



# HOKKAIDO UNIVERSITY

Title	QUICK FREEZING OF MOUSE AND BOVINE BLASTOCYSTS WITH GLYCEROL AND SUCROSE
Author(s)	KISHI, Masao
Citation	Japanese Journal of Veterinary Research, 38(2), 61-61
Issue Date	1990-07-20
Doc URL	<a href="https://hdl.handle.net/2115/3208">https://hdl.handle.net/2115/3208</a>
Type	departmental bulletin paper
File Information	KJ00002377361.pdf



## QUICK FREEZING OF MOUSE AND BOVINE BLASTOCYSTS WITH GLYCEROL AND SUCROSE

Masao KISHI

*Department of Theriogenology  
Faculty of Veterinary Medicine  
Hokkaido University, Sapporo 060, Japan*

The effects of glycerol and sucrose concentrations, and the duration and temperature of equilibration on the post-thaw *in vitro* survival of mouse blastocysts cryopreserved by the quick-freezing method (Takahashi, & Kanagawa, 1985) were studied. In addition, the osmolalities of the cryoprotectants were measured and the osmotic behaviour of mouse blastocysts during equilibration was examined. The highest survival rates of early blastocysts quickly frozen in 3M glycerol with 0, 0.125, 0.25 and 0.5M sucrose at 25°C were obtained after equilibration for 2, 2, 0.5 and 5 min (68.4, 74.3, 86.5 and 19.5%, respectively). It was observed that the survival rates of blastocysts frozen in 3M glycerol in combination with any concentration of sucrose were lower than 50.0%, and significantly lower compared to that of early blastocysts. In both embryo stages, the survival rates obtained at any equilibration time in 3M glycerol with 0.5M sucrose at 25°C were very low. It was further observed that the survival rates decreased with the increase in the duration of equilibration (up to 10 min). When blastocysts were frozen in 2 and 4M glycerol with 0.125 and 0.25M sucrose, respectively, low survival rates were obtained compared to those frozen in 3M glycerol with the same concentrations of sucrose. A significantly higher survival rate for frozen-thawed blastocysts was obtained when the embryos were equilibrated in 3M glycerol with 0.25M sucrose at 3°C rather than 25°C (66.7% and 46.7%, respectively,  $P < 0.05$ ). Results from this study indicated that the concentrations of glycerol and sucrose, as well as the duration and temperature of equilibration significantly affect the survival of frozen-thawed mouse blastocysts. Exposure of mouse blastocysts to freezing medium resulted in an initial reduction in mean volume due to the exit of water because of the initial hyperosmoticity of the cryoprotectant that was then followed by a gradual increase in size due to the permeation of glycerol. The quick freezing of bovine embryos was also attempted with 7 blastocysts using 3M glycerol with 0.125 or 0.25M sucrose. However, no embryo survival was obtained. Therefore, the optimal conditions for quick freezing of bovine blastocysts should be further investigated.