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

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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.