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

Panzani Duccio[1a], Vannozzi Iacopo[a], Marmorini Paola[b], Rota Alessandra[a], Camillo Francesco[a].

[a]Dipartimento di Scienze Veterinarie, Università degli Studi di Pisa, 56124 San Piero a Grado, Pisa (PI), Italy.

[b]Private practitioner, Cenaia, Pisa, Italy.

[1]Corresponding author: Panzani Duccio, Dipartimento di Scienze Veterinarie, Università di Pisa, San Piero a Grado, Pisa, 56124, Italy; tel. +390502210164, fax +390502210182, d.panzani@vet.unipi.it

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 recipents' 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 PGF2 α 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 (Procacillina®, 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

	Pregnancies/ET at 14 days (%)	**Pregnancies/ET at 40 days (%)**	**Foals born/ET (%)**	**Pregnancy loss 14 - 40 days (%)**	**Pregnancy loss after 40 days (%)**	**Overall pregnancy loss (%)**
Day of embryo recovery						
7	12/18 (66.7%)	10/18 (55.6%)	9/18 (50.0%)	2/12 (16.7%)	1/10 (10%)	3/12 (25%)
8	234/299 (78.3%)	211/299 (70.6%)	200/299 (66.9%)	23/234 (9.8%)[a]	11/211 (5.2%)[a]	34/234 (14.5%)[a]
9	22/26 (84.6%)	20/26 (76.9%)	15/26 (57.7%)	2/22 (9.1%)[a]	5/20 (25%)[b]	7/22 (31.8%)
10	5/8 (62.5%)	2/8 (25.0%)	2/8 (25.0%)	3/5 (60%)[b]	0/3 (0%)	3/5 (60%)[b]
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%)	0/1 (0.0%)	0/1 (0.0%)	1/1 (100%)	0/0 (0%)	1/1 (100%)
Embryo quality						
1-2	267/338 (79.0%)[a]	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%)[b]	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)						
150-399 µm	46/57 (80.7%)	30/57 (52.6%)	30/57 (52.6%)	13/46 (28.3%)[a]	3/30 (10%)	16/46 (34.8%)[a]
400-699 µm	57/75 (76.0%)	55/75 (73.3%)	53/75 (70.7%)	2/57 (3.5%)[b]	2/55 (3.6%)	4/57 (7%)[b]
700-1199 µm	51/67 (76.1%)	48/67 (71.6%)	45/67 (67.2%)	3/51 (5.9%)[b]	3/48 (6.2%)	6/51 (11.8%)[b]
1200-3000 µm	12/16 (75.0%)	10/16 (62.5%)	9/16 (56.3%)	2/12 (16.7%)	1/10 (10%)	3/12 (25%)
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

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

[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

	Pregnancies/ET at 14 days (%)	Pregnancies/ET at 40 days (%)	Foals born/ET (%)	Pregnancy loss 14 - 40 days (%)	Pregnancy loss after 40 days (%)	Overall pregnancy loss (%)
Reproductive career						
Nulliparous	136/175 (77.7%)	122/175 (69.7%)	114/175 (65.1%)	14/136 (10.3%)	8/122 (6.6%)	22/136 (16.2%)
Pluriparous	137/176 (77.8%)	121/176 (68.8%)	112/176 (63.6%)	16/137 (11.7%)	9/121 (7.4%)	25/137 (18.2%)
Recipient day post ovulation						
Anovulatory	10/11 (90.9%)	10/11 (90.9%)	9/11 (81.8%)	0/10 (0%)	1/10 (10%)	1/10 (10%)
3	2/2 (100.0%)	1/2 (50.0%)	1/2 (50.0%)	1/2 (50%)	0/1 (0%)	1/2 (50%)
4	9/12 (75.0%)	7/12 (58.3%)	7/12 (58.3%)	2/9 (22.2%)	0/7 (0%)	2/9 (22.2%)
5	82/113 (72.6%)	75/113 (66.4%)	69/113 (61.1%)	7/82 (8.5%)	6/75 (8.0%)	13/82 (15.9%)
6	70/87 (80.5%)	64/87 (73.6%)	59/87 (67.8%)	6/70 (8.6%)	5/64 (7.8%)	11/70 (15.7%)
7	70/86 (81.4%)	61/86 (70.9%)	56/86 (65.1%)	9/70 (12.9%)	5/61 (8.2%)	14/70 (20.0%)
8	28/37 (75.7%)	23/37 (62.2%)	23/37 (62.2%)	5/28 (17.9%)	0/23 (0%)	5/28 (17.9%)
9	2/2 (100.0%)	2/2 (100.0%)	2/2 (100.0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)
10	0/1 (0.0%)	-	-	-	-	-
Recipient selection and treatment						
Acyclic treated	10/11 (90.9%)	10/11 (90.9%)	9/11 (81.8%)	0/10 (0%)	1/10 (10%)	1/10 (10%)
Blind treated	47/63 (74.6%)	43/63 (68.3%)	40/63 (63.5%)	4/47 (8.5%)	3/43 (7%)	7/47 (14.9%)
Selected untreated	166/211 (78.7%)	146/211 (69.2%)	136/211 (64.5%)	20/166 (12.0%)	10/146 (6.8%)	30/166 (18.1)
Selected treated	50/66 (75.8%)	44/66 (66.7%)	41/66 (62.1%)	6/50 (12.0%)	3/44 (6.8%)	9/50 (18.0%)

