

# Effect of embryo transfer technique on the likelihood of pregnancy in the mare: a comparison of conventional and Wilsher's forceps-assisted transfer

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The aim of this study was to compare the success of embryo transfer (ET) performed by veterinarians with different degrees of experience using one of two ET techniques. Over three years, 179 embryos were transferred by three operators with moderate to high experience using a 'conventional' manual technique, and 170 embryos were transferred by four operators with little or no previous ET experience using a 'Wilsher' technique (Polansky speculum and Wilsher's cervical forceps). The pregnancy status of recipient mares at the first pregnancy diagnosis and the embryo loss by the last examination were compared between groups and within group among operators. More recipients became pregnant ( $P<0.005$ ) following ET with the Wilsher technique (157/170; 92.3 per cent) than with the conventional technique (127/179; 70.9 per cent), while the incidence of pregnancy loss did not differ between the groups (9.1 v 7.9 per cent, respectively). For the conventional technique, there was a significant operator effect ( $P<0.01$ ) on the percentage of pregnant recipients at the first examination (50.9–79.7 per cent); no operator effect was apparent for the Wilsher technique (90.9–93.4 per cent). In conclusion, the Wilsher technique yielded high pregnancy rates (>90 per cent).

## Introduction

The collection and transfer of horse embryos (ET) was first reported in 1972.<sup>1,2</sup> However, compared with bovine ET, uptake in equine clinical practice was relatively slow, until an upsurge in ET in Argentina during the 1990s, primarily as a result of increasing commercial demand for high-goal Polo Pony horses, combined with a shift to non-surgical instead of surgical transfer.<sup>3,4</sup> Uptake in other breeds was somewhat slower and, especially in the USA,<sup>5,6</sup> was delayed until the acceptance of ET offspring by most of the major horse registration authorities, with the continuing exception of the racing Thoroughbred. In 2015, the International Embryo Transfer Society reported a total of 20,924 horse ETs worldwide,<sup>7</sup> with over 87 per cent of recorded ETs occurring in Brazil,

followed numerically by Argentina (6.3 per cent), the USA (2.9 per cent) and France (2.4 per cent).

Historically, horse embryos were transferred using surgical techniques.<sup>8,9</sup> For obvious welfare and practical reasons, a switch to a transcervical non-surgical technique took place during the 1990s accompanying an increase in numbers of transfers performed. However, the success of early attempts to transfer embryos non-surgically was disappointing compared with surgical transfer.<sup>10,11</sup> Excessive manipulation of the cervix and subsequent contamination of the uterus and/or release of inflammatory products (ie, prostaglandins) were proposed as possible explanations for the poor success following transcervical transfer of horse embryos. For these reasons, many veterinarians continued to perform ET surgically during the 1990s. Nevertheless, it soon became clear that the transcervical route of transfer was not in itself responsible for the low pregnancy rates after ET; indeed, surveys began to report high pregnancy rates (81–83 per cent) when experienced practitioners performed non-surgical transfers.<sup>5,12</sup> These reports came from commercial ET centres in Argentina and the USA, where large numbers of transfers were performed every year in properly synchronised and selected recipients. From this progression, it can be inferred that the operator's skills

Veterinary Record (2018)

doi: 10.1136/vr.104808

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Provenance and peer review Not commissioned; externally peer reviewed.

Received December 5, 2017

Revised March 1, 2018

Accepted May 5, 2018

in performing the transcervical transfer significantly influence the likelihood of pregnancy after ET.

In an attempt to minimise the influence of the operator on the outcome of transcervical ET, Wilsher and Allen<sup>13</sup> developed a technique using vaginal speculum and cervical grasping forceps (Wilsher's forceps) to facilitate the passage of the transfer pipette through the cervix, minimising manipulation and reducing the risk of introducing contamination or misplacing the embryo (eg, in the cervix) when ET was performed by less experienced operators. Despite the ease of the technique and the promising results obtained in the preliminary<sup>13</sup> and follow-up<sup>14</sup> studies (over 80 per cent pregnancy), this technique has not been widely implemented in practice. This low spread is evidenced by the low percentage of veterinary surgeons reporting its use in a survey presented during a workshop on ET at the International Symposium of Equine Embryo Transfer held in Ghent in 2016 (PM McCue, F Riera and C Aurich, oral communication), and the lack of published reports on the use of the Wilsher's forceps since their first description in 2004. Perhaps the need for extra operators and specialised equipment to perform the transfer, and the common belief among experienced practitioners that the results would be similar whether the conventional technique or the Wilsher's forceps were used,<sup>15</sup> have slowed its uptake. In this respect, there are no published studies comparing the efficacy of the two techniques performed by operators of different degrees of experience and with recipients managed similarly.

