CALVES OBTAINED AFTER TRANSFER OF FROZEN-THAWED BOVINE IMMATURE OOCYTES CRYOPRESERVED IN VARIOUS CRYOPROTECTANTS

T. Suzuki, M. Takagi, and A. Boediono
United Graduate School of Veterinary Sciences,
Yamaguchi University, Yamaguchi 753, Japan

The objective of this study was to evaluate the fertilization and cleavage rates in vitro of frozen-thawed bovine oocytes at the germinal vesicle (GV) stage. Various permeating cryoprotective agents [1.8M ethylene glycol (EG), 1.3M ethylene glycol monomethyl ether (EME) and 1.6M 1,2-propanediol (PROH)] and different concentrations of trehalose (T) and polyvinylpyrrolidone (PVP) on post-thaw developmental capacity were examined. In the present study, the procedures for freezing the oocytes were described by Suzuki et al. (Theriogenology 40:651-659, 1993); and for maturation, fertilization and culture in vitro were described by Boediono et al. (Reprod. Fertil. Dev. 6:261-264, 1994). When bovine GV oocytes were frozen slowly in mixtures of 1.8M EG plus 5% PVP and 0.05M T, almost 80% of them developed to metaphase; 22% were degenerated after in vitro maturation, and none of those cryopreserved underwent parthenogenetic activation. The fertilization rate was higher ($P<0.05$) for oocytes frozen in a mixture of 1.8M EG plus 0.05M T or 0.1M T than in a mixture of 1.8M EG plus 0.2M T or without T (14/30, 47% and 16/30, 53% for 1.8M EG plus 0.05M T and 0.1M T vs 8/30, 27% and 10/30, 30% for 1.8M EG plus 0.2M T and without T, respectively). However, the number of oocytes that were normally fertilized or were polyspermic was not different. No significant differences were observed in subsequent development using EG, EME and PROH for GV oocytes. The addition of 0.05M or 0.1M of trehalose to the freezing solution yielded significantly better cleavage and blastocyst rates than the solutions containing 0.2M or no trehalose. Without frozen-thawed, GV oocytes yielded significantly higher ($P<0.01$) cleavage and blastocyst rates compared to frozen-thawed GV oocytes (83/100, 83% and 38/83, 46% vs 68/150, 45% and 3/68, 4%). We found that 5% PVP had a more beneficial effect as compared to either 10% or 20% PVP for the development of blastocysts (16/80, 20%; 4/42, 10% and 0/18, 0% for 5%, 10% and 20% PVP).

Transfer of six blastocysts derived from frozen-thawed GV oocytes into 3 recipient heifers (2 embryos each) resulted pregnancies in all recipients at 60 days. The twin and single calves were delivered from two recipients. One recipient resulted in abortion by day 92.