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Embryo quality and transcervical technique are not the limiting factors in donkey embryo transfer outcome

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Abstract

Embryo transfer (ET) in the donkey resulted in a very low recipient pregnancy rates. The aim of these studies was to investigate if nonsurgical transfer techniques or donkey embryo quality affect donkey recipient pregnancy failure. In Study 1, the impact of transfer technique was investigated by evaluating if cervical catheterization is associated with prostaglandin release and suppression of luteal function and if donkey recipients would become pregnant after nonsurgical transfer of horse embryos. Four jennies, from 5 to 8 d after ovulation, were submitted to a sham transcervical ET and to evaluation of PGFM and progesterone plasma concentrations. Five 8 d horse embryos were nonsurgically transferred into synchronized donkey recipients (HD). Cervical stimulation caused a transient $PGF_{2\alpha}$ release in two of four jennies in the absence of a significant decrease in progesterone plasma concentration. All transferred horse embryos resulted in pregnancies in the jenny recipients. In Study 2, donkey embryo viability was investigated by 1.2 meters, 6-diamidino-2-phenylindole (DAPI) staining of 10 embryos and by the transfer of 6 and 12 donkey embryos in synchronized mare (DH) and donkey (DD) recipients, respectively, of known fertility. The estimated proportion of dead cells in DAPI stained embryos was 0.9% (range 0–3.9%) and below what is considered normal (20%) for horse embryos. Three of six and six of 12 of the DH and DD ETs, respectively resulted in pregnancies at 14 and 25 d (50%), a higher pregnancy rate than previously reported after DD ET. The overall results of this study suggest that the transcervical technique for ET and donkey embryo viability are not the reasons for the low pregnancy rates that have previously been described in donkey recipients, and that nonsurgical ET in donkeys can result in acceptable results.

Keywords: Embryo transfer; Donkey; Embryo viability; Mare

1. Introduction

Embryo transfer (ET) could be a useful tool for the preservation of 52 equid species and breeds faced with the risk of extinction, including most donkey breeds of the Mediterranean area, and equid species, such as the Przewalski's Horses (*Equus przewalskii*) and Grant's zebra (*E. burchellii*) [1]. In the donkey, surgical and nonsurgical ET has resulted in low recipient pregnancy rates [2,3]. Camillo et al [3] suggested that possible reasons for poor results after transcervical ET were poor viability of donkey embryos or their susceptibility to flushing/holding media; poor quality recipients; suboptimal synchronization of donors and recipients; or a release of $PGF_{2\alpha}$ in the recipient during cervical manipulations, with consequent luteolysis and pregnancy failure.

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Between-species surgical transfers of horse and donkey embryos yielded pregnancy rates similar to horse-to-horse transfers [4]. Mares and jennies have been able to carry a pregnancy at least until 40 d after surgical transfer of donkey and horse embryos, respectively [4,5]. As it is not likely that a female of one species is a better recipient for embryos of a different species than for the homologous one, an effect of the technique can be advocated to explain the low pregnancy rate after donkey-to-donkey transcervical ET [2,3].

The cervix of the jenny is longer, smaller, and tighter than the cervix of the mare [6], and catheterization is more difficult and requires more manipulation [3]. For these reasons, a release of $PGF_{2\alpha}$, as seen during cervical manipulation in some mares [7,8], followed by a decrease of progesterone plasma concentration [8], was hypothesized for jennies subjected to transcervical ET [3].

The 4′, 6′-diamidino-2-phenylindole dihydrochloride (DAPI) staining method has been used for the evaluation of embryo damage (described as the percentage of dead per total cells) in horses [9] and other species [10–13], but not in donkeys.

The selection of reproductively healthy recipients has been considered one of the most important factors in the achievement of acceptable pregnancy rates after ET in the horse [14,15]. Recipient selection was not described in previous studies for nonsurgical ET in the donkey [2,3].

The aims of the present study were to investigate the effects of a nonsurgical transfer technique and embryo quality on pregnancy rates in donkey recipients after ET. The role of nonsurgical ET was investigated by evaluating prostaglandin release and subsequent luteal function after sham ET and pregnancy rates in donkey recipients after transcervical transfer of horse embryos (Study 1); donkey embryo viability was investigated by DAPI staining and by transfer of donkey embryos into synchronized mare and donkey recipients (Study 2).

2. Materials and methods

The studies were performed at the Dipartimento di Clinica Veterinaria of the Pisa University (Italy).

2.1. Animals

Amiata jennies (n = 10) and Standardbred mares (n = 16), evaluated as normal based on clinical findings [14,15], were maintained in dry lots and fed with hay *at libitum* and commercial horse feed. The mares, already included in a commercial ET program as recipients, were between 3 and 10 y and were used as donors or recipients. Nine of the jennies were between 2 and 10 y and were used as embryo donors and recipients, one jenny was 20 y and was only used as an embryo donor. Three Amiata jacks (8–12 y) and two Saddlebred stallions (10 and 14 y) were used to provide fresh semen.

2.2. Estrus control and mating

Ovarian activity was monitored in jennies and mares by ultrasound examination on a biweekly schedule during diestrus and daily during estrus until ovulation. Embryo donors were artificially inseminated with fresh-extended semen, from of one of the stallions or jacks of the same species, every second day during estrus and until ovulation. When needed, ovulations between donors and recipients were synchronized by a $PGF_{2\alpha}$ analogue (alfaprostol, 3 mg im; Gabbrostim, CEVA VETEM, SpA, Milano, Italy) and hCG (2,000 IU iv; Vetecor 2000, BIO 98 Srl, Milano, Italy).

2.3. Semen collection and AI

Semen was collected using a Colorado or Missouri artificial vagina, filtered and placed in a water bath at 37 °C. After evaluation of volume and motility, semen was diluted 1:2 with INRA96, and kept in a dark place at room temperature. Every insemination dose was prepared to have at least 500×10^6 progressively motile sperm. Artificial inseminations were performed within 2 h after semen collection.

2.4. Embryo recovery and transfer

Embryo recoveries were done 7 to 9 d after ovulation by a self-made, 2-way tubing system connected to a cuffed 32° Fr, silicon-made catheter (AB Technology, Pullman, WA, USA) [3]. Jennies' and mares' uteri were flushed 4 to 6 times with a total of 2 to 10 l of Lactated Ringer's solution (Galenica Senese S.r.l., Siena, Italy) depending on uterine capacity. After the flushing, alfaprostol was administered to donors to induce luteolysis. Recovered embryos, destined to be transferred, were washed 10 times in EmCare Holding Solution (EHS; ICPbio, Ltd., Auckland, New Zealand) and evaluated for quality [16]. After the quality evaluation, the embryos were gently aspirated into a 0.25 or 0.5 ml French straw preceded and followed by a small amount of holding solution and a bubble of air. The French straw was then inserted into a French Gun designed for bovine ET (IMV Technologies, Bicef, Piacenza, Italy) and nonsurgically transferred into synchronized recipients treated 10 min before transfer with acepromazine (3.3 mg/iv/100 kg; Prequillan, Fatro, Bo-