

Super Ovulation

The use to purified gonadotropins to artificially induce the ovulation of a significantly larger number of oocytes than would be collected by natural means was first described in 1956 A.H Gates (Gates 1956). Although these glycoprotein polypeptide hormones are normally produced within the pituitary glands for natural sexual development and reproductive function (Godine 1982), both Pregnant Mare Serum Gonadotropin (PMSG) and Human Chorion Gonadotropin (HCG) are collected and purified from placenta. The first hormone used for hyperstimulation is PMSG, which is a functional mimic of Follicle Stimulating hormone (FSH), which initiates ovarian follicular growth by affecting the granulosa cells, which later become the cumulus cells needed to support the oocyte and act as a spermatozoa chemoattractant (Sun 2015). To further mimic the function of gonadotropins during natural ovulation, the second administered hormone is HCG, which takes on the role of Luteinizing Hormone (LH). A spike in the production of this hormone, called the LH Surge, triggers ovulation and is produced for approximately 24-48hrs in response to FSH stimulation (Hecht 1989). The purpose of controlled ovarian hyperstimulation in this protocol is to recover a significantly larger cohort of embryos for manipulation than can be expected by relying on natural methods, therefore reducing the number of animals required per editing experiment. Additionally, by injecting gonadotropins, the animal's estrous cycle (Nelson 1981) is adjusted to a predictable mating time table that allows the researcher to have more control over the window of fertilization (Fox 1985). This procedure is based on the use of C57BL/6J mice, however, hormone stimulation has been optimized for other commonly used mouse strains which can easily be adopted to this protocol (Luo 2011).

<u>Required Material</u>	<u>Source</u>	<u>Stock</u>	<u>Working</u>
Male Mice (3-8mo)	C57BL/6J JAX 000664		
Female Mice (3-4wk)	C57BL/6J JAX 000664		
Pregnant Mare Serum Gonadotropin (PMSG)	Millipore: Cat# 367222	Lyophilized 1mg = 1000IU	100 IU
Human Chorion Gonadotropin (HCG)	Millipore: Cat# 230734	Lyophilized 1mg = 3000IU	100 IU
Phosphate Buffered Saline (PBS)	Gibco: Cat# 14190-144	1X	1X

Prepare Hormones:

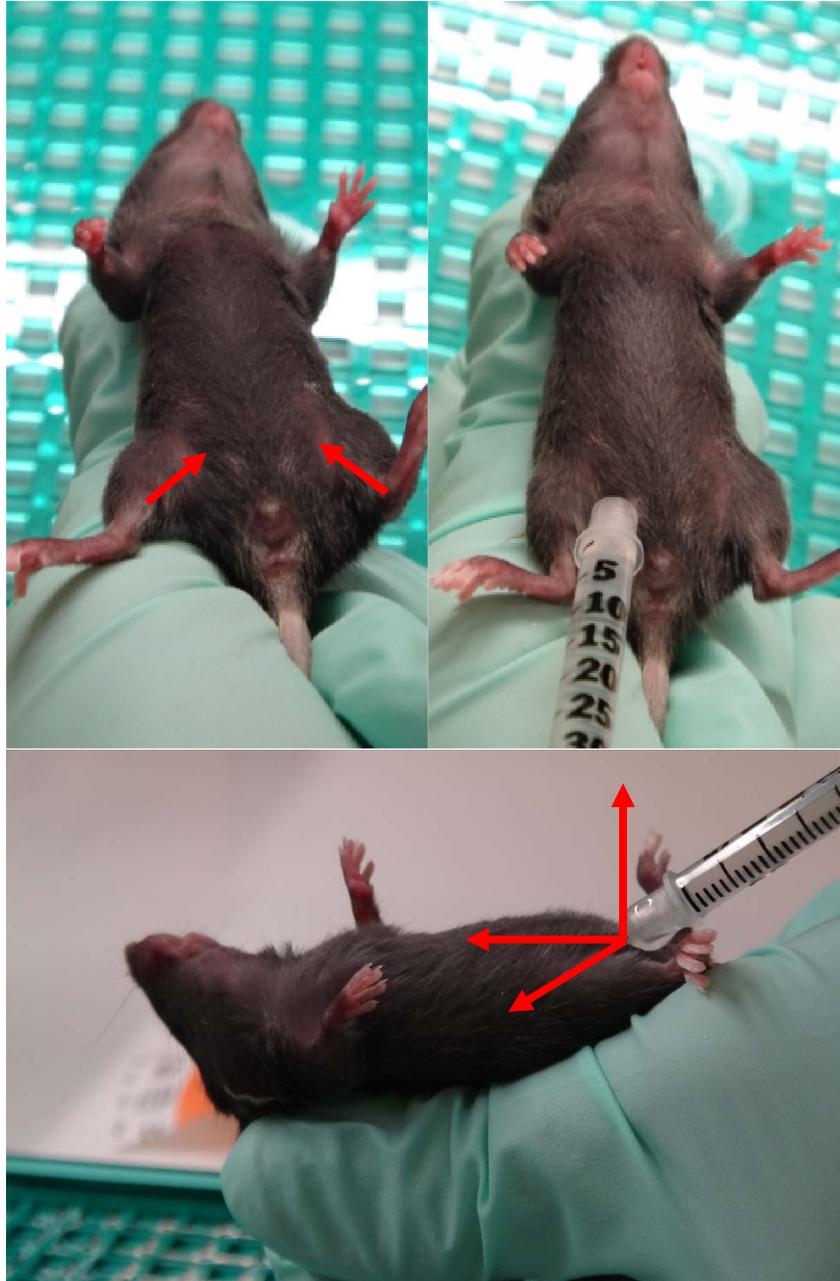
Note: Hormones should be kept cold throughout entire protocol. To minimize exposure to improper temperatures, pre-chill tubes and PBS on ice.

