

SUPEROVULATION

Procedure

1. Use prepuberal 3-4 week-old females. Older females can be used but have lower frequency of plugging.
2. Day 1: Inject 7.5 units of Gestyl IP between 10am and 3pm.
3. Day 3: inject 7.5 units of Pregnyl IP between 12 noon and 2pm.
4. After Pregnyl injection immediately mate one on one with a stud male.
5. Day 4: Collect 1 cell eggs from females that have plugged.

Note: The timing between Gestyl and Pregnyl is far less critical than the time between Pregnyl and collection of one- cell eggs that can be manipulated in the early afternoon.

Reagents:

I. **PMS:** Gestyl from Diosynth, Inc. Chicago. 10 vials, 1000 units/vial. Store in refrigerator.

1. Dilute to concentration of 2.5 units/01 ml with PBS plus BSA (PBS+1g/LBSA), i.e., 1000 units into 40 ml.
2. Break tops of ampules and add a small volume of BSA/PBS. Wait until Gestyl is dissolved. Take contents of ampule and add to total volume in a beaker, mix well.
3. Dispense 3 ml into Falcon 2063 tubes labeled with green tape. Store at -20 C .

II. **HCG:** Pregnyl from Sigma #CG-2. 2,500 units/vial. Store in refrigerator.

1. Dilute to concentration of 2.5 units/0.1 ml with PBS plus BSA, i.e., 2500units into 100 ml.
2. Pull rubber septum off vial and add a small volume of BSA/PBS. Wait until Pregnyl is dissolved. Take contents of vial and add to total volume in a beaker, mix well.
3. Dispense 3 ml into Falcon 2063 tubes labeled with pink tape. Store at -20 C .