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Efficacy of transvaginal ultrasound-guided twin reduction in the mare by embryonic or fetal stabbing compared to yolk sac or allantoic fluid aspiration

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Abstract

Transvaginal ultrasound-guided pregnancy reduction (TUGR) is a procedure described for the management of twins post-fixation in the horse. Success rates are often disappointing but reported to be more favourable for bilaterally-situated twins, and when intervention takes place before day 35 of gestation. This study aimed to determine whether stabbing the embryo/fetus rather than aspirating conceptus fluids improved the likelihood of success, measured as the birth of a normal live singleton foal. Data from 103 TUGR interventions were analysed by logistic regression analysis; method of treatment, relative conceptus location (i.e. uni- versus bilateral) and stage of gestation were included as inter-dependent factors that potentially influence the outcome. Overall, 34/103 (33%) TUGR interventions resulted in a single live foal. There was no significant difference ($p=0.14$) in outcome between TUGR based on fetal stabbing (12/28: 42.9%) versus fluid aspiration (22/75: 29.3%). There was also no significant influence ($p=0.11$) of the conceptuses being located unilaterally (19/65: 29.2%) versus bilaterally (15/38: 39.5%). However, TUGR was numerically more successful ($p=0.05$) when performed \leq day 35 of gestation (21/53: 39.6%), as opposed to $>$ day 35 (13/50: 26%). Day 45 may represent an even more critical time point since only 2 out of 15 TUGRs (13.3%) performed beyond this day resulted in the birth of a live foal, compared to 11/35 (31.4%) performed between days 36 and 45. While the numbers are low, this suggests that TUGR is not the method of choice for reducing $>$ day 45 twins. Four pregnancy losses were recorded 1-7 months post-TUGR (4/38: 10.5%) and, while it is tempting to attribute the losses to TUGR, this rate of late gestation pregnancy loss is normal. We conclude that TUGR by fetal stabbing does not offer significant advantages over fluid aspiration. However, TUGR should be performed before day 35 of gestation and considered primarily a salvage procedure to be used when re-breeding is not a viable alternative.

Keywords: Twins; Mare; Transvaginal pregnancy reduction; fetal stabbing.

1. Introduction

Transvaginal ultrasound-guided pregnancy reduction (TUGR) is a procedure described for the management of post-fixation twins [1,2]. The success of TUGR has been reported to be largely dependent on operator experience [1,2], and the gestational stage (before or after day 35 of gestation [3,4]) and relative position (uni- or bilateral [3]) of the two vesicles. TUGR was first reported in mares by Bracher et al. [3] who reported a viable singleton 10 days after the procedure in six of 13 (6/13; 46%) treated mares; in their study, the procedure had a higher success rate in the case of bilaterally-located twins (3/4; 75% versus 3/9; 33%) and, was more successful for unilateral twins, when performed before day 35 of gestation (2/5; 40% versus 1/4; 25%). By contrast, while Govaere et al. [5] achieved a similar success rate in terms of viable singleton pregnancies 10 days after TUGR (18/35: 51%) in mares with unilateral twin pregnancies, they did not see a significant difference in success depending on whether the procedure was performed before (12/21; 57%) or after (7/17; 41%) day 35. However, analyzing the results so soon after performing TUGR can be misleading because pregnancy losses can occur long after the intervention [6]; this explains why reported live foal rates are considerably lower than the 10 day post-TUGR results. In this respect, Jonker et al. [7] reported a success rate of 31% (5/16) in terms of live foals born, with all of the successful procedures having been performed before day 35 of gestation. Macpherson et al. [6] achieved a 16% (3/19) live singleton foal rate, and registered a higher percentage of success for bilateral pregnancies (2/8; 25% versus 1/11; 9%) while Morris et al. [8] obtained two live foals from 6 TUGR procedures (2/6; 33%). Mari et al. [4] reported a much higher success rate (14/24; 58%) after TUGR, with 67% (14/21) success for unilateral and 0% (0/3) for bilateral twins. However, it is almost certainly significant that they reported the highest likelihood of success (14/20; 70% versus 7/7; 0%) in unilateral twins if the procedure was performed between days 16 and 25 of gestation. This represents the period in which spontaneous

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reduction of unilateral twin pregnancies occurs; Ginther [9] reported spontaneous reduction rates of 59% (40/68) by day 20 and 85% (58/68) by day 30 of gestation.

