

Management of Twins

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Introduction

The establishment and loss of twins, often results in mare that is barren for a year and the associated economic loss. The incidence of twin births has been documented as occurring in 1-2% of the equine population¹ with twinning accounting for 6-30% of abortions in the mare.²⁻⁴ Abortion or still birth resulted in 64.5% of twin conceptuses that were maintained for ≥ 8 months, with only 14% of surviving foals reaching the second week of neonatal life.² In another study, only 11% of 130 mares carrying twins produced viable foals; and only 38% of these mares produced viable foals the following year.⁵ When twins are present, gestation proceeds normally until the conceptuses begin to compete for uterine space or placenta. With or without mummification, death of one fetus leads to abortion, usually between 5 and 9 months of gestation.⁶ Lactation commonly occurs after one foal dies and causes premature mammary gland development.⁶ If one or both foals are born alive, the mare may require assistance. Surviving foals are usually weaker, more susceptible to infection, and slower to develop than singletons. To avoid the potentially disastrous outcomes with twin fetuses, veterinarians have used various management practices such as: not breeding mares with two dominant follicles; breeding mares after the second ovulation; or short cycling mares that ovulate two follicles.⁷⁻⁹ These strategies result in the loss of valuable estrous cycles during the breeding season, resulting in decreased number of pregnancies and increased economic loss.⁹ Fortunately, with the use of ultrasound and an increased understanding of the mechanisms involved in twinning, better approaches to breeding management and twin reduction been developed.¹⁰⁻¹²

Manual Crushing during mobility phase:

Although pregnancies can be diagnosed with ultrasound as early as 9 days after ovulation, twin pregnancies are optimally detected between Days 13 and 15 of gestation when the embryonic vesicles are still mobile and two embryonic vesicles can be imaged.¹³⁻¹⁵ Natural twin reduction does not occur before Day 11, and it is negligible between Days 11 and 15.^{7,14,16} Therefore, twin pregnancies that are detected during the mobility phase (Days 9 to 15) are best managed by manually crushing one embryonic vesicle.⁶ When a double ovulation post breeding is noted, ultrasonography for pregnancy should be performed at day 13 to 15 post ovulation. This way recognition that twins are present is made early and there is ample opportunity before fixation occurs for the vesicles to naturally separate and the crushing of one with minimal manipulation performed. If the vesicles are too small or if they are in the body of the uterus close to the bifurcation it is difficult to manipulate them and reevaluation the next day or at a later point during the day when they have moved can be beneficial. The smaller vesicle or the vesicle that needs the least amount of uterine manipulation is preferentially destroyed.¹⁷ Survival rates of the remaining vesicle exceed 90%.^{5,17} If the embryonic vesicles are next to or superimposed on one another, again if they are still mobile re-examination that or the next day may present them separated and crushing of one feasible. When both embryonic vesicles continue to sit next to each other, movement of the vesicle closest to the tip of a horn can occur with either manual manipulation to separate them. Alternatively, the ultrasound probe can be placed between the vesicles and pressure is used to move one vesicle up the uterine horn for crushing. Continued pressure can be applied to the vesicle once it has reached the tip of a horn. This restricts its movement and rupture occurs. Using this technique, the fluid released after

rupture of the embryo dissipates far from the remaining vesicle preventing interference with endometrial contact of the remaining vesicle. If separation cannot be performed due to straining, an enlarged uterus or inability to differentiate the individual vesicles buscopan (3cc IV) or propantheline (15mg IV) can be administered. This relaxes smooth muscle allowing differentiation of the borders of the vesicles through the uterus and subsequent isolation and movement. Once a single embryonic vesicle remains there is much controversy and many opinions as to whether treatment ie. Flunixin Meglamine(Banamine) at 1mg/kg IV and/or Altrenogest(Regumate) at .044mg/kg orally should be initiated. Previous studies have shown that manipulation of the uterus for 20 minutes is necessary for prostaglandin release to occur and therefore induce lyses of the corpus luteum.¹⁷ It is the author's opinion that it is difficult to determine the true extent of uterine manipulation or amount of trauma inflicted on the existing vesicle and therefore it is prudent to initiate therapy not only for preventative measures but for owner comfort. Re-evaluation for continued growth and presence of the remaining vesicle should be performed 2-3 days post crushing. Usually loss of remaining vesicle will occur within this time frame if it is going to occur.

