Factors affecting recipients' pregnancy, pregnancy loss and foaling rates in a commercial equine embryo transfer program

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Abstract

During 11 breeding seasons, 351 seven to ten days old horse embryos were non-surgically transferred into recipients that ovulated between 3 and 10 days earlier. Pregnancy rates at 14 and 40 days and foaling rates were 77.8% (273/351), 69.2% (243/351) and 64.4% (226/351), respectively. Pregnancy loss between 14 and 40 days was 11% and between 40 days and delivery was 7%. The transfer of quality grade 3-4 embryos resulted in a significantly lower pregnancy rate at 14 days compared to the transfer of grade 1-2 embryos (46.2% vs 79%; P<0.05). Eight days old embryos resulted in significantly lower pregnancy losses than day 9 or 10 embryos, as occurred for embryos between 400 and 1200µm compared to embryos smaller than 400µm. Embryos recovered from mares older than 20 years resulted in a significantly higher pregnancy loss rate than those recovered from younger mares. The same happened for embryos coming from mares affected by reproductive pathologies compared to healthy mares performing sport activity. None of the evaluated parameters influenced significantly recipients’ foaling rate.

1. Introduction

Since the first successful attempt in 1974 [1], the equine embryo transfer (ET) technology has been studied and developed and today recipients' pregnancy rates at 14 days range 65-89% [2-6]. Several donor’s, embryo’s, recipient’s and technical factors have been analyzed to assess their effect on recipients pregnancy. Evaluated donors’ factors have been age, intrinsic fertility and sport activity [7-14], while embryo factors were age, quality and developmental stage [2-4,15-18]. Investigated recipient factors have been age, parity, day after ovulation, synchronization with the donor, treatments [2,4,6,8,16-23]. Technical factors studied, finally, were surgical or non-surgical ET procedures, month in which ET was performed and embryo flushing and holding media employed [4,15,17,24,25].

Foaling rate is one of the most common factors analyzed when evaluating Thoroughbred reproductive efficiency, and this parameter is mainly affected by mares fertility [26-32]. Surprisingly, foaling rate has been reported in only one study on equine ET [33].

The aim of this study was to retrospectively analyze donors’, embryos’, recipients’, technical and environmental factors that affected recipients’ pregnancy rates, pregnancy losses and foaling rates in a commercial equine ET program.

2. Materials and Methods

Data on the outcome of transfer of equine embryos performed in winter, spring or summer (winter,
from the 15th of February to the 21st of March; spring, from the 22nd of March to the 21st of June; summer, from the 22nd of June to the 15th of August) of 11 breeding seasons (2002-2012) at the former Dipartimento di Clinica Veterinaria of the Pisa University (Department) were retrospectively analysed.

2.1 Donors

The donors' were of different breed (Show Jumping Mares, Standardbred, Quarter Horses, Haflinger, Arab), age (2-10, 11-15, 16-20, 21-24 years old) and reproductive category (healthy donors performing sport activity, SHD; healthy donors not performing sport activity, NSHD; donors affected by reproductive pathologies, RPD; donors affected by non reproductive pathologies, NRPD) [34]. Sport activity was intended as: show jumping, reining or harness racing.

Embryo donors’ housing, estrus cycles monitoring, AI and post AI treatment were described in Panzani et al. 2014 [34].

2.2 Embryos

Three-hundred-fifty-one 7-10 days old equine embryos were recovered 7-10 days following ovulation using two different protocols described previously [34]; briefly, uteri were flushed either by DPBS added of 0.4% BSA (ZE067, IMV Technologies, Bicef, Piacenza, Italy) (PBS) or by ringer lactate (Galenica Senese, Siena, Italy) (RL).

PBS and ringer lactate recovered embryos were washed 10 times in DPBS added of 0.4% of BSA (PBS/PBS) or EmCare Holding Solution (ICPbio, Ltd., Auckland, New Zealand) (RL/EHS) respectively, evaluated for quality [16] by a 40x magnification stereo-microscope before being prepared for transfer. Embryo recovery, manipulation and transfers have been done in controlled temperature rooms (25 ± 2°C), with media at 37°C, embryo search and washing were done under a laminar flow hood.

Two-hundred-and-fifteen/351 recovered embryos were measured using the ocular microscope scale.

