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Twin Reduction Techniques

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Introduction

We have known three things about twins for a long time. Firstly twins are repeatable within the same mare, secondly twinning rate varies according to breed and thirdly, the more fertile the stallion the more twins he is expected to achieve.

Twins are avoidable and should not occur on well managed breeding farms. Clients understand this and are demanding the most sophisticated management programs available. Presented below are the management techniques in use at the Goulburn Valley Equine Hospital. The hospital is a major referral centre and each year is expected to handle multiple referral cases of twin pregnancy that are diagnosed after cessation of the mobility phase.

Historically twins have been the single most important cause of abortion in Thoroughbreds. As most twin pregnancies terminate in early foetal resorption or loss, late term abortions, or the birth of small growth retarded foals and mares aborting twins in late gestation frequently have foaling difficulties, damage their reproductive tracts and are difficult to rebreed, twins are disastrous financially. If foals born alive they are frequently small, have intra-uterine growth retardation and a poor survival rate with many needing expensive sophisticated critical care.

Origin, associations, and a general overview of twinning in the horse has been published elsewhere.

It is our responsibility to successfully manage early pregnancies such that no mare delivers or aborts twin foals. In consultation with farm managers, owners and clients we must to utilise available equipment and technology commensurate with economic constraints and other owner/manager preferences to diagnose twin pregnancies as early as practically possible. It is also the responsibility of a veterinary profession to adequately inform owners/managers/clients of reasons why twins may be not diagnosed.

Twins are still occasionally missed despite multiple examinations. Reasons why twins may not be detected, despite repeated examination are: 1) difficulty distinguishing structures (this may be related to a poor examination environment i.e., too much light, poor display characteristics of the ultrasonographic unit, mare movement and/or lack of restraint) 2) variable growth patterns 3) inability to detect heart beats of adjacent embryos 4) operator experience and 5) resolution of the equipment. The most common cause of misdiagnosing the presence of twin pregnancies is examination prior to the time period that a second pregnancy (asynchronous ovulation) may be reasonably expected to be detected. Another reason appears to be scanning too quickly.
The incidence of abortion has been reported as decreasing\(^5\) and in the German Thoroughbred industry has almost halved since the event of ultrasonography.\(^6\)

**Origin of Twins**

Twins in the horse are unlikely to be identical. Almost all cases of twin pregnancy are expected to be related to multiple ovulation, although at least two occurrences of monozygotic twin pregnancies have been suspected.\(^7,8\) The reason for lack identical twin formation in the horse is probably related to the capsule.\(^9\) The capsule forms in equine embryos aged around 6 days, shortly after their entry into the uterus.\(^10,11\) When embryos were cultured prior to the formation of the capsule, hatching occurred similar to as occurs in the bovine;\(^11\) however, when embryos were cultured after formation of the capsule the zona pellucida continued to become progressively thinner and finally fell away from the developing conceptus. Other species that do not have a capsule, such as sheep, cattle and humans, all have the ability to routinely give birth to identical twins which apparently form from pinching of a hatching embryo by the zona pellucida.

It has been shown that twins in mares are as likely to result from synchronous versus asynchronous ovulation\(^12\) and that pregnancy rate per follicle was identical for double ovulations on opposite ovaries to that obtained from single ovulations per cycle but was higher than the pregnancy rate per follicle when double ovulations occurred on the same ovary.\(^13\)

**The Outcome of Twin Pregnancies**

Understanding the outcome if twin pregnancies are left to develop is of great importance to veterinarians, farm managers and owners. Also important is when to intervene and what probability of success such interventions may be expected to achieve.

**What Happens if We Do Nothing?**

The mare is very efficient at reducing twins to a single pregnancy. This is done by a competitive absorption of nutrients that is related to size and position of the early pregnancy and later to orientation of the embryo proper within the developing conceptus.\(^14\) However, the initiation of any non-intervention program depends on the age of identification, the orientation of the vesicles and any disparity in size.

**Recognition \(\leq 16\) days from ovulation**

Embryo reduction before or on the day of fixation is not considered an important aspect of the natural correction of twins.\(^15\) The probability of a mare losing one or both vesicles of a set of twins from identification prior to fixation is minimal and approximates that of early embryonic death for the same time period (per vesicle). The recognition of twin pregnancies prior to fixation day (day 16) is dependant on the day of examination relative to the day of ovulation. Asynchronous ovulations occasionally result in a gross disparity in vesicle size, sometimes as much as 4-5 days i.e. identification of a day 11-12 and a day 16 vesicle concurrently (Fig. 1a). In instances such as this, examination one day earlier may have failed to detect the younger of the two pregnancies. Recognition that all twin pregnancies occur from multiple ovulation dictates mandatory re-examination of all mares.
that have two CL’s and only a single vesicle detected prior to fixation (day 16). Recognition prior to fixation is also dependent on operator experience, resolution of the equipment (≥ 5 MHZ preferred), monitor capabilities, restraint and other facilities (ability to darken the environment), the presence of uterine cysts and the skill of the examiner.