After fixation of the embryonic vesicles between 16 and 17 days of gestation, the success rate of reducing the twins to a viable singleton varies tremendously among procedures.

Natural reduction and the deprivation theory

Biological or natural reduction occurs when excess embryos are eliminated and one viable embryo continues to develop.¹⁸ The surviving embryonic vesicle continues to grow and develop normally in appearance, with the only potential difference from a singleton pregnancy being vesicle orientation. Orientation can be defined as rotation of the embryonic vesicle so that

the embryo proper is on the ventral aspect of the vesicle and the thickest portion of the wall of the conceptus assuming a ventral position. This is hypothesized to be a function of disproportionate strength of the yolk sac wall and thickening and encroachment of the dorsal uterine wall on the conceptus.¹⁸ The actual mechanism of natural reduction is not known.¹⁹ Whether twin embryonic vesicles fix unilaterally (in the same uterine horn) or bilaterally (in different uterine horns) will affect the incidence of natural reduction. Ginther et al presented landmark research^{9,18} suggesting that close apposition of the embryonic vesicles appeared to result in a compression of the vesicles against each other; subsequently, contact between the endometrial and trophoblastic surfaces of the vesicles was lost. Most unilateral reductions occurred before Day 26, potentially because the yolk sac is not efficient as a nutrient purveyor. Ginther determined the overall incidence of natural reduction, resulting in a single conceptus, to be 64%. Although 83 to 89% of twins with unilateral fixation naturally reduced to a singleton, only 4% of twins with bilateral fixation reduced to a singleton.²⁰ In this study, Ginther did not include mares that lost both conceptuses, which would result in 77% (17/22) of mares naturally losing one or both of the twin conceptuses. The probability of natural reduction decreases as gestational age increases to Day 40, with 53% of unilateral reductions occurring before Day 20 and 82% before Day 30.^{14,19} When twins were present at examinations between 40 and 42 days, only 6% of the pregnancies terminated in birth of one foal, 31% resulted in two foals, and 63% lost both fetuses.⁹ Factors that appear to influence natural reduction are: relative orientation of the embryonic vesicles, synchronicity of the ovulatory follicles, and size disparity between the vesicles. If a vesicle has the embryo proper adjacent to another vesicle it is more likely to be reduced, than if the embryo proper is in contact with the endometrium; in addition if

asynchronous ovulations occur and one embryonic vesicle is larger it can impede the movement and growth of the smaller leading to a higher likelihood of reduction.

Manual reduction after fixation

Manual reduction is preferentially and most successfully performed before fixation of the embryonic vesicles. If twin conceptuses are observed after fixation, manual reduction can be attempted, however manual reduction of unilaterally fixed twins is difficult without damaging both conceptuses. If the vesicles can be separated, 90% of unilateral twins can be manually crushed between days 17-20.^{11,21} An attempt at manual reduction of bilateral twins between Days 16 and 40 is a necessity if abortion at a later stage of gestation is to be avoided. Seventy-five percent of bilateral twins may be successfully reduced to a singleton pregnancy by crushing one vesicle before 30 days of gestation. However, with bilateral twins of gestational age > 35 days, there is a greater risk of abortion at a later stage if a vesicle is crushed,^{7,11} potentially because fluid released from the crushed vesicle gets between the chorioallantois and endometrium and causes a loss of contact.²² Manual manipulation transrectally, while damaging without rupturing of one of the vesicles between 28 and 42 days, resulted in 28% of the mares having a single viable foal.²⁰