P>0.05

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

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

P>0.05

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

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

P>0.05

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

[25].

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.

[1] Oguri N, TsutsumiY. Non-surgical egg transfer in mares. J Reprod Fertil 1974;41:313–20.
[2] Foss R, Wirth N, Schiltz P, Jones N. Non surgical embryo transfer in a private practice (1998). American Association of Equine Practioners 1999;45:210–2.
[3] Squires EL, McCue PM, Vanderwall D. The current status of equine embryo transfer. Theriogenology 1999;51:91–104.
[4] Carnevale EM, Ramirez RJ, Squires EL, Alvarenga MA, Vanderwall DK, McCue PM. Factors affecting pregnancy rates and early embryonic death after equine embryo transfer. Theriogenology 2000;54:965–79.
[5] Riera FL, McDonough J. Commercial embryo transfer in polo ponies in Argentina. Equine Veterinary Journal 2010;25:116–8.
[6] Camargo CE, Weiss RR, Kozicki LE, Duarte MP, Garcia Duarte MC, Lunelli D, et al. Some Factors Affecting the Rate of Pregnancy after Embryo Transfer Derived from the Brazilian Jumper Horse Breed. Journal of Equine Veterinary Science 2013;11:924-9
[7] Ball BA, Little TV, Hillman RB, Woods GL. Pregnancy rates at Days 2 and 14 and estimated embryonic loss rates prior to day 14 in normal and subfertile mares. Theriogenology 1986;26:611–9.
[8] Vogelsang SG, Vogelsang MM. Influence of donor parity and age on the success of commercial equine embryo transfer. Equine Veterinary Journal 1989;21:71–2.
[9] Carnevale EM, Ginther OJ. Relationships of age to uterine function and reproductive efficiency in mares. Theriogenology 1992;37:1101–15.
[10] Carnevale EM, Griffin PG, Ginther OJ. Age-associated subfertility before entry of embryos into the uterus in mares. Equine Veterinary Journal 1993;25:31–5.
[11] Panzani D, Rota A, Pacini M, Vannozzi I, Camillo F. One year old fillies can be successfully used as embryo donors. Theriogenology 2007;67:367–71.
[12] Uliani RC, Ramires Neto C, Dell'Aqua JA, Pessoa MA, Camargo AL, Alvarenga R, et al. Effect of mare breed and age on embryo transfer efficiency. Animal Reproduction Science 2010;121:303–4.
[13] Campbell MLH.Embryo transfer in competition horses: Managing mares and expectations. Equine Veterinary Education 2014;26:322–7.
[14] Aurich C, Budik S. Season Does Not Influence Embryo Recovery Rate and Conceptus Size Until Day14 After Ovulation in the Horse. Reprod Domest Anim 2015;50(2):299-303.
[15] Iuliano MF, Squires EL, Cook VM. Effectof age of equine embryos and method of transfer on pregnancy rate. Journal of Animal Science 1985;60:258–63.
[16] McKinnon AO, Squires EL. Equine embryo transfer. VetClin North Am Equine Pract 1988;4:305–33.

