Preparation of high calcium hTF medium for IVF

1.0 Equipment

1.1 Heat-Stir CB162 Magnetic stirrer

1.2 Magnetic follower

1.3 1L beaker

1.4 Spatulas

1.5 1 Litre Volumetric flask

1.6 Analytical Balance

1.7 Electric filter pump

1.8 Gilson pipette (P10mL, P1000, P200)

1.9 Brady label printer

1.10 Scissors

1.11 Vertical Laminar Air Flow

1.12 Recycling cabinet (LAF)

2.0 Supplies

2.1 Water for Embryo Transfer

2.2 10ml Diamond tips, 1000µl and 200µl tips

2.3 1.5ml Eppendorfs

2.4 Parafilm

2.5 Corning 1000ml filter unit

2.6 14ml Falcon tubes

2.7 Weighing paper

2.8 Brady Labels

2.9 Brady ribbon

2.10 NaCl

2.11 KCl

2.12 MgSO4·7H2O

2.13 KH2PO4

2.14 NaHCO3

2.15 Glucose

2.16 Na-lactate

2.17 Na-Pyruvate

2.18 Penicillin G

2.19 Streptomycin

2.20 CaCl2·2H2O

2.21 Phenol Red

2.22 BSA (Albumin Bovine Serum, Fraction V, Fatty Acid-Free)

2.23 Purified water

2.24 Mask

2.25 Gloves

2.26 Safety glasses

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.

3.1.2 Chemical reagents must always be added in order unless otherwise specified.

3.2 Preparing the media

3.2.1 Add approx. 500ml of Embryo Transfer Water into a 1L beaker and rinse an appropriate sized magnetic follower in purified water, dry with a tissue and place into the beaker.

3.2.2 Place the beaker onto the magnetic stirrer and begin to stir at approx. 2.5, without heat.

3.2.3 Weigh out and add the reagents to the beaker of embryo transfer water in the order specified in Table 1. Rinse the spatulas with purified water and dry them with a tissue between each chemical.

Table 1

Reagent Name	mg/1000ml
NaCl	5938.00
KCl	350.00
MgSO4·7H2O	49.00
KH2PO4	54.00
CaCl2·2H2O	755.00
Glucose	500.00
Na-lactate (ml)*	3.4ml
Na-Pyruvate	37.00
Penicillin G	75.00
Streptomycin	50.00

NaHCO3	2100.00
0.5% Phenol Red (ml)	0.2ml
BSA (Albumin Bovine Serum, Fraction V, Fatty Acid-Free)	4000.00

***NOTE:** when adding the Na-lactate pipette 4 x 0.85ml aliquots (for 1000ml hTF) very slowly, making sure to pick up and expel the full volume, as the liquid is very viscous. Pre-rinse the pipette tip when pipetting viscous liquids and change the tip each time it is used for cold liquids to increase accuracy.

3.2.4 Stop the magnetic stirrer. Weigh out and add the BSA to the solution then cover the beaker with parafilm and leave until the BSA has dissolved naturally (approx. 1 hr).

3.2.5 Once the BSA has dissolved, briefly stir the solution by switching the stirrer on to a low setting taking care not to introduce any bubbles.

3.2.6 Pour the solution into a 1L volumetric flask and make the volume of the solution in the flask up to 1L with embryo transfer water by rinsing the beaker with embryo transfer water 3-4 times and adding it to the solution. Continue adding until the bottom of the meniscus reaches the 1 litre mark.

3.2.7 Parafilm the opening of the volumetric flask or use a stopper then mix gently by inversion. Replace the solution into the beaker.

3.2.8 Filter the solution through a Corning 1000ml filter unit with electric pump.

3.2.9 Once filtered, in a deep cleaned LAF cabinet, wearing gloves and a mask, take a 1ml aliquot and place in an Eppendorf tube.

3.2.10 Check the osmolality of the hTF; it should be 300-310mOsm/kg.

3.2.11 In a clean LAF cabinet, wearing gloves and a mask, aliquot 8ml into 14ml Falcon tubes.

3.2.12 Label with "hTF" and date made. Parafilm each Falcon tube.

3.2.13 The hTF can be stored at 4-8°C for up to 3 months.

3.3 Testing the media

3.3.1 The hTF should be tested as soon after the preparation date as possible.

3.3.2 To test the hTF, select one IVF dish for the test.

3.3.3 Prepare the fertilisation medium (containing GSH) for that dish using the hTF to be tested. The corresponding wash dish should also be prepared using the hTF batch to be tested.

3.3.4 All other IVF dishes on that day should contain fertilisation media prepared using a proven tested batch of hTF. The corresponding wash dishes should also be prepared using the proven tested batch of hTF.

3.3.5 To pass the QC, the IVF dish containing the test batch of hTF should have a fertilisation rate similar to the tested batch of hTF. If the fertilisation rate for the test batch is significantly lower, or the cells are of a poor quality, the QC will have failed and a new batch will need to be prepared.