

SOP No: AHP 66

SUBJECT: Collection and storage of embryos in rodents

POLICY: This procedure may only be performed by a skilled operator

PRECAUTIONS: Surgery is carried out when the donor female is at gestation day 20.

EQUIPMENT: Euthanased pregnant female mice (20—60 hours p.c.)
70% Ethanol
M2 medium at room temperature

INSTRUMENTS (sterilized):-

Embryo-handling pipette consisting of handheld pipette assembly and pulled capillary
Flushing needle (either a 30- or 32-gauge hypodermic needle [end cut and/or ground to a blunt tip on an abrasive stone (oilstone) or sandpaper])
Forceps, fine
Forceps, watchmaker's #5, two pairs
Microdrop culture dish (see Protocol 4.5)
Organ culture dish (Falcon 3037) (*optional*)
Petri dishes (35-mm) or embryological watch glasses
Scissors, fine
Stereomicroscope with transmitted and reflected or fibre optics (*optional*) illumination (preferably a ground-glass stage) with 20x and 40x magnification
Syringe, 1-cc

PROCEDURE:

1. Open the abdominal cavity). Grasp the upper end of one of the uterine horns with fine forceps and gently pull the uterus, oviduct, ovary, and fat pad taut and away from the body cavity. Make a hole in the membrane close to the oviduct with the closed tips of a pair of fine forceps or scissors
2. Pull the oviduct, ovary, and fat pad taut with fine forceps and cut between the oviduct and ovary with fine scissors. Reposition the forceps and cut the uterus near the oviduct, leaving at least 1 cm of the upper part of the uterus attached if the collection is taking place on 2.5 dpc.
3. Transfer the oviduct and attached segment of uterus to a 35-mm petri dish or embryological watch glass containing M2 medium at room temperature. Oviducts from several mice can be collected in the same dish. Place dish under stereomicroscope.
4. Test the syringe to be sure that it is free of air bubbles and that the M2 medium is flowing smoothly before inserting the needle.
5. Use fine forceps to slide the end of the oviduct onto the flushing needle. Gently press the tip of the flushing needle against the bottom of the dish to hold it in place. Flush the oviduct with -0.1 ml of M2 medium.
6. Use pipettes to pick up the embryos and wash them through several drops of fresh M2 medium to rinse off the debris.

7. Transfer the embryos to a microdrop culture dish, rinse through several drops of equilibrated medium, and keep at 37°C, 5% CO₂ until needed. An organ culture dish with equilibrated embryo culture medium may be used as an alternative for short-term incubation

RECOMMENDATIONS:

- 1. To prevent the oviduct from moving while locating the infundibulum, it can be placed in a very small drop of medium or onto dry plastic if it is moved directly from a drop of M2.**
- 2. Because the tip of the flushing needle is blunt, it will not puncture the oviduct. Therefore, it is often possible to use it as a tool to press down the oviduct to the plastic and hold it in place inside the infundibulum while flushing.**
- 3. It is important to use only good-quality embryos for experiments and to distinguish them from delayed or fragmenting embryos**

DATE ISSUED: 04.12.2008

REVISED:

REFERENCES

- 1. Foley PL Common Surgical Procedures in Rodents. In: *Laboratory Animal Medicine and Management*, Reuter J.D. and Suckow M.A. (Eds.). International Veterinary Common Surgical Procedures in Information Service, Ithaca NY Nagy**
- 2. Nagy A, Gertsenstein M, Vintersten K, Behringer R.(2003) Manipulating the Mouse Embryo. A laboratory Manual – 3rd Ed.pp161-208**