2.3 Recipients

One-hundred-and-fifty-one Standardbred mares between 2 and 12 years old, multiparous or nulliparous, considered generally and reproductively healthy after clinical examination, were included as embryo recipients in the program. Pregnant mares were leased to the embryo owner from day 40 of pregnancy until the weaning of the foal and then came back to the Department to be re-included in the program; for this reason most of mares were employed as recipients for more
than one year. Thirteen Haflinger mares of the same age and sanitary status were also employed as
recipients, for Haflinger embryos only. Mares, maintained in dry lots, fed with hay ad libitum and
2-3 kg of mixed grain per day, were checked by ultrasound for ovarian activity throughout all the
year: weekly during anestrus, bi-weekly during transition and diestrus and daily during estrus and
until ovulation. When needed, recipients ovulations were synchronized with the donors’ ones using
PGF2α analogue alfaprostol (3mg, IM, in a single injection; Gabbrostim, Vetem, Spa, Monza-Brianza, Italy) and hCG (2000 UI, IV, in a single injection; Vetecor 2000, Bio98, Bologna, Italy).
Immediately before the transfer, recipients were submitted to three different regimes:
- Treated with 30000 IU IM of penicillin procaine (Proacillin®, Merial Italia, Milano, Italy) and 0.5 mg, EV of flunixin niglumine (Niglumine®, Bio98, Milano, Italy) once a day for 3 days, plus 0.044 mg/kg, OS of altrenogest (Regumate, Hoechst, Milan, Italy) once a day until pregnancy diagnosis and, in case of positivity, until the 100th day of pregnancy (blind treated recipients);
- Submitted to trans-rectal palpation and ultrasound examination and, if graded as acceptable [4], employed as embryo recipient without any treatment (selected untreated recipients)
- Submitted to trans-rectal palpation and ultrasound examination and, if graded as marginally acceptable [4], employed as embryo recipient and treated with altrenogest as described above (selected treated recipients)
In 11 cases embryos were transferred into acyclic recipients in spring transition showing, at ultrasound, an uterine edema of grade 2-3 [35] treated twice a day with altrenogest (0.044 mg/kg, OS, BID) from the third day after ovulation of the respective donor, until pregnancy diagnosis, and in case of positivity until day 100 of pregnancy.
Mares were removed from the recipients’ herd after 12 years of age, or after two consecutive negative pregnancy diagnoses, or after abortion.

2.4 Embryo Transfer
Embryos were gently aspirated into a French straw preceded and followed by an air bubble and a small amount of holding solution. Embryos <1 mm were transferred by a 0.25 ml straw, while embryos >1 mm were transferred by a 0.5 ml straw using a French Gun designed for equine ET (IMV Technologies, Bicef, Piacenza, Italy). Recipients were treated with acepromazine (4 mg, IV, in a single injection; Prequillan, Fatro, Bologna, Italy) 10 minutes before entering into a stock, than the rectum was evacuated from manure, the tail wrapped, perineum washed with povidone iodine soap and rinsed 3 times and, finally, dried with clean paper towels. The operator inserted the
guarded gun protected by a sanitary sheath through the vagina. The vaginal part of the cervix was
grabbed with three fingers and pulled backwards, the tip of the gun was blindly inserted in the
cervical os, the sanitary sheath was then broken, and the cervix was manipulated to aid the gun
insertion and progression. The embryos were released in the body of the uterus, without any trans-
rectal manipulation [11]

2.5 Pregnancy diagnoses
Pregnancy diagnosis was performed by ultrasound 14 days after donors’ ovulations and checked on
days 25 and 40. Thereafter, pregnant recipients were transported to the donors’ owner stud, where
private practitioners managed pregnancy and parturition. Data on pregnancy and foaling outcomes
were collected directly from the Veterinarians or the owners.

2.6 Statistical Analysis
Data were analyzed using the software IBM SPSS Statistics (version 22), and differences were
considered statistically significant when P values were lower than 0.05. The Fisher’s exact test was
employed to evaluate differences in between groups in pregnancy rates at 14 and at 40 days, foaling
rates, and pregnancy losses between 14 and 40 days of pregnancy and between 40 days and
parturition.
The embryo’s factors studied were: age, developmental stage, quality and diameter.
The donors’ factors compared were: breed, age, reproductive category and sport activity in mares
under 16 years old (SHD vs NSHD) [34,36]. The environmental and technical factors studied were:
breeding season, season of the year and flushing protocol/media (PBS/PBS vs. RL/EHS)
respectively [34,37].
The recipient’s factors studied were: parity, treatment/regime and day after ovulation.