**Fig 1.** Twins with a large disparity in age (~day 10 and day 15) (Fig 1a) Twins at ~ day 15. Note the indentation of the corpus cornual junction and the horizontal line that is lower and marks the junction between the two vesicles (Fig 1b).

**Recognition after fixation (after day 16)**

The recognition of unilaterally fixed twins from day 17 through to 21 (prior to clear recognition of the developing foetus within the vesicle) may be the most difficult time to determine if there are twins present. Ultrasonographically, all that is detectable is a thin line (the apposition of the two yolk sacks) running approximately in the middle of a slightly over-sized vesicle (Fig. 1b). Recognition of the fetus(es) within the vesicle a few days later makes differentiation easier (Fig. 2). Occasionally an inexperienced operator may confuse an abnormally orientated 28 to 30 day single pregnancy with 17 to 20 day unilaterally fixed twins (Fig. 3). From days 22 to 60 the presence of multiple foetuses, umbilical cords and general excess in the number of visible membranes should alert the practitioner to the likelihood of more than one pregnancy (Fig. 4). The junction between two developing foetuses (after 30 days) between the two allantochorions results in a common membrane from the area of apposition. This common membrane has been referred to as the *twin membrane* (Fig. 4)\(^{16}\) and has diagnostic potential, particularly late in pregnancy when it might not be possible to view both foetuses transrectally (>100 days). After 100 days, careful transabdominal ultrasonography may be necessary to determine the presence of twins.
Days 17 to 40

The outcome of pregnancies post fixation is dependent upon their size (diameter) and the nature of their fixation. Unilateral (both fixed together at the same corpus cornual junction) fixation reduction is much higher than bilateral (one on each side) fixation reduction. Fortunately, unilateral fixation is much higher (approximately 70%) compared to bilateral (30%). In 28 mares with known ovulatory patterns, synchronous ovulations did not affect the type of fixation (9/17 unilateral, 8/17 bilateral). However for asynchronous ovulation the frequency of unilateral fixation (10/11) was greater (p< 0.01) than the frequency of bilateral fixation (1/11). The incidence of embryo reduction was greater (p< 0.01) for unilateral fixation (14/19) than for bilateral fixation (0/9) and was greater (p< 0.05) for asynchronous ovulation (9/11) than for synchronous ovulation (5/17). Practically speaking this means that if asynchronous ovulations have resulted

Figure 3. An abnormally orientated ~30 day pregnancy (Fig 3a) should not be confused with twins wherein the vesicle has coalesced into a single vesicle with an ultrasonically visible line within (Fig 3b).
in significant age differences between vesicles (i.e. > 3 mm diameter at day 15) then rate of embryonic reduction is very high.\textsuperscript{14} In cases of unilateral fixation, 22 of 22 mares with vesicles of dissimilar size had reduction compared to 19 of 26 (73\%) with vesicles of similar size.\textsuperscript{17} As a result of work studying reduction of unilateral versus bilateral twin pregnancies in mares from days 17 to 40 Ginther proposed that the nutrient intake from the larger vesicle (before the foetus was present) prevented adequate nutrition of the smaller vesicle. Later the position of the foetus proper and its emerging allantoic sac seemed to determine whether a given conceptus survived or underwent late reduction. The foetus, the vascularised wall of the yolk sac adjacent to the foetus and the emerging allantoic sac were exposed to the endometrium (uterine lumen) in the surviving vesicles. In the vesicles that underwent reduction, much of the corresponding area of the vesicle wall was covered by the wall of the adjacent survivor and is thus deprived of adequate embryonal-maternal exchange and therefore regresses.

\textit{In summary, dissimilarity in diameter increases the likelihood of unilateral fixation, increases the incidence of reduction for unilateral fixed vesicles, hastens the day of occurrence of reduction and shortens the interval from initiation to completion of reduction.}

The incidence of reduction for bilaterally fixed vesicles is negligible and approximates that of standard early embryonic death in this period.

