Mouse Embryo Collection and Culture

Mutant Mouse Resource and Research Center

University of Missouri

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Hormone Preparation and Injection

Mouse Pregnant Mare Serum Gonadotropin (PMSG) and Human Chorionic Gonadotropin (hCG) preparation

Supplies:

- 1.5 mL Sterile Eppendorf tubes
- Small freezer storage box with lid
- Pipettor and pipette tips

Reagents:	**Company**	**Catalog #**
PMSG (1000 IU)	ProspecBio	HOR-272
hCG	Millipore-Sigma	230734
DPBS w/o $CaCl_2$, $MgCl_2$	Gibco	14190-144

Procedures:

1. Reconstitute each hormone to a concentration of 25 IU/mL.
2. Aliquot the reconstituted hormones and store at -80°C for up to three months.

Injection Procedure:

1. Inject 5 IU PMSG per mouse interperitoneally between 11:00 am-1:00 pm.
2. Approximately 48 hours post PMSG injections, administer 5 IU hCG per mouse for embryo collection.
   a. For oocyte collection, hCG injections should occur between 4:30pm-6:30pm.
3. Collect oocytes/embryos between 8:00am-11:00am the following day.

Note: Injection and collection times are based on a 12 hour light cycle, injection times may vary depending on light cycle and desired collection times.

Hyaluronidase stock (1 mg/mL) preparation for denuding of mouse zygotes

Supplies:

- 1.5 or 2.0 mL microcentrifuge tubes
- 1000 µl Pipette and pipette tips.

Reagents:	**Company**	**Catalog #**
Hyaluronidase	Sigma	H4272-30 mg
BSA	Sigma	A3311

Flushing handling medium (FHM) or comparable

Procedure:

1. Dilute hyaluronidase using FHM or comparable to a dilution of 1mg/mL.
2. Aliquot and store at -20°C for up to three months.

Embryo Collection

Standard Cell and Tissue Collection

Supplies:

- VersiDry Lab Soaker for benchtop
- 35 mm Petri dishes (Falcon 1008)
- Kimwipes
- Nitrile Gloves
- Micro-scissors, Fine forceps, Curved, serrated forceps
- Stereo microscope
- Disposal bags
- 70% Ethanol

Procedure:

1. Euthanize up to 4 female mice.
2. Place the animals on a VersiDry Lab Soaker on the benchtop and spray the abdominal area with 70% ethanol.
3. Grasp the abdominal skin with serrated forceps and make a lateral incision at the midline using scissors (see Figure 1). Push the intestines away to reach the reproductive tract to collect the desired tissue.
    a. For collection of zygotes, dissect out the oviduct and small part of the uterus (Figure 1).
    b. For collection of 2-cell embryos and later stages collect both oviduct and uterine horn (Figure 2).
4. Place the tissue into a petri dish containing warm FHM or comparable.

