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Factors affecting cryosurvival of nuclear-transferred bovine and swamp buffalo blastocysts: effects of hatching stage, linoleic acid–albumin in IVC medium and Ficoll supplementation to vitrification solution

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Abstract

The objective was to determine whether the hatching stage of cattle and swamp buffalo somatic cell nuclear-transferred (SCNT) blastocysts affected cryosurvival after vitrification, and whether addition of linoleic acid–albumin (LAA) to the IVC medium and Ficoll to the vitrification solution improves cryosurvival. Fused couplets were activated with ethanol and cycloheximide-cytochalasin D (day 0), and were allowed to develop in the presence of 0.3% BSA or 0.1% LAA + 0.2% BSA. Hatching blastocysts were harvested at day 7.0 (cattle) or day 6.5 (buffalo), and classified into one of three categories, according to the ratio of extruding embryonic diameter from zona to embryonic diameter inside the zona. The blastocysts were vitrified in 20% DMSO + 20% ethylene glycol + 0.5 M sucrose, with or without 10% Ficoll in TCM199 + 20% FBS, using Cryotop as a cryodevice. The post-thaw survival of the blastocysts was assessed by *in vitro* culture for 24 h. In cattle, when the LAA-supplemented IVC medium and the Ficoll-free vitrification solution were used, cryosurvival of the early-hatching blastocysts (77%) was not different from those of middle- and late-hatching blastocysts (74 and 80%, respectively). Inclusion of Ficoll in the vitrification solution did

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not improve the cryosurvival of SCNT blastocysts (54 to 68%). Early-hatching SCNT blastocysts produced in the absence of LAA were sensitive to the vitrification procedure (cryosurvival 56%; $P < 0.05$ versus 80% in the late-hatching blastocysts). The full-term developmental potential of SCNT blastocysts was proven only in the non-vitrified control group. In buffalo, the mean cryosurvival of hatching SCNT blastocysts produced with LAA (89%) was not different from that of those produced without LAA (87%). In conclusion, bovine SCNT blastocysts, regardless of their hatching stage, were relatively resistant to vitrification by the ultra-rapid cooling procedure when the blastocysts were produced in the presence of LAA. Furthermore, swamp buffalo SCNT blastocysts were more tolerant of vitrification than bovine SCNT blastocysts.

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