Twinning is an important cause of pregnancy loss in mares and is often associated with economic loss. Most twin pregnancies can be expected to result in early gestational death, late-term abortions, or delivery of small foals with retarded growth. These foals can be more susceptible to infections and slower to develop after birth, compared with singletons.\(^1\) Abortion in late gestation and the birth of twins often involve dystocia and trauma to the reproductive tract and are associated with poor rebreeding potential.\(^3\) Prior to 1997, reported incidence rates of twinning-associated spontaneous abortion in mares ranged from 112 of 516 (22%)\(^4\) to 689 of 1,788 (38.5%).\(^5\) Results of a 9-year study\(^6\) undertaken between 1988 and 1997 in the United Kingdom have shown that the rate of spontaneous abortion in mares was as low as 75 of 1,252 (6%). Although the direct effect of increased availability of ultrasound scanning on twin-associated spontaneous abortions has not been studied, it is possible that routine early scanning practices have contributed to this reduction. A report in 1973 indicated that only 9 of 62 (15%) twin pregnancies produced 2 live foals and that foals born alive were usually emaciated.\(^4\) In that study,\(^4\) 13 of 31 (42%) foals born of twin pregnancies had died by 7 days of age, compared with 15 of 1,665 (0.9%) singleton foals. Mares that give birth to twins are also likely to have an oversized uterus, which delays postpartum involution and has been associated with reduced live foaling rates in subsequent breeding seasons.\(^1,7\)

Undetected and unmanaged twin pregnancies have important implications, with negative effects on animal health and economic loss being the principle detriments. Reduction of twin pregnancies without subsequent loss of the remaining foal is particularly important in animals with high economic value, mares in poor subsequent breeding potential, cases where the diagnosis is made late in gestation or late in the breeding season, and situations where a stallion is not available for rebreeding.

Twin births in mares can and should be avoided, and it is common practice to reduce twin pregnancies to singleton pregnancies at the time of diagnosis. This article summarizes the most common techniques available to veterinary practitioners for reduction of a twin pregnancy in mares and reported success rates for each of these methods.

**Key Factors Associated With Twin Pregnanacies**

1. **Twinning appears to have a high degree of repeatability and heritability.**\(^6\) The rate of occurrence can vary according to breed and may be directly proportional to the fertility of a stallion.\(^9\)

2. **Multiple ovulations**—The prevalence of multiple ovulations is thought to be highest in Thoroughbreds, Warmblood breeds, and Irish draft horses and lowest in other draft breeds, Arabians, and pony breeds.\(^10\) A study\(^11\) investigating multiple ovulations in mares of different breeds found twin vesicles in 97 of 629 (15.4%) Thoroughbred mares, compared with 46 of 1,294 (3.6%) Standardbred mares. Multiple ovulations and twin pregnancies have also been found to occur more frequently in some familial lines than in others.\(^7\)

3. **Reproductive status**—An analysis of reproductive status at the time of breeding in mares treated with human chorionic gonadotropin revealed that previously barren and maiden mares were significantly \((P = 0.005)\) more likely to have multiple ovulations and twin pregnancies than were lactating mares.\(^12\) Negative feedback on the hypothalamic-pituitary axis caused by lactation has been hypothesized to contribute to this effect.\(^12\)

4. **Induction of ovulation**—Results of statistical analyses have suggested that 1% to 2% of pregnancies in mares involve twinning.\(^13\) However, the use of ovulation induction agents in breeding practice may influence these rates. In a large retrospective study\(^12\) to evaluate the effects of ovulation induction on the frequency of multiple ovulations and twinning in Thoroughbreds, twin pregnancy was diagnosed on day 14 after ovulation in 187 of 1,409 (13.3%) ovulatory cycles in which mares were bred (following administration of human chorionic gonadotropin). In that study,\(^12\) treatment with human chorionic gonadotropin significantly \((P < 0.001)\)

**Abbreviations**

- eCG: Equine chorionic gonadotropin
- PG: Prostaglandin
- TUA: Transvaginal ultrasound-guided aspiration
- TVUEVI: Transvaginal ultrasound-guided embryonic vesicle injection
- TVUEVP: Transvaginal ultrasound-guided embryonic vesicle puncture

**References**

- From the Faculty of Veterinary Science, University of Melbourne, Werribee, VIC 3030, Australia.
- Address correspondence to Dr. Krekeler (krekeler@unimelb.edu.au).
increased the odds of a mare having a twin pregnancy. Investigators in another study\(^9\) found no significant difference in multiple pregnancy rates between Thoroughbred mares that did and did not receive human chorionic gonadotropin to induce ovulation. These studies had technical differences in regard to doses and time of administration that may have accounted for their different results. However, there is clearly a need for meticulousness in evaluation of mares for twin pregnancies and for management of twin embryos.\(^{14}\)