The aim of this study was to compare the effect of transcervical ET technique (vaginal speculum and Wilsher's forceps v conventional) on the likelihood of pregnancy and of subsequent embryo loss in recipient mares after ET performed by operators with different levels of experience. The working hypothesis was that the percentage of pregnant recipients would be higher, and less variable between operators, when the speculum and Wilsher's forceps were used, whereas the incidence of early embryonic loss (EEL) would not differ.

## Materials and methods

### Mares

The data used in this study were collected retrospectively from the reproductive records of donor and recipient mares presented to or owned by Utrecht University's Department of Equine Sciences (The Netherlands) during three consecutive breeding seasons (2015–2017). More than 90 per cent of the recipient and donor mares were warmblood mares. Overall, data for 349 embryos recovered from 294 embryo flushes performed on 160 different donor mares and subsequently transferred to 263 different recipient mares were investigated. All recipients were maiden or barren at the time of transfer (no foaling mares were used as recipients). The ages of the recipient and donor mares

ranged from 3 to 17 (mean±standard error of the mean (SEM): 7.6±2.7) and from 3 to 23 (mean±SEM: 11.1±5.7) years old, respectively. Recipient mares were kept in small groups (n=5–20 mares) in grass paddocks (ad libitum feeding), or in individual boxes with daily exercise in a horse walker or sand paddock, and fed hay and concentrate with ad libitum access to freshwater.

During the first examination of the breeding season, the reproductive tract was palpated and scanned transrectally, and the cervix was palpated per vaginam to detect any cervical pathology and to aid in the determination of the stage of oestrous cycle. Mares found to be in dioestrus (presence of one Corpus Luteum (CL), absence of endometrial oedema and a tight cervix) were injected intramuscularly with 37.5 µg of d-cloprostenol (0.5-ml Genestranvet; Eurovet Animal Health BV, Bladel, The Netherlands) to induce luteolysis. Similarly, recipient mares that did not receive an embryo during a given dioestrus were either administered d-cloprostenol 8–10 days postovulation or allowed to return to oestrus spontaneously. Once in oestrus, recipient mares were scanned every 24 hours until ovulation was detected. Human chorionic gonadotrophin (Chorulon 1500 iu, Intervet Nederland BV, Boxmeer, The Netherlands) was administered to induce ovulation and ensure adequate synchronisation with donor mares in 51 per cent of cycles.

Donor mares were either inseminated at the university or at other locations by a referring veterinarian, using frozen-thawed or chilled-transported semen from various stallions selected by the mare's owner.

### Embryo handling and donor–recipient synchrony

The uterus of donor mares was flushed on days 8–9 after ovulation, depending on the time of insemination/ovulation within the day (AM v PM), using 3 litres of lactated Ringer's solution (3000-ml Ringer's lactate; Baxter Nederland BV, Utrecht, The Netherlands). Flushing was performed using a closed system, and recovered fluid was passed through an in-line embryo filter (Em Con Filter; Immuno Systems, Spring Valley, Wisconsin, USA). Embryos (324 day-8 and 25 day-9 embryos) were identified using a dissecting microscope (Olympus SZ60, Olympus Nederland BV, Leiderdorp, NL) and, after washing, were held in a holding medium (Syngro; Bioniche Animal Health, Athens, Georgia, USA) at room temperature for between 30 minutes and 4 hours before transfer. All recovered embryos were transferred regardless of the grade and size. The day-9 embryos ranged in diameter from 192 to 1800 µm (mean±SEM: 1048±42 µm; >85 per cent grade I), while the day-8 embryos ranged between 160 and 1120 µm (mean±SEM: 542±17 µm; >90 per cent grade I). All embryos were loaded into 0.5-ml straws containing holding medium interspersed with air bubbles as follows: column of medium-column of air-column of medium containing the embryo-column of air- and column of medium. In

each case, the straw containing the embryo was placed in a disposable transfer pipette with openings at both sides of the tip with a diameter of 3 mm, covered by a sterile plastic chemise (IMV Technologies Netherlands, Leeuwarden, NL). These sheaths were compatible with a stainless steel 'cassou'-type rigid transfer stylet for day-8 embryos (IMV Technologies Netherlands), which was introduced into the transfer pipette, pushing the straw to the tip.

