COMPARISON OF TWO VITRIFICATION PROTOCOLS FOR CROSSBRED *Bos indicus* X *Bos taurus* IN VITRO-PRODUCED EMBRYOS


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INTRODUCTION

Dairy herds in tropical countries are often maintained as crossbred *B. indicus* x *B. taurus* hybrids to take advantage of heterosis, such as resistance to heat stress. Creating crossbred *B. indicus* x *B. taurus* embryos by in vitro methods may offer a means of rapidly improving tropical dairy herds, especially if the embryos can be cryopreserved. The aim of this study is to compare the viability of in vitro-produced crossbred *B. indicus* x *B. taurus* embryos (1/2, 3/4) using two vitrification solutions and equilibration/dilution temperatures.

PROCEDURES

Cumulus oocyte complexes aspirated from ovaries of purebred *B. indicus* and crossbred (*B. indicus* x *B. taurus*) were *in vitro* matured and *in vitro* fertilized with a Holstein bull sperm. The presumptive zygotes were co-cultured with cumulus cells in CR2aa medium, supplemented with fetal calf serum (FCS), in 5% CO2, 95% of humidity and 38.8°C in air. Blastocyst stage embryos classified as good or excellent were vitrified using one of two vitrification solutions, a mixture of glycerol/ethylene glycol (GE) vitrification solution, or dimethylsulphoxide/ethylene glycol (DE) vitrification solution. Embryos (n=34) assigned to GE vitrification were equilibrated in a medium of PBS +20% FCS (HM1) containing 10% v/v G for 5 min., followed by 10% v/v G + 20% v/v E for 5 min., and then transferred to a vitrification solution of 25% v/v G + 25% v/v E in HM1 for 30 sec. The embryos were immediately aspirated into an Open Pulled Straw (OPS) and plunged into liquid nitrogen. Embryos vitrified in GE were warmed by immersing the OPS in HM1 containing 1M sucrose for 1 min (37°C), and then stepwise diluted in fresh HM1 containing 1M, 0.5M and 0.25M sucrose for 5 min., and finally washed in HM1. Stepwise equilibration and dilution of GE embryos were at 20°C. Embryos (n=43) assigned to DE vitrification were equilibrated in a medium of PBS + 5% FCS (HM2) containing 10% v/v D and 10% v/v E for 1 min, and then transferred to a vitrification solution of 20% v/v D + 20% v/v E in HM2 for 30 sec. The embryos were immediately aspirated into an Open Pulled Straw (OPS) and plunged into liquid nitrogen. Embryo vitrified in DE were warmed by immersing the OPS in HM2 containing 0.25M sucrose for 1 min (39°C), and then stepwise diluted in fresh HM2 containing 0.25M, 0.15M sucrose for 5 min, and finally washed in HM2. Stepwise equilibration and dilution of DE embryos was at 39°C. Diluted embryos from both groups and untreated control embryos (n=49) were cultured in TCM-199 with monolayer granulosa cells for 72 h. Blastocyst re-expansion and hatching was assessed and analyzed by Chi square test.

RESULTS and INTERPRETATION

Overall, 67% of the thawed embryos were expanded blastocysts (remainder were blastocysts) and 56% were excellent quality (remainder were good). No significant difference (P>0.05) was found between the rates of blastocyst re-expansion and hatching for the GE and DE vitrification procedures (Fig. 1). However the hatching rate of control embryos was significantly higher (P<0.05) than that of vitrified embryos (Fig 1). Some works have showed that in vitro produced bovine embryos are more susceptible to cryopreservation using standard methods than in vivo produced embryos (Enright et al., 2000; Massip, 2001), which may be due to their ultrastructural characteristics (Fair et al., 2001). Nevertheless, the result of the present work indicates that both vitrification procedures are promising for the cryopreservation of crossbred *B. indicus* x *B. taurus* in vitro produced embryos.

![Figure 1. Effect of two vitrification protocols on in vitro embryo survival after warming and embryo co-culture for 72 h. Columns with different letters differ significantly (P<0.05).](image)

REFERENCES


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