Veterinary Research Scholars Program
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Cryosurvival and fertility of mouse cumulus oocyte complexes by using a Cryoloop method

Lyndsy Gieche, Cansu Agca, Liga Wuri, and Yuksel Agca

Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Columbia, Missouri

College of Veterinary Medicine
University of Missouri

Introduction:

- Embryo and gamete cryopreservation is important to preserving rodent strains used as models for animal and human disease research.
- Cryopreservation of mammalian oocytes is less successful than that of sperm and embryos.
- Increased oocyte cryopreservation success would provide more complete genome banking of genetically modified rodent strains.

Objectives:

- To improve oocyte cryopreservation success by comparing mouse oocyte cumulus complexes (COCs) cryo-survival and post-thaw fertility rates between a vitrification procedure with a Cryoloop versus a 0.25 mL French straw.

Materials and Methods:

- **Animals and COCs collection:** Outbred CD-1 mice (n=22) were superovulated by an intraperitoneal injection of 10 IU PMSG, and a 10 IU HcG injection 48 hrs later.
- **Vitrification and thawing procedure:** COCs were exposed to PBS containing 15% ethylene glycol (EG) + 20% fetal calf serum (FCS) for 5 min. The COCs were then transferred into PBS containing 30% EG + 0.5 mol/L sucrose + 20% FCS for 1 min, loaded into Cryoloop or 0.25 mL French straw (8 per method) and immediately plunged into liquid nitrogen (LN_2) for vitrification. The vitrified COCs were thawed by transferring them into PBS containing 0.5 M sucrose and 20% FCS for 5 min at 37°C.
- **Post-thaw viability evaluation:** COCs were transferred into mouse embryo culture medium (FHM) containing 1 mg/mL hyaluronidase to remove cumulus cells surrounding the oocytes. Numbers of oocytes with intact and damaged oolemma were recorded based on morphological evaluation.
- **In-vitro fertilization (IVF) and embryo culture (IVC):** An IVF procedure was performed to compare the fertility of COCs vitrified by a Cryoloop method versus freshly collected COCs using fresh epididymal sperm. IVC were performed in KSOM media to determine developmental competence of Cryoloop-vitrified COCs up to blastocyst stage.
- **Statistical Analysis:** The data was analyzed by the general linear model procedure of SAS. The experiments were repeated at least six times for each experiment.

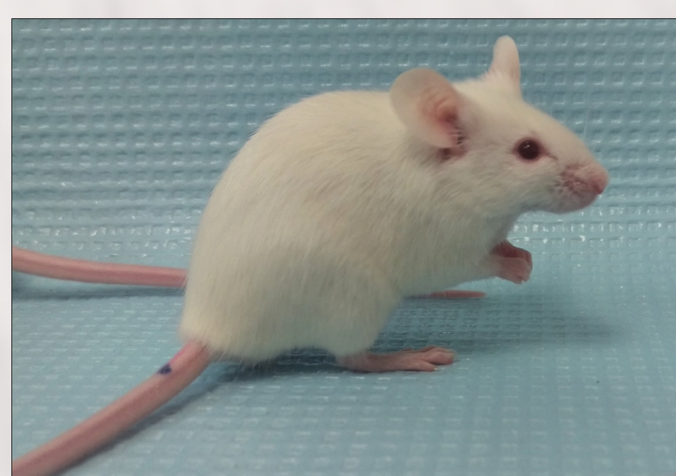

Figure 1: Outbred CD-1 mice

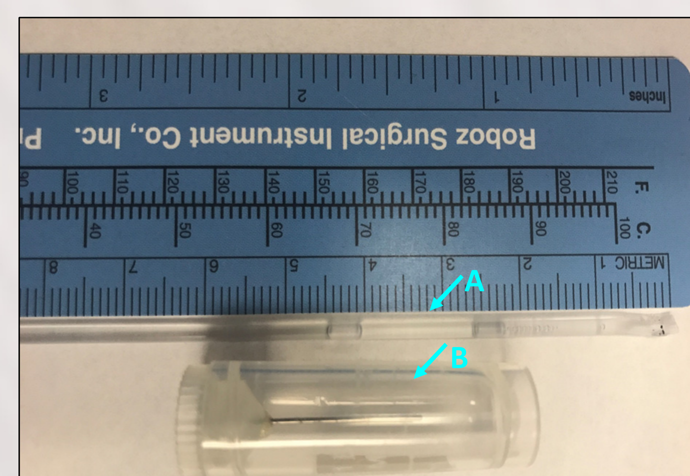

Figure 2: Arrow A shows cryoprotectant solution inside a 0.25 mL French straw; Arrow B shows a Cryoloop, approximately 2 cm long, inside the Cryoloop chamber, approximately 4 cm long by 1.5 cm in diameter