Embryos were transferred into recipient mares that had ovulated 3–10 days previously, with donor–recipient asynchrony ranging from –1 to +5 days, that is, the recipient ovulated between one day before (–1) and five days after the donor (+5). Potential recipient mares were examined by transrectal ultrasonography to confirm the absence of endometrial oedema, free intrauterine fluid, and the presence of uterine tone, a CL and a tight cervix. If considered suitable for ET, recipient mares were restrained in stocks and sedated with 3–4 mg of intravenous detomidine hydrochloride (0.3–0.4-ml Domosedan, Vetoquinol BV, 's-Hertogenbosch, The Netherlands). Next, the perineum was thoroughly scrubbed with a povidone iodine solution and, after rinsing with clean water, the entrance into the vestibule was cleaned with chlorhexidine gluconate-alcohol ketonatus solution (Spervasept forte, Spervital, Toldijk, The Netherlands) to remove any contamination that may have passed the vulva lips. No further routine pre-ET or post-ET supportive treatment was administered to recipients.

## Operators

Embryos were transferred by seven different veterinary surgeons with different levels of previous experience in ET. The level of previous experience in ET and the estimated number of transfers previously performed by each operator, along with the years in which they performed transfers and the position held in the clinic (specialist, resident or intern), are shown in Table 1.

The previous ET experience of each operator was scored as (1) none: when the veterinary surgeon had no formal training other than observation of routine ET performed by both the conventional and Wilsher techniques (operators 6 and 7)—the number of embryos transferred before the commencement of the study

was 0; (2) low: the veterinary surgeon had observed numerous ETs using the conventional technique and had 'hands on' experience in handling the dioestrous cervix during embryo flushing; however, the number of ET performed before the commencement of the study was less than 10—previous training with the Wilsher technique consisted of performing two sham transfers after observation of a film of the technique (operators 4 and 5); (3) medium: this veterinary surgeon had previous experience in embryo flushing (approximately 100 ET flushes) and 50 ETs performed before the commencement of the study (operator 1); and (4) high: a veterinary surgeon with extensive experience of embryo flushing and ET for clinical and research purposes with the conventional technique, with over 100 (operator 2) and 500 ETs (operator 3), respectively, performed before the start of the study period.

## ET technique

Three operators (1–3) transferred embryos by the 'conventional technique' (group 1, n=179 ETs). For this technique, the transfer device was introduced into the vagina with the tip covered by a gloved hand until the cervix was located. The tip of the transfer gun, covered by the sterile chemise, was introduced into the external os of the cervix and advanced by approximately 1 cm; next the chemise was pulled backwards and torn to free the tip of the transfer pipette, which was then advanced towards the uterine lumen, where the embryo was deposited. On some occasions, the operator introduced a hand into the rectum to help guide the transfer device into the uterus. The frequency with which rectal guidance was required or used was not recorded. One operator (number 3) used a double-gloved technique, in which an extra sterile glove was used to cover the inner glove and transfer device up to the vestibular-vaginal ring, to further protect the outer chemise from contamination.

