ABBREVIATED IVF PROTOCOL

This checklist can be used to ensure that each step in the IVF procedure is completed successfully.

DAY –2

☐ Preparation of Media
  - saline
  - OMM
  - OCM

☐ Check incubators for accuracy of temperature, O₂ and CO₂ readings

DAY –1

☐ Collection of ovaries from slaughterhouse

☐ Preparation for oocyte collection
  - Place OCM and saline in the oven
  - Prepare OMM microdrops and cover them with mineral oil
  - Place plates in the incubator
  - Set up for oocyte collection
    - Scalpel
    - Scalpel blades
    - Gloves
    - 400 ml beaker
    - container to discard ovaries
    - bench paper to cover surface

☐ Harvesting oocyte-cumulus complexes from ovaries
  - Add 100 ml of OCM to beaker
  - Slice ovaries
  - Swirl ovaries in the beaker
  - Pour OCM and oocyte mixture into 50 ml sterile centrifuge tubes and place into water bath

☐ Collection of cumulus oocyte complexes (COCs) from centrifuge tubes (5 min. sedimentation time)
  - Set up for oocyte collection
    - 100 µm cell strainer
    - sterile transfer pipets
    - 10 ml airtite syringe
    - 18 gauge needle
    - Grid plate
    - Set up cell strainer over 50 ml beaker
- Using transfer pipet, suck up pellet and place in cell strainer
- Flip cell strainer over into a grid plate on a slide warmer
- Fill needle/syringe with OCM and rinse the debris from the strainer into the grid dish

☐ Searching for cumulus oocyte complexes
  - X-plate
  - Dissecting microscope
  - Searching instrument (microdispensor, wiretrol, etc.)
  - Slide warmer
  - Transfer cumulus oocyte complexes to X-plate and rinse two times

☐ Oocyte maturation
  - After the last rinsing place cleaned cumulus oocytes complexes (10/drop) into a 50 μl microdrop of pre-equilibrated OMM covered in oil
  - Mature cumulus oocyte complexes for 18-24 h (place maturation plate in the back of the incubator)

☐ Prepare media for fertilization
  - H-SOF
  - 90% Percoll
  - SOF-FERT

DAY 0

☐ Preparation of media for fertilization (~2.5 h prior to fertilization)
  - ISOLATE (tighten cap and place in warm oven)
    1.5 ml 50% over 1.5 ml 90%
  - H-SOF (tighten cap and place in warm oven)
    1 centrifuge tube per person with 15 ml H-SOF and 1 tube with 10 ml H-SOF (labeled wash)
  - SOF-FERT (leave cap loose and place at 38.5°C in 5% CO₂)
    35 mm dishes containing 1700 μl SOF-FERT
    1-centrifuge tube with 3 ml SOF-FERT
  - PHE (place in oven)
  - Warm-up centrifuge canisters (place in oven)
  - Plug in citothaw

☐ Matured oocytes: setup for washing and fertilization
  - X-Plate with H-SOF
  - Searching instrument
  - Dissecting microscope
  - Heater
  - Scissors
  - Semen straw plunger
  - Inverted microscope
  - Small petri-dish
  - Rack for tubes (place in front of the heater)
  - Slide warmer
  - Plastic sterile Pasteur pipets
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- Pipette (25 ml)
- Pipet tips

□ Matured oocytes: washing and fertilization
  - Add 5 ml H-SOF to each well of X-plate.
  - Transfer about 200 COCs to each well.
  - Transfer COCs to 35 mm dish with 1700 µl/plate SOF-FERT.

□ Sperm preparation (working in front of the heater)
  - Place 1-3 straws of semen in citothaw.
  - Layer semen on top of ISOLATE gradient.
  - Place ISOLATE tube in a warmed centrifuge canister.
  - Centrifuge for 10 min at 1000 x g.
  - Collect semen pellet with a Pasteur pipet.
  - Place pellet into the 10 ml H-SOF tube.
  - Place H-SOF tube into a warmed centrifuge canister.
  - Centrifuge for 5 min at 200 x g.
  - Pipet off supernatant down to the pellet.
  - Add SOF-FERT and determine concentration.

□ Fertilization
  - Add 120 µl of semen to each fertilization plate containing COCs.
  - Add 80 µl of PHE to each plate.
  - Place plates back in the incubator and allow fertilization to proceed for 8-10 h.

□ Culture media
  - Prepare plates with 50 µl microdrops of SOF-BE1 and cover with mineral oil
  - Place plates in the incubator to equilibrate for at least 2 h

□ Setup for removal of oocytes/embryos from fertilization drop
  - Vortexer
  - slide warmer
  - Hyaluronidase (optional) -warm up
  - Sterile dolphin-nose microcentrifuge tubes (and holder)
  - Heater (in front of microscope)
  - X-Plate
  - H-SOF
  - Dissecting microscope
  - Searching instrument
  - Timer

□ Remove oocytes/embryos from fertilization drop
  - Rinse microcentrifuge tube with H-SOF and leave ~30-50 µl for collection of oocytes/embryos.
  - Transfer oocytes/embryos from the fertilization drop to the microcentrifuge tube.
  - Vortex the tube for 5 min in front of the heater.
  - Use Pasteur pipet to move contents of the microcentrifuge tube to a well of the X-plate.
- Search for cumulus-free oocytes.
- Wash 2X in H-SOF.
- Transfer in groups of up to 30 to the SOF-BE1 microdrops.
- Place culture plate in the back of the incubator.

**DAY 3**

- Pre-warm stage of inverted microscope and room
- Determine cleavage rate (be quick)

**DAY 7-9**

- Pre-warm stage of inverted microscope and room
- Collect data on blastocyst development