Of the 85\% of reductions by day 40 in cases of unilateral fixed twin pregnancies, 59\% of reductions had occurred between day 17 and 20, 27\% between day 21 and day 30 and 14\% between days 31 to 38. The majority of early reductions occurred spontaneously (by day 20) as compared to reductions after day 20 that were preceded by a gradual decrease in size of the eliminated vesicle. In addition when twins were dissimilar in diameter (4mm or more) they were more likely to undergo reduction by day 20.\textsuperscript{17} Other studies have demonstrated similar results. The hypophysis of an early embryonic reduction mechanism for elimination of excess embryo in mares was not new and had been suggested as early as 1982. However, ultrasonography was necessary to adequately document the occurrence and nature of the reduction.\textsuperscript{18}
Day 40 onwards

Ginther and Griffin\textsuperscript{16} examined the natural outcome of bilateral twins (one in each horn) that were viable on day 40 in 15 pony mares. Readers should be aware that pony mares are not necessarily a good model for larger breeds, as the incidence of twins is low and evidence is suggestive that the larger the breed (Draught, Thoroughbred and Warmblood) the higher the probability of maintaining twins. Fifteen pony mares were monitored by ultrasonography until the outcome of the pregnancy was determined. Sixty six \% (10/15) of the pregnancies resulted in either death of both (80\%) or death of one (20\%) during months two or three. Nothing occurred from then until month 8. Between months 8-11, two mares lost one foetus (foetal death was associated with mummification) and two mares lost both. The two mares that lost one pregnancy both delivered undersized weak foals at birth. One mare (7\%) delivered live twins at term and two normal foals were born from mares loosing the one pregnancy (absorption of the foetus rather than mummification) in month two. In this study six live foals were born (2 of normal size), from a total of 15 mares and 30 foetuses. This incidence is similar to previous reports wherein of 130 pregnant mares with twins, only 17 live foals (13\%) were produced.\textsuperscript{19} An interesting observation from the later report was that from the 102 mares that delivered live or dead twins in the previous year, only 37 produced live foals the next seasons and thus over two seasons there was an average of 23\% producing live foals.\textsuperscript{19} An earlier study,\textsuperscript{20} was extremely useful in categorising outcome of twins that managed to survive to later pregnancy. Twinning accounted for 22\% of the cases of abortion and still-birth between 1967-1970. Sixty two sets of twins and their placentas were examined from Thoroughbred mares. All were considered to be dizygous. Abortion or still-birth of both twins from 3 months of gestation to term occurred in 64.5\% of mares, although most (72.6\%) slipped from 8 months to term. In the remaining cases one twin (21\%) or both twins (14.5\%) were born alive. Most foals at term were stunted and emaciated and of the 31 alive at birth only 18 had survived to 2 weeks of age.\textsuperscript{20} In this study twin placenta

\hspace{1cm} was divided into three morphological groups according to the disposition of the chorionic sacks within the uterus. Type A placenta was seen in 79\% of cases (48 sets of twins). One foetus occupied one horn and most of the body (mean 68\% of the total functional surface area) while the other twin occupied only one horn and usually only a small part of the adjacent body. Where the chorions abutted there was a variable degree of invagination of the smaller chorion into the allantoic cavity of the larger twin. These pregnancies frequently ended in abortion or stillbirth of one or both twins. In this group 31/48 lost their pregnancies between 3 and 9 months (64.5\%). The gestation length in this group was frequently shorter and at birth the larger twin had a much greater chance of survival than the smaller one. In this group only 6 foals of 48 sets were born alive. Of the 6 foetuses born alive, 5 were the larger twin. Type B placenta occurred in 11\% of cases (7 sets) and the placentas were orientated such that the villous surface areas were more or less equally divided and each foetus occupied one horn and half of the body. Both foals were usually similar in size and were usually born alive. Nine foals survived to 2 weeks from 6 sets of twins that made it to term. In this group (7 total) one aborted at 7 months. Type C placenta was seen in 10\% of cases (6 sets of twins). In this group there was a greater disparity between the surface area of the 2 chorions. The smaller twin occupying only part of one horn, died earlier on and became mummified. The larger twin was usually born alive and a fair chance of survival. In this group 3 foals were born alive from 6 pregnancies at term.\textsuperscript{20} The authors attributed the loss of twin foetuses and poor survival rates to placental insufficiency.
It should be clear that our philosophy is that non intervention is only acceptable when twins are diagnosed as a unilateral occurrence between days 17 and day 40 and then the decision depends on factors such as the value of the foal, the potential for rebreeding and the ability of the veterinarian to manually intervene. Intervention in twin pregnancies is strongly recommended in all other circumstances (see below).

