ABBREVIATED IVP PROTOCOL

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

DAY -2

☐ **Preparation of Media**

- saline
- OMM
- OCM

☐ **Check incubators for accuracy of temperature, O_2 and CO_2 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% CO_2)
  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

- 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**

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