Introduction
The advent of transrectal ultrasonography has dramatically improved the ability of veterinarians to accurately diagnose and manage twin pregnancies in mares. As such the incidence of abortion due to twin pregnancy in mares has declined over the past 10 to 20 years [1]. In spite of the improved accuracy of detection and management of twin pregnancies in the mare associated with transrectal ultrasonography, twin pregnancies are occasionally not diagnosed until after the formation of endometrial cups at approximately 35 to 40 days of gestation. There are several potential reasons for failure to detect early twin pregnancies in mares:

1. Presence of (multiple) endometrial cysts
2. Too early detection of pregnancy such that one embryonic vesicle remains too small to detect with ultrasonography
3. Incomplete examination of the entire uterus
4. Poor image resolution
5. Embryonic vesicles that are immediately adjacent but not properly identified as twins.

Fortunately, the incidence of twin pregnancies identified after Day 35 to 40 in mares is low, and veterinary practitioners are infrequently required to deal with twin pregnancies in mares beyond Day 35 to 40. Nonetheless, the high rate of abortions, poor neonatal viability, growth-retarded fetuses, dystocia and reduced fertility in mares subsequent to twin abortions necessitates that twin pregnancies that are identified after Day 40 be managed in order to avoid these consequences.

There is little information available concerning the outcome of twin equine pregnancies after Day 40 beyond that concerning the incidence of observed twin abortions or twin births in later gestation. Although spontaneous reduction of twin pregnancies after Day 40 have been reported [2, 3, 4], the frequency of such spontaneous reductions appears to be low. In 15 twin fetuses examined in pony mares, death of one fetus (two mares) occurred during months 2 and 3 of gestation [3]. In the remaining mares, two mares lost both fetuses during months 8 to 11, two mares lost one fetus during months 8 to 11, and only one mare delivered twins at birth [3]. Although these observations are reported for pony mares, it appears that some additional reduction of twin to singleton pregnancies can occur in mares after Day 40 of gestation. The 13% incidence of reduction of twin to singleton pregnancies in this study suggests that further reduction of twin pregnancies can occur after Day 40 albeit at a low rate. These observations are further supported by the author's observation in clinical cases of later twin pregnancy.

Several methods have been utilized in attempts to reduce twin pregnancies detected during the fetal stage of pregnancy in mares. These methods include acute nutritional deprivation[5], transvaginal ultrasound guided puncture of the conceptus [6], manual trauma or ballottement of the fetus [7], surgical removal of the
conceptus [8], and transabdominal ultrasound guided injection of the fetus [7, 9, 10, 11]. This paper will focus only upon the use of transabdominal ultrasound-guided fetal puncture as a method to reduce twin pregnancy in mares.

**Transabdominal Procedure**

Transabdominal ultrasound-guided fetal puncture for twin reduction was originally reported by Rantanen and Kincaid in 1988 [9]. In the original description of the technique, a fetal intracardiac injection of potassium chloride was used to induce death of one fetus. Although effective, this approach is more technically challenging, as it requires accurate placement of the KCl into the fetal vascular space in order to induce cardiac arrest. Subsequently, McKinnon and Rantanen described the use of procaine penicillin G (PPG) for intrafetal injection in order to induce fetal death [7]. This technique does not require precise placement of the material in the fetal vascular space and fetal death can be induced by placement of the PPG into either the fetal thorax or abdomen. McKinnon and Rantanen cite other advantages of PPG injection compared to KCl to include: 1) reduced bacterial contamination, 2) PPG can be visualized ultrasonographically, 3) fetal death can be induced without precise intracardiac placement of the solution.

Although no controlled studies have been conducted relative to the effects of gestational age on the outcome of this procedure, Rantanen found a higher success rate when the procedure was conducted between day 115 and 130 of gestation [10]. Attempts earlier in gestation appeared to have a lower success rate whereas attempts later in gestation appeared to be associated with more neonatal problems in surviving foals [10].