**When and How Should We Intervene?**

**Recognition ≤ 16 days from ovulation**

The first technique for manual crush of the conceptus during the mobility phase utilised manual reduction with good results\(^{21}\) and was a variation from previously reported techniques for twin pregnancies.\(^ {22,23}\) The technique involved gentle manipulation of the embryonic vesicle to the tip of one uterine horn and manual rupture. When applied to single pregnancies it resulted in pseudopregnancy and when applied to twin pregnancies it resulted in a single pregnancy in 7 of 8 attempts.\(^ {21}\) Later utilising the same techniques, mares were treated with single or multiple progestagen administration (hydroxyprogesterone caproate), an anti-prostaglandin (flunixin meglumine) plus progestagen or given no treatment prior to manual embryonic rupture in the mobility phase.\(^ {24,25}\) Results were 10/10 (100%) mares maintaining pregnancy in the control group (no treatment, just manual rupture) and 37/40 (92.5%) for treated mares. The amount of PGF\(_{2α}\) released was directly correlated with the pressure required to cause embryonic rupture. Flunixin meglumine inhibited PGF\(_{2α}\) release after embryonic rupture. Treatment with progestagen plus flunixin meglumine or progestagen singly or multiply was not better than no treatment at all (although it was subsequently shown that the progestagen chosen had no ability to maintain pregnancy in ovariectomised mares and did not bind to progesterone receptors in the horse).\(^ {26}\) Another report\(^ {27}\) demonstrated that 60 of 66 mares (90.9%) maintained a single vesicle after manual reduction was attempted prior to fixation. Five of the six mares in which the procedure was not successful subsequently conceived. Since 1984\(^ {28}\) we have used a modification\(^ 4\) of the technique described originally.\(^ {21}\) With this technique the ultrasound probe is used to manipulate the vesicles while keeping one or both vesicles in view during the manipulation and more importantly the crushing or rupture of the vesicle (Fig. 5). Utilising this technique it is possible to more accurately and quickly separate vesicles. It was original proposed\(^ {21}\) that when vesicles were in apposition mares be re-examined approximately one hour later. By utilising the probe to manipulate vesicles, separation is achieved (pre-fixation) very quickly in most instances. Commonly the smaller foetus is destroyed despite the lack of evidence to support pre-fixation reduction. On occasion it is necessary to revisit the mare 24-48 hr after the original evaluation if the smaller of the two vesicles is less than 1 cm in diameter as sometimes these can be more difficult to destroy.

Separation of vesicles should always be possible if the vesicles are still able to be identified as two spherical non-coalesced structures. Briefly, the technique involves separation of the vesicles using the probe. A finger is placed on either side of the probe to help stabilise the vesicle to be moved. Gentle back and forth movement of the probe with pressure results in the two vesicles becoming separated (Fig. 6). The separation is identified by lack of a vesicle under the probe. The vesicle can be crushed as close as 0.5 cm from the other but it is generally best to separate them at least 2 cm. This is in case the
mare moves at the time of increasing pressure. The vesicle is crushed by gradually increasing the pressure using the probe. Occasionally refractory cases may need a sudden increase in pressure much like a quick flick of the end of the probe. This later technique is quite useful for smaller (day 11-13) vesicles.

Figure 5. The technique for simple crush of a vesicle pre-fixation. The vesicles to be separated are identified. Frequent back and forth scanning maybe necessary to continually confirm the location of each vesicle (Fig. 5a) and then gentle pressure is used to separate it from the other (Fig. 5b). As pressure is gradually increased the vesicle changes shape and starts to flatten (Fig. 5c). At the appropriate moment a little quick extra pressure completes ablation. It is quite common to see fluid immediately after the crush. (Fig. 5d).

When the vesicle is crushed it is not uncommon for fluid to surround the other. This is not a problem at this stage of pregnancy. Later (≥day 25) fluid surrounding then other vesicle is thought to be a potential problem.