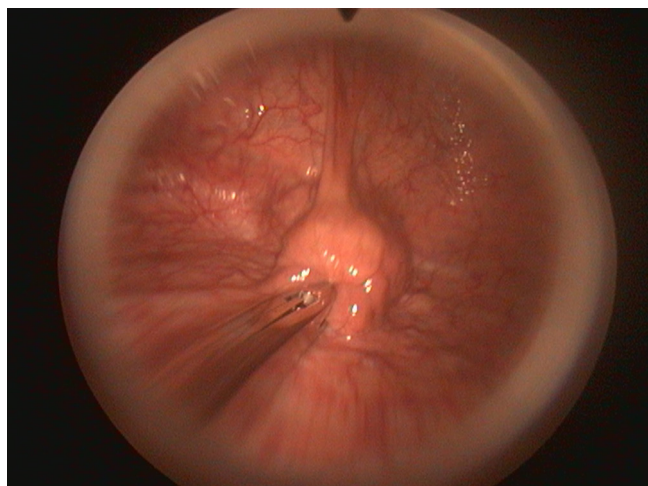
The remaining four operators (4–7) used the technique reported by Wilsher and Allen,<sup>13</sup> with the following modifications (Wilsher technique, group 2, n=170 ETs): only two people performed the transfer instead of three, and a transfer pipette with the embryo loaded in a 0.5-ml straw (as described above) was used instead of an AI pipette with the embryo loaded in 2.5 ml

**TABLE 1:** Characteristics and transfer outcome of the veterinarians performing the ET

Vet	Clinical position	Previous experience	Technique	Year(s) in the study	n	P (%)	EEL (%)
1	Resident	Medium	Conventional	2015	53	50.9	11.1
2	Specialist	High	Conventional	2015–2016–2017	52	78.8	4.9
3	Specialist	High	Conventional	2015–2016–2017	74	79.7	8.5
4	Specialist	Low	Wilsher	2016–2017	91	93.4	7.2
5	Specialist	Low	Wilsher	2016–2017	68	91.2	11.4
6–7	Intern	None	Wilsher	2016–2017	11	90.9	10.0

Vet: veterinary surgeon. Previous experience in ET: none=no transfers performed before the commencement of the study; low=less than 10 transfers; medium=50 transfers; high=more than 100 transfers performed before the commencement of the study. Year(s) in the study: temporal contribution of each veterinarian for the three years of the study (2015–2017). n: number of ETs. P (%): percentage of pregnant recipients at the first examination (four to six days after ET). EEL: early embryonic loss by the last examination (34–36 days after ET). ET, embryo transfer.



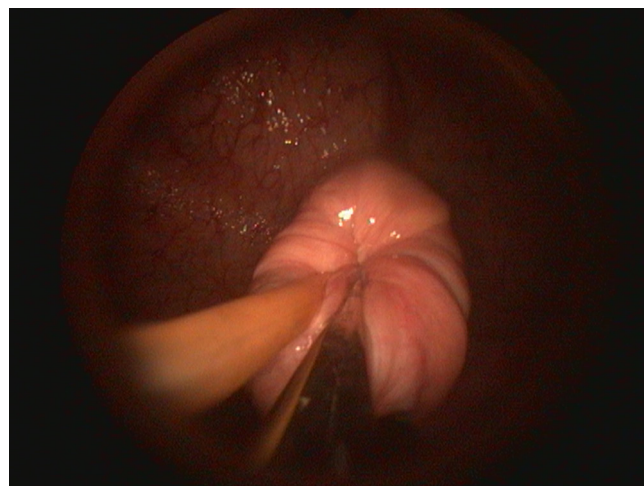


**FIG 1:** Endoscopic image of the Wilsher's cervical forceps grasping and pulling back slightly the cervix at 6 o'clock for preparation of embryo transfer.

of holding medium.<sup>14</sup> In brief, the operator performing the transfer introduced a 35-cm long duck-billed-type speculum (Polansky speculum: Surgical Holdings, Southend-on-Sea, Essex, UK) into the recipient's vagina and used a head torch to illuminate the cranial vagina and visualise the cervix. Once located, the assistant held the speculum in place while the operator used the cervical forceps as described<sup>13</sup> (Wilsher Equine Embryo Transfer Forceps; Surgical Holdings) to grab and straighten the cervix by exerting a slight pull (Fig 1). The forceps was left in place and handed to the assistant (Fig 2), while the operator removed the



**FIG 2:** Example of an embryo transfer performed by the Wilsher technique. The Polansky speculum and the Wilsher's cervical forceps are in place held by the assistant, while the operator wearing a head torch introduces the transfer pipette covered by the protective chemise.