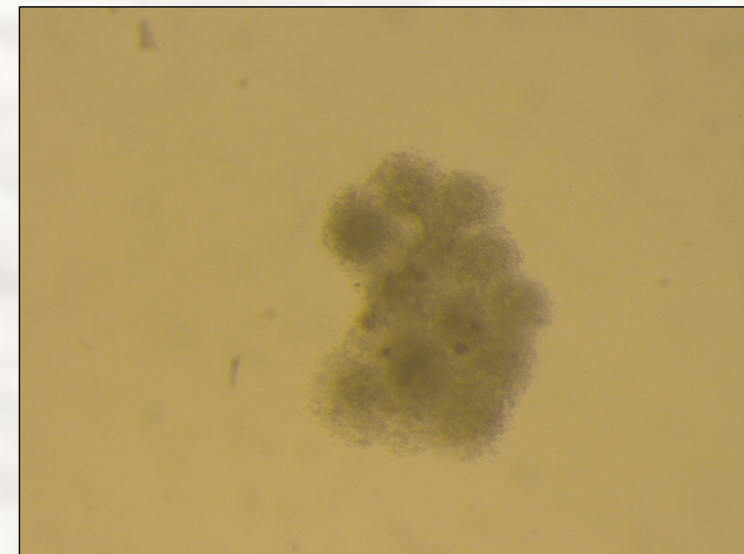

Figure 3: Mouse cumulus oocyte complex

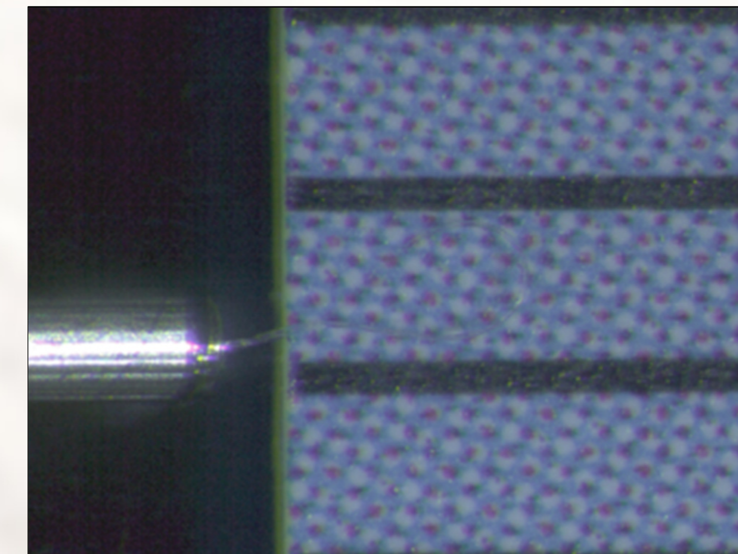

Figure 4: Nylon loop of Cryoloop over mm portion of a ruler; loop diameter is approximately 1 mm wide

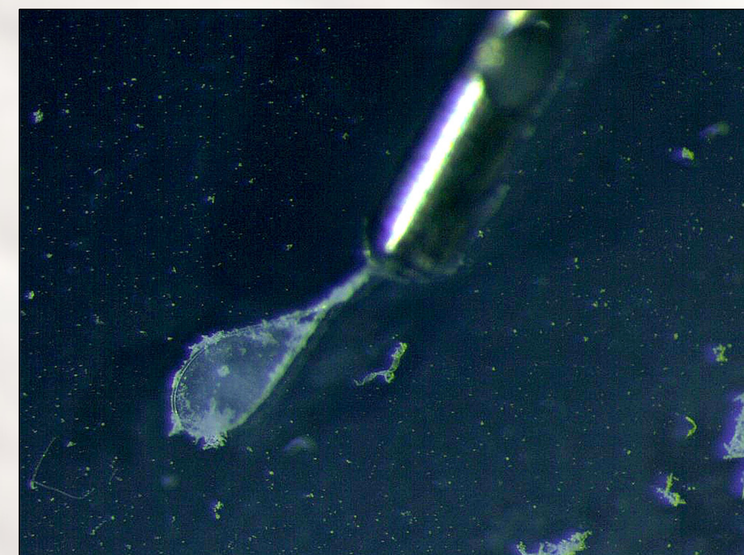

Figure 5: Demonstration of ice crystal formation of a water droplet in a Cryoloop plunged into LN_2

Figure 6: Vitrification achieved with a droplet of cryoprotectant in a Cryoloop plunged into LN_2