At the GVEH records (www.gvequine.com.au/breeding_efficiency.htm) were evaluated for 1716 Thoroughbred (TB) mare cycles and 1294 Standardbred mare (St B) cycles. Twins were diagnosed in 245 of 1716 cycles in TB mares (14.3% of cycles) and 46 of 1294 of St B cycles (3.5%). After twin reduction mares are not routinely examined until the next scheduled examination i.e. 21-25 days post ovulation (detection of the foetus). When mares were re-examined after pre fixation embryonic reduction 10/245 TB mares (4%) had lost the remaining pregnancy and 8/46 St B mares (17.4%) were empty. The
number of TB mares loosing the remaining pregnancy (4.0%) is similar to 3.7% (63/1716) which was the calculated rate of early embryonic death (EED) on the same farms for mares with a single pregnancy diagnosed at day 13-15 and then subsequently found to be empty at the next scan. Interestingly the number of St B pregnancies lost was much higher after twin reduction (8/46-17.4%) compared to the calculated rate of EED (7.1%). This we believe was likely related to economics wherein the Std B clients are unwilling to scan too early. Thus twins when detected are likely to be closer to final fixation or fixed already and harder to manage.

Figure 6. Separation of twins that are together is possible if they are able to assume a figure 8 shape (Fig. 6a). Gentle pressure results in them moving apart (Fig. 6b, 6c and 6d). When they are separated enough to crush the vesicle has disappeared from the screen. When vesicles are in close proximity to each other at the time of the crush it is quite common for fluid from the crushed vesicle to surround the other (Fig. 6e).
It is our contention that the procedure has developed to the stage that it is *always expected* a single pregnancy will exist after pre-fixation embryo reduction is attempted. Unless a mistake occurs and the other vesicle is ruptured at the time of initial manipulation, we feel that any failure to survive the procedure is more likely a result of uterine inflammatory changes and infection rather than a result of the procedure. We believe that this is the most reliable technique available but feel it is important to highlight the experience of the personnel involved. From discussions with farm managers and other veterinarians it is clear that only veterinarians involved with sophisticated reproductive management such as the routine use of ultrasonography can expect to achieve these types of results. Our strong recommendation to veterinarians and clients is that all mares are examined within 14-16 days of breeding. Expected time to ovulation after breeding will depend on frequency of examination and use of ovulation induction agents such as hCG or GnRH. Factors that may modify this decision are breed, mare value, ability of the stud master or owner to facilitate examination of the mare and on occasion education of the owner.

**Recognition after fixation (after day 16)**

**Days 17-20**

In all cases of *bilaterally fixed* twins one is destroyed immediately. The mare has an extremely efficient biological embryo reduction mechanism that operates when twins are in apposition (*unilaterally fixed*). Reduction occurred in 100% of 22 mares with asynchronous ovulation (vesicles size greater than 4 mm in diameter) and 19/26 (73%) of mares with vesicle size (0-3 mm difference). Because the rate of embryo reduction between day 17 and 20 is so high for unilateral fixation, equine practitioners frequently elect to leave these developing pregnancies and determine their outcome later. Our philosophies are that if the two vesicles have coalesced into one larger vesicle with an ultrasonographically visible line in division (Fig. 3b) they are left totally alone, however if the individual vesicles have still retained a spherical orientation or a spherical shape (like a figure ∞), then they can be separated gently with the probe and are crushed either in situ or after being manipulated apart (Fig. 6). Due to the nature of our practice, few mares present with this configuration in the Thoroughbred population, however, it is not uncommon in the Standardbred population wherein economics dictate that pregnancy diagnosis is often delayed past the time the mobility phase has ended. Results from our practice with twins in this configuration are reduced compared to pre-fixation intervention procedures. Others have reported good results post fixation. One group reported success in 49/50 cases post fixation. The work of Bowman (1986) more closely parallels our experiences. With bilateral embryo fixation and intervention, he reported almost no losses with 40/44 mares from day 16 to day 30 (90.9%) having a single pregnancy detected on day 45. With unilateral fixation the results were days 16 to 17 (16/18 - 89%) days 18 to 19 (23/24 - 95.8%) days 20 to 21 (8/13 - 61.5%) days 22 to 24 (4/9 - 47.4%) days 25 to 30 (1/4 - 25%). Because of the high incidence of embryo reduction with unilateral fixation and the low incidence with bilateral fixation, we have clear recommendations with twins in the day 17 to 20 period. Those that have rounded (figure ∞ shaped) twins, still retaining their vesicle turgidity, that can be separated, are crushed either in situ or after being manipulated apart. In all cases where the vesicles have apparently coalesced into a larger vesicle with an ultrasonographically single line dividing the two we leave them alone, more particularly so, if there is any unevenness in vesicle size. In all cases of bilaterally fixed twins one is destroyed immediately.
Day 21 to 30

All cases of bilaterally fixed twins of this age group are manipulated and one destroyed immediately (Fig. 7). In most cases we do not attempt to manually destroy one vesicle with unilaterally fixed twins (Fig. 8) of this age group until after day 30 and before day 35. At this age it is too easy to rupture both vesicles and the maximum success we believe we can expect is 50% (see previous section) which is less than or similar to the mares own biological reduction mechanism.

