How to collect 1-cell and 2-cell embryo

Immature females (21-day old)
(or older females can be used, or you can naturally mate without superovulation)
↓
PMSG 3:30 p.m.
↓ (48h)
hCG 3:30 p.m.
↓
Check plug 9:00 a.m. (0.5 dpc)
Collecting 1-cell embryo from oviduct 1:00 p.m. (1 dpc)
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If collecting 2-cell embryo, do it on the next morning 9:00 – 10:00
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Culture in media see if all embryos progress to blastocyst

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.