CMF_Superovulation & oviduct collection

1. Day -2 (Monday); Inject 3-6 week old female mice with 5 IU of pregnant mare serum gonadotrophin (PMSG) intraperitoneally. The hormone **MUST** be injected between 12:00 – 14:00.

2. Day 0 (Wednesday); Inject females with 5 IU of human chorionic gonadotrophin (hcG) intraperitoneally. The hormone **MUST** be injected between 12:00 – 13:00.

3. Day 0; Immediately after injection of hcG, add the female into a cage containing a stud male (proven breeder). It is best to add the female to the male cage.

4. Day +1, early am (~08:00); Check the females for the presence of a vaginal plug. Those mice that were successfully plugged should be marked.

Plugged females can be shipped to Bern at this time and the rest of the procedure (step 5) is carried out by the staff of the Clean Mouse Facility (CMF).

5. Day +2, early am (~08:00-09:00); Cull the females and collect the oviducts. Place into small Petri dishes containing M2 media (+ antibiotics). The harvested oviducts will then be flushed to obtain 2-cell stage embryos.

NB: Young females 3-6 weeks of age are normally the most responsive to superovulation. However, this can vary with strain. Females 6-8 weeks of age can also be used if necessary.

NB: Breeding pairs used to produce the donor females for superovulation should be kept on a high fat, or breeding diet.

Optional: To maximise the nutritional state of the females to be superovulated, the male littermates can be culled within 7 days of birth.

NB: Stud males should not be less than 10 weeks of age (can be used up to ~8 months of age). Stud males should be placed into individual cages 1 week before required (so the dominant male does not suppress testosterone production in littermates). New stud males should be set up with 'extra' females for one week for practise before being used as stud males. Stud males usually require ~3 days rest after mating to allow sperm production to increase.