**Considerations in Management of Twin Pregnancies**

Prior to examining the techniques available for twin pregnancy reduction, factors that may result in failure to detect the condition should be considered. These include difficulties in interpretation of ultrasonographic images, endometrial cysts obscuring visualization via ultrasonography, variability in embryo growth patterns, embryological abnormalities, inability to detect 2 fetal heart beats, and the presence of remnants of a nonviable early embryo.\(^{14}\) Some authors have proposed that the most common cause for failure to detect an early twin pregnancy is performing the ultrasonographic evaluation too soon after breeding to be able to recognize a second pregnancy following an asynchronous ovulation.\(^1\) It may also be necessary to perform multiple ultrasonographic examinations on consecutive days to identify reproductive tract abnormalities that may possibly be misclassified as embryos. Reflecting on concerns such as these may assist veterinarians in achieving continued success with diagnosis and management of twin pregnancies.

It has been reported that synchronous and asynchronous ovulations are equally likely to produce twins.\(^1\) The pregnancy rate per ovulation when double ovulation occurred bilaterally was reportedly similar to that for single ovulations per cycle and was higher than the pregnancy rate per ovulation when double ovulation occurred unilaterally.\(^{17}\) Fertilization rates as high as 27 of 28 (96%) have been reported in healthy mares,\(^{18}\) suggesting almost all multiple ovulations could potentially result in multiple pregnancies; however, this conclusion was based on results for a uniform study population of Standardbred mares of similar age and may not be accurate when extrapolated to other populations of mares. Pregnancy rates at evaluation will usually be lower than this, owing to death of defective embryos.\(^{18}\) Results of 1 large study\(^9\) suggested that most multiple pregnancies in mares result from multiple, rather than single, ovulations.

Following fertilization, the equine conceptus travels slowly through the oviduct and enters the uterus on day 6 after ovulation.\(^3\) In a study\(^{20}\) of 22 pony mares, embryos were detectable by use of a 5.0-MHz transducer on day 9, day 10, and day 11 after ovulation in 5 (23%), 8 (36%), and 20 (91%) mares, respectively. During this time, embryonic vesicles were located in the uterine body approximately 60% of the time and, thereafter, in either uterine horn with increasing frequency until day 16 to 17 after ovulation.\(^{20}\) Although PGF\(_2\alpha\), a luteolytic agent, can be produced by the endometrium of pregnant mares, this capacity is greatly reduced by the presence of a conceptus.\(^{21}\) In experiments that altered the amount of uterine tissue accessible to the conceptus, pregnancies failed when conceptus mobility was restricted to less than two-thirds of the endometrial surface; however, pregnancies were maintained when mares received exogenous progesterone, suggesting that luteolysis and a resulting decrease in circulating progesterone concentration was responsible for pregnancy failure.\(^{22}\) It has been proposed that the purpose of intrauterine mobility of the conceptus is to distribute antiluteolytic hormone onto the endometrium,\(^{23}\) interacting with the endometrial epithelium and preventing secretion of PGF\(_2\alpha\), thereby achieving luteostasis and maintenance of the pregnancy.\(^{3}\)

When the growing conceptus is physically unable to travel through the narrow uterine lumen at 16 to 17 days after ovulation, fixation occurs, commonly at the base of 1 uterine horn.\(^{24}\) The fixed embryo continues to grow and begins differentiation at this stage; this involves organogenesis, membrane development, and villous attachment of the trophoblast to the endometrial epithelium to achieve attachment.\(^3\) Attachment occurs around day 35 after ovulation, when the embryo establishes a nutritive placenta as fetal chorionic villi interdigitate with the maternal endometrial epithelium. Given that the total combined surface area of twin placentas has been shown to be only slightly greater than that of singletons, placental insufficiency has been proposed to be responsible for some of the ill effects of twinning.\(^4\)

Beginning on day 36 after ovulation, fetal trophoblast cells originating from the chorionic girdle start endometrial cup formation. The endometrial cups secrete eCG,\(^{25}\) which functions primarily as a luteinizing hormone–like hormone, causing development of accessory corpora lutea, which then produce progesterone for the maintenance of pregnancy.\(^{26}\) Equine chorionic gonadotrophin is produced until days 80 to 120 after ovulation, regardless of pregnancy status.\(^{27}\) Pregnancy loss while eCG is being secreted does not interrupt the formation of accessory corpora lutea, so that progesterone continues to be secreted, and estrus often fails to return, occasionally for an extended period beyond days 80 to 120 after ovulation, until endometrial cups are no longer functional.\(^{28}\) Therefore, any manipulation or treatment performed after endometrial cup formation may be associated with a substantial risk of loss because fetal death commonly results in inability to rebreed until the following season.

