COLLECTION AND CULTURE OF GRANULOSA-OOCYTE COMPLEXES (GOCs)

Required material:

MEM-HEPES

MEM-$NaHCO_3$

Collagenase

DNase

100x ITS (Sigma-Aldrich: 1 mg/ml I, 0.55mg/ml T, 0.5 µl/ml S)

Recombinant follicle stimulating hormone (rFSH) (stock concentration = 500 ng/ml)

(3) 35 mm Petri dishes

24-well plate with Type I collagen inserts

Before beginning:

1. 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 20 µl collagenase (10 mg/ml) and 20 µl DNase I (1 mg/ml) to one of the collection dishes, to make a concentration of 1x of each enzyme. Add 2 ml MEM-$NaHCO_3$ to a 35 mm Petri dish for washing. Preheat all three dishes to 37^{o}C for at least 30 minutes.

2. Prepare culture medium (1x ITS ± 10mIU/mL FSH).
   Per well of 24-well plate with collage inserts:

	Culture with FSH	Culture without FSH
100x ITS	10 µl	10 µl
rFSH (500 ng/ml)	1.48 µl	---
MEM-$NaHCO_3$	988.52 µl	990 µl
Total volume	1 ml	1 ml

   Preheat 24-well plate with inserts and culture medium (750 µl outside collagen insert, 250 µl inside collagen insert) to 37^{o}C for at least 30 minutes.

GOC retrieval:

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. Transfer ovaries to the second collection dish containing collagenase and DNase. Use forceps to break the ovaries into pieces and incubate in the enzymes for 30 minutes at 37^{o}C. Vigorously pipette up-and-down with 1000 µl pipette to help with the removal of the basal lamina.

4. Transfer GOCs by mouth-pipetting to MEM-$NaHCO_3$ in washing dish.

5. Transfer GOCs by mouth-pipetting into the collagen insert, making sure to spread them apart as much as possible on the insert. Up to 30 GOCs may be placed per insert.

6. For culture periods longer than 3 days, change medium every 3 days. Remove 250 µl medium from outside the collage insert and replace with fresh medium of the same composition.