3. Results:
Out of 351 embryos transferred, 273 (77.8%) and 243 (69.2%) resulted in a pregnancy at 14 and 40
days, respectively, while 226 (64.4%) gave birth to a healthy foal. Pregnancy losses were 30/273
(11.0%) and 17/243 (7.0%) between 14 and 40 days and between 40 days and parturition,
respectively. These results were similar between different breeding seasons (P>0.05).
The mean diameter (±SD) of 7, 8, 9 and 10 days old embryos was 404.9±306.5µm (n=12),
660.3±326.8 µm (n=191), 912.4±753.6 µm (n=8), and 1224.5±821.0 µm (n=4), respectively.
Although embryo quality affected significantly 14 days pregnancy rates, it had no effects on
pregnancy loss, which was influenced by embryo age and diameter instead (Table 1).

Donors’ age class and reproductive category also significantly affected 14-40 days and overall pregnancy loss rates. Pregnancy loss after 40 days, and overall pregnancy loss, were significantly lower in SHD mares under 16 years old compared to NSHD of the same age (Table 2).

Neither the analyzed recipients’ factors nor the employed media for embryo flushing and holding or season of the year (winter, spring or summer) had a significant effect on the outcome of embryo transfer (Tables 3, 4, 5).

Recipient’s 40 days pregnancy rate and foaling rate were not significantly influenced by the evaluated factors.

Table 1: Recipient’s pregnancy, foaling and pregnancy loss rates according to embryo’s factors

<table>
<thead>
<tr>
<th>Day of embryo recovery</th>
<th>Preg. ET at 14 days (%)</th>
<th>Preg. ET at 40 days (%)</th>
<th>Foals born/ET (%)</th>
<th>Pregnancy loss 14 - 40 days (%)</th>
<th>Pregnancy loss after 40 days (%)</th>
<th>Overall pregnancy loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>12/18 (66.7%)</td>
<td>10/18 (55.6%)</td>
<td>9/18 (50.0%)</td>
<td>2/12 (16.7%)</td>
<td>1/10 (10%)</td>
<td>3/12 (25%)</td>
</tr>
<tr>
<td>8</td>
<td>234/299 (78.3%)</td>
<td>211/299 (70.6%)</td>
<td>200/299 (66.9%)</td>
<td>23/234 (9.8%)</td>
<td>11/211 (5.2%)</td>
<td>4/234 (14.5%)</td>
</tr>
<tr>
<td>9</td>
<td>22/26 (84.6%)</td>
<td>20/26 (76.9%)</td>
<td>15/26 (57.7%)</td>
<td>2/22 (9.1%)</td>
<td>2/20 (10%)</td>
<td>3/22 (13.8%)</td>
</tr>
<tr>
<td>10</td>
<td>5/8 (62.5%)</td>
<td>2/8 (25.0%)</td>
<td>3/5 (60%)</td>
<td>0/3 (0%)</td>
<td>3/5 (60%)</td>
<td></td>
</tr>
</tbody>
</table>

Embryo stage

- Blastocyst: 263/339 (77.6%), 236/339 (69.6%), 219/339 (64.6%), 27/263 (10.3%), 17/236 (7.2%), 44/263 (17.5%)
- Early Blastocyst: 9/11 (81.8%), 7/11 (63.6%), 7/11 (63.6%), 2/9 (22.2%), 0/9 (0%), 2/9 (22.2%)
- Morula: 1/1 (100%), 0/1 (0%), 0/1 (0%), 1/1 (100%), 0/0 (0%), 1/1 (100%)

Embryo quality

- 1-2: 267/338 (79.0%), 239/338 (70.7%), 222/338 (65.7%), 28/267 (10.7%), 17/222 (7.1%), 45/267 (16.9%)
- 3-4: 6/13 (46.2%), 4/13 (30.8%), 4/13 (30.8%), 2/6 (33.3%), 0/4 (0%), 2/6 (33.3%)

Total: 273/351 (77.8%), 243/351 (69.2%), 226/351 (64.4%), 30/273 (11.0%), 17/243 (7.0%), 47/273 (17.2%)

Embryo diameter range (n=215 embryos)

