

Tutorial Article

Current methods for the diagnosis and management of twin pregnancy in the mare

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Summary

Historically, twin pregnancy has been one of the leading causes of pregnancy loss in the mare. Numerous studies have identified factors that increase the incidence of twinning. The identification and management of twins during the mobility phase remains the most effective method to control the potentially detrimental effects of twin pregnancy. Options exist for post fixation management of twins, although these methods are generally less successful than management during the mobility phase.

Introduction

Twinning has long been recognised as one of the most important causes of reproductive wastage in the mare. Mares carrying twins at Day 40 or later frequently lose one or both fetuses in mid to late gestation (Ginther and Griffin 1994) (Fig 1). Mares aborting in late gestation or giving birth to twins are likely to suffer dystocia (Roberts 1982; Frazer 2003; McKinnon 2007), are prone to fetal membrane retention (Frazer 2003) and experience decreased live foaling rates in the following season (Pascoe 1983), probably due to delayed involution of an oversized uterus (McKinnon and Rantanen 1998). At parturition, twin foals suffer a higher rate of stillbirth than singleton foals (Platt 1973). Twin foals born alive are frequently smaller, weaker, more susceptible to infection and slower to develop than singleton foals (Jeffcott and Whitwell 1973; Wolfsdorf 2006) (Fig 2). They also suffer a higher neonatal mortality rate (Jeffcott and Whitwell 1973). The total villous chorionic surface area of twin placentas combined is only slightly greater than that of singleton pregnancies (Jeffcott and Whitwell 1973), making it likely that the detrimental effects of twin pregnancy are mediated by relative placental insufficiency.

Jeffcott and Whitwell (1973) found twinning to be the largest single cause of fetal mortality, accounting for 56 of 258 abortions (22%) examined in a predominately Thoroughbred population. Whitwell (1980) stated that twinning was the...
largest single cause of abortion, responsible for between 22% and 29% of all abortions each season. Examination of Thoroughbred breeding records in Germany revealed 703 of 1817 abortions (38.6%) were attributable to twin pregnancy (Merkt and Jochle 1993). The authors of this study reported a decline in abortion due to twinning over time. A similar finding was reported by Giles et al. (1993) who reviewed 3514 abortions over a 6 year period in central Kentucky. In this study, which included neonatal deaths up to 7 days of age, 221 abortions, or 6.28% of total abortions, were due to twinning. This figure was similar to that reported by Smith et al. (2003), where 75 of 1252, or 6.0% of abortions were attributable to twinning. A review of abortions in Newmarket between 1996 and 2001 reported 6 of 210, or 2.9%, were attributable to twinning (Ricketts et al. 2003). The decline in the reported incidence of abortion due to twin pregnancy over time has largely been attributed to the widespread use of ultrasonography for early diagnosis and management of twin pregnancy in routine studfarm practice (Giles et al. 1993; Merkt and Jochle 1993; Ricketts et al. 2003; Smith et al. 2003).

Factors influencing the occurrence of twins

Ovulation rate

While reports of monozygotic twins (Rooney, 1970; McCue et al. 1998) and triplets (Meadows et al. 1995) exist, almost all cases of twinning in the mare are attributable to multiple ovulations (dizygotic or fraternal twinning). Identical twins are thought to arise when embryo bisection occurs in association with hatching from the zona pellucida. The most likely reason for the lack of identical twin formation in the mare is the embryonic capsule that forms around Day 6 and is thought to prevent bisection of the embryo by the zona pellucida (McKinnon and Rantanen 1998).

Greater rates of twin pregnancy occur in breeds with a higher incidence of multiple ovulations and the incidence of multiple ovulations has been shown to differ between breeds. A greater incidence of multiple ovulations occurs in Thoroughbreds (19%) than in Quarter Horses (9%) and Appaloosas (8%) (Ginther et al. 1982). More recent studies report a higher incidence of multiple ovulation in Thoroughbreds, ranging from 29.3% (Davies Morel et al. 2005) to 31.7% (Davies Morel and Newcombe 2007).

