Preparation TYH & Methyl-β-cyclodextrin (MBCD)

1.0 Equipment

1.1 Stuart CB162 Magnetic stirrer

1.2 500ml beaker

1.3 500ml volumetric flask

1.4 Spoons & spatulas

1.5 Funnel

1.6 Magnetic follower (medium size)

1.7 Designated plastic stopper

1.8 Analytical balance

1.9 10ml Gilson pipette

1.10 1000µl Gilson pipette

1.11 Scissors

1.12 Measuring cylinder

1.13 Safety glasses

2.0 Supplies

2.1 Embryo transfer water

2.2 0.20µm Filters

2.3 20ml Syringe

2.4 Sterile media bottles

2.5 Parafilm

2.6 Weighing paper

2.7 Sodium chloride (NaCl)

2.8 Potassium chloride (KCl)

2.9 Magnesium sulphate heptahydrate (MgSO4·7H2O)

2.10 Potassium phosphate monobasic (KH2PO4)

2.11 Sodium bicarbonate (NaHCO3)

2.12 D-(+)-Glucose

2.13 Sodium pyruvate

2.14 Penicillin G potassium salt

2.15 Streptomycin sulphate salt

2.16 Calcium chloride dihydrate (CaCl2·2H2O)

2.17 Polyvinyl alcohol (PVA)

2.18 Methyl-β-cyclodextrin (MBCD)

2.19 Purified water

2.20 1.5ml Eppendorf tubes

2.21 10ml Diamond pipette tips

3.0 Procedure

3.1 General Information

3.1.1 Safety glasses must be worn at all times by the person preparing the media, and by those working in the immediate area.

3.2 Preparing the media

3.2.1 Add a medium magnetic follower to a 500ml glass beaker and add 300ml embryo transfer water. Stir at setting ~2.5 with no heat.

3.2.2 Add chemicals in the order outlined in Table 1, rinsing spatulas with purified water and drying with white tissue between each.

3.2.3 Cover the beaker with parafilm and continue to stir until the PVA has dissolved (approx. 1 hour).

Table 1

Reagent Name	**g/100ml**	**g/500ml**	**Vendor**	**Cat. Number**
NaCl	0.6970g	3.4850g	Sigma	S5886
KCl	0.0356g	0.1780g	Sigma	P5405
$MgSO_4 \cdot 7H_2O$	0.0293g	0.1465g	Sigma	M1880
KH_2PO_4	0.0162g	0.0810g	Sigma	P5655
$CaCl_2 \cdot 2H_2O$	0.0251g	0.1225g	Sigma	C7902
Na-Pyruvate	0.0055g	0.0275g	Sigma	P4562
Glucose	0.1000g	0.5000g	Sigma	G6152
Methyl-β-cyclodextrin	0.0983g	0.4915g	Sigma	C4555
Penicillin G	0.0075g	0.0375g	Sigma	P4687
Streptomycin	0.0050g	0.0250g	Sigma	S1277
$NaHCO_3$	0.2106g	1.0530g	Sigma	S5761
Polyvinyl alcohol (PVA)	0.1000g	0.5000g	Sigma	P8136

3.2.4 Pour the solution into a 500ml volumetric flask slowly using a funnel to avoid creating bubbles. Rinse the beaker with embryo transfer water and add to volumetric flask and repeat 2-3 more times.

3.2.5 Make the total volume up to 500ml with embryo transfer water until the bottom of the meniscus reaches the 500ml mark. The last ~1ml can be added in smaller volumes using a 1000µl pipette to ensure the solution does not go over the line. Cover the opening with designated plastic stopper or parafilm and mix by inversion.

3.2.6 Filter the solution through a 1000ml corning filter. Label container housing the filtered MBCD solution with 'Filtered MBCD' and date prepared.

3.2.7 Under a deep cleaned LAF cabinet, aliquot out 0.5ml into an Eppendorf. Use this sample to test osmolality; this should be 283-293mOsm/kg.

3.2.8 Prepare labels with 'MBCD' and the preparation date in the format DD/MM/YYYY.

3.2.9 In a deep cleaned LAF cabinet, aliquot 1ml of solution into autoclaved Eppendorf tubes, label and parafilm Eppendorfs, and store at 4-8°C for up to 3 months.

3.2.10 Record weights and batch/lot numbers of all chemicals.

3.3 Testing the MBCD solution

3.3.1 To test the MBCD solution, make one dish (usually a control dish) containing the batch of MBCD to be tested with all other MBCD dishes containing the previously tested batch. Perform IVF following standard protocol. For the MBCD to pass the test, the fertilisation rate should be >60% for a frozen sperm sample or >80% for a fresh sperm sample. If the MBCD test fails the QC, the solution will need to be re- made.