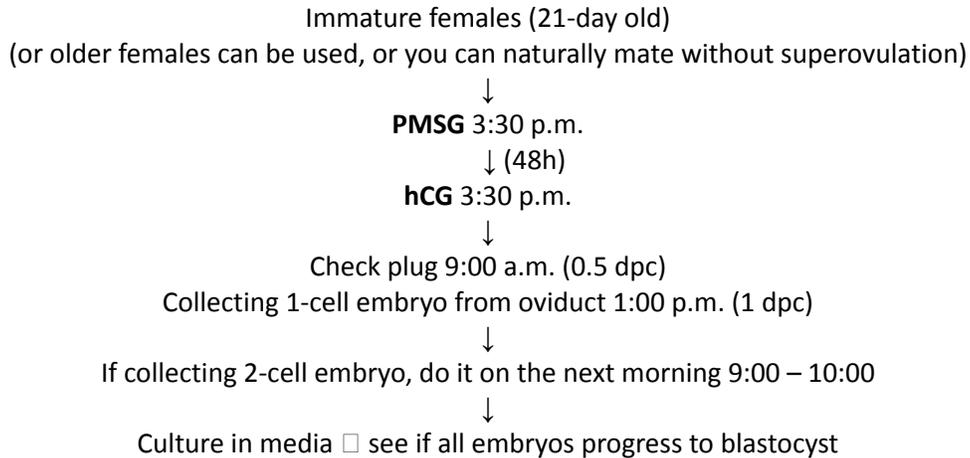


How to collect 1-cell and 2-cell embryo



Collecting 1-cell from the oviduct

- Prepare 0.1 % Hyarulonidase in PBS without Ca²⁺/Mg²⁺ (Aliquot and store at -20C)
 - Prepare media for collecting embryo, incubate at 37C
 - Prepare glass pipette
 - Open up the animal from the back (similar to the ovex incision)
 - Pull out the ovary, cut the oviduct out and put it in the media, do not nick the oviduct
 - Use the forceps to tear apart the oviduct, all the eggs with cumulus cells will come out together (or use the needle to tear the oviduct)
 - Make the drop of 0.1% Hyarulonidase and put all the eggs in by using pipette tip and leave them in Hyarulonidase for about 1-2 minutes
 - Use the mouth pipette, pipette up and down to break down the cumulus cells
 - Transfer the embryo to the media few times (washing out the hyarulonidase)
 - Count the embryo
- For this experiment, natural mating will be difficult to determine the time/stage of embryo because of the ovulation time is not similar in each dam. Using superovulation would be better.

For blastocyst collection: at 3.5 dpc (Similar protocol for superovulation and mating time)

- Cut the peritoneal muscle open, then remove ovaries and uterus all together and put it to the dish
- Use either saline, PBS, or media to flush the uterus with 25-guage needle and 6-mL syringe
- Insert the needle end into the junction between uterus and oviduct
- Use the forcep to hold the needle, then, slowly push the saline into the uterus
- Make sure that the fluid flow to the right direction (toward the cervix)
- Then strongly push the fluid approximately 1-2 mL per horn, do this under the light microscope
- All the morula, blastocyst or hatched blastocysts will be in the dish, transfer the uterus and ovaries into the other dish.