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EFFECTS OF PRECOOLING AND EQUILIBRATION TIME ON THE SURVIVAL OF MOUSE EMBRYOS CRYOPRESERVED BY VITRIFICATION

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Experiments were carried out to examine the effects of precooling the extracellular vitrification (EV) medium and equilibration time on the post-thaw survival of mouse 8–cell embryos, compacted morulae and blastocysts that were cryopreserved by vitrification.

Vitrification was achieved using an intracellular vitrification medium (IV; 10% glycerol+20% propylene glycol) and EV medium (25% glycerol+25% propylene glycol) in Dulbecco's phosphate-buffered saline +10% calf serum. Embryos from each embryo-stage group were equilibrated in the IV medium for 5, 10, or 20 min at room temperature (20°C). They were then pipetted and exposed to either precooled (4°C) or nonprecooled (20°C) EV medium (20 μl) within a 0.25 ml straw before being plunged directly into liquid nitrogen.

For 8–cell embryos, the highest survival rate was obtained when embryos were exposed to precooled EV medium rather than nonprecooled EV medium (77.8% vs. 50%, $p<0.01$) after 5–min equilibration in the IV medium. However, after 10–min equilibration in the IV medium, similar survival rates were observed when the embryos were exposed to nonprecooled or precooled EV media (73.4% and 72.6%, respectively). Compacted morulae showed the highest survival when the embryos were exposed to either precooled or nonprecooled EV medium (90.9% and 92%, respectively) after 10–min equilibration in the IV medium. The highest survival rate for blastocyst-stage embryos was obtained when embryos were exposed to precooled EV medium rather than nonprecooled EV medium (71.8% vs 42.9%, $p<0.01$) after 10–min equilibration in the IV medium.

Furthermore, for all developmental stages, the post-thaw embryo survival rates tended to decrease with increased length of equilibration in the IV medium, i. e. after 20 min.

The percentage of normal fetuses obtained after transfer to pseudopregnant recipients of vitrified-thawed compacted morulae exposed to either precooled or nonprecooled EV medium was the same (74.1%), and the latter did not differ from the percentage of normal fetuses achieved with the transfer of fresh (control) embryos (73.6%).

This study confirms the simplicity and efficiency of the vitrification technique for the cryopreservation of mouse embryos.