**EMBRYO CRYOPRESERVATION PROTOCOL**

Superovulate females with PMS (5 IU per female);
48 hours later, inject females with HCG (5 IU per female) & mate;
Embryo collection must be scheduled in 72 hours.

**Embryo Collection and Cryopreservation:**

1. Dissect the oviducts.
2. Flush the embryos using a dull needle by injecting Modified D-PBS (Specialty Media CAT# MR-006-C) through the infundibulum and Oviduct.
3. Collect the 8-cell embryos into cryovials containing 0.1 ml of M-DPBS, 25-30 embryos per vial.
4. Set on ice bath at 0°C.
5. Add 0.1 ml CPA (containing DMSO) and let the vials sit on ice for 30 min.
6. Place vials in –6°C ice bath for 2 min.
7. Seed vials (media in Pasteur pipettes for seeding must be -10°C).
8. Transfer vials to the controlled-rate freezer already set at -6°C & freeze to -80°C (approximate freezing time 2 1/2 hours, -1°C every 2 min).
9. Transfer vials to LN2 (liquid phase) for long-term storage.

**Thawing Procedure:**

1. Thaw each vial at room temperature for 10 min.
2. Slowly add 0.8 ml of M-DPBS to the vial containing the embryos (this process must be done gently, drop by drop). Mix the volume in the cryovial by pipetting the sample.
3. Draw up total volume containing the embryos and place in a 60mm. organ culture dish.
4. Place the dish on ice and transfer the embryos immediately.
5. Embryos should be transferred into the oviduct of the pseudopregnant recipients. We recommend that 10-12 embryos be transferred per female recipient. Our laboratory uses B6D2F1 females for this purpose.

Cryopreservation & Assisted Reproduction Laboratory
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March 2000