**Natural Reduction**

Multiple equine embryos can fix unilaterally (in the same uterine horn) or bilaterally (in separate uterine horns), and localization can influence decision making for management of twinning. The spontaneous or natural twin reduction rate has been shown to be negligible before day 15 after ovulation, and it is not known to occur at all prior to day 11.\(^{15}\) However, the diagnosis of a twin pregnancy should ideally be made prior to day 16 to allow intervention before chorionic fixation.\(^{16,17,29}\) One study\(^{30}\) showed that 233 of 923 (25.2%) multiple ovulations resulted in multiple pregnancies, while 349 of 923 (37.8%) resulted in singleton
pregnancies. The deprivation hypothesis was formulated to explain the occurrence of natural reduction, in which excess embryos are eliminated, leaving behind a single embryo.\textsuperscript{31} It has been suggested that close apposition of embryonic vesicles results in a loss of contact between endometrial and trophoblastic surfaces, reducing the nutritional supply to one of the vesicles and causing death of that embryo, with the other surviving to term.\textsuperscript{11} The actual mechanism for natural reduction remains unknown.

In a study\textsuperscript{32} exploring the deprivation hypothesis, unilateral fixation of twin vesicles was found to occur in 48 of 68 (71%) twin pregnancies, and 41 of 48 (85%) of these pregnancies had reduced naturally by day 40 after ovulation. Twenty-eight (58%) of these reductions occurred between days 17 and 20, 13 (27%) occurred between days 21 and 30, and 7 (13%) occurred between days 31 and 38 after ovulation. Investigators of another study\textsuperscript{33} found that, of 17 natural reductions of twin pregnancies with unilateral fixation, 9 occurred before day 20 and 14 occurred by day 30 after ovulation; only 3 of 17 occurred later than day 30. Greater dissimilarity of vesicular size (≥4 mm difference in diameter) was associated with a higher frequency of natural reduction before day 20 after ovulation.\textsuperscript{16} Similarly, in 22 mares with unilaterally fixed twins, natural reduction occurred in 100% of twin pregnancies with vesicles of dissimilar sizes (≥4 mm difference in diameter), whereas 19 of 26 (73%) twin pregnancies with vesicles of more similar size (0- to 3-mm difference in diameter) naturally reduced to a singleton pregnancy.\textsuperscript{15} These results suggest that for unilaterally fixed twins, allowing time for natural reduction to take place can be considered as an option in the management plan.

Occurrence of natural reduction is greatly dependent on whether fixation occurs unilaterally or bilaterally. Investigators of 1 study\textsuperscript{33} reported natural reductions in only 2 of 7 bilaterally fixed embryonic vesicles before day 40 after ovulation, and both of these occurred later than 30 days after ovulation. In another study\textsuperscript{34} of 15 pony mares pregnant with bilaterally fixed twins, 8 mares aborted both embryos; 2 aborted 1 embryo by 3 months; 4 aborted after 8 months, and only 1 mare delivered 2 live foals. The study\textsuperscript{34} was small, and the results may not be applicable for larger breeds because it has been suggested that mares of larger breeds may be more likely to maintain a twin pregnancy.\textsuperscript{11} However, these data suggest that benign neglect may not be a viable option for management of bilaterally fixed twins. Assessing the potential for natural reduction and the number of days in gestation of mares with a diagnosis of a twin pregnancy will thus aid in determining options available for management.

**Manual Reduction**

The frequent occurrence of twinning caused by multiple ovulations in mares necessitates early ultrasonographic examination of the uterus after breeding.\textsuperscript{35} Twin reduction is most successful from days 13 to 15 after ovulation.\textsuperscript{13} During this time, multiple conceptions resulting from asynchronous ovulations may be easily detected, as both vesicles are large enough to be visualized easily. Manual reduction by crushing of 1 vesicle at this stage has been reported to result in survival of the remaining conceptus to 42 days after ovulation in 37 of 40 (93%) pregnancies with unilaterally fixed twins.\textsuperscript{36,37} Similar success rates (where success is defined as reduction to a singleton pregnancy) were also reported (39/42 [93%]) when unilaterally fixed twin vesicles could be separated for 1 to be crushed between days 16 and 19 after ovulation.\textsuperscript{38} A study\textsuperscript{39} targeting the effects of operator, treatment, and mare age on live foaling rate reported no difference in success rates (P = 0.4) when manual reduction was performed between days 13 and 16 after ovulation, compared with those performed between days 17 and 20. Other factors, such as medical treatments (mares treated with flunixin meglumine and progesterone had higher foaling rates than untreated mares [P = 0.02]) and operator experience (significant effect for 4/10 operators), were more important when considering better prognosis for reduction procedures performed prior to day 21 after ovulation.

