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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目：
Title of Dissertation Studies on equilibrium vitrification of oocytes and embryos using low concentrations of cryoprotectants
(低濃度の耐凍剤を用いた卵子と胚の平衡ガラス化凍結法に関する研究)

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With original slow freezing, embryos are equilibrated with a solution containing low concentrations of cryoprotectants and are cooled slowly to -70°C before being cooled in liquid nitrogen (LN_2). They are dehydrated sufficiently and cryopreserved in a near-equilibrium state. Therefore, frozen embryos can be warmed slowly and transported with dry ice. In conventional vitrification, oocytes/embryos are vitrified in non-equilibrium state with high concentrations of glass-inducing solutes. Therefore, the embryos cryopreserved by vitrification must be stored in LN_2 and warmed very rapidly. To enable handy transportation of vitrified embryos with dry ice, we developed equilibrium vitrification, named EFS35c (2010, 2012). This method yielded high survival rates in mouse embryos at various stages. However, the high toxicity and osmolality caused by high concentrations of cryoprotectants could damage embryos. In addition, the method is not effective for vitrification of mouse oocytes. In the present study, we developed a new equilibrium vitrification method by which embryos are vitrified in a near equilibrium state using a solution containing low concentrations of cryoprotectants and thus with relatively low toxicity and osmolality.

We used two-cell embryos to develope new method. First, we vitrified embryos with 1-step or 2-step protocol in LN_2, and then kept at -80°C for various period. The survival and the developmental ability into blastocysts were higher when using 2-step. Next, we examined the optimum protocol for embryos vitrified in LN_2 and then kept at -80°C for 7 days. When embryos were pretreated with ED5/5 at 25°C for 2 min and treated with EDFS10/10a at 25°C for 1 or 0.5 min, vitrified in LN_2, and kept at -80°C for 4, 7, and 28 days, the survival (96-100%) and developmental ability (54-85%) were relatively high. Next, we assessed the developmental ability by *in vivo* using C57BL/6J mice. There were no significant differences in birth rates between vitrified and fresh embryos. Therefore, the vitrified 2-cell mouse embryos with EDFS10/10a had the ability to develop to term. Therefore, this new method is effective for vitrifying 2-cell mouse embryos.

Next, we examined whether this method is useful for embryos at other stages. When 4- and 8-cell mouse embryos to vitrified with this method and then kept at -80°C for 4, 7, and 28 days. The survival rates (97-100%) and the developmental ability *in vitro*

(68-89%) were quite high and no significant different with unvitrified control groups. This indicated that embryos at these stages were vitrified in a near-equilibrium state. However, when morulae vitrified with this method and then kept at -80°C, the survival decreased markedly (19-55%).

When blastocysts were vitrified and then kept at -80°C for 4 days, all of blastocysts were dead. This is would be due to the blastocoel, which contains a large amount of water and promotes the formation of ice during cooling/warming. After collapse of the blastocoelic cavity with a glass pipette, and then kept at -80°C for 4, 7, and 28 days. The survival rates were quite high (65-90%). Therefore, this method is effective for mouse blastocysts.

Finally, we examined whether this method is effective for mouse oocytes. The viability of oocytes vitrified with EDFS10/10a and then kept at -80°C for 4, 7, and 28 days were quite high. The rates of survival, fertilizations and ability to develop into blastocyst were also high (88-99%, 80-90% and 55-70%, respectively).

In conclusion, we developed a new equilibrium vitrification method using low concentrations of cryoprotectants (EDFS10/10a). This new method is effective for oocytes, 2-, 4-, 8-cell embryos and blastocysts of mouse. Further studies are needed to examin whether this method is effective for other species.