The majority of previous descriptions of TUGR concentrated on aspirating as much yolk-sac or allantoic fluid as possible. A possible alternative to aspiration is stabbing to disrupt the embryo/fetus accompanied by minimal or no aspiration of fluid. While positioning the embryo/fetus over the needle can be difficult, the period during which the needle is in place is shorter and it is less likely that the needle will need to be repositioned by reintroduction. This and the fact that the 'treated' conceptus will reduce less in size should reduce the risk of damaging or displacing the adjacent twin; on the other hand, the relatively large amount of fluid remaining in the treated conceptus could leak out and negatively influence the co-twin. Previously, the fetal stabbing technique was described for unilateral twin pregnancies by Govaere et al. [5] who observed no significant difference in the success 10 days after TUGR between fetal stabbing (12/23: 52.2%) and fluid aspiration (6/14: 42.9%)

The aim of the present retrospective study was to compare the success of TUGR focussed on aspirating conceptus fluid (yolk sac and/or allantoic) with TUGR focussed on fetal stabbing, in terms of live singleton foals born at term. The working hypothesis was that fetal stabbing would be more successful for unilateral twins because of a reduced risk of inadvertently disrupting the membranes or endometrial attachment of the non-punctured conceptus, e.g. due to fluid leakage after repeated or prolonged aspiration attempts or as a result of the intact conceptus 'falling' into the space created by aspiration.

2. Material and methods

The foaling results for 103 mares subjected to TUGR were reviewed retrospectively. All TUGR procedures were performed at Utrecht University's equine clinic by a single experienced operator helped by one of three assistants. All mares were Warmbloods aged

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between 4 and 21 years and pregnant with unilateral (n= 65) or bilateral (n= 38) twin pregnancies of 21-76 days. In 75 of the 103 mares, as much yolk-sac or allantoic fluid as possible was aspirated; in the remaining 28 mares the embryo/fetus was disrupted by stabbing accompanied by intra-amniotic or intra-fetal aspiration, with little or no allantoic or yolk-sac fluid recovery. In all cases, the mares were carrying twin pregnancies of similar size and with similar embryonic/fetal heart rates. For bilaterally situated twins, TUGR was always performed at first presentation (although the time of first presentation varied from 21-76 days). In the case of unilaterally-situated twins, first presentation was generally delayed until day 28-30. Moreover, in twins in which there was a noticeable discrepancy in embryo/fetal size or heart-rate at presentation, TUGR was delayed for 5-7 days to allow more time for possible spontaneous reduction.

Before the onset of TUGR, all mares received 1.1 mg/kg flunixin meglumine i.v. (Finadyne®; Schering-Plough, Oss, The Netherlands), and the majority were lightly sedated with 0.01 mg/kg of detomidine hydrochloride i.v. (Domosedan®; Pfizer, Capelle a/d IJssel, The Netherlands). In addition, the rectum was relaxed by instillation of 50 ml of 2% lidocaine (Lidocaine®; Dopharma BV, Raamsdonksveer, The Netherlands) through a soft, silicone catheter. The tail was then bandaged and the vulva and perineal area scrubbed. A 7.5 MHz transvaginal sector transducer (Parus 240®; Pie Medical, Maastricht, The Netherlands) secured in a sterile purpose-made plastic and stainless steel, 3-piece transducer containing a needle channel was used for the procedure. A biopsy line on the ultrasound screen was used to indicate the needle trajectory and ensure accurate placement in the yolk/allantoic sac or embryo/fetus of one conceptus. The transducer and transducer casing were introduced aseptically into the vagina and advanced to lie laterally to the cervix, on the side to which the uterus horn containing the conceptus destined for TUGR was located. A sterile 50 mm 19 gauge needle was mounted on a sterile 47 cm long stainless steel tube and connected to an

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aspiration pump. The needle was then introduced into the needle channel of the transducer casing and the aspiration pressure was set at 200 mm Hg. In the case of embryo/fetal stabbing, one of the embryos/fetuses was brought over the biopsy-line by careful manoeuvring of uterine horn and transducer. The needle was then introduced rapidly into amnion or embryo/fetus and aspiration was performed while rotating the needle vigorously within the fetus/embryo; no more than 10 ml of fluids was removed during fetal stabbing. In the case of fluid aspiration, the needle was introduced into either the yolk-sac or allantoic cavity of one conceptus; in the case of bilateral twins, as much fluid as possible was aspirated. In the case, of abutting twins the amount of fluid removed depended on the developmental stage; aspiration was stopped if the membranes from the other vesicle approached the needle, or when the vesicle had noticeably reduced in volume. After the procedure, all mares were treated with 0.088 mg/kg body weight/day altrenogest *per os* (Regumate Equine®; Intervet Nederland B.V., Boxmeer, The Netherlands). Ten to 15 days after TUGR, mares were reassessed by the referring veterinarian; if the mare was still pregnant the altrenogest treatment was continued until 100 days of gestation. If two vital conceptuses remained at this time, the TUGR procedure was repeated. The procedure was considered a success if a live normal singleton foal was born at term.

