print close

217 A NEW APPROACH TO CRYOPRESERVATION OF LARGE EQUINE EMBRYOS BY VITRIFICATION AFTER BLASTOCOEL MICROMANIPULATION

J. Scherzer, R. A. Fayrer-Hosken, L. Ray and G. Heusner

Abstract

Vitrified large equine embryos >800 μm recovered on Day 8 after ovulation have not been successfully transferred yet. In this study, we examined the effects of reduction of the blastocoelic fluid and microinfusion of a cryopreservative prior to vitrification on pregnancy outcome. In 2006, 33 embryos, recovered at the expanded blastocyst stage, were transferred fresh with an average pregnancy rate of 91% (30/33). However, suitable recipients are not always available. The sizes of embryos used for this vitrification project were 805 μm (embryo #1), 820 μm (#2), 1120 μm (#3), 1286 μm (#4), and 979 μm (#5). They were all morphologically graded excellent (according to IETS guidelines). These embryos were assigned to either no micromanipulation (embryos #1–#3) or microinfusion of VS1 (1.4 M glycerol in PBS; embryos #4 and #5) after additional aspiration of the blastocoelic fluid and before microinfusion for embryo #5. To facilitate aspiration and microinfusion, a laser system (XYclone; Hamilton Thorne Biosciences, Beverly, MA, USA) was applied. Approximately 20% of the total blastocoelic fluid was removed using a standard micromanipulator and microcapillary system (Eppendorf). All embryos were then vitrified as previously described (Eldridge-Panuska *et al*. 2005 Theriogenology 63, 1308–1319). In brief, embryos were exposed to VS1 and VS2 (1.4 M glycerol + 3.6 M ethylene glycol in PBS) for 5 min, and VS3 (3.4 M glycerol + 6.6 M ethylene glycol in PBS) for 1 min. Embryos in VS3 were then individually loaded into 0.25-mL straws, separated by 2 air bubbles from columns of 0.5 M galactose. Then straws were placed for 1 min into a cooled plastic goblet surrounded by liquid nitrogen. The goblet was finally plunged into liquid nitrogen. Digital images of all embryos were taken prior to and during the vitrification procedure, and also after thawing prior to embryo transfer. All expanded blastocysts initially decreased in size. After transfer to VS2 and VS3, they lost their spherical shape and blastocoels collapsed. Four vitrified embryos were transfered to recipients on Day 8 after ovulation. After thawing in air at room temperature for 5 s and then in water at 30°C for 15 s, straw contents were emptied into a Petri dish and mixed. After 5 min, single embryos were loaded into an AI pipette and nonsurgically transferred to recipients. The blastocoel of only one embryo re-expanded during the 5 min after thawing (#3) and one embryo was split into two halves (#4). One week after transfer of embryos, recipients were examined by ultrasonography. None of the control embryos nor the split embryo in the treatment group led to the formation of an embryonic vesicle. However, the blastocyst, which had undergone both aspiration of blastocoelic fluid and microinfusion of VS1 (#5), had formed an embryonic vesicle at Day 15 after ovulation. During a further exam on Day 28, the uterine tone in the recipient was still increased, but ultrasonography revealed resorption of the embryo, which was probably caused by heat stress. Nevertheless, we will test this protocol for the cryopreservation of large equine embryos on a larger scale during the next breeding season.

Reproduction, Fertility and Development 19(1) 225 - 226

Full text doi:10.1071/RDv19n1Ab217

© CSIRO 2007

Printed from **CSIRO PUBLISHING - Reproduction, Fertility and Development**
http://www.publish.csiro.au/paper/RDv19n1Ab217.htm