Transvaginal, ultrasound-guided twin reduction

Selective reduction of pregnancy using transvaginal ultrasonography has been examined in mares having both singleton^{23,24} and twin pregnancies.^{25 26,27} The technique involves a 5 or 7.5 MHz transvaginal ultrasound transducer designed for use in large animals. Typically, the transducer and casing are cold-disinfected or sterilized prior to placement in the mare. Some

individuals cover the transducer with a sterile latex cover (latex ultrasound transducer cover, Civco Medical Instruments, Kalona, Iowa) or sterile sleeve filled with sterile lubricating jelly. The mare's tail is wrapped and the perineal area cleansed. Ideally, chemical restraint is not used to perform the procedure because of the uterine relaxation induced by some agents (alpha agonists such as xylazine and detomidine hydrochloride). Local anesthetics (2 % lidocaine) can be infused directly into the rectum or mixed with rectal lube and carried into the rectum at the time of the procedure. Prior to performing the procedure, the mare often receives flunixin meglumine (1 mg/kg, IV) to counteract prostaglandin release during uterine manipulation. Wearing a sterile obstetrical sleeve, an operator carries the transducer into the anterior vagina. The operator's arm is then removed from the vagina and placed in the rectum for manipulation of the reproductive tract. The operator manually secures the pregnancy (transrectally) and the transducer is manipulated (transvaginally) until the pregnancy is imaged on the ultrasound screen. The fetal position is clearly identified. A puncture guide on the ultrasound screen is used to select a path for needle placement in the yolk or allantoic sac. An assistant passes a sterile, 16 to 18-gauge, 60 cm needle with an echogenic tip (Echogenic tip spinal needle, Cook Ob/Gyn®, Spencer, Indiana) through a needle channel in the transducer casing. A sharp jab of the needle is made for passage of the needle through the vaginal and uterine walls into the yolk or allantoic space. After ultrasonographic identification of the echogenic needle tip in the yolk or allantoic space, a 60-ml luer-tip syringe or suction pump is connected to the needle and fluid is aspirated. To facilitate complete fluid aspiration, the needle can be moved within the sac into areas of detectable fluid. The orientation of the twins (unilateral vs. bilateral) influences when aspiration is discontinued. For unilateral twins, aspiration is discontinued when there is danger of aspirating the fetal membranes between the twins, the conceptus can no longer be visualized

because of fluid removal, or it is no longer possible to obtain fluid. When performing the procedure on a bilateral twin, complete fluid evacuation is ideal. Trauma to the treated fetus is not a concern with bilateral twins, and may actually be advantageous.

The success rate of transvaginal ultrasound-guided twin reduction is highly variable and dependent on many factors. Day of gestation at the time of reduction appears to impact pregnancy outcome following the procedure. When examining success rates²⁶⁻²⁸, there appears to be an advantage to performing the procedure before 36 days of gestation, particularly in the case of unilateral twins. One could argue that unilateral twins prior to Day 40 might reduce naturally and intervention is not necessary.²⁰ However, by Day 25-30 a size discrepancy is often noted in unilateral twins that are in the process of natural reduction.¹⁸ When twin embryos are similar in size between 25 and 35 days gestation, aggressive management of twins is probably the best option. However, performing the transvaginal ultrasound-guided procedure in mares with unilateral twins as significant limitations due to the close proximity of the embryos/fetuses and associated membranes. One may inadvertently penetrate the adjacent vesicle, and possibly the embryo or fetus, if the placental membranes are not seen in the imaging plane. When aspirating placental fluids for termination of a unilateral twin one can easily aspirate placental membranes into the needle tip causing damage to the remaining fetus. Additionally, when fluid is withdrawn from a unilateral twin vesicle, the adjacent vesicle tends to pull from the endometrium and “fall” into the evacuated space (Macpherson, unpublished data). Fluid may also leak from the incompletely evacuated vesicle causing the placental membranes to separate from the endometrium.²⁴ More recently, one author (Macpherson) has attempted to damage the embryo,

directly, when performing this procedure in unilateral twins rather than aspirating fluid from the vesicle. This prevents the membranes from pulling away from the endometrium and terminating both pregnancies. Treating the mare with exogenous progestins to enhance uterine tone can also help prevent separation of the membranes from the endometrium when performing this procedure.

