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Reproductive Technology

Oocyte Activation after Intracytoplasmic Injection with Sperm Frozen Without Cryoprotectants Results in Live Offspring from Inbred and Hybrid Mouse Strains

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Re-establishment of mouse strains used for mutagenesis and transgenesis has been hindered by difficulties in freezing sperm. The use of intracytoplasmic sperm injection (ICSI) enables the production of embryos for the restoration of mouse lines using sperm with reduced quality. By using ICSI, simplified sperm-freezing methods such as snap freezing can be explored. We examined the capacity of embryos from the inbred C57Bl/6J and 129Sv/ImJ mouse strains, commonly used for transgenic and *N*-ethyl-*N*-nitrosourea mutagenesis purposes to develop to blastocysts in vitro and to term following ICSI with sperm frozen without cryoprotectant. The results were compared to F1 (C57Blx6J) hybrid embryos. Following freezing, sperm were immotile but could fertilize oocytes at similar rates to fresh sperm. However, embryo development in vitro to the blastocyst stage was reduced in all three strains. No pups were born from C57Bl/6J or 129Sv/ImJ embryos obtained from frozen sperm following transfer to foster females, and only a limited number of F1 embryos developed to term. Activation of oocytes injected with frozen sperm with 1.7 mM Sr²⁺ (SrCl₂) did result in the birth of pups in all three strains. We conclude that the inability of sperm frozen without cryoprotectants to effectively activate oocytes may affect embryo development to term and can be overcome by strontium activation. This may become an effective strategy for sperm preservation and the restoration of most popular strains

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used for genetic modifications.

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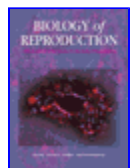
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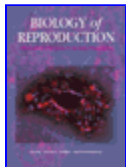
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