<table>
<thead>
<tr>
<th>Diameter range</th>
<th>Preg. ET at 14 days (%)</th>
<th>Preg. ET at 40 days (%)</th>
<th>Foals born/ET (%)</th>
<th>Pregnancy loss 14 - 40 days (%)</th>
<th>Pregnancy loss after 40 days (%)</th>
<th>Overall pregnancy loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>150-399 µm</td>
<td>46/57 (80.7%)</td>
<td>30/57 (52.6%)</td>
<td>30/57 (52.6%)</td>
<td>13/46 (28.3%)</td>
<td>3/30 (10%)</td>
<td>16/46 (34.8%)</td>
</tr>
<tr>
<td>400-699 µm</td>
<td>57/75 (76.0%)</td>
<td>55/75 (73.3%)</td>
<td>53/75 (70.7%)</td>
<td>2/57 (3.5%)</td>
<td>2/55 (3.6%)</td>
<td>4/75 (7%)</td>
</tr>
<tr>
<td>700-1199 µm</td>
<td>51/67 (76.1%)</td>
<td>48/67 (71.6%)</td>
<td>45/67 (67.2%)</td>
<td>3/51 (5.9%)</td>
<td>3/48 (6.2%)</td>
<td>6/51 (11.8%)</td>
</tr>
<tr>
<td>1200-3000 µm</td>
<td>12/16 (75.0%)</td>
<td>10/16 (62.5%)</td>
<td>9/16 (56.3%)</td>
<td>2/12 (16.7%)</td>
<td>1/10 (10%)</td>
<td>3/12 (25%)</td>
</tr>
</tbody>
</table>

Total: 166/215 (77.2%), 143/215 (66.5%), 137/215 (63.7%), 20/166 (12%), 9/146 (6.2%), 29/166 (17.5%)

*a,b*: Data designated by different superscripts differ significantly (P<0.05). Fisher’s exact test.
Table 2: Recipient’s pregnancy, foaling and pregnancy loss rates according to donors’ factors

<table>
<thead>
<tr>
<th>Donors' breed</th>
<th>Pregnancies/ET at 14 days (%)</th>
<th>Pregnancies/ET at 40 days (%)</th>
<th>Foals born/ET (%)</th>
<th>Pregnancy loss 14-40 days (%)</th>
<th>Pregnancy loss after 40 days (%)</th>
<th>Overall pregnancy loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arab</td>
<td>2/4 (50.0%)</td>
<td>2/4 (50.0%)</td>
<td>1/4 (25.0%)</td>
<td>0/2 (0%)</td>
<td>1/2 (50%)</td>
<td>1/4 (25%)</td>
</tr>
<tr>
<td>Haflinger</td>
<td>10/13 (76.9%)</td>
<td>10/13 (76.9%)</td>
<td>10/13 (76.9%)</td>
<td>0/10 (0%)</td>
<td>0/10 (0%)</td>
<td>0/13 (0%)</td>
</tr>
<tr>
<td>Jumping mares</td>
<td>175/226 (77.4%)</td>
<td>156/226 (69.0%)</td>
<td>141/226 (62.4%)</td>
<td>19/175 (10.9%)</td>
<td>15/156 (9.6%)</td>
<td>34/175 (19.4%)</td>
</tr>
<tr>
<td>Quarter Horses</td>
<td>47/54 (87.0%)</td>
<td>43/54 (79.6%)</td>
<td>42/54 (77.8%)</td>
