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

Preparation of Media and Reagents

A. Cook's IVF medium (used for IVF)

1. Cook's IVF medium is purchased in 50ml bottles from Cook Australia (Cat No: K-RVFE-50) and is used as supplied.

[We routinely use all 50ml of Cook's IVF medium for one IVF session but surplus can be frozen down for later use]

B. KSOM (used for embryo culture)

1. KSOM is purchased from Chemicon/Millipore (Cat No: MR-020P-5F) and supplied as 5 vials of powdered culture medium with 5 x 11.5ml sterile diluent (10ml is used to dissolve 1 vial of powder). It should be stored at +4°C.

2. Remove one vial of KSOM powder and one vial of diluent from the refrigerator. Check that they both have the same batch number and that the batch has not expired.

3. Remove 10ml of the diluent using a pipette.

4. Unscrew the top of the powder vial and carefully add the 10ml of diluent. Replace the lid and swirl to mix. DO NOT SHAKE. Allow to dissolve at room temperature with occasional swirling (which may take 15 minutes) or warm to 37°C to accelerate the process.

5. Using a non-pyrogenic, rubber-free 10ml syringe (e.g. B. Braun Injekt Luer Solo) filter the medium through a 0.2µm filter into a sterile 14ml Falcon tube. Push down the lid to seal tightly. Store at 4°C.

6. Discard the medium after 7 days.

C. M2 medium (used for embryo harvest and washing)

1. M2 medium is purchased from Sigma Chemical Co. (Cat. No. M7167) in 100ml bottles which are stored at +4°C until required.

2. 100 x stock solution of Penicillin/Streptomycin (10,000 units/ml Penicillin; 10mg/ml Streptomycin) is purchased from Sigma Chemical Co. (Cat. No. P0781). On arrival, it is thawed and divided into 1.3ml aliquots which are then re-frozen at -20°C until required.

3. Before use, thaw one aliquot of Penicillin/Streptomycin. Remove 1ml of M2 from the 100ml bottle and replace with 1ml Penicillin/Streptomycin stock solution. Final concentration of Penicillin/Streptomycin is: 100units/ml Penicillin; 0.1mg/ml Streptomycin

4. Using a non-pyrogenic, rubber-free 20ml syringe (e.g. B. Braun Injekt Luer Solo) filter the medium through a 0.2µm filter into sterile 14ml falcon tubes. Push down the lids to seal tightly. Store at 4°C.

5. Discard the medium after 7 days.

E. 1.5M Propylene glycol solution (ProH) - used as a cryoprotectant for embryo freezing

1. Accurately weigh 0.57g Propylene glycol (Aldrich, Cat No. 13,436-8) into a sterile 14ml Falcon tube.

2. Add 4.4ml M2 (with added Penicillin/Streptomycin) to the Propylene glycol.

3. Gently mix the two solutions by rocking.

4. Using a non-pyrogenic, rubber-free 10ml syringe (e.g. B. Braun Injekt Luer Solo) filter the Propylene Glycol/M2 mixture (ProH) through a 0.2µm filter into a sterile 14ml Falcon tube. Push down the lid to seal tightly. Store at 4°C.

5. Discard after 7 days.

F. 1.0M Sucrose solution - used to dilute the Propylene glycol during embryo thawing.

1. Measure 5ml M2 (with added Penicillin/Streptomycin) into a sterile 14ml Falcon Tube.

2. Add 1.71g Sucrose (VWR International (BDH), Cat No. 102745C).

3. Mix gently until the sucrose has dissolved.

4. Using a non-pyrogenic, rubber-free 10ml syringe (e.g. B. Braun Injekt Luer Solo) filter the Sucrose solution through a 0.2µm filter into a sterile 14ml Falcon tube. Push down the lid to seal tightly. Store at 4°C.

5. Discard after 7 days.

G. 18% Raffinose, 3% Skimmed Milk solution- cryoprotective agent (CPA) used for sperm freezing

1. Place 36ml of sterile water for embryo transfer (Sigma Chemical Co., Cat No. W1503) into a 50ml centrifuge tube and equilibrate to 60°C in a water bath.
2. Add 7.2g of Raffinose (Sigma Chemical Co. Cat No. R7630) and dissolve by gentle inversion. Replace tube in water bath whilst weighing Skimmed Milk powder.
3. Add 1.2g of Skimmed Milk powder (Becton Dickinson, Cat No. 232100) to the tube and mix by gentle inversion. Replace in water bath to dissolve further.
4. Aliquot into sterile 2ml microfuge tubes and centrifuge at 14,000rpm for 10 minutes.
5. Carefully decant the supernatant into universal tubes and discard the pellets.
6. Filter using a 0.45µm Nalgene syringe filter into a clean universal tube.
7. Either aliquot 5-10ml CPA into 15ml centrifuge tubes, or 1.1ml CPA into 1.5ml microfuge tubes.
8. Store at -20°C.