Another report\textsuperscript{40} has indicated that manual crushing of 1 embryonic vesicle does not decrease the live foaling rate of mares < 9 years of age but may result in a higher frequency of pregnancy loss in older mares. Reported success rates following manual reduction from day 20 to 30 after ovulation when twins are unilaterally fixed are also poorer (13 of 26 [50%]), compared with results of manual reduction prior to day 20, owing to difficulty in separating the vesicles.\textsuperscript{38} Manually crushing 1 of unilaterally fixed twin vesicles between days 30 and 35 after ovulation may have a success rate lower than the natural reduction rate.\textsuperscript{11} Performing the procedure prior to fixation at day 16 or 17 after ovulation is likely to result in successful reduction to a singleton pregnancy, and it has been proposed that success rates by this definition can reach 100% (with 10/10 successful reductions reported for such mares in 1 study).\textsuperscript{36}

Interestingly, investigators of a recent study\textsuperscript{41} concluded that the rupture of small embryonic vesicles prior to fixation (defined as a reduction on or before day 16 after ovulation) in multiple pregnancies does not carry an increased risk of abortion of the remaining twin, compared with the same risk for singleton pregnancies. In that study,\textsuperscript{41} 604 twin pregnancies were managed by manual reduction prior to fixation. The results indicated the frequency of pregnancy loss was greater for singletons than for manually reduced multiple pregnancies. Although debatable, a suggested explanation for this result was that reduction of a smaller and therefore less viable embryonic vesicle in twin pregnancies allowed the larger to thrive, while no such selection was possible in single pregnancies. Regardless, those data appear to suggest that there may be an advantage to correctly managed twinning, and that acceptable live foaling rates can be achieved.

Given that reported natural reduction rates for pregnancy with bilaterally fixed twins are low,\textsuperscript{31,32} these should all be reduced upon diagnosis. Recommendations have been made to eliminate one of the conceptions upon diagnosis up to day 30 after ovulation, and reduction to singleton pregnancies have been reported in up to 33 of 47 (70%) multiple pregnancies managed by this method.\textsuperscript{13,42} It has been suggested that a reason...
for poorer prognosis for survival of the remaining twin following reduction of a twin pregnancy on or after day 31 is that the fluid produced by rupturing 1 vesicle can interfere with physiologic exchanges between the remaining embryo and the dam. This was reflected in a study in which 3 mares with singleton pregnancies (with pregnancies at days 43, 50, and 55 after ovulation) received an injection of 250 mL of sterile placent al fluid into the nongravid horn of the uterus. In all 3 mares, fetal stress (identified ultrasonographically by clouding of the amniotic fluid with echogenic particulate matter and by excessive movement of the fetus) was detected ≤ 1 hour after the procedure. All 3 fetuses died ≤ 3 hours after the procedure. These results underscore that manual reduction of bilateral twin pregnancies should be performed without any delay to help reduce the possibility of adverse effects on the remaining twin. Furthermore, a recent retrospective study found no effect of position of embryonic vesicles at the time of manual reduction (on or before day 17 after ovulation), parity, or month bred on live foaling rates. More skill is often required to separate unilaterally fixed twins, and a less-experienced practitioner may choose to delay action, considering the potential for natural reduction in that scenario.

There has been some evidence that tissue trauma resulting from manual reduction increases PGF subalpha production by the endometrium. Several studies have yielded contrasting information regarding anti-inflammatory drug and progestogen treatments. Progesterone is produced by the corpus luteum in the first 100 days of pregnancy, and PGF subalpha production may result in luteolysis. A study in which mares did not receive any treatment at the time of twin pregnancy reduction showed no significant (P > 0.05) difference in pre- versus postcrushing concentrations of cortisol, progesterone, and PGF subalpha in plasma samples. Another study also showed that 20 minutes of palpation was necessary for a significant (P < 0.05) increase in plasma PGF subalpha concentration. Investigators of that study concluded that if reduction is performed before day 30 after ovulation without complications, the administration of exogenous progesterone or flunixin meglumine is not essential for the survival of the remaining conceptus. A retrospective case-control study showed that plasma progesterone concentrations did not differ between mares that lost their pregnancy and those that delivered a live foal, but this result was confounded because many mares had received exogenous progestogens. In another study, a dose of flunixin meglumine (1.0 mg/kg [0.45 mg/lb], IV) was shown to effectively inhibit all PGF subalpha synthesis and release during rupture of embryonic vesicles. Altrenogest (0.088 mg/kg [0.040 mg/lb], PO), a synthetic progestogen, has also been used as an alternative treatment for the prevention of embryonic death.

Survival and growth of the remaining embryonic vesicle should be evaluated 2 to 3 days after crushing of the excess vesicle; embryonic loss usually occurs within this period. Examination should be repeated 1 week later, and then every other week for a month, to confirm the death of 1 embryo and normal growth of the other. Despite the many factors that have been reported to affect outcomes following manual reduction of twin pregnancies, the success rate of this procedure with respect to live foal production has been shown to be greater than that achieved by other techniques. Therefore, it often remains the treatment of choice.