Reconstitute lyophilized hormones. (Hormone arrives as a flaky white powder).

1. Add pre-chilled PBS to glass bottle containing hormone.
2. Mix by gentle inversion and pipetting to ensure recovery of hormone, as the lyophilized powder maybe be difficult to locate in glass bottle.
3. For PMSG: Adjust volume to 20mL with PBS (Now 50 IU/mL)
4. For HCG: Adjust volume to 60mL with PBS. (Now 50 IU/mL)
5. Since our mice are housed in cages of 5, we aliquot our hormones into “single cage” aliquots of 600uL (Additional 100uL to account for volume loss in syringe).
6. Aliquots are distributed into pre-chilled 1.5mL centrifuge tubes and immediately exposed to “Flash Freezing” conditions. (Dry-Ice immersed in 100% EtOH). Liquid nitrogen bath is also adequate. Effort should be made to prevent EtOH from entering the cap of the tube by capillary action.
7. Once frozen, hormones are places directly into -70°C (or colder) freezer conditions.

Hormone Induced Ovulatory Response

Hormones are administered via a 26-28 gauge needle into the intraperitoneal (IP) cavity of the female mouse. Proper injections are carried out by first restraining the mouse, then inverting the mouse to expose the lower abdomen, with the animal's head facing away at approximately a 230° angle. This allows for an unobstructed view of the injection site as well as adjusting the animal's internal organs away from the entry site to prevent sharps related internal wounding. To either the left or right side of the abdomen, injections are made between the two nipples presented on either side (see image).



Hormone Injection Schedule Matrix (*In Utero Culture*)

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
PMSG	No Action	HCG	Oo/Zyg	2C/4C	8C/Mor	Blast
			e0.5	e1.5	e2.5	e3.5
Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday	Monday
Wednesday	Thursday	Friday	Saturday	Sunday	Monday	Tuesday
Thursday	Friday	Saturday	Sunday	Monday	Tuesday	Wednesday
Friday	Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday
Saturday	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday
Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday

Injections:

1. Refer to Table 1 in order to plan injection schedule.
2. Remove PMSG from freezer and allow to reach Room Temperature (RT).
3. Use PMSG within 15 minutes of reaching RT.
4. IP inject female mice with 5 IU PMSG (100uL) between 1-2pm.
5. Approximately 46-48 hours later, remove HCG from freezer and allow to reach RT.
6. Use HCG within 15 minutes of reaching RT.
7. Inject same female mice with 5 IU HCG (100uL), 46-48 after PMSG, to induce ovulation approximately 11 hrs after injection.
8. Once injected, place hormone stimulated female into mating cage of stud (1:1) with proven fertility.
9. The following morning, collect plugged females (See Image). Presence of copulation plug suggests successful breeding.