As a result of differences in the incidence of multiple ovulation between breeds, more twins occur in Thoroughbreds, draught horses and Warmbloods than in Standardbreds and Ponies (McKinnon and Rantanen 1998). In a study of early pregnancies diagnosed with ultrasound between 12 and 20 days after ovulation, 97 of 629 (15.4%) Thoroughbred mares had twin vesicles whereas only 39 of 634 (6.1%) Standardbred mares were pregnant with twins (Bowman 1986). Twins were diagnosed in 245 of 1716 cycles in Thoroughbred mares (14.3%) and in 46 of 1294 cycles in Standardbred mares (3.5%) (McKinnon 2007). In addition, a high degree of repeatability of multiple ovulations and twin pregnancies has been reported both in individual mares and within certain family lines (Ginther et al. 1982). Researchers in Poland examined 12,648 pregnancies in 2033 Thoroughbred mares and estimated the heritability of twinning in this population to be from 0.24–0.29, depending on the model used (Wolic et al. 2006).

Ovulatory pattern

Several studies have examined the effect of ovulatory pattern on the occurrence of twin embryos. Unilateral double ovulation has been shown to result in the recovery of fewer embryos in comparison to mares in which an ovulation occurs from each ovary (bilateral double ovulation). In a review of 1300 double ovulatory cycles, the proportion of embryo recovery attempts yielding 2 embryos was significantly less in unilaterally ovulating mares (247/699; 35%) than in bilaterally ovulating mares (290/601; 48%) (Riera et al. 2006). This was similar to the finding of Squires et al. (1987) who reported the recovery of fewer twin embryos from unilateral than from bilateral double ovulators. It has been hypothesised that some mechanism at the level of either the ovary or oviduct may interfere with ovulation or oocyte pickup in unilateral, double ovulating mares (Riera et al. 2006).

Reproductive status

The reproductive status of the mare at the time of breeding has been shown to affect the occurrence of multiple ovulations and twin pregnancies. Barren and maiden mares have been shown to have a greater incidence of multiple ovulation and twin pregnancy in comparison to lactating mares. It is hypothesised that this is due to a feedback effect of nursing on the hypothalamic-pituitary axis (Perkins and Grimmett 2001). Ginther et al. (1982) demonstrated a reduction in multiple ovulation rates of lactating mares by between 42 and 67% in comparison with barren and maiden mares. A later study (Ginther 1983a) demonstrated double ovulations and twin embryos more frequently in barren mares (11 and 6%, respectively) than in lactating mares (5 and 1%, respectively). In West Germany between 1976 and 1985, 33% of twin pregnancies occurred in lactating mares, yet lactating mares represented 58% of the broodmare population (Merkt and Jochle 1993). In a review of 3373 Thoroughbred mares, Allen et al. (2007) identified a significantly greater number of twin and triplet pregnancies at Day 15 in maiden mares (14.4%) and barren mares (16.4%) in comparison to foaling mares (8.0%).

Season

As the breeding season progresses, there is an increase in the multiple ovulation rate and in the percentage of mares conceiving twins. As diagnosed via palpation per rectum during 478 oestrous cycles, twin ovulations increased from 12% of all cycles in May, to 19% in June (Jeffcott and Whitwell 1973). Of all mares who conceived twins, 61.3% conceived twins in May and June (Jeffcott and Whitwell 1973). This seasonal effect on
the incidence of twinning was also demonstrated in a review of German Thoroughbred breeding records. Twin pregnancy was diagnosed in 0.9% of 3229 conceptions during February and March, which was significantly different from the rate of 1.76% which occurred in 2157 conceptions in June and July (Merkt and Jochle 1999).