[17] Stout TAE. Equine embryo transfer: Review of developing potential. Equine Veterinary Journal 2006;38:467–78.
[18] Jacob JCF, Haag KT, Santos GO, oliveira JP, Gastal MO, Gastal EL. Effect of embryoage and recipientasynchrony on pregnancyratesin a commercial equine embryo transfer program. Theriogenology 2012;77:1159–66.
[19] Hinrichs K, Sertich PL, Kenney RM. Use of altrenogest to prepare ovariectomized mares as embryo transfer recipients. Theriogenology 1986;26:455–60.
[20] Hinrichs K, Sertich PL, Palmer E, Kenney RM. Establishment and maintenance of pregnancy after embryo transfer in ovariectomized mares treated with progesterone. Reproduction 1987;80:395–401.
[21] Squires EL, Carnevale EM, McCue PM, Bruemmer JE. Embryo technologies in the horse. Theriogenology 2003;59:151–70.
[22] Koblischke P, Budik S, Müller J, Aurich C. Practical experience with the treatment of recipient mares with a non-steroidal anti-inflammatory drug in an equine embryo transfer programme. Reproduction in Domestic Animals 2010;45:1039–41.
[23] Lopes EP, Siqueira JB, Pinho RO. ReproductiveParameters of Mangalarga Marchador Mares in a Commercial Embryo Transfer Programme. Reproduction in Domestic Animals 2011;46(2):261-7.
[24] Allen WA, RowsonLE. Surgical and non-surgical egg transfer in horses. J Reprod Fertil Suppl 1975:525–30.
[25] Alvarenga MA, Alvarenga FCL, Meira C. Modifications in the technique used to recover equine embryos. Equine Veterinary Journal 1993;25:111–2.
[26] Brück I, Anderson GA, HylandJH.Reproductive performance of thoroughbred mares on six commercial studfarms. AustVet J 1993;70:299–303.
[27] Morris LHA, Allen WR. Reproductive efficiency of intensively managed Thoroughbred mares in Newmarket. Equine Veterinary Journal 2002;34:51–60.
[28] Hemberg E, Lundeheim N, Einarsson S. Reproductive Performance of Thoroughbred Mares in Sweden. ReprodDomestAnim 2004;39:81–5.
[29] Allen WR, Brown L, Wright M, Wilsher S. Reproductive efficiency of Flatrace and National Hunt Thoroughbred mares and stallions in England. Equine Veterinary Journal 2007;39:438–45.
[30] Baker CB, Little TV, McDowell KJ. The live foaling rate per cycle in mares. Equine Veterinary Journal 2010;25:28–30.
[31] Sharma S, Dhaliwal GS, Dadarwal D. Reproductive efficiency of Thoroughbred mares under Indian subtropical conditions: A retrospective survey over 7 years. Animal Reproduction Science 2010;117:241–8.
[32] Davies Morel MCG, Lawlor O, Nash DM. Equine endometrial cytology and bacteriology: effectiveness for predicting live foaling rates. Vet J 2013;198:206–11.
[33] McCue PM, Wall JJ, Brink ZA, Seidel GE Jr. Live foal rate: effects of embryo transfer and donor mare age. In: Proceedings of the Annual Conference of the International Embryo Transfer Society, Córdoba, Argentina, 2010 Reprod Fertil Dev 2010;22:247.
[34] Panzani D, Rota A, Marmorini P, Vannozzi I, Camillo F. Retrospectivestudy of factors affecting multiple ovulations, embryo recovery, quality, and diameter in a commercial equine embryo transfer program. Theriogenology 2014;82:807–14.
[35] Ginther OJ. Reproductive Efficiency. In: Reproductive Biology of the Mare. Ed. Equiservices Publishing, Cross Plains, Wisconsin 53528, USA; 1992:508.
[36] Carnevale EM, Coutinho da Silva MA, Panzani D, Stokes JE, Squires EL. Factors affecting the success of oocyte transfer in a clinical program for subfertile mares. Theriogenology 2005;64:519–27.
[37] Rambags B, van Boxtel D. Oocyte mitochondrial degeneration during reproductive ageing

in the mare. Havemeyer Foundation Monograph Ser 2006;18:25–7.

