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## Establishment of a transport system for mouse epididymal sperm at refrigerated temperatures.

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### Abstract

The exchange of genetically engineered mouse strains between research facilities requires transporting fresh mouse sperm under refrigerated temperatures. Although sperm generally maintains fertility for 48 hours at cold temperatures, in vitro fertilization rates of C57BL/6 mouse sperm are low after 48-hour cold storage. Furthermore, 48 hours is often not sufficient for the specimens to reach their destinations. To increase the availability of this technology, we aimed to extend the cold storage period while maintaining sperm fertility. In this study, we determined the optimal medium for sperm preservation and evaluated the effect of reduced glutathione in the fertilization medium on sperm fertility after cold storage. We found that higher fertility levels were maintained after 72-hour cold storage in the preservation medium Lifer compared with storage in paraffin oil, M2 medium, or CPS-1 medium. In addition, 1.0 mM glutathione enhanced sperm fertility. After transporting sperm from Asahikawa Medical University to our laboratory, embryos were efficiently produced from the cold-stored sperm. After transfer, these embryos developed normally into live pups. Finally, we tested the transport system using genetically engineered mouse strains and obtained similar high fertilization rates with all specimens. In summary, we demonstrated that cold storage of sperm in Lifer maintains fertility, and glutathione supplementation increased the in vitro fertilization rates of sperm after up to 96 hours of cold storage. This improved protocol provides a simple alternative to transporting live animals or cryopreserved samples for the exchange of genetically engineered mouse strains among research facilities.

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PMID: 22722060 [PubMed - as supplied by publisher]

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