<td>4/47 (8.5%)</td>
<td>1/43 (2.3%)</td>
<td>5/47 (10.6%)</td>
</tr>
<tr>
<td>Standardbred</td>
<td>39/54 (72.2%)</td>
<td>32/54 (59.3%)</td>
<td>32/54 (59.3%)</td>
<td>7/39 (17.9%)</td>
<td>0/32 (0%)</td>
<td>7/39 (17.9%)</td>
</tr>
<tr>
<td>Donors' age class</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-10</td>
<td>59/76 (77.6%)</td>
<td>56/76 (73.7%)</td>
<td>51/76 (67.1%)</td>
<td>3/59 (5.1%)(^a)</td>
<td>5/56 (8.9%)</td>
<td>8/59 (13.6%)</td>
</tr>
<tr>
<td>11-15</td>
<td>79/101 (78.2%)</td>
<td>72/101 (71.3%)</td>
<td>65/101 (64.4%)</td>
<td>7/79 (8.9%)(^a)</td>
<td>7/72 (9.7%)</td>
<td>14/79 (17.7%)</td>
</tr>
<tr>
<td>16-20</td>
<td>78/105 (74.3%)</td>
<td>71/105 (67.6%)</td>
<td>69/105 (65.7%)</td>
<td>7/78 (9.0%)(^a)</td>
<td>2/71 (2.8%)</td>
<td>9/78 (11.5%)</td>
</tr>
<tr>
<td>21-24</td>
<td>57/69 (82.6%)</td>
<td>44/69 (63.8%)</td>
<td>41/69 (59.4%)</td>
<td>13/57 (22.8%)(^b)</td>
<td>3/45 (6.7%)</td>
<td>16/57 (28.1%)</td>
</tr>
<tr>
<td>Donors' reproductive category</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSHD</td>
<td>173/218 (79.4%)</td>
<td>154/218 (70.6%)</td>
<td>140/218 (64.2%)</td>
<td>19/173 (11.0%)</td>
<td>14/154 (9.1%)</td>
<td>33/173 (19.1%)</td>
</tr>
<tr>
<td>SHD</td>
<td>35/42 (83.3%)</td>
<td>34/42 (81.0%)</td>
<td>34/42 (81.0%)</td>
<td>1/35 (2.9%)(^a)</td>
<td>0/35 (0%)</td>
<td>1/35 (2.9%)</td>
</tr>
<tr>
<td>NRPD</td>
<td>16/20 (80%)</td>
<td>16/20 (80%)</td>
<td>15/20 (75.0%)</td>
<td>0/16 (0%)</td>
<td>1/16 (6.3%)</td>
<td>1/16 (6.3%)</td>
</tr>
<tr>
<td>RPD</td>
<td>49/71 (69.0%)</td>
<td>39/71 (54.9%)</td>
<td>37/71 (52.1%)</td>
<td>10/49 (20.4%)(^b)</td>
<td>2/39 (5.1%)</td>
<td>12/49 (24.5%)</td>
</tr>
<tr>
<td>Healthy donors under 16 years old performing or not sport activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSHD</td>
<td>95/126 (75.4%)</td>
<td>89/126 (70.6%)</td>
<td>77/126 (61.1%)</td>
<td>6/95 (6.3%)</td>
<td>12/89 (13.5%)(^a)</td>
<td>18/95 (18.9%)</td>
</tr>
<tr>
<td>SHD</td>
<td>34/40 (85.0%)</td>
<td>33/40 (82.5%)</td>
<td>33/40 (82.5%)</td>
<td>1/34 (2.9%)</td>
<td>0/33 (0%)(^b)</td>
<td>1/34 (2.9%)</td>
</tr>
</tbody>
</table>