![Figure 7. Twins at ~ day 24 (Fig 7a and b). Firm pressure results in flattening of the vesicle (Fig 7c) and ultimately rupture (Fig 7d).](image-url)

Day 30 to 35

During the period prior to the formation of endometrial cups, gentle pressure may be placed on one vesicle. We do not attempt total ablation at this time as resulting fluid sometimes surrounds the other foetus and effectively separates placental (chorionic girdle/trophoblast cells) attachments to the uterus. In these cases (total rupture of the vesicle),
Figure 8. Twins at day 30 (Fig 8a and 8b). The membrane between the twins can be clearly detected (Fig 8c). Rupture of the vesicle is demonstrated by multiple membranes (Fig 8d). It is not ideal to rupture membranes in unilateral pregnancies or bilateral pregnancies after ~day 35.

definition of the remaining vesicle is very common. Between days 30-35 we attempt to pinch one vesicle and create a ‘snow flake’ effect which is the shedding of cells from the membranes. Demonstration of this effect almost always results in gradual loss of the effected conceptus. The pinching of the vesicle can be likened to membrane slipping of a bovine pregnancy except that we use the probe to produce the effect. Occasionally in mares with multiple cysts twins may be missed and then identified at a later time. In general if they are unilateral it is best to leave them to the mare’s natural embryonic reduction method, however ablation can be attempted (Fig 9).

Day 36 to 60

From day 36 onwards it is a reasonable assumption that endometrial cup formation and subsequent eCG secretion will prevent many mares from returning to heat after early embryonic death. Abortion after 35 days is commonly associated with difficulties recycling the mare. In one study when mares were aborted either between day 26 and 31 or between day 30 and 50, 8/11 became pregnant versus 2/7, respectively. This is similar to the work of Pascoe who concluded that the administration of a prostaglandin analogue < 35 days of gestation was outstandingly successful as a method of treatment for twin pregnancy.
Manual intervention at this time in our experience is very good in bilateral twin pregnancies (<45 days) but only approximately 50% successful in unilateral twin pregnancies. Success improves with use of more subtle pressure and damage to the chorioallantoic membrane rather than complete rupture in one attempt. Demonstration of the ‘snowflake effect’ without vesicle rupture consistently results in a gradual (48hr) stress of the foetus and ultimate loss of heartbeat for the conceptus. These pregnancies have the foetal fluids that become progressively more hyper echoic and reduce in size without interfering with the survival of the other foetus (Fig 10). Early pregnancies tend to be
resorbed without a major increase in echogenicity of the fetal fluids (Fig 11). It is important with these foetuses to always attempt to damage the same one. Multiple attempts, i.e. everyday or every other day for 5 to 10 sessions maybe necessary to elicit the correct response; (Fig. 12), however, quite frequently we are unable to create sufficient damage for foetal destruction. In these cases rather than creating major trauma (rupture of the vesicle) an alternative approach is sought after day 60. A combination of membrane slip and or oscillation of the foetus are used. If we are unsuccessful in establishing a response at this stage then the pregnancy is left until after day 100 (see below). Our anticipated success rates are 50% reduction and ~50% of cases result in further examination after day 100. With careful manipulation the demise of both fetuses should occur less than 5% of the time.

Figure 11. Normal 48 day pregnancy on the left and resorption of fluids and the fetus on the right.