[38] Squires EL. Collection and transfer of equine embryos. Bulletin / Animal Reproduction Laboratory 1995;8:24-6

[39] Pessoa MA, Cannizza AP, Reghini MFS, Alvarenga MA. Embryo Transfer Efficiency of Quarter Horse Athletic Mares. Journal of Equine Veterinary Science 2011;31:703–5.

[40] Marinone AI, Losinno L, Fumuso E, Rodríguez EM, Redolatti C, Cantatore S, et al. The effect of mare's age on multiple ovulation rate, embryo recovery, post-transfer pregnancy rate, and interovulatory interval in a commercial embryo transfer program in Argentina. Animal Reproduction Science 2015;158:53–9.

[41] Chevalier-Clément F. Pregnancy loss in the mare. Animal Reproduction Science 1989;20(3):231-44

[42] Papa FO, Lopes MD, Alvarenga MA, Meira C De, Luvizotto MCR, Langoni H, et al. Early embryonic death in mares: clinical and hormonal aspects. Braz J Vet Res Anim Sci 1998;35(4):170-3.

[43] Vanderwall DK. Early Embryonic Loss in the Mare. Journal of Equine Veterinary Science 2008;28:691–702.

[44] Nath LC, Anderson GA, McKinnon AO. Reproductive efficiency of Thoroughbred and Standardbred horses in north-east Victoria. AustVet J 2010;88:169–75.

[45] Silva LA, GintherOJ. An early endometrial vascular indicator of completed orientation of the embryo and the role of dorsal endometrial encroachment in mares. Biology of Reproduction 2006;74:337-43

[46] Ginther OJ, Garcia MC, Bergfelt DR, Leith GS, Scraba ST. Embryonic loss in mares: Pregnancy rate, length of interovulatory intervals, and progesterone concentrations associated with loss during days 11 to 15. Theriogenology 1985;24:409–17.

[47] Carnevale EM, Bergfelt DR, GintherOJ.Agingeffects on follicular activity and concentrations of FSH, LH, and progesterone in mares. Animal Reproduction Science 1993;31:287-99

[48] Ball BA, Little TV, Weber JA, Woods GL. Survival of day-4 embryos from young, normal mares and aged, subfertile mares after transfer to normal recipient mares. J Reprod Fertil 1989;85:187–94.

[49] Ginther OJ, Bergfelt DR, Leith GS, Scraba ST. Embryonic loss in mares: Incidence and ultrasonic morphology. Theriogenology 1985;24:73–86.

[50] Woods GL, Baker CB, Hillman RB, SchlaferDH.Recent studies relating to early embryonic death in the mare. Equine Veterinary Journal 1985;17:104–7.

[51] Bergfelt DR, Woods JA, GintherOJ.Role of the embryonicvesicle and progesterone in embryonic loss in mares. J Reprod Fertil 1992;95:339–47.

[52] Wilsher S, Allen WR. The effects of maternal age and parity on placental and fetal development in the mare. Equine Veterinary Journal 2003;35:476–83.

[53] Wilsher S, Clutton-Brock A, Allen WR.Successful transfer of day 10 horseembryos: influence of donor-recipient asynchrony on embryo development. Reproduction 2010;139:575–85.

[54] Wilsher S, Allen WR. An improved method for non surgical embryo transfer in the mare. Equine Veterinary Education 2004;16:39–44.

[55] American College of Obstetricians and Gynecologists Committee on Gynecologic Practice and Practice Committee. Female age-related fertility decline. Committee Opinion No. 589. Fertil Steril 2014;101:633–4.

[56] Mortensen CJ, Choi YH, Hinrichs K, Ing NH, Kraemer DC, Vogelsang SG, et al. Embryo recovery from exercised mares. Animal Reproduction Science 2009;110:237–44.

[57] Kelley DE, Smith RL, Gibbons JR, Vernon KL, Mortensen CJ. Effect of exercise on ovarian blood flow and embryo recovery rates in mares. Animal Reproduction Science

2010;121:284–5.
[58] Squires EL, Imel KJ, Iuliano MF, Shideler RK. Factors affecting reproductive efficiency in an equine embryo transfer programme. J Reprod Fertil Suppl 1982;32:409–14.