With bilateral twin pregnancies, there is significantly less likelihood that penetration of the conceptus and surrounding membranes will occur. As a consequence, one can more aggressively aspirate fluid from the selected conceptus or induce fetal damage with the needle. Also, the time limitations seen with unilateral twins are not as stringent when using this procedure for bilateral twin pregnancies. However, age of the mare, parity, size of the mare, position of the uterus, tone of the uterus can all negatively impact the success of the procedure. The procedure is significantly more difficult if the pregnant uterus is pendulous within the abdomen in aged mares or advanced pregnancy. As a consequence, performing the procedure in older, multiparous mares after 45 or 50 days can be challenging.

Cranio-cervical dislocation

Cranio-cervical dislocation (CCD) is described as the dislocation of the first cervical vertebrae from the cranium, disrupting the ligamentous attachments and severing the spinal cord. This new procedure can be performed using transrectal or transabdominal techniques between 60 and 110 days of gestation to produce a single foal.²⁹ The basis for this procedure is to eliminate

one twin before placental formation is complete, allowing the remaining fetus to utilize the entire endometrial surface for nutrient and oxygen exchange and to grow to its full potential.

Transrectal manipulations have been performed between 60 and 90 days of gestation. The mare is restrained in stocks or twitched in the doorway. Sedation using detomidine HCL at a dose of 10-20mg/kg IV can be administered as needed. However, after sedation, the uterus may relax, and the fetuses may move cranially in the abdomen and out of reach. Relaxation of the smooth muscle in the uterus and rectum can be achieved by the administration of propantheline bromide (30mg, IV (Compounded at Hagyard Pharmacy-30mg/ml) or Buscopan (5cc, IV); this will allow easier identification and manipulation of the fetuses. To help inhibit prostaglandin release, flunixin meglamine (Banamine) at a dose of 1mg/kg is administered before the procedure. The smaller fetus or the fetus that has less contact with the endometrium and minimal space to grow is preferentially reduced. This fetus is usually identified in the more cranial aspect of the uterine horn in unilateral twins. Once the identified fetus is located, the head must be isolated by finding the dome-shaped head and palpating the mandible or moving caudally and locating the cervical vertebrae. Cranio-cervical dislocation is performed by stabilizing the head between the thumb and forefinger and bending the head from side to side. This will damage the ligaments attaching the head and neck. Dislocation is then created by placing the thumb at the base of the cranium and applying pressure proximal and dorsally. A distinctive pop is felt if dislocation is achieved, and the thumb and forefinger can be placed in the space created between the head and neck. Mares should be placed on Altrenogest(Regumate) at .088mg/kg once a day once daily for 3 to 4 weeks. After cranio-cervical dislocation, death with loss of the fetal heart beat is usually evident within 24 hours to one week. Fetal viability should

be evaluated in one week later and every two weeks for a month to establish normal growth of the continuing fetus and demise of the other.

If transrectal reduction can not be achieved, a surgical procedure can be used. To date, this procedure has been used for twins between gestational ages of 58 and 150 days.

Transabdominal ultrasound is used to identify the horn in which the most viable fetus is located, as determined by fetal size and by the fetus that has the greatest surface area for attachment to the endometrium. A standing flank laparotomy is performed ipsilateral to the horn containing the fetus that has been identified for reduction. Preoperative medications include: Propantheline bromide (30mg IV, compounded at the Hagyard Pharmacy), flunixin meglumine (Banamine) 1 mg/kg IV, Procaine Penicillin G at a dose of 22,000 mg/kg, IM, twice daily and gentamicin at a dose of 6.6mg/kg IV once a day. Propantheline bromide is essential for relaxing and preventing uterine contractions while finding and manipulating the fetus. A standing flank incision is made ipsilateral to the uterine horn containing the fetus that has been identified for reduction.

