



Hosted by the Frozen Embryo and Sperm Archive, MRC- Harwell

Harvest and cryopreservation of mouse sperm

1. For each mouse to be sperm frozen label 8 x Nunc 1.8ml cryotubes (Scientific Laboratory Supplies, Cat. No. 363401), 1 x Kleenex tissue and 1 x 35mm Falcon 351008 Petri dish with the appropriate sample code.
2. Prepare the cooling apparatus by placing a platform, (e.g. the insert from a 200µl pipette tip box), into a polystyrene box. This acts as a support for the cryotube rack.
3. Carefully pour liquid nitrogen into the polystyrene box so that it reaches the top of the platform. Place a cryotube rack on top of the platform so that it is suspended in liquid nitrogen vapour.
4. Replace the lid on the polystyrene box and allow it to fill with vapour. Replenish the liquid nitrogen as necessary during the freezing session, but do not allow the level to rise above the platform.
5. Thaw one aliquot (1.1ml) of cryoprotective agent (CPA) for each male and bring to 37°C in the incubator or hot block. Mix by inversion if there is any precipitation.
6. Pipette 1.0ml CPA into one side of a 35mm Petri dish and place on the hot block at 37°C.
7. Sacrifice the male and swab the abdomen with 70% alcohol.
8. Cut through the abdominal skin, and then cut through the body wall, to reveal the internal organs.
9. Dissect the vasa deferentia and cauda epididymides from the mouse and clean off all adipose and vascular tissue. This is best achieved by placing the organs on a tissue and examining them under a dissecting microscope lit from above.
10. Transfer the organs into the culture dish containing the CPA.
11. Using a 0.5ml insulin syringe and needle cut the surface of one cauda epididymis 6 to 8 times and gently palpate the tissue to expel the sperm. Then gently extrude sperm out of the vas deferens by “walking” along it with two pairs of watchmakers forceps. Repeat for the other side.
12. To disperse the sperm, shake the dish gently for ~30sec and then incubate at 37°C for 10 minutes.
13. Keeping the dish at an angle, shake the dish briefly and move the cauda epididymides and vasa deferentia to one side of the dish using a pipette tip.



EMMA - Cryopreservation training course



14. Using a wide bore pipette tip aliquot 100µl of the sperm suspension into each of 8 cryotubes. Replace the screw cap and tighten to seal the cryotube.
15. Place the cryotubes in the pre-cooled freezing apparatus and leave for 10 minutes.
16. Remove the cryotubes from the rack and plunge into liquid nitrogen.
17. Place the cryotubes in the appropriate compartment of a pre-cooled Nalgene system 100 cryobox, dividing the tubes equally between two boxes so that they can be stored in two separate freezers.
18. Ensuring the cryoboxes and their contents remain frozen at all times place the appropriately labelled cryobox into each of the sperm freezers.

NB: For sperm sample thawing protocol, see IVF procedures.