Transvaginal Ultrasound-Guided Reduction

First reported in 1993, TUA involves aspiration of yolk sac or allantoic fluid. Dutch Warmblood mares with twin pregnancies between days 20 to 43 after ovulation were used in that study. Flunixin meglumine (350 mg, IV) and 2% lidocaine solution (50 mL, administered into the rectum through a flexible catheter) were given prior to ultrasound-guided penetration and aspiration with an 18-gauge needle and syringe through the vaginal fornix. In that study, the success rate (where success was defined as the presence of 1 viable conceptus ≥ 10 days after the procedure) when twins were unilaterally fixed was 3 of 9 for procedures performed between gestational days 20 and 45 and 2 of 3 for procedures performed prior to day 35. When twins were fixed bilaterally, reduction to a singleton pregnancy was successful in 3 of 4 mares that had the procedure performed prior to day 35 after ovulation and in 1 of 4 mares that underwent the procedure after day 35. A similar study in which twin pregnancy reduction via ultrasound-guided puncture of the embryonic vesicle was performed prior to day 35 after ovulation produced comparable results, with 9 of 15 mares having a viable singleton detected 7 days after the procedure and 5 of 16 mares delivering live foals. In another study of mares that underwent this procedure, 2 of 8 mares initially carrying bilaterally fixed twins delivered a live foal, whereas 1 of 11 mares with unilaterally fixed twins produced a live foal. Among 7 of 10 mares with unilaterally fixed twins that had the procedure performed later than day 35 after ovulation, none delivered live foals. It was noted that in most cases, fetal death occurred ≤ 14 days after the procedure, but some mares carried apparently viable singletons for 3 to 9 months before aborting. However, sample size was particularly small in each of these studies, and as such, the findings should be interpreted cautiously.

More encouraging live foaling rates were reported in another study of mares that underwent TUA; 14 of 20 (70%) mares pregnant with unilaterally positioned twins delivered a live foal when the procedure was performed between days 16 and 25 after ovulation, although 0 of 4 mares (1 and 3 with unilaterally and bilaterally fixed twins, respectively) treated later than day 40 gave birth to live foals. The interval after ovulation explored in that study coincided with the period in which natural reduction typically occurs; however, a possible indication for reduction via aspiration methods during this period may be detection of similarly sized vesicles, where natural reduction is deemed unlikely to occur.

A recent clinical trial in which reduction of 44 twin pregnancies in mares (39 and 5 with unilaterally and bilaterally fixed twins, respectively) was attempted via TUA (with or without embryonic or fetal puncture)
between 25 and 62 days after ovulation has provided the most promising live foaling data to date. In that study,50 20 of 41 (49%) mares that underwent the reduction procedure between days 25 and 62 after ovulation delivered live singleton foals (3 mares that underwent additional procedures when twin pregnancy persisted 10 days after the procedure were excluded from these calculations). Further analysis revealed a successful outcome (delivery of 1 live foal) for 7 of 14 mares that underwent TUA between days 25 and 30 after ovulation, 9 of 14 mares that underwent the treatment between days 31 and 35, and 4 of 8 that had the procedure performed between days 36 and 42. None of the 5 mares that underwent TUA between gestational days 43 and 62 delivered a live foal. In that study, flunixin meglumine (1.1 mg/kg [0.5 mg/lb], IV) and cefquinome (1.0 mg/kg, IV) were administered prophylactically against PGF2α release and bacterial infection, respectively. Those investigators found a significant ($P = 0.027$) negative effect on live foaling rate when TUA was combined with puncture of the embryo or fetus and attributed that result to the need for greater manipulation of the uterus. One conclusion of the study50 was that the optimal time for reduction of twin pregnancies via TUA is between days 30 and 33 after ovulation, which would allow an opportunity for natural reduction to occur and avoid eCG production starting on day 35, potentially allowing a mare to be rebred in the same season if death of the remaining twin occurs.

