THE EFFECT OF THREE CRYOPROTECTIVE AGENTS ON ZONA-INTACT AND ZONA-FREE MOUSE EMBRYOS USING THE QUICK FREEZING METHOD

Othman Abas Mazni

Department of Theriogenology
Faculty of Veterinary Medicine
Hokkaido University, Sapporo 060, Japan

The effect of various concentrations of glycerol, ethylene glycol and dimethylsulphoxide (DMSO) in the presence of either 0.25M lactose or sucrose on the survival of frozen-thawed zona-intact and zona-free embryos was studied. The quick freezing method in which embryos were directly transferred to liquid nitrogen vapor at – 170°C before being plunged in liquid nitrogen was used.

High survival rates of frozen-thawed zona-intact embryos were observed after 48 hours of *in vitro* culture when the freezing medium contained 3M ethylene glycol and 0.25M lactose (76.5%) or 3M glycerol and 0.25M sucrose (73.5%). As for zona-free embryos, survival rates of 62.7% and 60.8% were observed when freezing mixture of either 2M glycerol or 3M ethylene glycol with 0.25M lactose were used, respectively. However, when sucrose was used, a significantly lower survival rate of zona-free embryos was observed ($P<0.05$). A significant difference in the survival rates between zona-intact and zona-free embryos was observed when 3M glycerol was used in the presence of either 0.25M lactose or sucrose ($P<0.05$). Similarly, a considerable difference was observed when 2M and 3M ethylene glycol were used with 0.25M lactose and sucrose, respectively. However, low viabilities of frozen-thawed zona-intact and zona-free embryos were obtained with DMSO. This study indicated that the effect of these cryoprotective agents varied with its concentration and the type of sugars used.

Zona-free decompacted morulae were bisected using a fine glass needle and cultured between 8 to 48 hours before they were frozen using 2M glycerol and 0.25M lactose. However, none of the frozen-thawed bisected embryos developed to eublastocysts after 48 hours of *in vitro* culture.