Normal Rates of Pregnancy and Foaling Achieved Following Transfer of Large Expanded Equine Blastocysts Vitrified After Blastocoele Collapse

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The vitrification of embryos collected from donor mares is integral to advanced horse breeding programs. However, the size of equine blastocysts varies greatly on the days they are most readily recovered, and poor pregnancy outcomes have previously been reported when expanded blastocysts greater than 300 µm in diameter were vitrified. The findings of several studies indicate that the cryotolerance of large expanded blastocysts can be improved by aspirating the fluid from the blastocoele, which reduces the volume of the blastocyst and prevents damaging ice crystal formation. Therefore, the objective of this study was to determine the viability of large expanded blastocysts after blastocoele collapse and vitrification by assessing the pregnancy outcomes following transfer to recipient mares.

A total of 28 blastocysts were recovered from donor mares on day 7 or 8 after ovulation using a standard embryo flushing procedure and evaluated morphologically according to the guidelines of the International Embryo Technology Society (IETS). Sixteen blastocysts (Grade 1: n=11; Grade 2: n=5) were less than 300 µm in diameter (mean of 204.8 ± 8.9 µm; range of 150-260 µm), and twelve blastocysts (Grade 1: n=7; Grade 2: n=5) were greater than 300 µm in diameter (mean of 502.0 ± 49.8 µm; range of 300-900 µm). All blastocysts greater than 300 µm in diameter were punctured and had blastocoele fluid aspirated via micromanipulation using an injection pipette (the larger the blastocyst, the greater the amount of fluid aspirated). All manipulated, collapsed blastocysts (>300 µm group) and all non-manipulated blastocysts (<300 µm group) were vitrified using the Cryotop method according to the manufacturer's instructions (Vitrification and Thawing Kit; Kitazato Corporation, Shizuoka, Japan). All embryos in both groups re-expanded after warming and were each transferred to a recipient mare at 5 days after ovulation.

The Day 14 pregnancy rate for both groups was 75% (<300 µm: 12/16 recipients; >300 µm: 9/12 recipients). The percentages of recipient mares in each group that were diagnosed as pregnant at Day 45 (63% vs 42%) and that gave birth to a live foal (63% vs 42%) did not differ significantly (P>0.05; <300 µm: 10/16 recipients; >300 µm: 5/12 recipients). There was no effect of embryo classification at collection (Grade 1 vs Grade 2) on any of the pregnancy outcomes examined (P>0.05).

The results of this study show that in vivo-recovered, large expanded equine blastocysts (>300 µm in diameter) can be effectively vitrified after blastocoele collapse. The birth of foals reported here extends the findings of previous equine embryo transfer studies by demonstrating that an acceptable proportion of such manipulated embryos retain full-term developmental potential.