins to enhance uterine tone can also help prevent separation of the membranes from the endometrium when performing this procedure. Progestin therapy is generally continued until establishment of placentation (Day 100-120). Mares maintained on exogenous progestins require frequent monitoring to verify fetal viability.

With bilateral twin pregnancies, there is significantly less likelihood that penetration of the conceptus and surrounding membranes will occur. As a consequence, one can more aggressively aspirate fluid from the selected conceptus or induce fetal damage with the needle. Also, the time limitations seen with unilateral twins are not as stringent when using this procedure for bilateral twin pregnancies. However, age of the mare, parity, size of the mare, position of the uterus, tone of the uterus can all negatively impact the success of the procedure. The procedure is significantly more difficult if the pregnant uterus is pendulous within the abdomen in aged mares or advanced pregnancy. As a consequence, performing the procedure in older, multiparous mares after 45 or 55 days can be challenging.

Advantages to using a transvaginal ultrasound-guided approach for twin reduction in mares include twin reduction prior to placentation, minimal trauma to the mare and standing, out patient procedure. Disadvantages of this approach include expense, need for specialty equipment and success variability.

Cranio-cervical dislocation
Cranio-cervical dislocation (CCD) is described as the dislocation of the first cervical vertebrae from the cranium, disrupting the ligamentous attachments and severing the spinal cord. This new procedure can be performed to resolve twin pregnancy using transrectal or transabdominal techniques between 60 and 110 days of gestation. The basis for this procedure is to eliminate one twin before placentation is complete, allowing the remaining fetus to utilize the entire endometrial surface for nutrient and oxygen exchange.

This procedure has been performed using a transrectal approach between 60 and 90 days of gestation. The mare is restrained in stocks or twitched in the doorway. Sedation can be administered as needed, but is generally not
recommended due to uterine relaxation and difficulty in reaching the fetuses. Relaxation of the smooth muscle in the uterus and rectum is generally achieved by the administration of propantheline bromide (15-30mg, IV) or N-butylscopolammonium bromide. These agents facilitate easier identification and manipulation of the fetuses. To help inhibit prostaglandin release, flunixin meglumine (1mg/kg, IV) is administered before the procedure. The smaller fetus or the fetus that has less contact with the endometrium and minimal space to grow is preferentially reduced. This fetus is usually identified in the more cranial aspect of the uterine horn in unilateral twins. Once the targeted fetus is located, the head is isolated by finding the dome-shaped head and palpating the mandible or moving caudally and locating the cervical vertebrae. Cranio-cervical dislocation is performed by stabilizing the head between the thumb and forefinger and bending the head from side to side. This will damage the ligaments attaching the head and neck. Dislocation is then created by placing the thumb at the base of the cranium and applying pressure proximal and dorsally. A distinctive pop is felt if dislocation is achieved, and the thumb and forefinger can be placed in the space created between the head and neck. Mares should be treated with altrenogest at a dose of .088mg/kg once a day once daily for three to four weeks. After cranio-cervical dislocation, death with loss of the fetal heart beat is usually evident within 24 hours to one week. Fetal viability should be evaluated in one week and every two weeks for a month to establish normal growth of the continuing fetus and demise of the other. Using this procedure in eight mares between 55 and 90 days gestation, five (63%) delivered live, singleton foals.

While successful, transrectal cervico-cranial dislocation is technically challenging due to limited proximity of the fetus and potential for rectal injury. A similar procedure for fetal termination has been used via a flank approach (surgical). To date, this procedure has been used for twins between gestational ages of 58 and 150 days. Preoperative medications include: propantheline bromide (30mg IV), flunixin meglumine (1 mg/kg IV), broad spectrum antibiotics (such as penicillin and gentamicin) and exogenous progestins (altrenogest). Propantheline bromide is essential for preventing uterine contractions while finding and manipulating the fetus. Transabdominal ultrasound is used to identify the horn in which the targeted fetus is located. Fetal size and positioning relative to the endometrium are factors considered when selecting a fetus to terminate. A standing flank laparotomy is performed ipsilateral to the horn containing the fetus that has been identified for reduction. Identification of the preferred uterine horn is not always possible because of fetal movements and imaging capabilities. If this occurs, the incision is made in the right flank of the mare, allowing more access to the reproductive tract without intestinal interference. The uterus is located within the abdominal cavity with one arm, and the twin is isolated as described for transrectal dislocations. Cranio-cervical dislocation is performed by manipulating the fetus through the uterus, without incising or invading the uterine lumen. The flank incision is then routinely closed. With this technique, death of the manipulated twin may not be evident until 24 hours to seven weeks. Mares are administered antibiotics and anti-inflammatory agents for 5-7 days and exogenous progestins for at least 30 days after the procedure. Cranio-cervical dislocations, using the surgical procedure, have been performed between 58 and 150 days of gestation. Manipulations were only performed once. Cranio-cervical dislocations, using the intra-abdominal surgical procedure, produced a single normal healthy foal in 24 of 38 (63%) of mares. With this technique, death of the manipulated twin was evident from one to eight weeks after the procedure. One fetus that underwent CCD never died and abortion was induced at seven months in order not to affect the mare’s present or future fertility. Re-evaluation of fetal viability is performed with transrectal or transabdominal ultrasonography every two weeks until demise of one twin is observed. Signs of impending death of a fetus include: loss of thoracic shape, with the fetus becoming more convex; loss of definition of abdominal organs; and irregular, weak heartbeats.