A variety of methods have been reported as used to treat twins at this stage. Manual crushing was originally reported and results suggested that earlier crushing was better and if possible crushing should occur prior to day 31 because after day 35 sometimes manual rupture was not possible. The author quoted the following results between day 35 and 45; 60% resorption of both, 20% single foaling and 20% survival of both. Pascoe demonstrated that needle puncture of one twin combined with non steroidal antiinflammatory treatment (meclomenic acid) resulted in no foals born. A more elaborate and invasive approach, such as intra-foetal injection with saline via a laparotomy has been reported or removal of one foetus via a video endoscope (abandoned as being not practical). An interesting report was the surgical technique for removal of one conceptus from mares with twin concepti more than 35 days of gestational age. Eight mares had bicornuate pregnancies and 7 mares had uni-cornuate twin concepti. Five of six surviving mares with bicornuate twin concepti, delivered a single viable foal and none of the 7 mares originally with uni-cornuate twin concepti, produced a foal. The poor survival rate of uni-cornuate twin concepti was attributed to disruption of the remaining chorioallantois during surgery. Transvaginal ultrasound guided foetal puncture for destruction of one of a set of twin pregnancies has been reported. Foetal fluids from one foetus were aspirated while observing the relationships of the needle foetus yoke sac and/or allantochorion between days 20 and 45. Three of four bicornuate twin pregnancies
resulted in a single pregnancy 10 days or greater after interference (similar to or less than our ability to manually destroy one conceptus in this configuration). Three of nine (33%) still had a viable single pregnancy after 10 days when twins were fixed together (between day 20 and 45). These results were disappointing, however they may be improved with experience and/or antibiotic therapy at the time of intervention. Ultrasound guided fluid withdrawal between day 50 and 65 was studied in single pregnancies; however, the study did not involve any twins. Our experiences with transvaginal ultrasound guided foetal reduction are small (N=5) however, between 45 and 60 days the foetus within the vesicle was difficult to position. We only have attempted to directly puncture the foetus, not aspirate fluid and are unlikely to persevere with this technique (foetal puncture at this age) due to difficulties involved. All cases ended in loss of both foetuses, usually within three days of interference. A more recent report suggested around 20% success with trans vaginal procedures.

Figure 12. Increased debris and echogenicity of the fetal fluids (Fig 12a) are good signs of fetal demise. Occasionally an increase in size of the amniotic cavity (Fig 12b) is observed with fetal demise.

Day 60 to 100

Between day 60 and 100 it becomes more difficult to damage the chorioallantois. In these cases we identify the most conveniently located (always the smallest) of the twins and repeatedly traumatise it by oscillation, or membrane slip, or attempt to damage the cranium with multiple attempts of single digit percussion. Similarly to the previous scenario approximately 50% succumb to this procedure, however it is tedious and time consuming and thus we avoid this time period most commonly.

Days 100 onwards

Probably the most common reason for being presented mares at this late stage of gestation with twins is failure of the aforementioned techniques. Less frequently twins have been missed in earlier diagnostic attempts and more recently there has been an increase in diagnosis of twins at this stage due to the widespread use of fetal sexing procedures. Frequently, mares have been identified with twins late in the breeding season and the owner has adopted a non-intervention approach. Because the possibility of foetal reduction after 100 days is very low and the probability of abortion or stillbirth is extremely high, an approach was developed to eliminate one pregnancy at a later stage of gestation. The technique involved transabdominal ultrasonographic identification of the
twins and intracardiac injection of a lethal substance. The smaller twin was always identified. Initial results with saline and air were unsuccessful but when the solution was replaced with potassium chloride, 7/18 mares (39.9%) had single live foals. We have been utilising this technique since 1988 and can report similar experiences. Our initial success was not very promising (2/10 live foals) until the potassium chloride solution was replaced with 10ml-20ml of procaine penicillin which has resulted in a live foal rate of 56%. The current procedure at the GVEH is to tranquilise the mare with Detomidine and to identify the smaller foetus or in the case of evenly sized fetuses, the one with more potential for placental expansion. A 6-10 inch, 16 needle with a tip designed for ultrasonographic enhancement (Cook, Australia) is passed through the needle guide biopsy channel into either the heart, lungs or abdomen of the identified foetus (Fig. 13). Penicillin is injected and the foetus monitored for the next 5 to 10 minutes. If the needle is in the chest or abdomen the demise of the foetus still occurs however it just takes a little longer (up to 5 minutes). The apparent advantages of penicillin as we see them are: 1) it reduces iatrogenic bacterial contamination, 2) it can be visualised ultrasonographically as it is injected and 3) foetal death can still be obtained without intracardiac needle placement. We have attempted to place mares on Regumate and long term oral antibiotics, however have found no difference in foetal survival rates with either treatment compared to those that have received no treatment. At the time of needle puncture mares are treated with systemic antibiotics for 3 days and intravenous phenylbutazone. There has been much discussion about the production of small dysmature foals from successful use of this technique. While this occasionally does occur, care in correct fetus identification (biggest fetus with the most potential for placental expansion) has lowered the occurrence of this outcome dramatically. This is more readily possible when there have been multiple opportunities to observe fetal size, position with the pregnant horn (s) and uterine body and placental arrangements. After injection and death of the fetus it gradually becomes mummified (Figs. 13g and 13i). Commonly these fetal remnants can be detected within the placenta at birth (Fig 14).
Figure 13a. Twins at ~110 days (trans rectal). The ribs, lungs, liver, aorta and abdominal contents can be clearly identified. Figure 13b. Transabdominal scan demonstrating the line of needle puncture. Figure 13c. Immediately after injection of penicillin. The needle is visible to the left of the puncture line guide. The fetal chest is not as easy to see as it is filled with penicillin. In addition some penicillin has escaped outside the chest. Figure 13d. The surviving twin one day after injection. The fetal heart, lungs, liver ribs and aorta are all clearly visible. Figure 13e. The injected twin one day after injection. It is difficult to accurately see anatomy. Figure 13f. Fetal head and eyes of the surviving twin 10 days after injection. Figure 13g. The injected twin showing signs of advanced mummification at 10 days after injection Figure 13h. Same as Figures 13d and 13f at 22 days after injection. Figure 13i. Same as Figures 13e and 13g at day 22 after injection.

