

VITRIFICATION OF COOLED EQUINE EMBRYOS

J.J. Hudson, S.A. Welch, E.L. Squires, E.M. Carnevale, P.M. McCue

Colorado State University, Fort Collins, CO 80523, USA

Embryo transfer (ET) is a now a common assisted reproductive technique in the equine breeding industry. The ability to collect embryos on a farm or at a veterinary clinic and ship them to a referral center for transfer has made embryo transfer more available to both horse owners and veterinary practitioners. Equine embryos can remain viable for 12–24 h when cooled and stored at 5 °C. Freezing of embryos would allow for long-term preservation of genetic material and allow for more efficient management of embryo recipients. The goal of the present study was to determine if pregnancies could be obtained following transfer of equine embryos cooled for 12 or more hours prior to vitrification.

Mares ($n = 640$) were superovulated using equine follicle stimulating hormone (eFSH) at a dose of either 25 mg once-per-day or 12.5 mg twice per day. Ovulation was induced by administration of 2500 IU of human chorionic gonadotropin (hCG) once a cohort of follicles >35 mm was detected. An embryo flush procedure was performed on day 6.5 post-ovulation. Embryos recovered were evaluated for size, grade and morphology. Forty morula or early blastocyst stage embryos with a grade of 1–2 and <300 μm in diameter were randomly assigned to one of two treatment groups. Embryos in Group 1 ($n = 620$) were washed three times in a commercial holding medium and then vitrified. Embryos in Group 2 ($n = 620$) were washed three times and then stored in holding medium at 5–8 °C for 12–19 h before being vitrified. Embryos were warmed by holding the straw in air at room temperature for 10 s and then submerging the straw in a water bath (20–22 °C) for an additional 10 s. The fluid columns in the straw (galactose diluent and glycerol plus ethylene glycol cryoprotectants) were mixed and allowed to equilibrate for 6 min. The contents of the straw were transferred directly into a recipient that ovulated 1–3 days after the donor mare using a Cassou gun and a standard non-surgical technique. Pregnancy rates following transfer were compared using Chi-square analysis.

The optimal ovulation rate (4.1 ± 1.9 follicles) and embryo recovery rate (2.6 ± 1.9 embryos) per cycle was for mares administered 12.5 mg eFSH twice daily with a one day 'coast' period between the end of eFSH treatment and administration of hCG. There were no significant differences in embryo size, grade or morphology score between treatment groups prior to vitrification. Pregnancy rates (day 16) following nonsurgical direct transfer were not significantly different ($p > 0.05$) between embryos vitrified immediately after collection (15 of 20; 75%) and embryos cooled for 12–19 h prior to vitrification (13 of 20; 65%). Results of this study indicate that small equine embryos (<300 μm) can be stored at 5–8 °C for 12–19 h prior to vitrification without a significant loss of viability. The clinical significance is that practitioners can ship embryos to a referral center for cryopreservation, storage in liquid nitrogen and transfer at a later date.

Keywords: Equine; Embryo transfer; Cooling; Vitrification