

## IN VITRO FERTILISATION (IVF)

### Preparation

3 days prior to performing the IVF: (Date: \_\_\_\_\_)

- stimulate females with HyperOva or PMSG injection (5 IU-i.p.) at 19:00h (5-10 females per sperm sample)

1 day prior to IVF: (Date: \_\_\_\_\_)

- Stimulate females with hCG 5 IU-i.p. injection (hCG 5 IU-i.p.) at 18:00 h.
- DO NOT MATE FEMALES.**
- On the same evening, prepare IVF media drops containing 200 µl HTF medium (CosmoBio) under oil in 6 cm dish and leave in the CO<sub>2</sub> incubator to equilibrate.

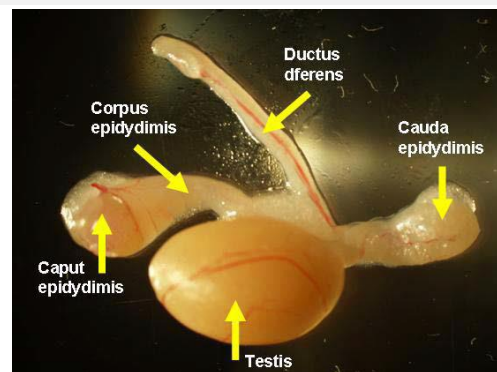
### Procedure

#### Performing IVF using **frozen** sperm

- Remove straw from LN<sub>2</sub> and thaw for 1 minute in air and 10 minutes in 37 °C water bath.
- Dispense all of sperm suspension into a 100 µl drop of FertiUp medium (CosmoBio) in a 3 cm petri dish (w/o oil) and incubate for 40-60 minutes at 37 °C with 5% CO<sub>2</sub>.
- Take 20 µl of capacitated sperm solution from the edge of the FertiUp drop and add to the IVF drop prepared the day before.
- Proceed directly to step 5 (below)

#### Performing IVF using **fresh** sperm

- Remove the cauda epididymis, and vas deferens (with the least amount of fat attached) altogether as shown below.
- Pinch the epididymis and puncture its membrane. Squeeze the tissue to press out the sperm mass.
- Collect the sperm mass with forceps and place it in a tube containing 400 µl of FertiUp medium (CosmoBio). Incubate at 37°C with 5% CO<sub>2</sub> for 45-60 minutes to capacitate the sperm.
- Put 2-3 µl of capacitated sperm in the IVF drop



- Prepare the females:
  - Stimulated and unfertilized females are used. Euthanize the mice and expose the reproductive tracts.
  - Dissect out the ampullas with minimal oviduct fragments and place them in mineral oil
  - One by one, place the ampoule and the oviduct in the oil surrounding the sperm IVF drop.
  - Scratch out the oocyte clutches directly into the IVF drop, make sure no tissue makes contact with the IVF drop before removing the tissue from the dish.
- Incubate the IVF Incubation dish for 5.5 hours at 37°C with 5% CO<sub>2</sub>.
- At the end of the IVF add 1ul of hyaluronidase medium and incubate for 2-3 minutes to dissociate remaining cumulus cells.
- Wash the cells three times in M16 medium.
- Transfer the eggs to the KSOM medium for overnight incubation.  
Perform embryo transfers the following day on 2-cell stage embryos.