
Protocol Section:	Unassisted IVF	Policy No:	P-CMMR4-IVF-01
Protocol Subject:	Unassisted IVF	Effective Date:	
		Date Reviewed:	15 May 2003
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Hormonal induction of oocyte donor mice

Three - Four weeks old mice are superovulated by intraperitoneal (ip) injection of PMSG followed by HCG 48-52 h later. The dose for these two hormones are strain-dependent and it ranges from 2.5 IU to 5.0 IU.

Culture dish preparation

It is convenient to prepare culture dishes the day before an IVF. The following types of dishes are used:

1. Fresh sperm dish: 1 dish (35X10mm) for each strain w/ 1ml of HTF medium with BSA (4 mg/ml)
2. Fertilization dish (35X10mm): 1 dish per 3 females; one 250 ml drop of HTF medium with BSA
3. Wash dish (60X15mm): 1 dish per fertilization dish; five 200ml drops of HTF medium with BSA
4. Culture dish (30X10mm): 1 dish per fertilization dish; one 100ml drops of HTF or KSOM (1 mg/ml) medium with BSA

Mineral oil (pre-gassed) is added to cover the drops, and the dishes are transferred to an incubator at 37 °C in an atmosphere of 5% CO₂.

Fresh egg collection for IVF

The females are euthanized by cervical dislocation 13 hours after the HCG injection and their oviducts removed into a plastic culture dish placed containing 2.5 ml of HTF (Human Tubal Fluid) medium. The cumulus - oocyte complexes are released from the ampulla into the HTF by rupturing with the aid of fine forceps, and then moved into the 250 µL HTF fertilization drop using a wide bore pipette tip. Prepared IVF dishes are placed and overlaid with mineral oil sealed in modular incubator chamber at 37°C in a humidified atmosphere of 5% CO₂.

Fresh sperm collection for IVF

Sacrifice a male by cervical dislocation 15 to 45 minutes before the females reach 13 hours after the HCG. Dissect out the epididymides along with the vas deferentia. Place them in the sperm dish and mince the epididymis, making 5-7 slashes with the needle of an insulin syringe. Then, using forceps, "walk down" the vas to push out any remaining sperm. Place sperm dish in the incubator under gas and allow sperm to "swim out" for 15 minutes. Count the sperm; you will want to have somewhere between 15 - 50 x 10⁶ sperm per ml (depending on age and strain). Essentially, in each IVF drop should be added to the four oocyte donor with a minimum count of 10⁶ progressively motile sperm per ml both fresh and frozen sperm.

Thaw sperm (if using frozen sperm)

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Thaw frozen samples rapidly by removing from liquid nitrogen storage and place into a water bath at 37 °C until all ice crystals are melted (approximately 2 min). Centrifuge thawed sperm samples at 900 g for 4 min. Discard the supernatant and replace with 50- μ L of HTF. Mix gently sperm sample (by tapping the tube with the fingertips). Incubate the resuspended sperm sample for 15 min at 37 °C to allow a minimal "swim up". After the incubation time, take 40- μ L aliquot for the IVF and add a fertilization drop.

Washing eggs post-IVF

Maintain the fertilization drop for 4-6 hours in incubator and then wash eggs with HTF to eliminate excess sperm. After, wash the eggs to remove the excess of sperm. Using a capillary transfer pipette, transfer the eggs from the IVF drop to one of the drops of medium in the "wash dish", leaving behind as much debris as possible. All viable cells should be transferred to a second drop. Finally, the embryos should be distributed among the remaining drops of medium. Culture eggs overnight in a 100- μ L drop of HTF.

Transfer embryos to Pseudopregnant Females or to KSOM for culture to later stages

At this point the embryos can be counted and transferred to pseudopregnant foster mothers, or they can be cultured to later stages. For this latter step, transfer 2-cell embryos from each wash dish to one of the drops of KSOM medium from the corresponding culture dish. They are then washed 2-3 times and then distributed to the rest of the drops (to remove the HTF) for culture to blastocyst stage

Reference

Sztein JM, Farley JS, Mobraaten LE (2000) In vitro fertilization with cryopreserved inbred mouse sperm. Biol Reprod 63, 1774-1780

Issued by Lab Manager: _____ **Date:** _____

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