

Animal Preparation for Microinjection:

SUPEROVULATION:

Pregnant mare's serum (PMS) is used to mimic follicle-stimulating hormone (FSH) and human chorionic gonadotropin (hCG) is used to mimic luteinizing hormone. The times that the PMS and hCG are administered relative to each other and to the light cycle of the mouse room will affect both the developmental uniformity and the number of eggs that are recovered from superovulated female mice. For most strains, a 42- to 48-hour interval between the PMS injection and the hCG injection has been found to be optimal in terms of egg yield. Generally, ovulation takes place between 10 and 13 hours after injection of hCG, but to control the time of ovulation precisely it is important to administer the hCG prior to the release of endogenous LH (luteinizing hormone).

The recommended dose of PMS for most strains is 5 IU injected intraperitoneally. This hormone is generally supplied as a lyophilized powder. For administration, the PMS is resuspended at 50 IU/ml in sterile 0.9% NaCl and then divided into convenient aliquots. It can be stored in this form at -20 C for at least a month but does not last indefinitely. A dose of 0.1ml is injected into each animal.

hCG is the second gonadotropin that is administered to induce superovulation. Generally, injections of 5 IU are administered intraperitoneally (it is important that the hCG get into the circulation quickly, before the release of endogenous LH). hCG is also supplied as a lyophilized powder. It is resuspended at 500 IU/ml in sterile water, divided into 100 ml aliquots, lyophilized, and then stored, protected from light, at -20 C. To administer the hormone, an aliquot is resuspended in 1 ml of 0.9% NaCl to give a final concentration of 50 IU/ml; 0.1ml is then injected into each animal.

On a 5 am-7pm light cycle, PMS is administered between 1 and 2 pm and the hCG 46-48 hours later usually between noon and 1pm, and thus at least 2-3 hours before endogenous LH release. ¹

¹ From "Manipulating the Mouse Embryo A Laboratory Manual"; Hogan et. al; QL959.M265 1986 ISBN:0-8769-175-1.

EXAMPLE SET UP OF MICE IN PREPARATION FOR MICROINJECTION:

If microinjection of embryos is to take place on a Thursday:

- MONDAY: PMS injections given at *about* 1pm.
- WEDNESDAY:

1. hCG injection given at *about* 12pm and females are placed in mating cages with stud males. These females will serve as embryo donors.

2. Estrus females are selected and placed in mating cages with vasectomized males. These females will serve as foster mothers.

- Thursday:

1. At *about* 10 am all females are checked for plugs. Non-plugged females are returned to stock cages. It is a good idea to keep non-plugged superovulated females aside for a couple of weeks before repeating the hormone treatments a second time.
2. Embryo isolation, microinjection and implantation takes place on this day.