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The Influence of Polyvinylpyrrolidone on Freezing of Bovine IVF Blastocysts Following Biopsy

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A study was conducted to develop a better freezing protocol for *in vitro* developed biopsied bovine blastocysts. Biopsied blastocysts were exposed to 1.8 *M* ethylene glycol (EG) + 0.05 *M* trehalose (T) and different concentration (5, 10, and 20%) of polyvinylpyrrolidone (PVP). Exposure to the solutions alone did not affect their *in vitro* development (Experiment 1). Experiments 2, 3, and 4 tested the viability of biopsied blastocysts cryopreserved in 1.8 *M* EG + different concentrations of T (0, 0.05, 0.1, and 0.3 *M*), 1.8 *M* EG + different concentrations of PVP (0, 5, 10, and 20%), and 1.8 *M* EG + 0.05 *M* T + different concentrations of PVP (0, 5, 10, and 20%), respectively. The proportion of biopsied blastocysts that reexpanded following cryopreservation in 1.8 *M* EG + 0.05 *M* T (38.5%) and 1.8 *M* EG + 0.1 *M* T (36.1%) was significantly ($P < 0.05$) higher than the proportion that reexpanded in 1.8 *M* EG + 0.3 *M* T (13.9%) (Experiment 2). The viability and the percentage of embryos that developed to >250 μm in diameter in the 5, 10, and 20% PVP groups (77.8 and 50.0%, 78.1 and 43.8%, 76.9 and 65.4%, respectively) were significantly higher than those that developed cryopreserved without PVP (55.1 and 20.7%) (Experiment 3). Optimum development of *in vitro* culture of frozen–thawed biopsied blastocysts was obtained using 1.8 *M* EG + 0.05 *M* T and 20% PVP. Analysis of blastocysts >250 μm in diameter showed that the number of ICM cells of biopsied blastocysts cryopreserved in 1.8 *M* EG + 0.05 *M* T with or without PVP was not different from the number of unfrozen biopsied blastocysts. These results indicate that PVP has some beneficial effect on freezing of biopsied bovine blastocysts.

At present, the most practical and efficient livestock embryo-sexing method is the one utilizing the polymerase chain reaction (PCR) using Y-specific primers (14). In the context of bovine embryo sexing, PCR has been used to amplify male-specific DNA from embryo biopsies with a successful amplification product indicating a male biopsy (embryo) (2, 7, 14, 18, 19, 25). Since microsurgical techniques are required to obtain blastomeres from the embryo and around 4 h is required to obtain the test results by PCR, it would be desirable to keep these biopsied embryos at −196°C before transfer. However, results to date indicate that the viability of the biopsied or bisected bovine embryo is reduced after cryopreservation (22). In particular, bisection of frozen–thawed bovine embryos resulted in decreased survival (13, 24). The use of bovine embryo bisection in conjunction with sexing has been reported (3, 19), but there is little information about the viability of frozen–thawed IVF bovine embryos without a zona pellucida following biopsy. Embryos frozen in ethylene glycol (EG) or propylene glycol (PG) can be rehydrated directly in holding medium without stepwise dilution of the cryoprotectant (21, 26). However, even for such permeable cryoprotectants as EG and PG it may be necessary to include a low concentration of sugar in the holding medium to protect the embryos from osmotic shock. Recently, Leibo and Oda (10) reported that polyvinylpyrrolidone (PVP) was effective for mouse embryo freezing. The present study was aimed at developing a freezing protocol for *in vitro* developed biopsied blastocysts without a zona pellucida and evaluating the viability of these biopsied blastocysts following freezing in different cryoprotectant solutions.

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MATERIALS AND METHODS

In Vitro Blastocyst Production

Ovaries were obtained from a local slaughterhouse. Oocytes with a compact cumulus were cultured for 20 to 22 h (38.5°C, 5% CO_2 in air) in medium-199 (TCM-199 with Earle's salts, L-glutamine, 2.200 mg/ml sodium bicarbonate,

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