\(^{a,b}\): Data designated by different superscripts differ significantly (P < 0.05). Fisher’s exact test
Table 3: Recipient's pregnancy, foaling and pregnancy loss rates according to recipient's factors

<table>
<thead>
<tr>
<th>Reproductive career</th>
<th>Pregnancies/ET at 14 days (%)</th>
<th>Pregnancies/ET at 40 days (%)</th>
<th>Foals born/ET (%)</th>
<th>Pregnancy loss 14 - 40 days (%)</th>
<th>Pregnancy loss after 40 days (%)</th>
<th>Overall pregnancy loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulliparous</td>
<td>136/175 (77.7%)</td>
<td>122/175 (69.7%)</td>
<td>114/175 (65.1%)</td>
<td>14/136 (10.3%)</td>
<td>8/122 (6.6%)</td>
<td>22/136 (16.2%)</td>
</tr>
<tr>
<td>Pluriparous</td>
<td>137/176 (77.8%)</td>
<td>121/176 (68.8%)</td>
<td>112/176 (63.6%)</td>
<td>16/137 (11.7%)</td>
<td>9/121 (7.4%)</td>
<td>25/137 (18.2%)</td>
</tr>
</tbody>
</table>

Recipient day post ovulation

<table>
<thead>
<tr>
<th>Anovulatory</th>
<th>Pregnancies/ET at 14 days (%)</th>
<th>Pregnancies/ET at 40 days (%)</th>
<th>Foals born/ET (%)</th>
<th>Pregnancy loss 14 - 40 days (%)</th>
<th>Pregnancy loss after 40 days (%)</th>
<th>Overall pregnancy loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10/11 (90.9%)</td>
<td>10/11 (90.9%)</td>
<td>9/11 (81.8%)</td>
<td>0/10 (0%)</td>
<td>1/10 (10%)</td>
<td>1/10 (10%)</td>
<td></td>
</tr>
<tr>
<td>2/2 (100.0%)</td>
<td>1/2 (50.0%)</td>
<td>1/2 (50.0%)</td>
<td>0/1 (0%)</td>
<td>1/2 (50%)</td>
<td>1/2 (50%)</td>
<td></td>
</tr>
<tr>
<td>4/12 (75.0%)</td>
<td>7/12 (58.3%)</td>
<td>7/12 (58.3%)</td>
<td>2/9 (22.2%)</td>
<td>0/7 (0%)</td>
<td>2/9 (22.2%)</td>
<td></td>
</tr>
<tr>
<td>8/211 (37.2%)</td>
<td>75/113 (66.4%)</td>
<td>69/113 (61.1%)</td>
<td>7/82 (8.5%)</td>
<td>6/75 (8.0%)</td>
<td>13/82 (15.9%)</td>
<td></td>
</tr>
<tr>
<td>70/87 (80.5%)</td>
<td>64/87 (73.6%)</td>
<td>59/87 (67.8%)</td>
<td>6/70 (8.6%)</td>
<td>5/64 (7.8%)</td>
<td>11/70 (15.7%)</td>
<td></td>
</tr>
<tr>
<td>70/86 (81.4%)</td>
<td>61/86 (70.9%)</td>
<td>56/86 (65.1%)</td>
<td>9/70 (12.9%)</td>
<td>5/61 (8.2%)</td>
<td>14/70 (20.0%)</td>
<td></td>
</tr>
<tr>
<td>8/283 (75.7%)</td>
<td>23/37 (62.2%)</td>
<td>23/37 (62.2%)</td>
<td>5/28 (17.9%)</td>
<td>0/23 (0%)</td>
<td>5/28 (17.9%)</td>
<td></td>
</tr>
<tr>
<td>2/2 (100.0%)</td>
<td>2/2 (100.0%)</td>
<td>2/2 (100.0%)</td>
<td>0/2 (0%)</td>
<td>0/2 (0%)</td>
<td>0/2 (0%)</td>
<td></td>
</tr>
<tr>
<td>10/1 (0.0%)</td>
<td>-</td>
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<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Recipient selection and treatment

<table>
<thead>
<tr>
<th>Acyclic treated</th>
<th>Pregnancies/ET at 14 days (%)</th>
<th>Pregnancies/ET at 40 days (%)</th>
<th>Foals born/ET (%)</th>
<th>Pregnancy loss 14 - 40 days (%)</th>
<th>Pregnancy loss after 40 days (%)</th>
<th>Overall pregnancy loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10/11 (90.9%)</td>
<td>10/11 (90.9%)</td>
<td>9/11 (81.8%)</td>
<td>0/10 (0%)</td>
<td>1/10 (10%)</td>
<td>1/10 (10%)</td>
<td></td>
</tr>
<tr>
<td>2/2 (100.0%)</td>
<td>1/2 (50.0%)</td>
<td>1/2 (50.0%)</td>
<td>0/1 (0%)</td>
<td>1/2 (50%)</td>
<td>1/2 (50%)</td>
<td></td>
</tr>
<tr>
<td>4/12 (75.0%)</td>
<td>7/12 (58.3%)</td>
<td>7/12 (58.3%)</td>
<td>2/9 (22.2%)</td>
<td>0/7 (0%)</td>
<td>2/9 (22.2%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Recipient's pregnancy, foaling and pregnancy loss rates according to flushing/holding media employed

<table>
<thead>
<tr>
<th>Flushing media</th>
<th>Pregnancies/ET at 14 days (%)</th>
<th>Pregnancies/ET at 40 days (%)</th>
<th>Foals born/ET (%)</th>
<th>Pregnancy loss 14 - 40 days (%)</th>
<th>Pregnancy loss after 40 days (%)</th>
<th>Overall pregnancy loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS/PBS</td>
<td>31/43 (72.1%)</td>
<td>25/43 (58.1%)</td>
<td>25/43 (58.1%)</td>
<td>6/31 (19.4%)</td>
<td>0/25 (0%)</td>
<td>6/31 (19.3%)</td>
</tr>
<tr>
<td>RL/EHS</td>
<td>242/308 (78.6%)</td>
<td>218/308 (70.8%)</td>
<td>201/308 (65.3%)</td>
<td>24/242 (9.9%)</td>
<td>17/218 (7.8%)</td>
<td>39/242 (16.1%)</td>
</tr>
</tbody>
</table>

Table 5: Recipient's pregnancy, foaling and pregnancy loss rates according to ET season

