

Reagents, supplies, and equipment:

- IVF Media K-RVFE-50 Cook Medical
- 35mm tissue culture dishes
- PMS
- 60 mm tissue culture dishes
- hCG
- mouth pipet
- syringe
- dissecting instruments
- Mineral oil
- KSOM
- organ culture dishes
- Mixed gas (5%CO₂, 5% O₂, 90% N₂)

Keywords: in vitro fertilization IVF

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IVF procedure:

1. Day One: Administer PMS via IP injection to females of appropriate strain and age for ova donors at about noon.
2. Day Three: Administer hCG via IP injection to females ~48 hours post PMS injection.
3. Prepare dishes for egg collection, sperm collection and IVF.

- a. One dish of 1 ml IVF medium per male for fresh sperm IVF.



- b. One dish of 3 ml IVF medium per three females.



- c. One IVF/wash dish per three females with 400 μ l drop for fertilization and four 150 μ l drops for wash post fertilization covered with oil – no lids on these dishes. Keep at 37° C under mixed gas. (mixed gas is 5%CO₂, 5% O₂, 90% N₂)



4. Day Four: IVF


- a. Collect sperm – See sperm thawing protocol.
- b. Add 5 – 10 μ l of sperm to IVF drop in dishes. (Frozen = 10 μ l and fresh = 5-10 μ l depending on motility.) Incubate under mixed gas for one hour (fresh be can used immediately). Record time sperm was added.
- c. 13-14.5 hours post hcg collect oviducts from superovulated females. Work quickly on this step, trying to take no more than five minutes per group of three females. Dissect out clutches of eggs and transfer into IVF+sperm drop with as little extra media as possible using 10 μ l pipetman. Record time of addition to each dish.
- d. Repeat until eggs have been added to all IVF dishes. Incubate for 4-6 hours at 37° C under mixed gas.
- e. Using mouth pipette, wash eggs/zygotes into first 150 μ l drop. Move best looking ones into next drop, leaving behind dead, fragmented cells. Divide healthy ones between the last two drops. Record number of live, fragmented, dead, and zona free cells. Again, try to take less than five minutes for this step and return promptly to incubator.
- f. Incubate overnight under mixed gas.

5. Day Five: Score IVF


- a. For each IVF dish, count and record number of two-cell embryos, one cell, fragmented and dead oocytes. These embryos can be cryopreserved, transferred to 0.5 dpc pseudopregnant females or kept in culture up to the blastocyst stage. KSOM

medium or other culture medium is used if culturing beyond the two-cell stage.

Attachments

 [image.png](#) - Added on March 8, 2018 at 8:43 AM by Jennifer Skelton



 [IVF_and_wash_dishes.png](#) - Added on March 8, 2018 at 8:52 AM by Jennifer Skelton



 [Three_ml_IVF_dish.png](#) - Added on March 8, 2018 at 8:50 AM by Jennifer Skelton