**Induction of oestrus and ovulation**

The effect of ovulation induction using human chorionic gonadotropin (hCG) or oestrus induction using cloprostenol on twinning rates has been investigated. New Zealand researchers reviewed 2119 ovulatory cycles in Thoroughbred mares over a 7 year period, and demonstrated a 3-fold increase in the odds of a diagnosis of twins on ultrasound examination performed 14 days post ovulation following ovulation induction with hCG compared to cycles in which no ovulation induction was performed (Perkins and Grimmel 2001). A similar result was obtained in a study of 680 Thoroughbred mares in Italy, where the percentage of mares diagnosed with twins via transrectal ultrasound 16 days after last being mated was significantly higher in those mares treated with 5000 iu hCG i.m. (n = 221; 13.1%) than untreated controls (n = 324; 6.5%) (Veronesi et al. 2003). The increased incidence of twin pregnancy in hCG-treated mares is thought to be due to an increase in the multiple ovulation rate (Perkins and Grimmel 2001).

Veronesi et al. (2003) also found an increased twinning rate in mares treated with 0.5 mg cloprostenol to shorten the luteal phase (n = 86; 17.4%) and in those mares who received both cloprostenol and hCG (n = 49; 30.6%) compared with untreated controls (n = 324; 6.5%). In a review of 3373 Thoroughbred mares, Allen et al. (2007) identified a significantly higher incidence of multiple pregnancies in mares associated with the hormonal induction of oestrus (13.0% vs. 10.6%). These same authors found no increase in twin pregnancy rate associated with the use of ovulation-inducing drugs. A similar finding was reported by Davies-Morel and Newcombe (2007) who reviewed the breeding records of 1239 Thoroughbred mares obtained over a 3 year period. In this study, the incidence of multiple ovulation and subsequent multiple pregnancy was not significantly different between mares who received 750 iu hCG subcut. (n = 536) and those that received no ovulation-inducing agent (n = 703).

**Stallion effect**

Ginther et al. (1982) reported the results of a survey of veterinarians in which many believed that twins were more likely to occur in mares bred to highly fertile stallions. It is possible that with extended longevity of viable spermatozoa in the reproductive tract that there is a greater possibility of fertilisation of asynchronous ovulations (Frazer 2003).

**Diagnosis of twin pregnancies**

It is important to diagnose and manage twin pregnancy as early as possible. More pregnancies will go to term and result in the birth of a single, live foal if one of the twin vesicles is eliminated sooner rather than later (Roberts 1982). While pregnancy may be detected as early as 9 days after ovulation using ultrasonography (Ginther 1986), the first pregnancy check is usually conducted 14–16 days after ovulation in routine stud practice (Lofstedt and Newcombe 1997). This is because both singleton and twin vesicles can usually be easily detected at this time. Twin vesicles arising from synchronous ovulations (ovulations occurring within 24 h of each other) will be of similar size and should be easily identified.

Twins may also arise from asynchronous ovulations (ovulations occurring >24 h apart). In this instance, the embryonic vesicles will be of dissimilar size and the size disparity will depend upon the time between ovulations (Fig 3). Asynchronous ovulations can occur up to 4 days apart and may result in a second embryonic vesicle that is too small to detect if early pregnancy diagnosis is conducted in relation to the time of the first detected ovulation. Examination of the ovaries for the presence and number of corpora lutea is important at the time of early pregnancy diagnosis because of the high correlation between the number of ovulations and the number of embryonic vesicles (Ginther 1987).

Diagnosis of twin pregnancy after fixation can be difficult in cases of unilateral fixation. Before the appearance of the embryo proper on about Day 21, unilaterally fixed twin vesicles may appear as a single, large over-sized vesicle, with only a thin line visible in the middle (McKinnon 2007) (Fig 4). The later appearance of embryonic heartbeats, umbilical cords and excess fetal membranes will further aid in the diagnosis of twin pregnancy.

