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The Cryodropper: A Novel Device for Mouse Embryo and Sperm Vitrification

Barbara J Stone and Sarah J Srodulski
ParaTechs Corporation, Lexington, Kentucky, USA

Abstract

Cryopreservation of animal models as germplasm is ubiquitously favored by the biomedical research community for its ability to safeguard against catastrophic animal loss, provide effective vivarium management options, and as a convenient method to transport and share animal models.

A novel tool for rodent embryo and sperm cryopreservation, the Cryodropper has a user-friendly design and employs simple but effective vitrification and thaw protocols. Embryos or sperm are vitrified in the device along with compatible thaw medium in liquid Nitrogen vapor. Thawed germplasm is dispensed from the device by flushing with thaw medium allowing quick and complete recovery of material. By providing compatible thaw medium in the device, errors and confusion are mitigated during material retrieval. The system is compatible with standard storage and shippers commonly used for world-wide distribution, providing convenience for researchers and facilities.

CD1 mouse sperm cryopreserved in the Cryodropper recovered motility as well as sperm cryopreserved in conventional sperm straws. In vitro fertilization (IVF) was used to determine sperm viability of samples vitrified in Cryodroppers and was not statistically different from IVF using fresh sperm samples. For embryos vitrified in the Cryodropper, development of thawed 2-cells to blastocyst stage was assessed in tissue culture as a measure of embryo health. 91% of embryos developed to blastocyst stage.

These data indicate that germplasm vitrified in the Cryodropper are viable for embryo culture and IVF with further applications in rodent assisted reproductive techniques.

Methods

Embryo Vitrification

2-cell embryos were collected from the oviducts of >8 week old female CD1 mice at 1.5 days post coitum after mating with CD1 male mice. Embryos were flushed with M2 medium (Millipore Sigma, St. Louis, MO, USA). The embryo Cryodropper was preloaded with 200µl embryo recovery medium in the bulb (0.5M sucrose in M2) and 2µl drop of vitrification media in the center of the pipette portion. Embryos for cryopreservation were transferred from M2 to pre-vitrification media (10% ethylene glycol, 10% DMSO, 80% M2) and vitrification media (15% ethylene glycol, 15% DMSO, 10% M2, 60% FS (Ficoll PM70 and sucrose) at 30 second time intervals as previously described[1]. Embryos were then loaded into the vitrification drop in the Cryodropper. The Cryodropper was sealed with a heat sealer and plunged directly into liquid Nitrogen. The Cryodropper was stored in a 4ml cryovial (USA Scientific, Ocala, FL, USA) in liquid Nitrogen vapor for at least 3 weeks.

Embryo Thaw

The Cryodropper was immersed in a 37˚C water bath until the media in the dropper was thawed (@10 sec). With scissors, the tip of the Cryodropper was cut off and the embryos deposited onto a culture dish. The embryo recovery medium was gently tipped from the dropper to the pipette and expelled into the embryo drop, minimizing bubbles. The embryos were immediately collected from the drop, transferred to a new drop of recovery medium, and incubated for 2 min. Embryos were then transferred to a drop of M2 medium containing 0.25 M sucrose incubated for 2 min, followed by incubation in M2 for at least 1 min. Embryos were then cultured in KSOM medium (Millipore Sigma) under oil at 37°C with 5% CO_2 for 2 days (until development to blastocyst stage for healthy embryos).

Sperm Vitrification

Sperm was collected from the cauda epididymides of > 8 week old male CD1 mice with proven fertility. Vitrified samples were prepared from 2 donors to reduce variability between samples. The sperm Cryodropper was preloaded with 90µl of preincubation medium (TYH medium with 0.75mM methyl-β-cyclodextrin, pre-gassed in 5% CO_2) in the bulb. Cryopreservation, thaw, and IVF procedures were performed as described[2]. Sperm was released into 120µl of cryoprotectant medium (18% raffinose, 3% skim milk, 100mM L-Glutamine) at 37˚C for 3 minutes. Using a gel loading pipette, 10µl sperm suspension was loaded into the center of the pipette portion of the Cryodropper and sealed. The Cryodropper was vitrified in the vapor phase of liquid Nitrogen. The Cryodroppers were stored in 4ml cryovials in liquid Nitrogen vapor for at least 3 weeks.

Methods (continued)

Sperm Thaw

The Cryodropper was immersed in a 37˚C water bath for 10 min. With scissors, the tip of the Cryodropper was cut off and the sperm deposited onto a Falcon IVF culture dish (Corning, Corning, NY, USA). The preincubation medium was gently tipped from the dropper to the pipette and expelled into the sperm drop, minimizing bubbles. The sperm was then incubated at 37°C with 5% CO_2 for 45 min to capacitate.

IVF

Female CD1 oocyte donors were superovulated with 5IU pregnant mare serum gonadotropin and human chorionic gonadotropin (Prospec, Ness Ziona, Isreal) at 47-48hr intervals. Oocytes were collected from oviducts 15-17hrs after the last injection into fertilization medium (Human Tubal Fluid medium with 5.14mM calcium and 1mM or 0.25mM reduced glutathione, for frozen or fresh sperm, respectively). 10µl sperm suspension was added to the oocytes and incubated for 4 hours. Presumptive fertilized embryos were collected, washed and incubated in KSOM medium at 37°C with 5% CO_2 for 24hrs. 2-cell stage embryos were counted and % fertilization is indicated as the number of 2-cell embryos recovered per oocytes collected.

Results and Conclusions

Embryo Vitrification and Thaw

CD1 embryos have an 90.8% ± 8.1 development rate using the Cryodropper (N=110), indicating that thawed embryos are healthy.

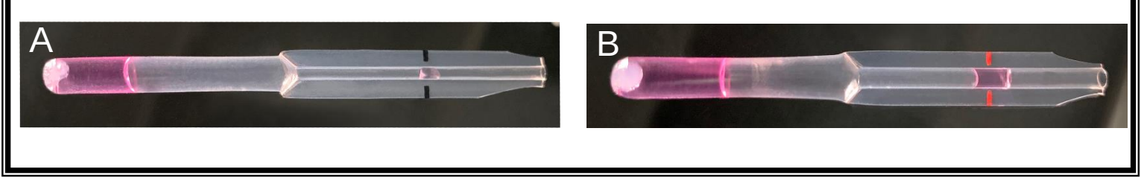
Therefore the embryo Cryodropper (Figure 1 A) is suitable for vitrification and recovery of mouse 2-cell embryos.

Sperm Vitrification and Thaw

Vitrification samples (N=7) and fresh sperm samples (N=3) were compared for % fertility by IVF assay. Sperm vitrified in the Cryodropper were able to fertilize oocytes as well as fresh sperm for CD1 mice (49% ± 21.4 and 46% ± 20.1, respectively) and at rates comparable to those found in the literature[3].

Therefore the sperm Cryodropper (Figure 1 B) is suitable for vitrification and recovery of mouse sperm.

Figure 1. The Cryodropper. The embryo Cryodropper (A) and sperm Cryodropper (B).



References

[1]Tsang WH, Chow KL. 2009. Mouse embryo cryopreservation utilizing a novel high-capacity vitrification spatula. BioTechniques 46:550-552. PMID:19594455.

[2]Behringer R, Gertsenstein M, Vintersten K, Nagy A. 2014. Manipulating the mouse embryo: a laboratory manual, 4thed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press.

[3]Vasudevan K, Raber J, Sztein J. 2010. Fertility comparison between wild type and transgenic mice by in vitro fertilization. Transgenic Res. 19(4):587-94. PMID:19844803.

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