STUDIES ON THE EFFECT OF VARIOUS CRYOPROTECTIVE AGENTS ON THE SURVIVAL OF FROZEN-THAWED MOUSE EMBRYOS

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The effects of various cryoprotective agents, namely dimethyl sulfoxide, methyl alcohol, ethylene glycol, glycerol, erythritol, adonitol and xylitol, on the survival of frozen-thawed mouse embryos were examined. The effects of the stage of development and the dilution method of cryoprotective agent were also examined.

Eight-cell embryos, morulae, early blastocysts and mid-blastocysts were recovered from superovulated immature ddY mice. Cryoprotective agent was added to the embryos in 5 equal increments at 5 minutes apart, giving a final concentration of 1.5 M. The embryos were cooled to −6°C at 1°C/min and seeding was induced at −6°C. After being held for 5 minutes at the seeding temperature, the samples were cooled to −35°C at 0.3°C/min and then transferred into liquid nitrogen.

Rapid thawing was done by placing the straws in a 37°C water bath. The cryoprotectants were diluted in one of two ways: (a) embryos were pipetted into phosphate buffered saline (PBS) containing 0.5 M sucrose and were kept there for 5 minutes before transferring them into PBS (sucrose method), or (b) they were directly pipetted into PBS (PBS method).

In the present experiment, methyl alcohol and adonitol were found to be less effective as compared to ethylene glycol, glycerol and DMSO. With the use of erythritol or xylitol, the embryos could survive only when the sucrose method was carried out.

Significant difference in the survival rate among the four stages of development was observed when glycerol, erythritol, xylitol or DMSO were used.

In this study, it was observed that the sucrose method was superior to the PBS method, especially when erythritol or xylitol was used. The same tendency was observed with the other cryoprotective agents, except for ethylene glycol, which was effective in both methods.