Transabdominal rather than transrectal ultrasonography may be more useful for the diagnosis of twins beyond 100 days of gestation (McKinnon 2007) (Fig 5). Beyond Day 70, transrectal diagnosis and monitoring of twin

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Fig 3: A pregnancy check conducted 14 days after a detected ovulation. The size of the vesicle on the left is consistent with a Day 14 pregnancy, while the vesicle on the right arose from a later ovulation. Asynchronous ovulations such as occurred in this example result in embryonic vesicles of different sizes. Vesicles of dissimilar sizes typically fix unilaterally and are more likely to undergo natural reduction to a single pregnancy.
pregnancy may be difficult due to the increasing volume of allantoic fluid and to the descent of the gravid uterus into the abdomen. In a study that followed 15 bilaterally fixed twins beyond Day 40 (Ginther and Griffin 1994), the only structure indicative of twins that was consistently detected during all transrectal ultrasound examinations was the ‘twin membrane’, which represented the area of apposition between the 2 allanto-chorions (Fig 6).

In undiagnosed cases of twin pregnancy that advance into later gestation, the first indication of twin pregnancy may be premature mammary development and lactation or the discovery of 2 aborted fetuses. The diagnosis of abortion due to twinning is aided by placental examination. Areas where the 2 allanto-chorions were in contact with each other are devoid of villi and appear grossly as large, pale areas sharply demarcated from the surrounding villous chorion (Jeffcott and Whitwell 1973) (Fig 7).

Twins may be missed for numerous reasons and despite multiple examinations (McKinnon and Rantanen 1998; McKinnon 2007). Due to the mobility of the embryonic vesicle in the uterus before fixation, a second vesicle may be missed if the entire uterus is not examined thoroughly. Twins resulting from asynchronous ovulations may also be missed, if examination for pregnancy is conducted before the time that a second vesicle can be detected. If more than one corpus luteum is detected at the time of early pregnancy diagnosis, a second examination should be conducted 2–3 days later to examine for the presence of a second embryonic vesicle. Reliance on rectal palpation alone to diagnose pregnancy may miss twins, especially in cases of unilateral fixation. The presence of endometrial cysts often confuses the diagnosis of twins, especially if their size and location has not been recorded previously. Endometrial cysts may be differentiated from embryonic vesicles by their lack of mobility, failure to increase in size during serial examinations and by their failure
to develop an embryo. If closely apposed, 2 embryonic vesicles may be mistaken for one if examination is performed too quickly. A thorough and systematic examination of the entire uterus with good quality ultrasound equipment in a well restrained mare and an optimal environment will minimise the chances of misdiagnosing twin pregnancy in the mare.

Management of twin pregnancies

The strategy chosen to manage twin pregnancy in the mare will depend on the age of the pregnancies at identification, the orientation of the vesicles and the type of fixation. It may also be important to consider the potential for rebreeding, the potential value of any offspring and the ability and experience of the veterinarian.

Before the advent of ultrasound to diagnose pregnancy prior to fixation, mares with 2 or more large preovulatory follicles on palpation were typically withheld from breeding in an attempt to prevent twin pregnancy. This management strategy however did not result in a decreased rate of twin pregnancy (Ginther et al. 1982).

Recognition prior to or on Day 16

While the mare is very efficient at reducing twins to a single pregnancy, the probability that a mare with twins will lose one or both vesicles before Day 16 is minimal and equates to that of early embryonic death for the same period (Ginther 1984). Embryo reduction before Day 11 is not considered an important aspect of the natural correction of twins (Ginther and Bergfelt 1988). Embryo reduction was also not detected during the mobility phase (Days 11–15) or on the day of fixation (Day 16) in any of 38 mares with multiple embryos (Ginther 1984).