H. Hyaluronidase (for removing adherent cells from IVF-produced embryos)

1. 30mg Hyaluronidase Type IV-S (EC 3.2.1.35) powder is purchased from Sigma Chemical Co. (Cat no. H4272). Enzyme activity should be between 750-1500 units/mg, but each batch has a different activity which should be checked.
2. Prepare a 10 mg/ml solution of hyaluronidase in M2 (with added penicillin/streptomycin) as follows.
   - 2.1. Label and date 12 x 1.5ml microfuge tubes.
   - 2.2. Tap the 30 mg bottle of Hyaluronidase gently, to make the powder go to the bottom of the bottle.
   - 2.3. Unscrew the lid and add 3ml of M2.
   - 2.4. Replace the lid and swirl gently to dissolve.
   - 2.5. When in solution, slowly aspirate into a 10 ml syringe using a 21G needle. Invert the syringe and aspirate some air to empty the needle. Remove the needle.
   - 2.6. Filter sterilize through 0.2µm filter into new 14 ml tube.

2.7. Aliquot 250µl into each of the microfuge tubes.

2.8. Store at –20°C.

3. Prepare a working solution with a final concentration of 300µg/ml, as follows:

3.1. Thaw a 250µl aliquot of Hyaluronidase and dilute 1:33 with M2 (30µl hyaluronidase + 960µl M2, or 200µl Hyaluronidase + 6.4ml M2).

3.2. Mix gently.

I. Preparation of Pregnant Mare's Serum Gonadotrophin (PMS)

1. Pregnant Mare's Serum Gonadotrophin (PMS) is purchased from the National Hormone and Peptide Program (NHPP), Harbour-UCLA Medical Centre, 1000 W. Carson Street, Torrance, CA 90509, USA. Each ampoule contains 2000iu.

2. To prepare a working solution of PMS, at 50iu/ml, measure 40ml sterile phosphate buffered saline (PBS) into a sterile 50ml centrifuge tube.

3. Withdraw 1.0ml of the PBS using a sterile syringe and 21G needle and inject the 1.0ml PBS, by piercing the needle through the rubber cap, into the ampoule of powdered PMS.

4. Swirl the mixture gently until the powder has dissolved.

5. Invert the ampoule and using the same syringe and needle remove the hormone solution and add it to the remaining 39ml PBS.

6. Rinse the ampoule with 1.0ml of the diluted hormone, (using the original syringe and needle). Swirl again to remove any remaining hormone, and return the solution to the large tube.

7. Gently mix the PMS solution by inversion.

8. Dispense the PMS solution into either 1.3ml aliquots (enough for 10 females) stored in sterile microfuge tubes, or 5.2ml aliquots (enough for 50 females) stored in sterile Universal vials.

9. Freeze at -20°C until required.

J. Preparation of Human Chorionic Gonadotrophin (hCG)

1. Human Chorionic Gonadotrophin (hCG) is manufactured by Intervet under the trade name Chorulon and supplied by W+J Dunlop Ltd; each ampoule contains 1500iu.

2. To prepare a working solution of hCG, at 25iu/ml, measure 60ml sterile water for embryo transfer (Sigma Chemical Co., Cat. No. W1503) into a sterile 50ml centrifuge tube (it just fits!).

3. Withdraw 1.0ml of the H_2O using a sterile syringe and 21G needle and inject the 1.0ml H_2O into the ampoule of powdered hCG, by piercing the needle through the rubber cap.

4. Swirl the mixture gently until the powder has dissolved.

5. Invert the ampoule and using the same syringe and needle remove the hormone solution and add it to the remaining 59ml H_2O.

6. Rinse the ampoule with 1.0ml of the diluted hormone, (using the original syringe and needle). Swirl again to remove any remaining hormone, and return the solution to the large tube.

7. Gently mix the hCG solution by inversion.

8. Dispense the hCG solution into either 1.3ml aliquots (enough for 10 females) stored in sterile microfuge tubes, or 5.2ml aliquots (enough for 50 females) stored in sterile plastic Universal vials.

9. Freeze at -20°C until required.