

COLLECTION AND CULTURE OF CUMULUS-OOCYTE COMPLEXES (COCs)

Required materials:

MEM-HEPES

MEM-NaHCO₃

Recombinant epidermal growth factor (rEGF), stock concentration = 100 µg/ml

Collagenase

DNase I

Mineral oil

(3) 35 mm Petri dishes

(1) 60x15 mm centre-well organ culture dish (catalog number: 353037)

1cc insulin-gauge needles

Forceps and dissection scissors

Before beginning:

1. At least 4 hours before collection, prepare IVM medium by diluting rEGF to a concentration of 10 ng/ml in MEM-NaHCO₃. Place 40 µl drops of IVM medium in culture dish and cover with mineral oil. Preheat dish to 37°C in 5% CO₂ in air.
2. At least 30 minutes before collection, preheat media for collection/washing. Add 2 ml MEM-HEPES to each of two (2) 35 mm Petri dishes for collection. Add 2 ml MEM-NaHCO₃ to a 35 mm Petri dish for washing. Preheat all three dishes to 37°C for at least 30 minutes.

COC retrieval and IVM:

1. Sacrifice mice and dissect out the ovaries.
2. Place ovaries in MEM-HEPES in the first collection dish. Under a microscope, remove fat from ovaries.
3. Add 20 µl collagenase (10 mg/ml) and 20 µl DNase I (1 mg/ml) to the second collection dish and transfer ovaries to this dish. Use 30-gauge needles to tear out the biggest follicles (which appear to be bulging out of the ovary), allowing the COC to ooze out of the follicle.
4. Transfer COCs by mouth-pipetting to MEM-NaHCO₃ in washing dish.
5. Place COCs into drops of IVM medium in organ culture dish. Incubate for 16 hrs at 37°C in 5% CO₂ in air to allow cumulus expansion.