<table>
<thead>
<tr>
<th>Season</th>
<th>Pregnancies/ET at 14 days (%)</th>
<th>Pregnancies/ET at 40 days (%)</th>
<th>Foals born/ET (%)</th>
<th>Pregnancy loss 14 - 40 days (%)</th>
<th>Pregnancy loss after 40 days (%)</th>
<th>Overall pregnancy loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>50/62 (80.6%)</td>
<td>45/62 (72.6%)</td>
<td>42/62 (67.7%)</td>
<td>5/50 (10.0%)</td>
<td>3/45 (6.7%)</td>
<td>8/50 (16.0%)</td>
</tr>
<tr>
<td>Spring</td>
<td>175/225 (77.8%)</td>
<td>154/225 (68.4%)</td>
<td>145/225 (64.4%)</td>
<td>21/175 (12.0%)</td>
<td>9/154 (5.8%)</td>
<td>30/175 (17.1%)</td>
</tr>
<tr>
<td>Summer</td>
<td>48/64 (75.0%)</td>
<td>44/64 (68.8%)</td>
<td>39/64 (60.9%)</td>
<td>4/48 (8.3%)</td>
<td>5/44 (11.4%)</td>
<td>9/48 (18.8%)</td>
</tr>
</tbody>
</table>
4. Discussion:

Which is the probability to have a foal is the first question an owner asks before deciding if to include or not his mare into an ET program. There is plenty of literature about foaling rates, especially in the Thoroughbred [4,11,21,26-32,34], but, in spite of many reports on thousand of recipients pregnancy rates up to 40-50 days [2-6,15,38,39], only one study describes foaling rates following embryo transfer in the mare [33].

Recipient’s pregnancy and pregnancy loss rates observed in this study were similar to what commonly reported in literature for surgical or non-surgical equine ET programs: 60-89% at 14 days and 58-70% at 50 days of pregnancy [2-4,6,21,33,38,40].

Pregnancy losses were also in the range of what reported in literature for mares carrying their own pregnancies between 14 and 40 days (2.6 to 24%) [27,41], and between 40 days and parturition (7-9.1%) [27,41-44]. Pregnancy losses were higher before day 40 than after, confirming that most of the pregnancies not resulting in a parturition of a live foal end during the embryonic phase, probably due to reduced embryonic viability [45,46].

In this study, as largely expected from the literature [3,4,6,8,16], the embryo quality had a significant influence on recipient’s pregnancy rate at 14 days. Probably only due to the very low number of embryos evaluated as quality grade 3 or 4, recipient’s foaling rate and overall pregnancy losses resulted not significantly different from that of quality 1 or 2 embryos.

Day of embryo recovery (embryo age) affected recipient’s pregnancy losses, but not recipient’s pregnancy or foaling rates. In particular day 9 and day 10 embryos resulted in a pregnancy loss rate after 40 days significantly higher than day 8 embryos. Day 10 embryos resulted also in a significantly higher overall pregnancy loss rate than day 8 embryos.Flushes for embryo recovery were performed on the 9th and 10th day post ovulation only in donors not providing embryos for at least three cycles or that produced very small embryos in flushes at days 8 or 9.

In the group of the embryos that have been measured, a higher pregnancy loss rate was observed after transfer of small embryos. In particular, embryos <400 µm resulted in significantly higher pregnancy loss rates compared to larger embryos (between 400 and 1199 µm). Most of the embryos <400 µm were 8 to 10 days old (8 days = 47/57; 9 days = 2/57; 10 days = 1/57). A “small for age” embryo may imply a delay in embryo development, as reported in literature for older mares [4,7,47], and the underdeveloped embryos could lead to a high risk of pregnancy loss [4,8,48].

Similarly, it is commonly accepted that the evidence of an underdeveloped embryo at ultrasonographic pregnancy diagnosis 14 days post ovulation is a negative prognostic factor for the
prosecution of a normal pregnancy [16,49-51].

In this study, only 12 large embryos (>1200 µm of diameter) were transferred and yielded intermediate results between small and normal embryos without statistical differences. Squires et al. [38,52] suggested that the increased fluid volume-to-surface area ratio of embryos > 2 mm made them more prone to damage during the collection and transfer procedures. On the other hand, Wilsher et al. [53] reported a 63 to 75% pregnancy rate at 25 days after nonsurgical transfer of ≥3 mm embryos in recipients that ovulated 5 to 8 days earlier. These authors used a different transfer method [54], employing an insemination pipette with a larger lumen that may have avoided damage during manipulation and transfer. This last study obtained pregnancy rates at 14 days similar to what observed with our larger embryos. Wilsher et al. [53], however, terminated all pregnancies at day 25 and thus no data on later pregnancy losses with these large embryos is available.