**FIG 3:** Endoscopic image of the transfer pipette freed from protective chemise (left part of the image) passing the cervical canal while the cervix is grasped by the Wilsher's cervical forceps (right part of the image).

transfer pipette from its plastic cover and cut off the tip of the chemise using sterile scissors. The tip of the pipette was then advanced 8–15 cm to pass through the cervix and into the uterine lumen (Fig 3), where the embryo was deposited. Immediately after deposition of the embryo, the forceps was released, and the transfer pipette, forceps and speculum were all removed from the vagina. Finally, any air remaining in the vagina was removed by pushing it out transrectally. When more than one ET had to be performed on the same day, the Polansky speculum and cervical forceps were rinsed with tap water, dried with tissue paper, sprayed with a 90 per cent alcohol solution and rinsed again with sterile lactated Ringer's solution before the next use. At the end of the day, all equipment was sterilised by autoclaving.

### Pregnancy diagnosis

Recipient mares were examined four to six days after ET for the presence of an embryonic vesicle. If an embryonic vesicle was not found at the first examination, the recipient was rechecked two days later. In addition, the quality of the CL, the degree of endometrial oedema and the presence of free intrauterine fluid were recorded at the time of the first examination. Mares with an embryonic vesicle but signs of luteal regression (ie, a small or inactive CL with no colour Doppler signal), presence of endometrial oedema and reduced uterine tone were considered to have low progesterone concentrations and were treated once daily with 0.05-mg/kg altrenogest per os (Regumate Equine; Intervet Nederland BV), until the development of secondary CL or loss of the pregnancy. Premature luteal regression after ET was diagnosed if the recipient showed signs of luteal regression (absence of a CL, presence of endometrial oedema with or without free intrauterine fluid) at or before day 13 after ovulation. The number of mares with premature luteolysis was recorded for each ET technique. Maintenance of

**TABLE 2: Effect of embryo transfer technique on pregnancy and early embryonic losses**

Technique	n	P (%)	EEL (%)	Recipient's age (years)	Donor's age (years)	Asynchrony D-R (days)	Mean embryo size (µm)
Conventional	179	70.9	7.9	7.3±0.2	11.0±0.4	1.1±0.1	598±24
Wilsher	170	92.3	9.1	7.6±0.2	11.1±0.4	1.1±0.1	586±24
P values	—	<0.005	NS	NS	NS	NS	NS

n: number of embryo transfers. P: percentage of pregnant recipients at the first examination (four to six days after ET). EEL: early embryonic loss by the last examination (34–36 days after ET). Asynchrony D-R: mean difference in days between the day of ovulation of the donor and the recipient mare (–1=the recipient ovulated one day before the donor; +5=the recipient ovulated five days after the donor). ET, embryo transfer; NS, not significant.

pregnancy and presence of a heartbeat were confirmed 34–36 days after ET.

### Experimental design

The experimental unit was considered to be the oestrous cycle of any recipient receiving an embryo. For each experimental unit, the age of the donor mare producing the embryo, the size of the embryo, the age of the recipient mare receiving the embryo, the synchrony (–1 to +5) between embryo age (day 8 or 9) and recipient's day of ovulation (days 3–10), and the month and year on which the embryo was transferred were recorded.

Between February 2015 and June 15, 2016, embryos were transferred by conventional technique only (n=132 transfers), by whichever of the three veterinary surgeons was on duty on a given day (operators 1–3). Between June 16, 2016 and the end of the study (end of November 2017), embryos were transferred by either the conventional (n=47; operators 2 and 3) or Wilsher technique (n=170; operators 4–7), according to the preferred ET technique of the veterinarian on duty on that day.

The outcome of ET was classified as positive (1) or negative (0), presence or absence of an embryonic vesicle at the first pregnancy examination, respectively. EEL was calculated as the number of losses between the first and last pregnancy examination.

### Statistics

The difference in pregnancy status and EEL in recipient mares to which an embryo was transferred using either the Wilsher's forceps or the conventional technique was tested using a chi-squared test. For each ET technique group, the mean age of donor and recipient mare, the mean size of the embryo, and the mean degree of synchrony were compared using unpaired *t* tests. Anderson-Darling test was used to test data for normality. All sequential data were normally

distributed ( $P>0.1$ ). In addition, chi-squared tests were used to compare differences in ET outcome and EEL among operators for a given ET technique and the percentage of ETs performed in the first part of the season (February–June) between each technique. The degree of significance was set at  $P\leq 0.05$ . All means are reported as  $\pm$ SEM. All data were analysed in the statistical software Systat 13 (Systat Software, San Jose, California, USA).