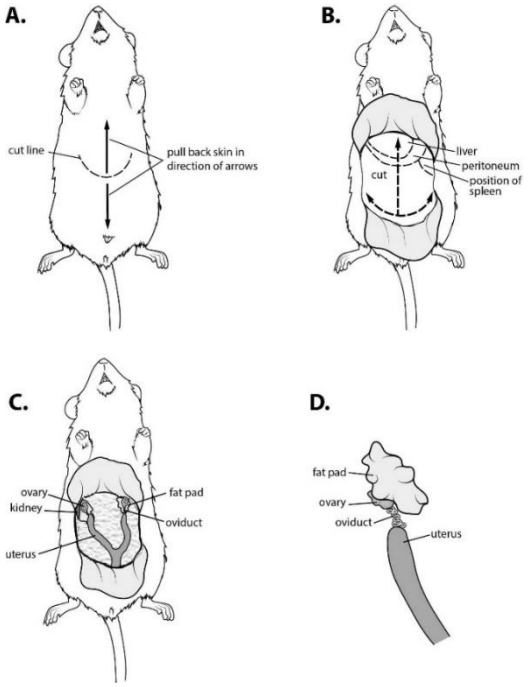

Figure 1. Reproductive organ dissection from a female mouse. Cut the skin with a scissors as indicated by dashed line and then pull the skin apart in the direction of solid arrows (A). Cut the body wall (peritoneum) as indicated by dashed line in (B); the layout of internal reproductive organ of a female mouse (C). An enlarged view of one side of internal reproductive tract of a female (adapted from Behringer et al., 2014. Manipulating the mouse embryo (4th edition), Cold Spring Harbor Laboratory Press, New York).

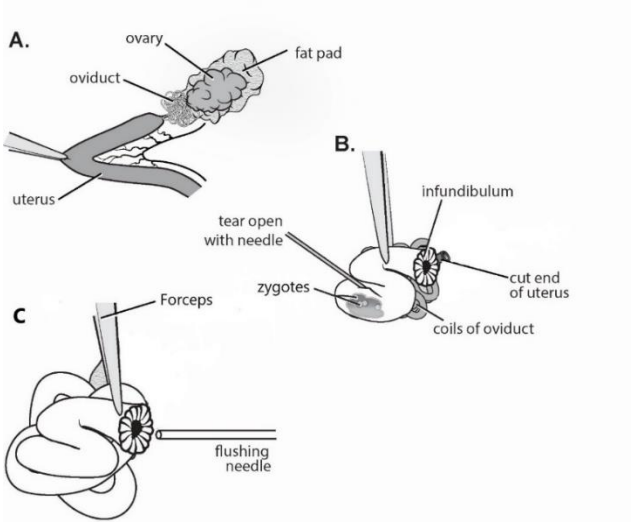

Figure 2. Collection of zygotes and cleavage stage embryos from oviduct. Excise oviduct by cutting first at oviduct and ovary junction and then a small section of uterine horn as illustrated in (A). Hold oviduct coil with a serrated forceps and release zygotes and cumulus mass by tearing the swollen ampulla with an insulin needle as indicated in (B). Cleavage stage embryos (2-cell to morula) are collected by flushing oviduct through the infundibulum (C) (adapted from Behringer et al., 2014. Manipulating the mouse embryo (4rd edition), Cold Spring Harbor Laboratory Press, New York).

Zygote-cumulus complex collection from the oviduct

Supplies:

- FHM or comparable
- Insulin syringe with needles
- Fine forceps
- Stripper pipette tips (Origio MXL3-125)
- Hyaluronidase (1 mg/mL)
- Mouth or hand micropipette

Procedures:

1. Move the oviducts into approximately 100 microliters of hyaluronidase (1 mg/mL) , hold the oviducts using fine tweezers and tear the ampulla using a 27 G needle to release the cumulus zygote complexes (Figure 2b).
2. Let the cumulus zygote complexes sit for no longer than five minutes. Using a micropipette, pick up the embryos and gently wash them 2-3 times in FHM or comparable and transfer to a culture dish of KSOM.
3. Transfer plates with KSOM and culture in 37°C, 5% CO_2 incubator.

Embryo collection by flushing oviducts

Supplies:

- FHM or comparable
- Hamilton blunt needle, 30 G
- Fine forceps
- Mouth or hand micropipette

Procedures:

1. Under the stereo microscope, using fine tweezers, gently grab the infundibulum, insert a 30 G Hamilton needle attached to a 1cc syringe (pre-loaded with FHM) and push the plunger to release 500 µl of the solution per horn to expel cleavage stage embryos prior to blastocysts (Figure 2C). Blastocysts can be collected by puncturing the uterus close to the utero-tubal junction and flushing with 1 mL of the medium (Figure 2).
2. Collect the embryos into a clean Petri dish containing FHM or comparable. Wash them 3 times in FHM and transfer to a culture dish of KSOM.
3. Transfer plates with KSOM and culture in 37°C, 5% CO_2 incubator.