Alternatives to aspiration techniques have been investigated, and an experimental model for assessing the effectiveness of other transvaginal reduction techniques has been described.51 In that study, the effectiveness of TVUEVI with amikacin (25 mg, once) for reduction of experimentally induced twin pregnancies in mares was compared with that of TVUEVP. Groups of 4 or 5 Standardbred-cross mares were inseminated following simultaneous induction of estrus. Subsequently, 1 mare in each group was selected to receive a 7-day-old embryo from a donor within the same group. Mares found to be carrying twins on day 28 after ovulation underwent 1 of 2 randomly assigned reduction procedures (TVUEVI or TVUEVP). Mares that were not pregnant were placed in a new group, and the experimental process was repeated. On day 33, 2 of 5 mares that had the TVUEVI procedure performed carried 1 viable embryo, compared with 1 of 5 mares in which TVUEVP was performed. The difficulty of the technique and operator ability may have contributed to the apparent differences in results between the experiments, and further studies are needed before conclusions can be reached.51 More importantly, the experimental method for inducing twin pregnancies resulted in 23 of 43 (53%) mares pregnant with twins on day 16 after ovulation, and 10 of these 23 (43%) maintained twin pregnancies on day 28. On day 16, the twin vesicles were located unilaterally in 15 of 23 (65%) mares and bilaterally in 8 (35%) mares. On day 28, 2 of 10 mares still pregnant with twins carried unilaterally fixed embryos, and the remaining 8 carried bilaterally fixed embryos. The proportion of mares with twin pregnancies established experimentally in the study was considerably higher than that expected to occur naturally (10%),52 possibly making this a useful model for further studies investigating twinning reduction techniques.51

In a study52 performed to evaluate the possible differences in efficacy between TUA and embryonic or fetal puncture techniques for reduction of twin pregnancies in 103 mares between days 21 and 76 after ovulation, investigators found no significant difference ($P = 0.14$) in success rates (with delivery of a healthy foal considered successful) for the puncture method (12/28 [43%]), compared with TUA (22/75 [29%]). Location of the embryonic vesicles at the time of the procedure also had no significant ($P = 0.11$) influence on outcome, with a healthy foal born to 19 of 65 (29%) mares that had unilaterally fixed twins and 15 of 38 (39%) that had bilaterally fixed twins.52

Transcutaneous Ultrasound-Guided Reduction

This technique was first applied as a method for reduction of twin pregnancies in a group of 19 mares between days 66 and 168 after ovulation.53 Following ultrasound-guided needle insertion into the fetal heart, potassium chloride (2 mEq/mL) solution was injected in 1-mL aliquots until cessation of cardiac activity; up to 16 mL of the potassium chloride solution was administered without affecting the remaining fetus.53 Mares were treated with flunixin meglumine (500 mg, IV, q 24 h) for 4 days beginning on the day of the procedure. Seven of 18 mares subsequently delivered live singleton foals. In a further study by those investigators,1 the same technique of transcutaneous ultrasound-guided fetal intracardiac injection was performed on mares with pregnancies beyond day 113 after ovulation, and 29 of 59 (49%) delivered live foals. The investigators concluded that the procedure is best performed between days 115 and 130 after ovulation.

The authors of another study35 reported that 9 of 24 (38%) mares that underwent the same procedure later than day 120 after ovulation delivered live foals. However, 3 of 24 (13%) mares delivered foals that were weak and failed to grow adequately and were therefore considered to have unsuccessful outcomes. It is important to note that apparent differences in success rates between the various studies may have been attributable to operator technique and experience.

Another variation of transcutaneous ultrasound-guided reduction involves injection of 10 to 20 mL of penicillin G procaine into the fetus in the region of heart, thorax, or abdomen and monitoring for fetal death immediately afterward.3 The use of this technique resulted in delivery of a live singleton foal in 8 of 13 mares.1 The authors indicated that the advantages of this technique included the possibility of reduced risk of iatrogenic bacterial infection, improved visualization on ultrasonography, compared with the use of potassium chloride, and the fact that injection into either the thoracic or abdominal region of the fetus resulted in fetal death. This technique appears to be reasonably successful in reduction of twin pregnancies. Unfortunately, to our knowledge, success rates for its use to eliminate one of unilaterally versus bilaterally fixed twins have not been reported. Most mares in the report1 that underwent this procedure were prophylactically treated with anti-inflammatory drugs, antimicrobials, and a progestogen after the procedure. Follow-up examina-
tions throughout the pregnancy are necessary to detect complications (ie, signs of an impending abortion following treatment), which may not be obvious immediately after the procedure.1

Use of a biopsy guide may help to improve the accuracy of needle placement in fetal injection techniques. Depending on the location of the fetus, 18- to 20-gauge spinal needles up to 6 inches in length may be required for the procedure. The most easily accessible fetus should be selected for elimination, unless there is a large size disparity with the more accessible fetus deemed the most viable. Minimizing trauma to the uterine tissues and to the remaining fetus may be largely dependent on operator technique, which accordingly may influence the success rates of this procedure.

Although data are lacking to draw conclusions about success rates of the procedure, the clinical report indicates that use of the technique can result in successful reduction of a late-term monozygotic twin pregnancy. Furthermore, although incisional infections can occur, these are not significantly associated with outcome (birth of both fetuses 30 to 60 days after the treatment).56

Reduction of twin pregnancies through this procedure involves dislocating the first cervical vertebrae from the cranium, disrupting the ligamentous attachments, and severing the spinal cord of a fetus. Given a diagnosis after formation of endometrial cups and the start of eCG production, reduction procedures should be performed before placental formation is complete (day 80 after ovulation)56 if possible, to allow the remaining fetus greater access to the endometrial surface for optimal placental contact and growth. 