Statistical analyses were performed using logistic regression so that the effects of a number of potential explanatory variables could be assessed simultaneously. The birth of a single live foal at term was considered as the binary, dependent variable while method of treatment (yolk/allantoic fluid aspiration versus embryo/fetal stabbing), relative conceptus location (i.e. uni- versus bilateral), and stage of gestation (i.e. \leq day 35 versus $>$ day 35 of gestation; or \leq day 35 versus day 36-45 versus $>$ day 45) were considered as explanatory or independent variables. Differences were considered significant if $p < 0.05$.

3. Results

The overall percentage of live normal singleton foals born was 33% (34/103); according to the referring veterinarians all of the foals born at term were apparently normal. In most of the unsuccessful TUGR procedures, the co-twin was no longer viable at the first pregnancy check 10-15 days after the procedure (65/69: 94%). In four cases (4/65: 6%) however, the death of the remaining twin occurred later in gestation; three mares aborted between the second and third month of pregnancy (two had had unilateral and one bilateral twins, and the gestational stages at the time of TUGR were 45, 54 and 43 days respectively; in all cases TUGR had been performed by fetal stabbing). The last mare aborted at 9 months of pregnancy (this mare had had unilateral twins treated by aspiration of allantoic fluid at day 70 of gestation).

The success rates relative to the technique used, the relative location of the 2 conceptuses and the gestational age are displayed in Table 1. There was no significant difference ($p=0.14$) in outcome depending on whether TUGR was performed by aspiration of the yolk/allantoic sac (22/75: 29.3%) or by embryo/fetal stabbing (12/28: 42.8%). Similarly, no statistically significant difference ($p=0.11$) was observed in success rate between unilateral (19/65: 29.2%) or bilateral (15/38: 39.5%) twin pregnancies. The outcome also did not differ statistically ($p=0.99$) whether one (32/97%: 33%) or more than one (2/6: 33%) attempts were necessary to ensure death of one fetus. However, the outcome very nearly reached statistical significance ($p=0.05$), between TUGR performed \leq day 35 (21/53: 39.6%) as opposed to $>$ day 35 (13/50: 26%) of gestation. Indeed, only 2/15 (13.3%) twins subjected to TUGR after day 45 of gestation resulted in a live foal, whereas 11/35 (31.4%) of the procedures performed in the day 36-45 window were successful. In the current study only 3 unilateral twins were treated by TUGR before 25 days of gestation, however the success rate did not differ ($p=0.99$) between these mares (1/3: 33%) and unilateral twin mares treated after day 25 (18/62: 29%).

4. Discussion

The overall percentage of viable foals born (33%) after TUGR treatment in this study is similar to those reported previously by Jonker et al. [7] (31%) and Morris [8] (33%), and higher than the 16% reported by Macpherson [6]. Other studies reported higher success rates (46-51%) based on an examination performed 7-10 days after the TUGR procedure [3, 5]. Delaying the post-TUGR examination to 10-15 days in the current study may have helped to reduce the number of 'false positive' successes, i.e. positive at the first post-treatment examination but failing to produce a live foal. In addition, the finding that four mares carried a viable singleton for 1-7 months after TUGR and then aborted, concurs with Macpherson's [6] suspicion that TUGR-related pregnancy loss can occur long after the intervention; unfortunately, no information on the appearance of the placenta of the aborted foetuses was available, so it is not possible to prove that the delayed losses were a consequence of TUGR and not due to some other cause of abortion (e.g. EHV infection, placentitis, endometrial insufficiency). In this respect, however, Allen et al. [10] recently reported post day 42 pregnancy losses in Thoroughbreds in Newmarket of between 6.3 and 8.2% while Bosh et al. [11] reported day 40-term reproductive losses in Thoroughbreds in Kentucky as between 10 and 13.5%. In short, the overall rate of late gestation pregnancy losses after TUGR (4/38; 10.5%) were no different to those seen in a normal broodmare population. Nevertheless, at least for the 2-3 month losses a delayed effect of TUGR is a plausible contributory factor. In the case of transabdominal ultrasound-guided twin reductions, it is accepted that the placenta of the surviving twin is never able to regain the endometrial attachment area 'lost' to its co-twin, leading to an increased risk of placental insufficiency later in gestation that can result in abortion or the birth of a emaciated foal [12,13] despite mummification of its twin; placental insufficiency following TUGR has not been reported, but could conceivably be an issue if

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TUGR is performed significantly after the time of chorioallantoic placental formation, i.e. beyond day 42 of gestation [14].

