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Cryopreservation and recovery of sperm
Cryopreservation & Assisted Reproduction Laboratory
Frederick National Laboratory for Cancer Research (FNL)

Sperm cryopreservation: As previously described (Ostermeier et al, 2008), a cryoprotective media (CPM) containing 18% (w/v) Raffinose (Sigma Aldrich; cat # R7630) and 3% (w/v) skim milk (BD Diagnostics; cat # 232100) is clarified, filtered and then supplemented with 477 µM alpha-monothioglycerol (MTG; FW=108.16; D=1.295g/mL; Sigma Aldrich; cat # M6145). Two mL of the CPM is used to collect the sperm from the epididymides and vas deferentia of two 3- to 6-month-old mutant males.

The sperm+CPM is loaded into 0.25 mL French straws (IMV; Maple Grove, MN; cat# AAA201) by first creating a 1.5 cm column of CPM, a 1.5 cm column of air and four 0.5 cm columns of sperm sample (~10 µL) each separated by an approximate 0.5 cm column of air and the last being followed by a final column of air. The straws are heat sealed and then exposed to LN_2 vapor before being stored in the liquid phase.

***In vitro* fertilizations (IVF)**: The *In vitro* fertilization medium, Research Vitro Fert (RVF; Cook Medical; Brisbane, Australia; cat# K-RVFE-50), is used for sperm incubation, IVF and zygote culture. The IVF dishes contain one 500 µL fertilization drop and three 150 µL wash drops, which are covered by mineral oil (Sigma cat #M8410). The dishes are equilibrated overnight in a humidified 37° C incubator maintained at 5% CO_2.

The straw containing sperm is transferred directly from LN_2 into a 37°C water bath and incubated for ~30 to 60 sec. The straw is removed and lightly dried with a kim wipe. The sealed end of the straw is cut off and a stylet used to push a single 10 µL aliquot of CPM+sperm into a fertilization drop. This can be repeated up to four times per straw. The sperm are incubated in the fertilization drop for ~1

hr prior to the addition of cumulus oocyte complexes from five superovulated 17- to 27-day-old female mice. Superovulation is achieved by intraperitoneal injections of 5 IU PMSG (Calbiochem (EMD Millipore); cat # 367222) followed 48 hours later by 2.5 IU hCG (Sigma; cat # C1063).

After 4 hrs of co-incubation of the sperm and oocytes, the presumptive zygotes are washed and only those appearing normal are cultured overnight in one of the 150 µL RVF wash drops.

Approximately 18 hrs after washing, two cell embryos are collected and transferred into B6D2F1 pseudopregnant recipients. We commonly do single sided transfers and recommend that 10-15 embryos be transferred per recipient.

References:

Ostermeier GC, Wiles MV, Farley J, Taft RA. Conserving, distributing and managing genetically modified mouse lines by sperm cryopreservation. PLoS ONE. 2008;3(7): e2792.