Two variations of the fetal craniocervical dislocation procedure have been investigated.56 With the first variant, manual dislocation is performed transrectally while the fetus can still be accessed via this route; use of this method between days 55 and 90 after ovulation resulted in 3 of 8 mares delivering a healthy singleton foal.56 This was reported in a retrospective study56 performed between 2001 and 2008. Some fetuses in which the procedure was performed survived (as evidenced by the presence of a fetal heartbeat) for up to 7 weeks afterward. The 3 remaining mares in that study aborted both fetuses 30 to 60 days after the treatment.56

The second variant enables access to the fetus through a laparotomy with a standing flank approach, and in the same retrospective study,25 24 of 38 (63%) mares delivered a healthy singleton foal. Gestational age of the fetus at the time of the procedure ranged from day 58 to 110 after ovulation, and this variable was not significantly associated with outcome (birth of a healthy singleton foal versus death of both fetuses). Preparation for the procedure included preoperative medications of propantheline bromide (30 mg/mare, IV), flunixin meglumine (1 mg/kg, IV), penicillin G procaine (22,000 U/kg [10,000 U/lb], IM, q 12 h) and gentamicin sulfate (6.6 mg/kg [3 mg/lb], IV, q 24 h). Mares continued to receive the latter 3 medications for 2 days after surgery; then the antimicrobial agent was changed to trimethoprim-sulfadiazine (24 mg/kg [10.9 mg/lb], PO, q 12 h) for 1 week, and altenogest (0.088 mg/kg, PO, q 12 h) was given for 1 month. Some fetuses in which the procedure was performed survived (as evidenced by the presence of a fetal heartbeat) for up to 8 weeks afterward. Although the findings were limited because of small numbers and the retrospective study design, the results underscored the need for regular ultrasonographic examinations until death of the fetus is confirmed. If the manipulated fetus does not die, an alternative solution such as induced abortion may be indicated.

The transrectal approach to isolating the fetus can be difficult to perform, and multiple manipulations may be required to achieve craniocervical dislocation.45 To our knowledge, no association between the number of attempts for fetal manipulation and pregnancy outcomes has been published, although duration of manipulation has been hypothesized to affect the outcome.45 For this reason, and given the apparent similarity in the proportion of mares that delivered live singleton foals after either procedure, laparotomy with a standing flank approach may be the preferred method for isolation of a fetus for craniocervical dislocation. Although we are unaware of any studies to evaluate the effects of tissue trauma or PG release associated with these procedures, the extent of trauma and inflammation are likely dependent on proficiency of the individual performing these tasks. Flank incisions typically heal without complications, and although incisional infections can occur, these are often easily resolved.56 When performed correctly by an experienced veterinarian, craniocervical dislocation does not involve disrupting uterine tissues or leakage of fluids from the manipulated conceptus (which can affect nutrient exchange and survival of the remaining fetuses as previously mentioned) and also may prevent the risk of contamination resulting in placentitis.56

Surgical Removal of a Conceptus

Surgical removal of one conceptus can be performed to allow normal development of the remaining conceptus. This technique should be performed later than 45 days after ovulation to ensure complete endometrial cup development and allantoic association with the chorion,57 and postsurgical care and regular examinations are required according to standards of care.

In a study58 of 15 mares that underwent reduction of twin pregnancies by this method between days 41 and 65 after ovulation, 5 of 7 mares with bilaterally fixed twins and 0 of 7 mares with unilaterally fixed twins subsequently delivered a live foal (1 mare was euthanized because of a femoral fracture several days after surgery). Preparation for the procedure included administration of progesterone (200 mg/mare, IM, q 24 h) for 2 weeks beginning the day before surgery; although the formulation and dosage changed, progesterone treatment was continued until day 100 after ovula-
tion. Antimicrobial agents were administered prophylactically for 5 days, including the day of surgery, and anti-inflammatory agents (flunixin meglumine [1 mg/kg, IV, q 12 h] or phenylbutazone [2 g/mare, IV, q 24 h]) were administered for various durations after the procedure. Ultrasonographic examination was performed a week before surgery and was repeated just before surgery to identify the conceptus to be removed (typically the smaller twin). The surgical times were long, with a mean anesthetic time (evaluated for 12/15 mares) of 100 minutes. Subsequent death of the remaining twin in 1 of 7 surviving mares that had bilaterally fixed conceptuses was attributed to inadvertent separation of the remaining chorioallantois from the endometrium during surgery. Death of the remaining twin in all 7 mares that had unilaterally fixed conceptuses was thought to be caused by disruption of the remaining chorioallantois during removal of the selected conceptus. Thirteen mares were successfully bred the following season.58

**Induction of Abortion**

Inducing abortion is an effective and inexpensive method for terminating twin pregnancies. Whereas the other techniques described in this report may require specialized equipment and specialized training, neither is required for a veterinarian to perform this procedure.