A method for prefixation embryo reduction was first described by Ginther (1983b) and was a variation of techniques described for the reduction of post fixation, bilaterally fixed embryos (Roberts 1982). This technique utilised ultrasound to identify the vesicles on Days 12–14 and if they were sufficiently separated, one was manually ruptured. Using the thumb and index finger, the vesicle was ruptured either in place or following movement to the tip of the uterine horn. Smaller vesicles (9–11 mm in diameter) were more difficult to rupture and a distinct popping sensation was felt upon rupture of the vesicle in 8 of 15 mares. For those vesicles that were together on initial examination, it was recommended that the mare be re-examined approximately 1 h later. A modification of this technique was later reported that eliminated the need to wait for vesicles to separate and utilised the ultrasound probe to manipulate the vesicles and manually reduce one in isolation from the other while visualising one or both throughout the procedure (McKinnon et al. 1993; McKinnon 2007). If 2 separate, noncoalesced vesicles can be visualised, then separation should be possible (McKinnon 2007). The probe is positioned where the 2 vesicles are in contact and gentle back and forth movement of the probe with pressure results in the 2 vesicles becoming separated. A vesicle may be manually reduced as close as 0.5 cm from the other but it is generally best to separate them by at least 2 cm (McKinnon 2007). Elimination of an embryo is achieved by gradually increasing the pressure on the vesicle with the probe, using the pelvis to stabilise the uterus. Smaller vesicles (11–13 days post ovulation) are more difficult to eliminate using this technique and may slip away when pressure is applied, so additional stability may be necessary. This may be achieved by placing fingers on either side of the probe in an attempt to keep the vesicle in one position. Alternatively, a sudden increase in pressure, as opposed to a more gradual increase in pressure, may be useful (McKinnon 2007). Fluid from the crushed vesicle may surround the remaining vesicle, but does not appear to be detrimental at this time, as opposed to later stages of gestation (Pascoe and Stover 1989).

Manual reduction techniques have been demonstrated to cause the release of PGF$_{2\text{a}}$ for up to 90 min, with the amount being directly correlated to the pressure required to effect rupture (Pascoe et al. 1987). This increase in PGF$_{2\text{a}}$ did not, however, cause a significant change in plasma progesterone concentrations. Other studies have failed to demonstrate a significant change in PGF$_{2\text{a}}$ concentrations following either fixed or mobile reduction of one member of a twin set (Veronesi et al. 2005). While these results suggest that anti-inflammatory and/or progestin therapy are unnecessary following manual twin reduction, some practitioners continue to utilise these treatments as part of their twin management protocol.

Manual reduction of one of twin vesicles prefixation does not result in a higher rate of embryonic death in the remaining vesicle than that encountered with singleton pregnancies. Twins were diagnosed prefixation in 245 of 1716 Thoroughbred mare cycles and subjected to manual reduction of one of the vesicles (McKinnon 2007). When mares were examined between 7 and 10 days later, 10 of 245 mares (4.0%) had lost the remaining pregnancy. This was similar to the rate of early embryonic death in mares with a single pregnancy diagnosed at Day 13–15 and then found to have lost the pregnancy at the next examination (63/1716; 3.7%). Pascoe et al. (1987) reported a 96% survival rate for the remaining conceptus (up to Day 42) after manual reduction of one member of a twin set in 100 mares, using either a mobile or fixed technique.

Recognition between Days 17 and 40

Fixation of the embryonic vesicle occurs at around Day 16 in the mare and is thought to be due to the increasing size of the embryonic vesicle and to an increase in uterine tone (Ginther 1992). In mares with twins, the vesicles may fix either together at the base of a uterine horn (unilateral fixation) or one vesicle may fix at the base of each uterine horn (bilateral fixation). In a study of 31 mares with twin embryonic vesicles, unilateral fixation was more frequent (71%) than bilateral fixation (29%) (Ginther 1989a). In 28 mares with known ovulatory patterns, synchronous ovulation did not affect the type of fixation.
(9/17 unilateral, 8/17 bilateral) (Ginther 1989a). However, this study also found that the rate of unilateral fixation was much higher than bilateral fixation (10/11 and 1/11 respectively). In 2 studies, the incidence of embryo reduction was found to be much higher for unilaterally fixed embryos than for bilaterally fixed embryos (14/19 vs. 0/9 [Ginther 1989a] and 17/19 vs. 1/9 [Ginther 1984]). Twins resulting from asynchronous ovulations (ovulations not occurring on the same day) were also more likely to undergo reduction to a singleton (9/11; 82%) as compared with those resulting from synchronous ovulations (5/17; 29%) (Ginther 1989a). Asynchronous ovulations are likely to result in embryos of different gestational ages and therefore different sizes. In 22 mares with unilaterally fixed twins, reduction occurred in 100% with vesicles of dissimilar size (>4 mm difference in diameter), whereas 19/26 twin pregnancies (73%) with vesicles of similar size (0–3 mm difference in diameter) underwent reduction to a singleton (Ginther 1989b). When reduction occurs with unilateral fixation, it most commonly occurs prior to detection of both embryos. Reduction was complete before detection of both embryos in 10/14 twin pregnancies (Ginther 1989a). Embryo reductions were complete by Day 20 in 29 of 49 (59%) unilaterally fixed twins, with reductions occurring in one day (Ginther 1989b). Reductions that occurred after Day 20 were usually preceded by a gradual decrease in size of the vesicle undergoing reduction (Ginther 1989b). A proposed mechanism for this high rate of embryo reduction in unilaterally fixed twins was proposed by Ginther (1989a). The deprivation hypothesis proposes that when the vascularised wall of an embryonic vesicle (trilaminar omphalopleure) is in contact with the bilaminar wall of the other vesicle rather than the endometrium, embryonal-maternal physiological exchange is severely reduced and the deprived vesicle undergoes reduction due to an inability to exchange nutrients across the yolk sac placenta.