Identification of the preferred uterine horn is not always possible, because of fetal movements and imaging capabilities. If this occurs, we prefer to make an incision on the right flank of the mare, allowing more access to the reproductive tract without intestinal interference. The uterus is located within the abdominal cavity with one arm, and the twin is isolated as described for transrectal dislocations. Cranio-cervical dislocation is performed by manipulating the fetus through the uterus, without incising or invading the uterine lumen. The flank incision is then routinely closed. With this technique, death of the manipulated twin may not be evident until 24 hours to 7 weeks. Mares should be placed on flunixin meglumine at 1mg/kg IV, Procaine Penicillin at 20-50iu/kg IM Q 12-24 hours and gentamicin at 6.6mg/kg IV or IM Q 24 hours for

the next 2 days, then trimethoprim sulfamethoxazole at 24mg/kg orally twice daily for one week and altrenogest at .088mg/kg orally once daily for the next month.

Ultimate success of the transrectal procedure at 55 to 90 days has had five of the eight mares (63%) deliver a single fetus of normal size. Unfortunately, the transrectal procedure is technically difficult, and multiple manipulations of the fetus are sometimes necessary before dislocation is achieved and therefore only the transabdominal procedure has continued to be performed to date. Cranio-cervical dislocations, using the surgical procedure, have been performed between 58 and 150days of gestation. Manipulations were only performed once. Cranio-cervical dislocations, using the intra-abdominal surgical procedure, produced a single normal healthy foal in %63.16 (24/38) of mares. With this technique, death of the manipulated twin was evident from one to 8 weeks post procedure. One fetus that underwent CCD never died and abortion was induced at 7 months in order not to affect the mare's present or future fertility. Re-evaluation of fetal viability is performed with transrectal or transabdominal ultrasonography at every 2 weeks until demise of one twin is observed.

Signs of impending death of a fetus include: loss of thoracic shape, with the fetus becoming more convex; loss of definition of abdominal organs; and irregular, weak heartbeats. Placentas from mares delivering singleton foals have a small sack attached to the allantoic surface. The nonviable fetus is marsupialised forming a small pouch, with a stalk protruding from the allantoic surface. This pouch contains the mummified fetal bones. Examination of the chorionic surface reveals minimal evidence that a twin was present, with microvilli present along the entire attachment of the placenta.

Cranio-cervical dislocation has advantages when compared to other procedures for reducing post-fixation twins. Results of this procedure may have a better outcome than

transcutaneous ultrasound-guided reductions in which some single foals have been reported to be undersized and weak (Johanna Reimer; Lexington, KY, personal communication, April 2001).

Transcutaneous, ultrasound-guided reductions are done later in gestation than cranio-cervical dislocations, and placental function and growth may be limited when twin reductions occur later in gestation. When performing cranio-cervical dislocations at a later stage of gestation than transvaginal ultrasound-guided aspirations, the uterus is not punctured with a needle, and fluid does not leak from the reduced conceptus, potentially disrupting the fetal membranes of the remaining conceptus. Additionally, not transversing the abdomen and entering the lumen of the uterus reduces the chance of bacterial contamination resulting in placentitis.

Disadvantages of cranio-cervical dislocation mostly pertain to isolation of the fetus. When the mare is at this stage of gestation, identifying the correct fetus within the uterus is similar to “bobbing for apples”. It is absolutely imperative that the uterus is relaxed enough for identification of fetal anatomy. No documented evidence is available at this time of gestation regarding the extent of trauma or prostaglandin release that occurs with manipulation of the fetus through the uterus, although previous studies have demonstrated 20 minutes of uterine manipulation is necessary for significant prostaglandin release.³⁰ The procedure is relatively rapid from isolation of the fetus to cranio-cervical dislocation; therefore, severe inflammation should not result, but proficiency and time of manipulations may affect the outcome. The incision site of the flank laparotomy heals with few complications. If a seroma or cellulitis develops, the incision can be opened on the ventral end and drained. Scarring was minimal, and all of the mare’s incisions healed normally. The duration of time that the manipulated fetus survives is inconsistent, and an explanation is unknown.²⁹