With the aim of producing a viable foal in the same breeding season, early induction of abortion is a reasonable alternative to other treatments and can allow for the mare to be bred again. The treatment of choice is a single dose of PGF2α (eg, dinoprost tromethamine, 5 to 10 mg, IM) or its analog (cloprostenol sodium, 250 µg, IM), which reliably lyses the corpus luteum and terminates pregnancy when administered between days 5 and 35 after ovulation. Monitoring for adverse effects, such as profuse sweating, mild colic, hypothermia, or diarrhea, is important following treatment, but these effects have been reported to be transient.59 Typically, mares can be expected to return to estrus 5 to 7 days after administration of PGF2α in the presence of a mature corpus luteum, with ovulation occurring 9 to 11 days after the treatment.11 Rebreeding early in the season and the associated mild delay in foaling is unlikely to result in an economic disadvantage, especially if mares have no reproductive abnormalities, are otherwise healthy, and are expected to conceive again easily.

For twin pregnancies diagnosed on day 35 after ovulation or later, repeated injections of PGF2α (eg, dinoprost tromethamine, 2.5 to 5 mg/mare, IM, q 12 to 24 h) or fluprostenol sodium (250 µg/mare, IM, q 12 to 24 h) can be required for successful induction of abortion.60 Mares can be expected to abort 2 to 5 days from the start of treatment.61,62 The presence of endometrial cups in mares treated from day 35 onward prevents the return of estrus for a prolonged period, occasionally beyond days 80 to 120 after ovulation, until endometrial cups are no longer functional.28

**Dietary Manipulation**

Dietary deprivation has been described as a possible method for reduction of twin pregnancies. Some husbandry practices, among other environmental factors, have been postulated as possible causes of twinning.63 In a small-scale study,41 41 mares pregnant with twins as determined by rectal palpation between 21 and 49 days after ovulation were treated by complete removal of free-fed grass, oats, feed concentrates, and alfalfa and received only grass hay. Full feed was resumed when a singleton pregnancy was confirmed, and 26 of 41 (63%) mares delivered live singleton foals. Although the outcomes suggested that dietary restriction may be helpful in reduction of twin pregnancies, the study did not include mares that received a control treatment. The small number of animals in the study also indicates the results should be interpreted cautiously, and it is likely that extreme feed depletion, which would be detrimental to the mare’s health, is necessary to be effective.

**Conclusions**

Although challenging, an early diagnosis and manual reduction by crushing of 1 embryonic vesicle during the mobility phase is still the most effective method available for the management of twin pregnancies in mares. When possible, twin pregnancies should be reduced prior to fixation (approx day 16 to 17 after ovulation).24 Experienced veterinarians can expect good outcomes with this technique, and techniques intended for reduction of later-term pregnancies should be reserved for cases where manual reduction can no longer be performed.

Use of TUA has resulted in delivery of a live singleton foal in up to 14 of 20 (70%) cases when performed before day 35 after ovulation, but results are typically poor after this time period.39,47,48,50 Recent reports35,45,53,56 suggest that elimination of a fetus by craniocervical dislocation or transcutaneous ultrasound-guided reduction procedures may be the most promising methods for reduction of twin pregnancies diagnosed relatively late in gestation, especially if detected following eCG production (from 35 days up to, and occasionally beyond, 120 days after ovulation).23,27,28 Although optimal intervals have not been established for use of these methods, craniocervical dislocation between days 55 and 110 after ovulation or transcutaneous ultrasound-guided reduction techniques performed between days 115 and 130 have provided good results, with delivery of live foals in up to 24 of 38 and 29 of 59 mares, respectively.1,60

Existing data are typically skewed, reflecting more mares pregnant with unilaterally fixed than with bilaterally fixed twins, owing to the nature of most equine multiple pregnancies. Although results for attempted reduction of bilaterally fixed twins have been reported for various methods, the relatively small numbers of mares with this condition may result in some inaccuracies when interpreting these results.

The data suggest that manual reduction, transcutaneous ultrasound-guided reduction, craniocervical dislocation, surgical removal of a conceptus, and induction of abortion have identified time frames where their use may be appropriate. However, optimal gestational times for use of TUA and dietary reduction techniques are less clear. Future efforts should be geared toward further identifying the best gestational intervals for the use of each method to provide live foaling rates comparable to those reported for manual reduction during the mobility phase of the embryo.
References


