Vitrification of mouse oocytes and embryos using ethylene glycol as the sole cryoprotective agent

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A study was made to determine if the vitrification of mouse oocytes and embryos can be simplified by using ethylene glycol as the sole cryoprotective agent. In study 1, ethylene glycol was used in a simple solution to vitrify mouse 8-cell embryos and the effect of the temperature at exposure before vitrification was determined. The results showed that the minimum concentration of ethylene glycol that vitrified on cooling was 7 M and that the vitrification of mouse 8-cell embryos can be achieved by exposure to 2 M ethylene glycol for 2 to 10 min and in 7 M ethylene glycol for 2 min at 18 to 22°C temperature. The exposure of the embryos to the vitrification solution at temperatures over 24°C was detrimental to their development *in vitro*.

In study 2, mouse zygotes were vitrified in 7 M ethylene glycol and the effect of renewing the culture medium on the *in vitro* viability of vitrified embryos was determined. The results showed that without medium change, exposure to ethylene glycol for 1 to 5 min at 18 to 22°C had no harmful effect but vitrification had a detrimental effect on the viability of vitrified zygotes to the expanded blastocyst stage ($p<0.01$). Study 2 also showed that mouse zygotes can be vitrified after exposure to 7 M ethylene glycol in 1 or 2 min and that medium change improves their viability *in vitro*.

In study 3, the one- or two-step method of exposure before vitrification with 7 M ethylene glycol in PB1 was used to vitrify mouse oocytes. The effects of the duration of dilution of ethylene glycol (5 vs. 10 min) with 1 M sucrose solution and the addition of 0.5 M sucrose to the vitrification solution were also determined. Study 3 showed that oocytes can be vitrified with 7 M ethylene glycol and the viability of oocytes after one-step exposure before vitrification can be improved by 10 min dilution time. The addition of 0.5 M sucrose did not improve the viability of oocytes vitrified in one step and diluted for 10 min.

This study has shown that the vitrification of mouse oocytes and embryos can be simplified using ethylene glycol as the sole cryoprotective agent.

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