



## **Procedures**

1. Hormones should be kept at -20°C until the day of use.
2. WEAR GLOVES when handling the hormones for injection. Do not recap needles.
3. Use a new syringe and needle for each injection.
4. The PMSG and hCG dose for a mouse is 2.5 to 5.0 IU given IP. Start with 5.0 IU and adjust the dosage downward, if needed.
5. To inject a mouse with PMSG or hCG, thaw a vial(s) of 50 IU/ml PMSG or hCG at room temperature or by gently rolling the vial between your hands. Do not shake the vial.
6. Draw 0.1mL of 50 IU/mL (5 IU) into a 1mL syringe with a 27-gauge needle.
7. Restrain the mouse for an IP injection by scruffing the mouse to minimize movement. You may find that tilting the mouse slightly head downward will help as this will move the gut and bladder away from the injection site.
8. Inject into the animal's lower right (your lower left) quadrant. Draw back on the syringe plunger before injecting to make sure you are not in the bladder or the gut. When drawing back on the plunger, if you see yellow you are in the bladder; red, you have hit a blood vessel; brown or green you are in the gut. If this should occur discard the entire syringe. Load a new syringe and try again. Training is available from OLAR veterinary staff. Contact OLAR directly at 304-293-3737.
9. Discard any unused PMSG and hCG. DO NOT RE-FREEZE.
10. After the hCG injection on day 2, immediately place the hormone-primed female with the designated fertile stud male. Do not have more than one female per male, as this would reduce egg fertilization efficiency.
11. Plug check the female(s) the next morning before 9 am. All females will be used for embryo collection even if a plug is not visible, as the plug may have fallen out.

## **Generic answers for some IACUC protocol form questions**

*Name of the procedure:* The superovulation of donor, female mice for embryo collection.

*Details of procedure:* To induce superovulation, donor, female mice will be hormone-primed using intraperitoneal (IP) injections of PMSG, pregnant mare serum gonadotrophin, and hCG, human chorionic gonadotrophin. Following the IACUC-approved SOP for the superovulation of donor female mice, the PMSG and hCG will each be given once. The hCG injection will be given 46-48 hours after the PMSG injection.

*Approximate possible percentage morbidity and mortality due to the described procedure, including likely causes and how they will be addressed.* The intraperitoneal injection procedure utilized for the hormone priming of the embryo donor females is well documented with very few clinical problems reported. We anticipate very good tolerance of the donor females to hormone priming, with less than 1% morbidity and mortality.