Journal of Equine Veterinary Science 37 (2016) 17–23

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Factors Affecting Recipients' Pregnancy, Pregnancy Loss, and Foaling Rates in a Commercial Equine Embryo Transfer Program

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

[a] Dipartimento di Scienze Veterinarie, Università degli Studi di Pisa, Pisa, Italy
[b] Private practitioner, Pisa, Italy

ARTICLE INFO

Article history:
Received 17 August 2015
Received in revised form 20 October 2015
Accepted 28 October 2015
Available online 5 November 2015

Keywords:
Embryo transfer
Pregnancy rate
Foaling rate
Recipient selection

ABSTRACT

During 11 breeding seasons, 351 7- to 10-day-old horse embryos were nonsurgically 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 to 4 embryos resulted in a significantly lower pregnancy rate at 14 days compared with the transfer of grade 1 to 2 embryos (46.2% vs. 79%; $P < .05$). Eight-day-old embryos resulted in significantly lower pregnancy losses than day 9 or 10 embryos, as occurred for embryos between 400 and 1200 μm compared with embryos <400 μm. Embryos recovered from mares >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 with healthy mares performing sport activity. None of the evaluated parameters influenced recipients' foaling rate significantly.

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% to 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], whereas 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, and treatments [2,4,6,8,16–23]. Technical factors studied, finally, were surgical or nonsurgical ET procedures, month in which ET was performed and embryo flushing, and holding media used [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 February

* Corresponding author at: Duccio Panzani, Dipartimento di Scienze Veterinarie, Università di Pisa, San Piero a Grado, Pisa 56124, Italy.
E-mail address: d.panzani@vet.unipi.it (D. Panzani).

0737-0806/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jevs.2015.10.011

15 to March 21; spring, from March 22 to June 21; summer, from June 22 to the August 15) of 11 breeding seasons (2002 to 2012) at the former Dipartimento di Clinica Veterinaria of the Pisa University were retrospectively analyzed.

2.1. Donors

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

Embryo donors' housing, estrus cycles monitoring, artificial insemination (AI) and post-AI treatment were described in Panzani et al 2014 [34].

2.2. Embryos

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

Phosphate-buffered saline and RL recovered embryos were washed 10 times in DPBS added of 0.4% BSA (PBS/PBS) or Emcare holding solution (ICPbio, Ltd, Auckland, New Zealand) (RL/EHS), respectively, evaluated for quality [16] by a $\times$40 magnification stereo-microscope before being prepared for transfer. Embryo recovery, manipulation, and transfers have been done in controlled temperature rooms ($25 \pm 2^{\circ}$C), with media at 37°C; embryo search and washing were done under a laminar flow hood.

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

2.3. Recipients

One hundred fifty-one Standardbred mares between 2-and12-year-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 reincluded in the program; for this reason, most of the mares were used as recipients for more than 1 year. Thirteen Haflinger mares of the same age and sanitary status were also used as recipients, for Haflinger embryos only. Mares, maintained in dry lots, fed with hay ad libitum and 2 to 3 kg of mixed grain per day, were checked by ultrasound for ovarian activity throughout the year: weekly during anestrus, biweekly during transition and diestrus, and daily during estrus and until ovulation. When needed, recipients' ovulations were synchronized with the donors' ones using prostaglandin-2-alpha (PGF2α) analogue alfaprostol (3 mg, intramuscularly [IM], in a single injection; Gabbrostim, Vetem, Spa, Monza-Brianza, Italy) and human Chorionic Gonadotropin (hCG) (2,000 UI, intravenously [IV], in a single injection; Vetecor 2000; Bio98, Bologna, Italy). Immediately before the transfer, recipients were submitted to three different regimes as follows:

- Treated with 30,000 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 transrectal palpation and ultrasound examination and, if graded as acceptable [4], used as embryo recipient without any treatment (selected untreated recipients)
- Submitted to transrectal palpation and ultrasound examination and, if graded as marginally acceptable [4], used as embryo recipient and treated with altrenogest as described previously (selected treated recipients)

In 11 cases, embryos were transferred into acyclic recipients in spring transition showing, at ultrasound, an uterine edema of grade 2 to 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, whereas embryos $>$1 mm were transferred by a 0.5-mL straw using a French gun designed for equine ET (IMV Technologies). Recipients were treated with acepromazine (4 mg, IV, in a single injection; Prequillan, Fatro, Bologna, Italy) 10 minutes before entering into a stock, then the rectum was evacuated from manure, the tail wrapped, perineum washed with povidone iodine soap and rinsed three 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 backward, 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 transrectal manipulation [11].

2.5. Pregnancy Diagnoses

Pregnancy diagnosis was performed by ultrasound 14 days after donors' ovulations and checked on days 25