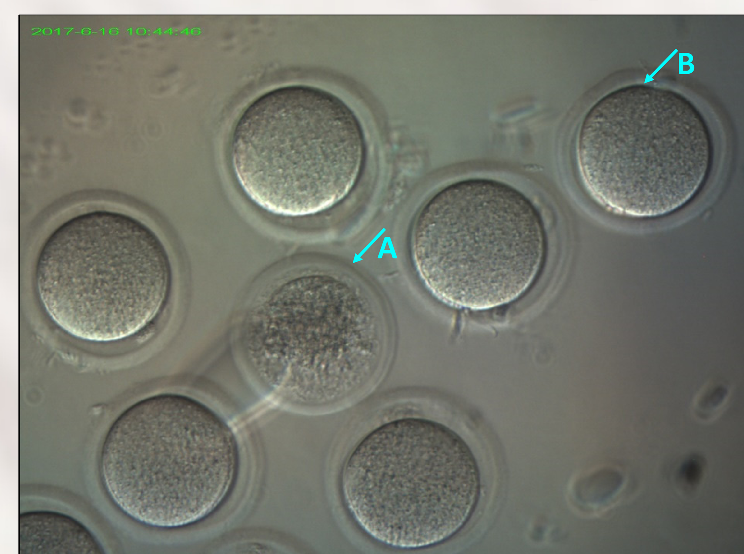

Figure 7: Mouse oocytes after vitrification and thawing of COCs with the Cryoloop method; Arrow A shows a dead oocyte, determined by the lack of oolemma integrity and cytoplasmic content; Arrow B shows a viable oocyte with intact oolemma

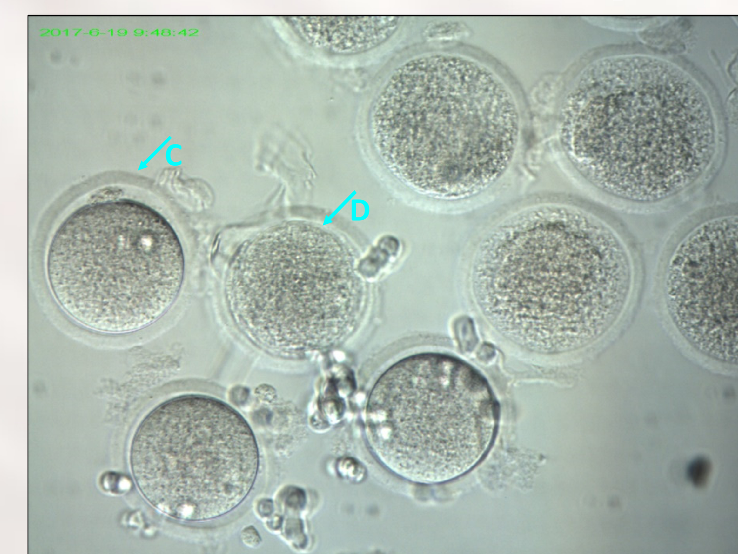

Figure 8: Mouse oocytes after vitrification and thawing of COCs with the 0.25 mL French straw method; Arrow C shows a viable oocyte with intact oolemma; Arrow D shows a dead oocyte, determined by the lack of oolemma integrity and cytoplasmic content

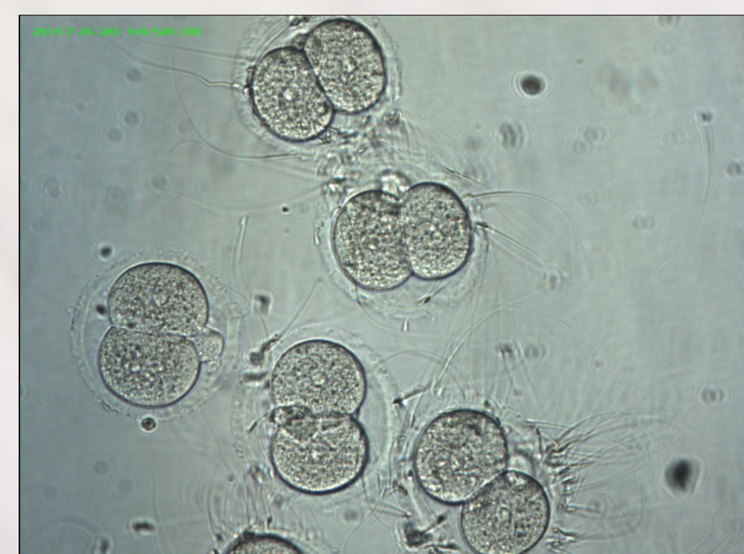

Figure 9: Mouse 2-cell stage embryos resulting from freshly fertilized COCs using fresh sperm

