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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.

Keywords: Cryotop; Ficoll; Hatching; LAA; Nuclear transfer

1. Introduction

Recent advances in embryo cryopreservation in bovine species include high survival rates after vitrification by ultra-rapid cooling procedures. With IVF-derived bovine embryos, ultra-rapid cooling in cryodevices such as open-pulled straws (OPS) [1] and gel-loading tips [2] has made it possible to cryopreserve embryos at various developmental stages ranging from 1-cell zygotes to expanded blastocysts. Embryos produced by nuclear transplantation have mechanical slits in their zonae pellucidae, and therefore initiate hatching earlier than non-manipulated embryos. Nguyen et al. [3] were the first to achieve high in vitro survival of bovine somatic cell nuclear-transferred (SCNT) blastocysts cryopreserved by a combination of partial dehydration and vitrification. They used conventional 0.25-mL French straws as embryo containers and a vitrification solution consisting of 40% ethylene glycol (EG) + 18% Ficoll + 0.3 M sucrose (EFS40), originally reported by Kasai et al. [4]. Gong et al. [5] used the same EFS40 solution for vitrification of SCNT embryos and successfully produced a cloned calf following transfer of nine vitrified-warmed embryos. Another cloned calf has been delivered from a SCNT blastocyst vitrified in a solution consisting of 20% EG + 20% DMSO + 0.6 M sucrose, using the OPS system [6].

Removal of serum from the IVC medium for culturing presumptive zygotes improved the resistance of blastocysts to cryopreservation [7–9]. Abe et al. [9] reported that cytoplasmic lipid droplets were highly accumulated in the bovine morulae and blastocysts when the IVF zygotes were cultured in IVC medium that contained serum. Supplementation with the unsaturated fatty acid-conjugated BSA (linoleic acid–albumin, LAA), to the IVM and IVF media [10] and IVC medium [11–13] produced bovine zygotes and embryos resistant to the conventional two-step freezing procedure.

In contrast to the highly reproducible SCNT procedure in cattle, efficiency in producing SCNT buffalo embryos is not satisfactory [14,15], despite an increased demand for cloned buffaloes. We have previously reported that efficiency in producing SCNT blastocysts in swamp buffalo (19–22%) was approximately half of that in cattle (39–41%) [15]. On the other hand, both bovine- and buffalo-enucleated oocytes receiving buffalo fibroblasts equally developed into blastocysts (33 and 39%, respectively) [16]. There have been only a few reports on the cryopreservation of buffalo embryos [17–21], including the birth of calves after transfer of vitrified-warmed water buffalo IVF-derived embryos [21].