Delivery of a singleton foals following this procedure is uneventful. Placentas from mares delivering singleton foals have a small sack attached to the allantoic surface. The nonviable fetus is marsupialized into the placenta forming a small pouch, with a stalk protruding from the allantoic surface. This pouch contains the mummified fetal bones. Examination of the chorionic surface reveals minimal evidence that a twin was present, with microvilli present along the entire attachment of the placenta.

Cranio-cervical dislocation has advantages when compared to other procedures for reducing post-fixation twins. The procedure is performed prior to complete placentation formation. As such, placental compromise does not occur for the remaining singleton. Anecdotal reports following transcocuteaneous cardiac puncture for twin reduction suggest that live born singleton foals may be undersized and weak (Johanna Reimer; Lexington, KY, personal communication, April 2001). Additionally, using cervico-cranial dislocation, the uterus is not penetrated with a needle via the abdomen thus reducing the risk of peritonitis and/or fluid leakage which might disrupt the endometrial contact for the remaining twin. Disadvantages of cranio-cervical dislocation include isolation of the fetus and surgical approach. Identifying the correct fetus within the uterus is similar to “bobbing for apples”. It is absolutely imperative that the uterus is relaxed enough for identification of fetal anatomy. Additionally, the long period between performing the procedure and fetal death, in some cases, is disconcerting to the mare owner. Little explanation for continued survival of the fetus with a dislocated spinal cord exists.
Transcutaneous, ultrasound-guided twin reduction

The use of transcutaneous ultrasonography to aid in twin reduction in the mare was pioneered by Rantanen and Kincaid in 1988.24 In experienced hands, an average of 50% of mares undergoing transcutaneous twin reduction will deliver one live foal.23,24,31 The suggested time to perform this procedure is between 115 and 130 days gestation.25 As with other described procedures, mares are often treated peri-operatively with broad spectrum antibiotics, anti-inflammatory agents, and exogenous progestins. The procedure is performed in the standing, heavily sedated mare. Sedation promotes movement of the fetuses into the cranial abdomen for easier accessibility and minimizes fetal movement during the procedure. The mare is examined with a 2.5-3.5 MHz transducer to determine fetal position and size. The most accessible fetus is selected for reduction, or when possible, the smaller fetus is targeted. The mare’s abdominal area adjacent to the fetuses is clipped and surgically prepared prior to the procedure. The transducer is placed in a sterile obstetrical sleeve containing sterile lubricant. Some veterinarians prefer to infiltrate the area adjacent to the targeted fetus with 2% lidocaine hydrochloride to provide anesthesia prior to passage of the needle. The use of a biopsy guide to perform the procedure is at the discretion of the operator. A biopsy guide on the transducer coordinates with software in the ultrasound which allows the operator to know the expected placement of the needle. Some operators find the use of a biopsy guide limiting when the fetuses and/or mare change position. Typically, a 6 inch, 18-gauge needle with a stylet and echogenic tip (Cook Veterinary Products, Brisbane, Australia) can be used to allow for better visualization of the needle tip on the ultrasound image. The needle is passed through the skin and abdomen in one motion. Once the needle is passed into the peritoneal space, the needle tip is located on the ultrasound image, advanced through the uterine wall and into the uterine lumen using a quick, thrusting motion. Penetration of the fetal thorax and heart can be challenging as the fetus frequently moves away from the needle if rapid penetration is not achieved. Free flow of blood from the needle after removal of the stylet indicates needle placement within the fetal heart. Potassium chloride (KCl, 2 mEq/ml, up to 32 mEq KCL) or procaine penicillin (10-20 ml)11 is injected into the fetal heart, thorax or abdomen. Potassium chloride generally results in rapid fetal death, particularly with intracardiac needle placement. Proposed advantages of injecting procaine penicillin for transcutaneous ultrasound-guided twin reduction include: 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. A disadvantage of using procaine penicillin to induce fetal death is that it may take up to a few days for the fetus to die (also dependent on injection site).

Cardiac activity of the treated fetus is monitored immediately after the procedure. The fetus does not always die immediately in which case the mare is monitored over subsequent days to assess the status of both the treated and untreated fetuses. Generally, mares are administered flunixin meglumine (1 mg/kg, IV) at the time of the procedure and for up to four additional days (twice daily). Progestin therapy (altrenogest, 0.044 to 0.088 mg/kg, PO, daily) and prophylactic antibiotics are prescribed at the referring veterinarian’s discretion.

Success rate of fetal cardiac puncture for twin reduction averages 50%. Factors that contribute to success of the procedure include proximity of the fetus to the adjacent twin (and placentation involved), operator experience and conditions for performing the procedure. As with cranio-cervical dislocation, the terminated fetus is mummified and delivered in a small placental sac along with the live fetus. The terminated fetus rarely interferes with the development of the remaining fetus. However, because the procedure is performed after placental formation is complete, some have speculated that placental insufficiency may result in small, unthrifty foals (Johanna Reimer, personal communication). Other disadvantages of the procedure include potential complications such as peritonitis (rare), need for special equipment and expense.

Elective termination of both pregnancies

When other procedures are not elected or fail to reduce twins to a singleton after endometrial cup formation has occurred, aborting both pregnancies is the last option. Mares carrying twin fetuses have an increased risk for abortion, dystocia, cervical tears and retained fetal membranes. While some mares successfully carry twin pregnancies to term, the risks of complications during pregnancy or delivery warrant termination of one or both fetuses.

Conclusions

Manual reduction of twin embryonic vesicles to a singleton is the most viable option for managing twin pregnancies in mares. In the event that twins are not identified during the period when manual reduction is a
feasible option, several other procedures are available to the mare owner. Pros and cons of all procedures, including success rates and stage of gestation, should be considered prior to selecting a method of management for late embryonic or fetal twins.

References