Because the mares’ own biological reduction mechanism is so efficient, no attempt is often made to reduce post fixation unilateral twins. If the individual vesicles retain their spherical shape and have not coalesced, it may be possible to manually separate unilaterally fixed twins between Days 16 and 20 (McKinnon 2007). Although the procedure was described as time-consuming and tedious, 39 of 42 unilaterally fixed twin pregnancies between Days 16 and 20 were successfully reduced in this manner (Bowman 1986). Reported success rates for manual reduction of unilaterally fixed twins after Day 20 range from 25% (Mari et al. 2004) to 50% (McKinnon and Rantanen 1998), which is much lower than the rate of natural reduction. Instead, the pregnancy is monitored and if no reduction has occurred by Day 30, both pregnancies are terminated before the establishment of endometrial cups either by manual reduction or with prostaglandin.

The incidence of reduction for bilaterally fixed twins is negligible. No reductions occurred in a review of 20 bilaterally fixed twin pregnancies monitored with ultrasound until Day 40 (Ginther 1989b). Only 1 of 9 (11%) bilaterally fixed embryos reduced to a singleton by Day 40 in another study (Ginther 1984). Because of the very low incidence of spontaneous reduction following bilateral fixation, it is recommended to eliminate one of the twins immediately upon detection up to Day 30 (McKinnon and Rantanen 1998). The ability of the remaining pregnancy to survive to term has been demonstrated to decrease if the reduction occurs after Day 31. In a review of 181 mares with bicornuate twin pregnancies in which one was manually eliminated (Roberts 1982), the success of the procedure before Day 31 (33/47: 70%) was considerably greater, in terms of live foals born, than that achieved after Day 31 (31/134: 23%). The author also reported the inability to manually reduce some twins after Day 35, even if extreme pressure is applied. The likely reason for the reduced likelihood of the remaining pregnancy to survive to term following manual reduction after Day 31 was the allantoic fluid from the ruptured pregnancy surrounding the other pregnancy and undermining the ventral surface of the choioallantois, elevating it off the endometrium and disrupting maternal-embryonal physiological exchange. Pascoe and Stover (1989) demonstrated this with the rapid death within 2–3 h of 3 pregnancies (Days 45, 50 and 55) following the injection of 250 ml of sterile placental fluid into the nongravid horn of the uterus.

Because of the potential to disrupt the remaining pregnancy if manual reduction is performed after Day 30, it has been suggested to instead disrupt one of the vesicles by application of manual pressure and oscillation in order to create a ‘snowflake’ effect without rupturing the choioallantois (McKinnon and Rantanen 1998). Demonstration of this effect almost always leads to a gradual loss of the affected conceptus.