### Results

The likelihood of pregnancy in recipient mares at the first examination was higher ( $P<0.005$ ) after transfer of an embryo using the Wilsher technique (92.3 per cent) than by the conventional technique (70.9 per cent; Table 2). EEL was not different between groups ( $P>0.05$ ). For all factors investigated (Table 2), only the percentage of transfers performed within the first part of the season differed significantly ( $P<0.001$ ) between the Wilsher and conventional groups.

More transfers (76.0 per cent) performed using the conventional technique occurred earlier in the season (February–June) than those transferred by the Wilsher technique (55.3 per cent). Overall, the likelihood of pregnancy in recipients following ET was lower in 2015 ( $P<0.01$ ) than in the subsequent years (Table 3). The percentage of recipients per operator that became pregnant differed ( $P<0.005$ ) within the conventional ET group, but not the Wilsher group ( $P>0.05$ ). In the conventional group, the operators with more previous experience (2 and 3) obtained a higher ( $P<0.005$ ) percentage of pregnant recipients (78.8 and 79.7 per cent, respectively) than the operator with moderate experience (50.9 per cent). When the operator with the lowest number of pregnant recipients (operator 1) is removed from the analysis, the pregnancy rate in recipients transferred by the Wilsher technique (157/170; 92.3 per cent) is still significantly higher

**TABLE 3: Effect of year on pregnancy and early embryonic losses**

Technique	2015			2016			2017		
	n	P (%)	EEL (%)	n	P (%)	EEL (%)	n	P (%)	EEL (%)
Wilsher	—	—	—	56	92.8	7.8	114	92.1	9.8
Conventional	98	63.3	8.1	54	83.3	6.8	27	74.1	10.0
Overall	98	63.3 <sup>a</sup>	8.1	110	88.2 <sup>b</sup>	7.4	141	88.6 <sup>b</sup>	9.8

Within row, different superscripts (a,b) indicate a significant difference ( $P<0.01$ ) in the percentage of pregnant recipients (P) at the first examination (four to six days after ET). n: number of embryo transfers. EEL: early embryonic loss by the last examination (34–36 days after ET). ET, embryo transfer.



( $P<0.005$ ) than in the conventional group (100/126; 79.3 per cent). Moreover, during the second half of the study (June 2016–November 2017) when the two ET techniques were performed contemporaneously, the percentage of recipients confirmed to be pregnant was also higher ( $P<0.01$ ) when using the Wilsher technique (92.3 per cent; 157/170) than the conventional technique by the two experienced operators (78.7 per cent; 37/47).

Of all transfers attempted using the Wilsher's forceps, two could not be completed (2/172; 1.2 per cent); following an unsuccessful ET attempt, the embryos were transferred into other suitable mares. The reasons for cancelling the transfer were either that the external os of the cervix could not be visualised and grasped with the forceps, or the transfer pipette got stuck in the cervical canal and could not be passed through, despite several attempts involving pulling slightly on the cervical forceps and changing the angle and position of the pipette. In addition, on four occasions (4/170; 2.3 per cent) per rectum manipulation of the transfer pipette was performed to release it from where it was lodged in the cervix to enable passage into the uterus; after two of these difficult transfers, the recipients did not become pregnant (2/4), and in another one, EEL occurred (1/2). Unfortunately, data on the percentage of cancelled or difficult transfers using the conventional technique were not accurately recorded to allow meaningful comparison between the ET techniques.

From all recipients diagnosed pregnant ( $n=284$ ), two mares (0.7 per cent) showed signs of luteal regression at the first pregnancy examination (one for each technique). Despite treatment with altrenogest, both recipients suffered EEL. These recipients had, respectively, received a day-8 and a day-9 embryos on days 9 and 10 after ovulation. For the recipients diagnosed as non-pregnant ( $n=65$ ), six mares showed premature luteolysis (9.2 per cent), four from the conventional and two from the Wilsher's forceps group.