The likelihood of success in the current study was not significantly influenced by the technique used for TUGR, which is in agreement with the observations of Govaere et al. [5] for mares with unilateral twins. One of the biggest risks of performing allantoic/yolk-sac fluid aspiration in unilateral twins is damage to the developing membranes or to the chorioallantoic-endometrial attachment of the co-twin as a result of puncture, accidental aspiration, leakage of fluid and/or collapse of the manipulated vesicle [1]. Moreover, fluid aspiration can be time-consuming because the needle can become obstructed by membranes forcing the operator either to stop or reposition the needle in order aspirate the desired amount of fluid. By contrast, stabbing and disrupting the fetus is generally much faster and requires fewer punctures of the membranes such that there is a lower risk of fluid leakage and therefore a lower risk of accidentally damaging the abutting twin; it may therefore be a useful option for unilateral twins beyond about day 30 of gestation. Fluid leakage following TUGR was not specifically monitored in the current study. Indeed, post-TUGR the mares' uteri were deliberately not examined because the pressure exerted during examination might increase the risk of fluid leakage. Fluid aspiration should theoretically be more appropriate for bilateral twin pregnancies, where the operator has more flexibility with regard to how the conceptus is penetrated since the risk of directly damaging the other twin [1] is low, and removal of as much fluid as possible will remove the risk of leaked fluid bathing and potentially compromising the remaining pregnancy.

This study narrowly failed to confirm previous reports of an influence of the stage of gestation on the success of TUGR. While the likelihood of success was not statistically improved when TUGR was performed \leq day 35 of gestation, it appeared that day 45 is a more relevant cut-off point for performing TUGR if a reasonable chance of success is to be offered,

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since the success rates for TUGR performed at \leq day 35, day 36 to day 45 and $>$ day 45 were respectively, 21/53 (39.6%), 11/35 (31.4%) and 2/15 (13.3%). Therefore, even late in the season it would appear prudent to perform TUGR before day 45 of gestation. If this cannot be achieved, for example because the mare is not presented for twin reduction until 6-7 weeks, it may be preferable to wait until day 65-90 when, assuming owner compliance, the recently described technique of cranio-cervical dislocation of one fetus may offer a better chance of success; early results were promising with 5/8 (62.5%) resulting in a live foal [15].

Despite the fact that the difference in outcome between unilateral and bilateral twin pregnancies was not statistically significant in this study, it is interesting to note that the highest success (61%: 8/13) was achieved in early (\leq 35 days of gestation) bilateral twins. Of course, these are also the type of twins that are more likely to respond favourably to other methods of reduction such as crushing, or pinching or disruption of the membranes or the umbilical attachment [16].

In the current study, the success rate for TUGR performed before day 25 of gestation was not significantly better than later reduction (numbers were low because this is only attempted if the owner or referring veterinarian were insistent that they did not want to wait for possible spontaneous reduction). We therefore conclude that, in the case of unilateral twins, it is preferable to wait until approximately day 30 before attempting TUGR, to maximize the possibility of natural embryo reduction intervening [2] or for one pregnancy to show clear signs of compromise. In addition, because of the potential risk of delayed pregnancy loss following TUGR, we recommend avoiding TUGR early in the breeding season when it is generally more prudent to terminate the pregnancy with a PGF2 α analogue and re-breed the mare.

In conclusion, TUGR by embryo/fetal stabbing is a valid alternative to fluid aspiration in the case of unilaterally located equine twins. However, TUGR may be best reserved as an option

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for reducing unilateral twins to singleton pregnancies at around day 30, late in the breeding season or in other situations when re-breeding is not a viable option. Performing TUGR up to about day 45 appears to offer a reasonable chance of success, beyond that time other options should be considered.

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Table 1: Live singleton foals obtained after transvaginal ultrasound guided twin reduction (TUGR) in 103 mares treated between days 21 and 76 of gestation. To give a complete overview of the various potential influences, the results are subdivided on the basis of; (a) stage of gestation (\leq day 35, day 36 to day 45, $>$ day 45), (b) relative location of the two vesicles (i.e. unilateral versus bilateral) and (c) technique used to terminate one conceptus (aspiration versus stabbing). Results are presented as both numbers of procedures that were successful over number performed, and percentage resulting in a live singleton foal. None of the individual factors examined significantly affected the likelihood of success.

Stage of gestation	Conceptus location	Treatment	Live singleton foals born
\leq Day 35	Unilateral	Aspiration	9/30 (30%)
		Stab	4/10 (40%)
	Bilateral	Aspiration	5/10 (50%)
		Stab	3/3 (100%)
Day 36 - Day 45	Unilateral	Aspiration	3/11 (27.3%)
		Stab	2/4 (50%)
	Bilateral	Aspiration	4/14 (28.6%)
		Stab	2/6 (33.3%)
$>$ Day 45	Unilateral	Aspiration	0/6 (0%)
		Stab	1/4 (25%)
	Bilateral	Aspiration	1/4 (25%)
		Stab	0/1 (0%)