Transvaginal aspiration of fetal fluids has been described as a method for reducing twin pregnancies to a singleton. Pascoe (1979) first reported this technique in which fetal fluid was aspirated per vaginam from one conceptus in 6 mares found to be carrying bicornuate twins at 42 days post breeding. While the results of this study were disappointing (all mares subsequently lost both pregnancies), better results were obtained in subsequent studies which utilised transvaginal ultrasonography. Of 13 mares subjected to the procedure between 20 and 45 days of pregnancy, 6 of the mares (46%) were found to be pregnant with a single conceptus 10 days later (Bracher et al. 1993). The success rate was higher for mares with bicornuate pregnancies as opposed to uniconnurate pregnancies (75% vs. 33% respectively). In contrast, Mari et al. (2004) reported the loss of both fetuses in 2 of 3 mares when transvaginal ultrasound-guided aspiration (TUGA) of fetal fluids was conducted on one of 2 bilaterally fixed twins between 40 and 50 days of gestation. Successful reduction and birth of a single viable foal was achieved in 14 of 20 (70%) uniconnurate twins when the procedure was performed between 16 and 25 days gestation. Based on these results, the authors concluded that the procedure was best performed before 25 days of gestation. MacPherson and Reimer (2000) suggested that the procedure is best performed prior to Day 36, particularly in cases of unilateral twins. In this study, the gestational age at the time of reduction in 7 of 10 mares with unilateral twins was >35 days and all resulted
in loss of both pregnancies. The successful application of transvaginal ultrasound guided aspiration of unilateral twins in the mare appears to require its use during a period in which the rate of natural reduction of twin pregnancies is high. Therefore in many cases it may be more prudent to monitor these pregnancies carefully for spontaneous reduction rather than intervene with transvaginal aspiration.

**Recognition from Day 60 onward**

A technique for the attempted reduction of twin pregnancies between Days 60 and 100 has been described in which the smaller of the 2 fetuses is identified and repeatedly traumatised by oscillation or percussion with the ultrasound probe, membrane slip or single digit percussion of the cranium (Rantanen and Kincaid 1988; McKinnon and Rantanen 1998). Multiple attempts at the procedure may be necessary to create sufficient damage for fetal death, and a success rate of approximately 50% should be expected (McKinnon and Rantanen 1998). Successful application of this technique requires that the examiner be able to identify which fetus was traumatised during earlier attempts.

Recently, cranio-cervical dislocation has been reported as a method for the reduction of twin pregnancies identified between 60 and 110 days of gestation (Wolfsdorf et al. 2005; Wolfsdorf 2006). The procedure involves identification of the smaller of the 2 fetuses, or the fetus that has less contact with the endometrium. Isolation of the fetus may be difficult and uterine relaxation is considered imperative to enable accurate identification of fetal anatomy (Wolfsdorf 2006). Once the fetus for reduction is selected, the head is isolated and stabilised between the thumb and forefinger. The head is then bent from side to side to damage the ligaments attaching the head to the neck. Dislocation of the head is then achieved by placing the thumb at the base of the cranium and applying pressure proximally and dorsally. If dislocation is successful, a distinctive pop is felt, and the thumb and forefinger is able to be placed between the head and neck.

By performing the procedure before placentation is complete, it is believed that the remaining fetus is better able to grow to its full potential, thus avoiding the birth of a small and weak foal, as can be observed when twin reduction is performed transabdominally at a later time (MacPherson and Reimer 2000). Cranio-cervical dislocation can be performed either transrectally or via flank laparotomy. Of 8 sets of twins which were reduced with transrectal manipulation, 5 mares delivered a normal sized single foal, while the other 3 mares aborted both fetuses between 30 and 60 days later (Wolfsdorf 2006). The procedure had to be repeated on 3 of the 8 mares before cranio-cervical dislocation was successful. Once cranio-cervical dislocation was achieved, it took from 1–3 weeks before the reduced fetus had no viable heartbeat. Of the 5 sets of twins reduced via flank laparotomy, 3 mares delivered a normal sized single foal. The authors concluded that the earlier death of the reduced fetus increased the chance of a single pregnancy going successfully to term (Wolfsdorf et al. 2005).