**Technique**

Careful evaluation of the twin pregnancy (Fig. 1) by transabdominal evaluation can be conducted with either a 5.0-mHz or 3.0-mHz transducer, although the 5.0 mHz transducer may not give adequate penetration to image both fetuses particularly if one fetus is located more dorsal within the abdominal cavity (more likely with earlier gestational age). Ultrasonographic evaluation of the twin pregnancy via transabdominal ultrasonography requires that hair be removed (#40 blade) from an area between the udder to approximately 25 cm cranial to the mare's umbilicus. Many twin pregnancies between 90 to 130 days will be imaged in the caudal abdomen, just cranial to the udder; however, sedation of the mare, particularly with acepromazine, results in a profound uterine relaxation. After sedation, the fetal location often shifts cranially. Although it may be useful to try to map the relative placental distribution of twins via transabdominal ultrasonography, this is technically difficult. Most often, we infer the relative placental distribution of the two fetuses by measuring fetal size. During this stage of gestation, a number of parameters may be used to assess relative fetal size. These include diameter of the orbit, biparietal diameter, and thoracic diameter. Relative size and location of the two fetuses should be carefully determined along with the orientation (cranial - caudal presentation) of the fetuses in order to ensure accurate identification of the fetuses during and after the procedure. The equine fetus is remarkably mobile during this stage of pregnancy and both fetuses can rotate freely from cranial to caudal presentation. Size may be the best discriminator if there is a detectable difference in the size between the two fetuses. If not, relative location should be determined, but keep in mind that these locations will change.

Figure 1. Twin fetuses (arrows indicate thorax of each fetus) imaged by transabdominal ultrasonography. To view this image in full size go to the IVIS website at www.ivis.org .

After the fetuses have been evaluated for size, location and fetal heart rate, the mare should be sedated. Originally, Rantanen described the use of a combination of acepromazine (10 mg), xylazine (100 mg) and butorphanol (10 mg) as a sedative/analgescic for this procedure. This combination works well and appears to give good uterine relaxation. This may be particularly helpful in those pregnancies that are carried high in the caudal abdomen in order to facilitate better access to both fetuses.

Once the fetal parameters have been established, and the twin for reduction has been selected, the ventral
abdomen overlying the twin is surgically prepared. In addition to the sedation and analgesia, a local infiltration of lidocaine is used to provide analgesia at the puncture site. Although injection of the fetus may be accomplished via a “free-hand” procedure using ultrasound, we prefer a 3.5-MHz ultrasound transducer fitted with a biopsy guide (Fig. 2). An 18-gauge, 8-inch spinal needle with stylet can be used for most fetal injections. The use of specialized needles with echogenic tips has been recommended by some authors to facilitate visualization of the needle tip via ultrasound [7]. Once the fetal thorax is visualized, the needle is introduced through the skin, abdominal wall and uterus into either the fetal thorax or abdomen. Injection of 10 to 20 ml of procaine penicillin G has been suggested to induce fetal death [7]. We typically use 15 to 20 ml of PPG to induce fetal death in our practice (Fig. 3 & Fig. 4). Intrafetal injection (into either the abdomen or thorax) does not result in immediate death of the injected fetus. In some cases, fetal death was not detected (confirmed) until the day following the injection (Fig. 5).

Figure 2. Ultrasonogram of needle placement (arrow) into the fetal thorax. To view this image in full size go to the IVIS website at www.ivis.org.

Figure 3. Injection of procaine penicillin G along diaphragm of the fetus (arrow indicates hyperechoic location of injected PPG). To view this image in full size go to the IVIS website at www.ivis.org.

This video clip depicts the injection of penicillin into the abdominal cavity of an equine fetus. The fetus is oriented with its head to the right. The ribs are visible as two dotted lines. The needle is introduced from the top-right corner of the image and penetrates through the chest and the diaphragm into the abdominal cavity (indicated by the arrow in Figure 2). Penicillin is injected and accumulates under the diaphragm as a bright white area that lines the curvature of the diaphragm (indicated by the arrow in Figure 3). - To view this video go to the IVIS website at www.ivis.org.