Figure 14. Membranes containing mummified bones (Figure 14a), the client was kind enough to arrange the bones they collected (Figure 14b) and the foal was of normal size at birth (Figure 14c).
A few years ago it was reported that a new technique to manage twins by dislocation of the head from the neck was associated with good success. Two techniques were described; firstly dislocation of fetal head per rectum and secondly via a standing flank laparotomy. The authors currently favour flank laparotomy. The technique is straightforward using standard standing flank laparotomy procedures. Immediately prior to grasping the fetus the mare is administered Probantheline as a uterine relaxant. The correct fetus is grasped and then the head is removed from the neck by pinching it between the thumb and first finger (15c). A distinct popping sensation is evident. In all cases the fetus that has been manipulated has been alive immediately after and for 24 hours or more. Ultrasonography is useful in demonstrating that the head is well separated from the neck after the surgery (Figs 15a and 15b). The potential benefits are a technique that was available for use earlier in gestation than trans-abdominal needle injection thus fetal death would occur at an earlier time (less prostaglandin inflammatory reaction) and possibly an improved ability of the placenta to compensate for the fetus that is left to grow.

After an initial success rate reported of 2 live foals from 4 mares the authors currently report a success rate overall of 64% from 44 cases with the most recent figures of 8 foals from the last 12 procedures. Another referral centre reported 2 foals from 4 attempts.

We can report one foal from 5 attempts. One mare aborted at 3-4 months after having a discharge and three had no foals. One was checked negative a few weeks after the procedure and that was one we had great difficulty grabbing the foal as the uterus started to contract and the foal was only 60 days old. This resulted in some trauma to the uterus. The heads separated easily and all cases had two live foals around 3-4 days after decapitation with a clear separation of head and neck. None aborted live twins to our knowledge. It is not known why in our hands such a simple technique did not meet the same results as others reported. It is possible that a more traumatic neck separation might result in a quicker death of the manipulated fetus. We plan on continuing to work with the procedure.

Figure 15a. Demonstration of correct response to dislocation. A blind ending stump at the end of the neck is clearly visible. Figure 15b. The dislocated head can be seen well away from the vicinity of the neck of the same foal depicted in 15a. Figure 15c. Technique for head dislocation (Courtesy of Dr. K. Wolfsdorf).
Late in gestation twins maybe difficult to recognise with trans rectal ultrasonography except in those cases with observation of the twin membrane.\textsuperscript{16} Abdominal ultrasonography is useful to diagnose twins.\textsuperscript{40,43}

In the event of failure to diagnose twin pregnancies, occasionally abortion is heralded by lactation late in gestation (>7 months). There are reports of successful maintenance of pregnancy despite premature lactation (>1 month prior to foaling) in cases with twin gestation. Apparently the premature lactation is induced by foetal death and the beginning of mummification of one foetus and thus is threatening to the remaining live foetus. Four mares with apparent impending twin abortion were able to deliver live single foals concurrent with a mummified twin after supplementation with progesterone was initiated upon recognition of inappropriate lactation late in gestation.\textsuperscript{44,45} In these cases although foals were born small they survived and thrived normally. Further work will be necessary to determine which mares will respond best or even at all to supplementation with progesterone for initiation of premature lactation.

From all of the preceding discussion it should be obvious to readers that in our opinion the best method of handling twins is early identification and destruction of one (day 11 to 16).

**References and Footnotes**


a. Dr. R. Holder, personal communication.
b. Dr. K. Wolfsdorf, personal communication 2008.
c. Dr. B. Woodie, personal communication 2008.