Transabdominal ultrasound guided twin reduction was first described as a method for elimination of one member of a twin set in pregnancies beyond 60 days of gestation in 1988 (Rantanen and Kincaid 1988). The procedure involves transabdominal ultrasonography of a sedated mare with either a 3 MHz (Rantanen and Kincaid 1988) or 5 MHz (MacPherson and Reimer 2000) transducer to identify the presence of twins and to select the most easily accessible and preferably smallest of the 2 fetuses for reduction (Frazier 2003). Sedation of the mare and the administration of clenbuterol cause uterine relaxation and move the fetuses cranially, which may improve visualisation and accessibility (Ball 2000). The area of the mares’ abdomen adjacent to the fetus to be reduced is surgically prepared and infiltrated with local anaesthetic. With the assistance of a biopsy guide attached to the transducer or using a ‘free hand’ technique (Ball 2000), a 15–25 cm, 16 or 18 gauge needle with a stylet is passed through the skin, abdomen and uterine wall. Following identification of the needle on the ultrasound image, the needle is then passed into the fetus (Fig 8). Initial reports of injecting air or saline into the fetal heart were unsuccessful at causing fetal death (Rantanen and Kincaid 1988). Subsequently, injection of the fetal heart with KCl (Rantanen and Kincaid 1988; Rantanen 1990; MacPherson and Reimer 2000), or injection of the fetal heart, lungs or abdomen with 10–20 ml of procaine penicillin (McKinnon and Rantanen 1998) have resulted in rapid fetal death, although the later technique appears to take longer to cause fetal death than the former. Fetal death should be confirmed the following day (Fig 9). Reported advantages of using procaine penicillin for transabdominal twin reduction include reducing iatrogenic bacterial contamination, ultrasonographic visualisation during fetal injection (Fig 10) and eliminating the need for intracardiac placement, as fetal death can be achieved with placement in either the thorax or abdomen (McKinnon and Rantanen 1998).
The initial report of the procedure involved 18 mares diagnosed as carrying twins between 66 and 168 days of gestation (Rantanen and Kincaid 1988). Following the intracardiac injection of KCl, 7 of these mares (40%) subsequently delivered single live foals. Based on these results and subsequent attempts at the procedure (Rantanen 1990), it was concluded that the procedure was best performed between 115 and 130 days of gestation. This was emphasised by subsequent results in 59 mares with twin pregnancies that went on to deliver 29 single live foals (49.2%) when the procedure was performed after Day 115 (McKinnon and Rantanen 1998). MacPherson and Reimer (2000) reported the birth of 9 normal, live, single foals from 24 mares (38%) following the procedure at >120 days gestation. In addition, 3 of 24 foals (12%) were born alive but were weak and small. This growth retardation has been ascribed to the lack of effective macrovillous placentation on that portion of the allantochorion of the surviving fetus which grows to occupy much of the uterine horn previously occupied by the dead twin (Ball et al. 1993) (Fig 11). An increased success rate, measured in terms of single live foals born, was reported with the use of 10–20 ml of procaine penicillin injected into the fetal heart, thorax or abdomen (McKinnon and Rantanen 1998). Using this technique, 8 of 13 mares (62%) with twin pregnancies went on to deliver single, live foals.

Follow-up ultrasonography of the remaining fetus has been recommended, although an unsuccessful outcome for the remaining fetus is usually not immediately apparent (McKinnon and Rantanen 1998). Of the mares subjected to this procedure that subsequently aborted both fetuses, most aborted 1–2 months after the procedure (MacPherson and Reimer 2000). Most reports of the procedure include post operative treatment with NSAIDs, systemic antibiotics and progestin therapy, although some have found no difference in fetal survival rates with long-term antibiotic and Regumate therapy (McKinnon and Rantanen 1998).

**Conclusion**

While the incidence of abortion due to twinning has declined subsequent to the routine use of ultrasonography for pregnancy diagnosis, the early and accurate diagnosis and management of twins remains an important part of routine equine breeding management. While options exist for the management of twins post fixation, more mares will give birth to a single foal if one of the vesicles is eliminated during the mobility phase.
References


