Vitrification of *in vitro* produced Zebu embryos

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Abstract

Survival and pregnancy rates achieved after traditional, slow-cooling-rate cryopreservation of *in vitro* produced Zebu (*Bos indicus*) embryos are generally poor. Vitrification is considered an alternative to traditional methods for preservation of embryos. The aim of the present experiment was to evaluate the addition of sucrose to vitrification medium and the influence of embryonic diameter on survival rates of *in vitro* produced Zebu embryos within each treatment. Oocytes recovered by ovum pick-up (OPU) were matured for 24 hours, fertilized with frozen-thawed spermatozoa from a Nelore bull, and cultured *in vitro* in controlled conditions (5% CO_2, 5% O_2, 90% N_2, and saturated humidity). Two treatments were used to evaluate the effect of sucrose addition to the vitrification medium. All of the embryos were measured before vitrification to evaluate the influence of diameter on survival rate after warming. Day 7, excellent-quality blastocysts and expanded blastocysts were equilibrated for 40 seconds in either 25% ethylene glycol (EG) and 25% dimethylsulfoxide (DMSO; Treatment 1; n = 30) or 20% EG, 20% DMSO, and 0.5 M sucrose (Treatment 2; n = 34) and then loaded into open pulled straws (OPS) and immersed into liquid nitrogen. At warming, the open end of the OPS was immersed into 0.5 M sucrose solution, and embryos were subsequently rehydrated in decreasing concentrations of sucrose. Re-expansion and hatching rates were determined at 24 and 48 hours after warming and culture, respectively. There were no differences in the rates of re-expansion (30.0% vs. 44.1%) or hatching (13.3% vs. 23.5%) of embryos that had been cryopreserved using either of the two treatments. No differences were observed between diameters of viable embryos after vitrification and those that did not survive after treatments. Although more studies should be carried out to improve the viability of *in vitro* produced Zebu embryos after cryopreservation, at the present time, vitrification is probably the best method for cryopreservation of sensitive embryos.

Keywords: cryopreservation, sucrose, embryo, bovine, vitrification, Zebu.