Transcutaneous, ultrasound-guided twin reduction

The use of transcutaneous ultrasonography to aid in twin reduction in the mare was pioneered by Rantanen and Kincaid in 1988.³¹ In experienced hands, an average of 50% of mares undergoing transcutaneous twin reduction will deliver one live foal.^{21,25,31} The suggested time to perform this procedure is between 115 and 130 days gestation.³² The procedure is performed in the standing, heavily sedated mare to promote movement of the fetuses into the cranial abdomen for easier accessibility and to minimize fetal movement during the procedure. The mare is examined with a 2.5-3.5 MHz transducer to determine fetal positioning and size. The most accessible fetus is selected for reduction, or when possible, the smaller fetus is targeted. The mare's abdominal area adjacent to the fetuses is clipped and surgically prepared prior to the procedure. The transducer is placed in a sterile obstetrical sleeve containing sterile lubricant. Some veterinarians prefer to infiltrate the area adjacent to the targeted fetus with 2 % lidocaine hydrochloride to provide anesthesia prior to passage of the needle. The use of a biopsy guide to perform the procedure is at the discretion of the operator. A biopsy guide on the transducer coordinates with software in the ultrasound which allows the operator to know the expected placement of the needle. Some operators find the use of a biopsy guide limiting when the fetuses and/or mare change position. Typically, a 6 inch, 18-gauge spinal needle is used to perform the procedure. The needle is passed through the skin and abdomen in one motion. Alternatively, a 6 inch, 18 gauge needle with a stylet and echogenic tip (Cook Veterinary Products, Brisbane Australia) can be used to allow for better visualization of the needle tip on the ultrasound image. Once the needle is passed into the peritoneal space, the needle tip is located on the ultrasound image, advanced through the uterine wall and into the fetal heart using a quick, thrusting motion. Free flow of blood from the needle after removal of the stylet indicates needle placement within the

fetal heart. Potassium chloride (KCl, 2 mEq/ml, up to 32 mEq KCL) or procaine penicillin (10-20 ml)²¹ are injected into the fetal heart, thorax or abdomen. Proposed advantages of procaine penicillin for transcutaneous ultrasound-guided twin reduction include Ten to 20 ml of procaine penicillin were injected into the area and the fetus monitored for 5-10 minutes. McKinnon reported that fetal death took slightly longer when injecting penicillin into the thorax or abdomen than directly into the heart. The reported advantages of using procaine penicillin vs. KCl are 1) reducing the possible risk of iatrogenic infection, 2) better visualization of the agent as it is injected, and 3) fetal death even in the absence of cardiac placement. A disadvantage of using procaine penicillin to induce fetal death is that it may take up to a few days for the fetus to die (also dependent on injection site).

Cardiac activity of the treated fetus is monitored immediately after the procedure. The fetus does not always die immediately in which case the mare is monitored over subsequent days to assess the status of both the treated and untreated fetuses. Generally, mares are administered flunixin meglumine (1 mg/kg, IV, BID) at the time of the procedure and for up 4 additional days. Progestin therapy (altrenogest, 0.044 mg/kg, PO, daily) and prophylactic antibiotics are prescribed at the referring veterinarian's discretion.

As with cranio-cervical dislocation, the terminated fetus is mummified and delivered in a small placental sac along with the live fetus. The terminated fetus rarely interferes with the development of the remaining fetus. However, because the procedure is performed after placental formation is complete, some have speculated that placental insufficiency may result in small, unthrifty foals (Johanna Reimer, personal communication). As such, owners should be

advised of the pros (good success rate) and cons (potential infection; small foals) of electing this method of twin reduction before making the financial investment.

Elective termination of both pregnancies

When other procedures fail to produce a single viable conceptus and endometrial cup formation has occurred, aborting both conceptuses is the last option. Most mares that are carrying twins will naturally abort between 7 and 9 months. However, if natural abortion is allowed to occur, the breeding season will be lost, and the next breeding season may be affected. More importantly, mares have increased risks for dystocia, cervical tears, retained placentas and associated complications. While some mares successfully carry twin pregnancies to term, the risks of complications during pregnancy or delivery warrant termination of one or both fetuses.

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