Figure 4. Injection of procaine penicillin G into the caudal abdomen of an equine fetus (arrow indicates hyperechoic location of injected PPG). In this case fetal death was not induced subsequent to the injection and a second injection was necessary to induce fetal death. To view this image in full size go to the IVIS website at www.ivis.org.

Figure 5. Ultrasonogram of an equine fetus 24 hours after fetal injection. Note the hyperechoic region in the cranial abdomen of the fetus (arrow). To view this image in full size go to the IVIS website at www.ivis.org.

Mares that are candidates for transabdominal fetal twin reduction are treated with oral altrenogest (beginning on the day of the procedure), systemic antibiotics as well as flunixin meglumine for three days. Oral altrenogest is continued until the recheck examination two weeks after the procedure. The benefits of supplemental progestin therapy are uncertain, and some authors suggest equal success without the use of supplemental progestin [7]. If mares are placed on supplemental progestin, it is imperative that periodic monitoring of fetal viability be conducted to ensure that two dead fetuses are not retained in utero because of the exogenous progestin therapy - a circumstance that we have observed in one of our clinical cases. Most pregnancy losses subsequent to this procedure occurred within 2 to 4 weeks after the procedure [7].
Outcome

Success rates (measured as viable singleton foals born at term) vary across publications with this technique; however, success rates of 32-57% have been cited in previous studies [7,10] (Ball unpublished). Although there are a number of potential reasons for death of both fetuses following the procedure, one likely cause appears to be related to the frequency of intervascular connections between twin fetuses in the equine. Based upon the incidence of blood chimerism in equine twins, it appears that 44 to 50% of heterosexual twin pregnancies in the horse have vascular anastomoses [12,13]. It would seem likely that the presence of vascular anastomoses at the time of fetal twin reduction would result in death of the adjoining twin fetus due to the circulation of either the injected solution or other tissue degradation products. Because there is no known means to identify the presence of these chorionic anastomoses via ultrasonography, there is no method to identify which twin pregnancies might be susceptible to loss of both fetuses due to shared placental vasculature.

Anecdotal information regarding the size and vitality of singleton fetuses born after a successful twin reduction suggests that such fetuses may suffer some in utero growth retardation even after successful twin reduction by intrafetal injection. Unfortunately, data regarding birth weights of foals born to this procedure is not available in current literature. Evaluation of the placenta from foals born subsequent to twin reduction, however, suggests that placental microvillous formation may be sub optimal in that portion of the placenta that expands after death of one twin fetus [11] (Fig. 6 & Fig. 7). Because placental microvillous formation is well-established by around Day 150 [14,15], it appears that although the remaining viable fetus may gain an increased total placental area, the microvillous exchange surface may not be appreciably increased in these pregnancies [11].

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The fetus that dies after intrafetal injection becomes mummified and is typically present within an invaginated pouch protruding into the allantoic space of the remaining live fetus at term [11] (Fig. 8). Fetal bones may or may not be present depending upon the stage of gestation at which fetal death was induced (Fig. 9 & Fig. 10).

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**Figure 6.** Chorionic surface of the placenta from a singleton foal born subsequent to a fetal twin reduction at Day 115 of gestation. Note that part of the uterine body and horn have a very sparse formation of microvilli. To view this image in full size go to the IVIS website at www.ivis.org.

**Figure 7.** Close up of normal microvillous chorion (A) and avillous chorion (B) from the placenta shown in Figure 6. To view this image in full size go to the IVIS website at www.ivis.org.

**Figure 8.** Remnant of the placenta from the dead twin fetus is present along the chorionic surface of the remaining singleton placenta at term as a linear band (arrows). To view this image in full size go to the IVIS website at www.ivis.org.

**Figure 9.** Fetal mummy present as an invaginated sac in the allantoic cavity of the remaining normal singleton placenta at term (arrow). To view this image in full size go to the IVIS website at www.ivis.org.

**Figure 10.** Radiograph of fetal mummy (Fig 9) showing bones present within the mummified fetal remnant. To view this image in full size go to the IVIS website at www.ivis.org.
References


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