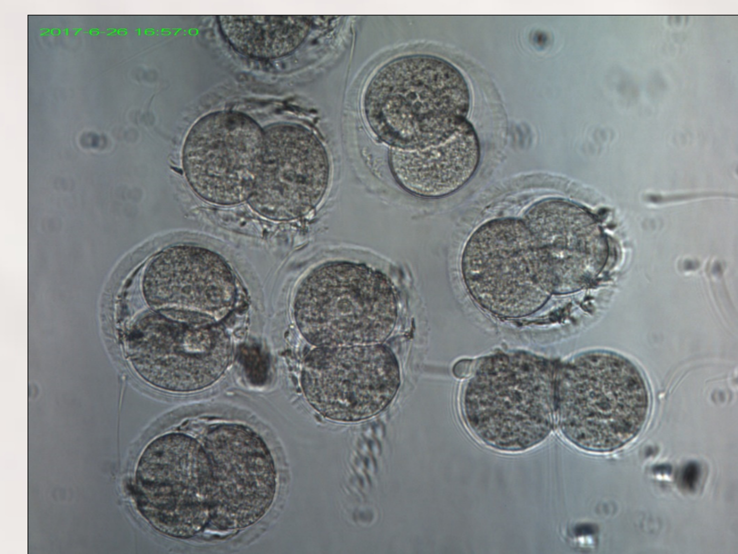

Figure 10: Mouse 2-cell stage embryos resulting after Cryoloop-vitrification and thawing

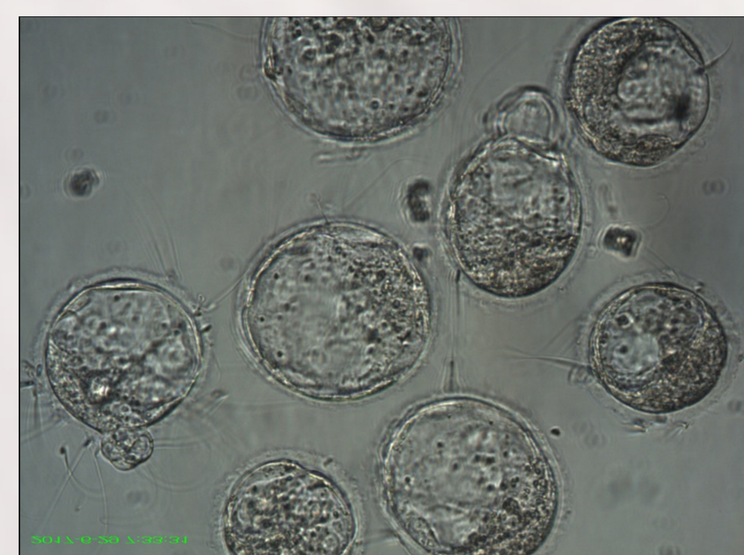

Figure 11: Mouse blastocyst stage embryos resulting from freshly fertilized COCs

Figure 12: Mouse blastocyst stage embryos resulting after Cryoloop-vitrification and thawing

Table 1. Post-thaw morphologic integrity of mouse oocytes following vitrification with Cryoloop or 0.25 mL French straws (n=8).

	No. oocytes vitrified	No. oocytes survived (%)
0.25 mL French straw	129	25 (19±2.0)[a]
Cryoloop	131	99 (75±2.0)[b]

[a,b] Values with different letters within a column are significantly different (P<0.05).

Table 2. In-vitro fertilization and pre-implantation embryonic development competence of fresh and Cryoloop-vitrified mouse COCs to blastocyst stage (n=14).

	No. oocytes	No. oocytes fertilized (%)	No. 2-cell (%)	No. blastocysts (%)
Fresh	82	64 (78±2.5)[a]	63 (99±2.1)[a]	58 (90±2.8)[a]
Vitrified	115	82 (71±2.2)[b]	64 (78±1.8)[b]	46 (56±2.4)[b]

[a,b] Values with different letters within a column are significantly different (P<0.05).

Results:

- Cryosurvival rates of COCs vitrified using 0.25 mL French straws were significantly lower (19%) than those vitrified with the Cryoloop method (75%) (P<0.05).
- In-vitro fertilization rates of COCs vitrified by the Cryoloop method was lower (71%) than those freshly fertilized COCs (78%) (P<0.05).
- Embryo development rates following IVF of Cryoloop-vitrified mouse COCs (56%) were lower than those freshly collected COCs (90%) (P<0.05).

Conclusions:

- Despite some reduction, acceptable in-vitro fertility and embryo development rates can be achieved following cryopreservation of mouse COCs with a Cryoloop vitrification method.
- This method may be a successful way to preserve mouse strains for genome banking.

Future Studies:

- Future testing will focus on fetal development competence of the embryos that were derived from Cryoloop-vitrified mouse COCs.

Acknowledgements/Funding:

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Mutant Mouse Regional Resource Centers