Preparation of CPA with L-Glutamine

1.0 Equipment

1.1 Spatula

1.2 Analytical Balance

1.3 1000ul Gilson pipette

1.4 Brady Printer

1.5 Scissors

1.6 Vortex mixer

1.7 Centrifuge

1.8 Safety glasses

1.9 LAF Cabinet

1.10 Water bath

1.11 Countdown timer

1.12 Tube rack

2.0 Supplies

2.1 Embryo Transfer Water

2.2 Raffinose Pentahydrate 99% or 98%

2.3 Skimmed milk Powder

2.4 L-Glutamine

2.5 50ml Corning centrifuge tube

2.6 1.5ml Eppendorfs

2.7 Sterile 1.5ml Eppendorfs

2.8 0.2µm Filters

2.9 20ml Syringe

2.10 Parafilm

2.11 Weighing paper/boat

2.12 Brady Labels

2.13 Brady ribbon

2.14 1000ul pipette tips

2.15 Gloves

2.16 Purified water

3.0 Procedure

3.1 Media preparation

3.1.1 Heat water bath to 60°C.

3.1.2 Add 40ml embryo transfer water to a 50ml Corning centrifuge tube and place in 60°C water bath.

3.1.3 Using the analytical balance weigh out 0.584g of L-Glutamine.

3.1.4 Remove the 50ml Corning centrifuge tube from the water bath and add the L-Glutamine to the embryo transfer water.

3.1.5 Vortex the solution for 3 minutes, then replace the tube back into the 60°C water bath.

3.1.6 Weigh out 7.2g of Raffinose pentahydrate.

3.1.7 Remove the 50ml Corning centrifuge tube from the water bath and add the Raffinose pentahydrate to the embryo transfer water solution. Return the tube to the water bath.

3.1.8 Weigh out 1.2g of skimmed milk powder. Remove the tube from the water bath and add the 1.2g of skimmed milk powder to the embryo transfer water solution.

3.1.9 Vortex for a further 3 minutes.

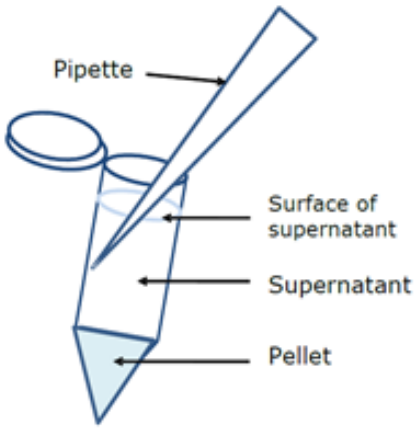
3.1.10 Incubate the solution in a water bath at 60°C for a total of 90mins. Vortex the solution at 30min intervals for duration of 3mins each. The vortex time is in addition to the 90mins.

3.1.11 At the end of the incubation period, aliquot 1ml of the solution into 1.5ml Eppendorf Tubes wearing gloves.

3.1.12 Centrifuge these samples at 10,000g or 10,100g (depending on the model of the centrifuge) for 60 mins, ensuring that the centrifuge is balanced. It may be necessary to centrifuge the solution in two batches, one after another. Alternatively the batches may be divided between two centrifuges run side by side.

3.1.13 Once the full centrifuge cycle has completed, carefully remove 0.7ml supernatant from the centre of each Eppendorf (Picture 1). Combine in a new 50ml Corning tube.

Picture 1



3.1.14 In a deep cleaned LAF cabinet, wearing gloves, filter the supernatant using a disposable 20ml syringe and 0.2µm filters into a fresh 50ml Corning tube.

3.1.15 In the LAF cabinet, remove ~500µl CPA and place into a clean Eppendorf. Use this sample to check the osmolality; this should be 500-540 mOsm/kg.

3.2 Aliquoting

3.2.1 Once the osmolality has been tested, in the deep cleaned LAF cabinet, aliquot 1.0ml per Eppendorf tube. Use autoclaved Eppendorfs.

3.2.2 Label the Eppendorfs with the solution name and date of preparation. Parafilm each Eppendorf and store at room temperature, away from light – in a drawer, for up to 3 months.

3.3 Testing the CPA

3.3.1 To test the CPA, make up 2 x 60µl dishes of CPA overlaid with silicone fluid. One dish should be made with tested CPA (control) and the other dish should be made with the CPA that needs testing.

3.3.2 Dissect a WT male and freeze the sperm of 1 epididymis in the tested CPA and the other in the CPA that needs to be tested. Determine pre-freeze progressive motility of both.

3.3.3 Thaw 1 straw from each test group into a warm 90µl MBCD dish, incubate at 37°C for 30 mins and determine post-freeze progressive motility of both.

3.3.4 Sperm from both test groups should have similar results. If all sperm are dead, repeat the test. CPA may need to be re-made.