

## Comparisons of Three Different Cryoprotectants for Freezing of Mouse Sperm

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The cryopreservation of sperm has been greatly contributed to animal breeding and reproduction. This study was examined to affect of raffinose, sucrose, or trehalose as cryoprotectant for freezing of mouse sperm. These cryoprotectants were supplemented in freezing solution contained with 3% skim milk for freezing of mouse sperm. Semen samples for cryopreservation were collected from four mouse strains (ICR, FVB, C57BL/6 and CBA). Sperm samples from individual males were distributed in aliquots of cryotubes. The cryotubes were immediately placed in the liquid nitrogen vapor phase of a storage container for 10 min before storage under liquid nitrogen. For thawing, the frozen cryotubes were removed from liquid nitrogen and put into a water bath kept at 37°C for approximately 2 min until the ice melted. Survival rate of mouse sperm was measured by vital staining. The survival rates (ICR : 51.0%, FVB : 27.6%, C57BL/6 : 25.7% and CBA : 23.3%) of frozen-thawed sperms in freezing solution supplemented with raffinose were higher than those supplements with sucrose (35.8, 19.6, 12.3 and 19.7%) or trehalose (16.0, 25.4, 25.3 and 24.7%). After *in vitro* fertilization with frozen-thawed sperms in freezing solution supplemented with raffinose, the cleavage rates were 61.1 (FVB : 217/355 eggs), 59.4 (C57BL/6 : 165/278 eggs), and 57.1 (CBA : 144/252 eggs)%, respectively. Moreover, these cleaved embryos (FVB : 163, C57BL/6 : 137 and CBA : 134) were transferred into each 7, 5, and 6 pseudopregnant females, and these pseudopregnant females were successfully showed each 4, 3, and 4 pregnant females and delivered 17 (10.4%), 17 (12.4%) and 18 (13.4%) offspring, respectively. Therefore, this result suggested that raffinose might be a good cryoprotectant for freezing of sperm for production of inbred mouse.