In this study, transfer of embryos recovered from donor mares older than 20 years resulted in a significantly higher overall and between 14 and 40 days pregnancy loss rates, despite 14 and 40 days pregnancy rates and foaling rate were comparable to the other age categories. These results are consistent with the high pregnancy loss rate affecting old mares, both if carrying their own pregnancies or after transfer of their embryos in recipients [12]. The lower embryo quality in old mares, compared to that of young ones, has been correlated to adverse effects of the aged uterus or oviduct or to inherent defects in embryos deriving from old mares’ oocytes [7,48] as it’s well known in the human species [55]. Carnevale et al. [36] confirmed that part of the problem is related with the quality of oocytes: when recipient mares received oocytes from donors <20 years or >20 years old the 16 day pregnancy rate was not different, but the pregnancy loss rate between 16 and 50 days was higher for older mares’ oocytes. Moreover, it was reported that the number of mitochondria of in vitro matured oocytes was significantly lower in oocytes from aged versus young mares [37].

In this study, a significantly higher pregnancy loss between days 14 and 40 was observed in recipients receiving embryos from donors affected by reproductive pathologies compared with those receiving embryos from healthy donors performing sport activity. These findings are not surprising based on literature [8] and on the consideration that most of the donors affected by reproductive problems had a history of repeated early embryo loss. Once again, the embryo loss could be due to early embryo or oocyte defects that can’t be overcome by the transfer in a recipient. Moreover, the lowest, although not statistically different, recipients’ foaling rates was achieved after transfer of embryos derived from donors affected by reproductive pathologies; in this category the
recipient's foaling rates were respectively 12%, 23% and 29% lower than foaling rates of mares receiving embryos from healthy donors, donors affected by non reproductive pathologies or donors performing sport activity.

Embryos collected from healthy donors performing sport activity resulted in the higher, although not statistically different, foaling rates, in a within 40 days pregnancy loss rate significantly lower than donors with reproductive pathologies (mentioned above) and in an overall embryo loss rate significantly lower than healthy donors not performing sport. In the group of healthy mares under 16 years old, the ET outcome was not affected by sport activity.

The effect of exercise on ET outcome previous studies is controversial: it has been reported to lower embryo recovery [56,57] or to have no effect on embryo recovery and pregnancy rates after transfer [39]. In our clinical experience [34] embryo recovery rate was not affected by sport activity, and the results of the current study on pregnancies after transfer seem to indicate that mares performing sport activity should not be discriminated as embryo donors.

In this study, no differences between pregnancy and foaling rates have been found between nulliparous and pluriparous recipients, probably as an accurate selection before inclusion into the program was done. It is well known that primiparous mares give birth to smaller foals due to a lower "microcotyledon surface density" [52]; however, as the recipients foaled far from the Department, placental and fetal weights were not compared between these two categories.

No differences between donor-recipient synchrony and recipient day after ovulation have been found in this retrospective study. Almost all ETs have been performed in recipients that ovulated between 5 and 8 days earlier, and with a synchrony between 0 and -3. These ranges are described to give the best results in pregnancy rates after surgical or non surgical ET [2,4,8,16,17,21,38,53,54].

In this study no advantage has been observed of a blind anti-inflammatory, antibiotic and progestinic treatment of recipients at ET as proposed by Foss et al.[2]. The results of this treatment protocol have been similar to those of recipients selected following the guidelines described by Carnevale et al.[4] or for recipients found “marginally acceptable” at the pre ET clinical examination, and treated with altrenogest only. Acyclic altrenogest treated recipients, although a small number, resulted having pregnancy and foaling rates comparable to cycling recipients, confirming what previously described in literature for ET [4,19,20] and for oocyte transfer [36] procedures.

Media employed for uterine flushing and embryo washing did not affect pregnancy or foaling rates.
Lower pregnancy rates after ETs were observed by Squires et al. [58] in winter, and by Carnevale et al.[4] during summer, in both cases at the Colorado State University Equine Reproduction Lab. In this study no effects of season were observed, possibly due to the mediterranean climate conditions of our region.

In conclusion, donors' age and reproductive category, and embryo quality, age and diameter significantly affected the outcome of ET at different end points, while they had no effect on recipients foaling rate.


Rambags B, van Boxtel D. Oocyte mitochondrial degeneration during reproductive ageing