## Discussion

The principal hypothesis that the likelihood of pregnancy would be higher after transferring horse embryos using the vaginal speculum and Wilsher's cervical forceps was substantiated by the results of this study. However, it is difficult to know how much of this effect could be attributed to the technique itself. In this respect, there was a marked difference in the pregnancy outcome among operators performing ET using the conventional technique (50.9–79.7 per cent), which clearly shows a strong influence of the skills and/or experience of the operator on the likelihood of success using this ET technique, as was previously reported.<sup>16</sup> On the other hand, the 79.7 per cent of recipients that were pregnant after transfer by the operator with the most experience was very close to the figures reported previously (81–83 per cent) for skilled practitioners

using adequately synchronised recipients from a large pool of preselected mares.<sup>5,12</sup> It is therefore possible that the Wilsher technique reported here yields an 8–12 per cent increase in the likelihood of pregnancy in recipient mares compared with the conventional technique, at least in this clinical setting.

The number of transfers performed using the two ET techniques was, however, not temporally evenly distributed, since most transfers performed by the conventional technique took place in the first half of the study (February 2015–June 2016). In contrast, more of the transfers performed using the Wilsher's forceps occurred late in the season (July–November), a period which is often reported to yield poorer results due to a shrinking pool of high-quality recipients available,<sup>17</sup> and yet this technique showed the highest results. This is an interesting observation for commercial ET programmes with a high turnover of staff, where relatively inexperienced operators could take on the responsibility of transferring embryos using the Wilsher technique with little previous training, with no reduction in the success of the programme. On the other hand, it appears that some small maiden mares with a small vaginal vault may not be ideal candidates for the Wilsher ET technique, because they strain hard and collapse the vaginal wall as the speculum is inserted. In these mares, it is recommended to sedate the mare more profoundly and be as fast as possible, only opening the Polansky speculum as much as needed to visualise and grasp the cervix. Some recipients ( $n=2$ ) could not be transferred even with the help of the Wilsher's forceps owing to the impossibility to grasp or pass the cervix. The exact number of cancelled transfers with the conventional technique was unknown, and therefore no comparisons between techniques could be made.

Bearing in mind these preliminary data, in which only one ongoing pregnancy resulted from the four difficult transfers using the Wilsher's forceps, it is logical to conclude that it is better to cancel a difficult transfer (regardless of the ET technique used) and move to another recipient (if available) rather than continuing to manipulate the cervix and transfer pipette (with per rectum assistance) until the uterine lumen is reached by 'any means'. In this regard, a recent study<sup>18</sup> in women receiving in vitro-produced embryos showed that the likelihood of pregnancy was negatively influenced by a difficult transfer, with 48 per cent ( $n=838$ ) and 35 per cent ( $n=84$ ) of transfers considered 'easy' or 'difficult' resulting in pregnancy, respectively. Difficult transfers may induce an inflammatory reaction which may be detrimental to embryo survival.<sup>19,20</sup>

It is more difficult to explain the apparent difference in pregnancy rate between the two ET techniques. One possibility is a higher risk of introducing contaminants into the uterus with the transfer pipette or the hand of the operator with the conventional technique. However, the cleaning of the recipient's perineum and vulva

was similar for both groups and, while the tip of the transfer gun does not touch the vestibular or caudal vaginal wall when using the Polansky speculum and Wilsher's forceps, a double sterile glove technique used by one of the two experienced operators performing the conventional technique did not improve the likelihood of pregnancy. Furthermore, the number of recipient mares with signs of premature luteolysis at the first pregnancy examination was low and did not differ between methods. Alternatives are that the conventional technique more often results in failure to enter the uterine lumen and/or more irritation and consequent release of inflammatory products during the transfer procedure that compromise subsequent embryo survival, especially in inexperienced operators.

The main limitation of this study was that data were collected retrospectively and the different ET techniques were not performed in a randomised fashion.

In conclusion, these results demonstrate that high pregnancy rates (>90 per cent) can be achieved when this modified Wilsher technique is used to transfer horse embryos to preselected, synchronised mares from a large pool of available recipients, without use of any routine supportive treatments such as altrenogest or NSAIDs at or around the time of transfer. In addition, the influence of the experience of the operator performing the ET on pregnancy rates appears to be greatly reduced with the Wilsher's forceps compared with the conventional technique.

**Competing interests** None declared.

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