Blastocyst Genotyping

1.0 Equipment

1.1 P200 Gilson pipette

1.2 EZ Grip Handling pipette

1.3 Dissecting Microscope

1.4 Heated Stage

1.5 Incubator (5% CO_2, 37°c)

1.6 Powerpette

1.7 Laminar airflow (LAF) cabinet

1.8 Vacuum aspirator with pump

1.9 Multi-channel pipette

1.10 37°C Waterbath

2.0 Supplies

2.1 200µl pipette tips

2.2 Gloves

2.3 200µl EZ tips

2.4 Silicone Fluid

2.5 10ml Stripette

2.6 Embryo Culture Dishes (35:3004)

2.7 Parafilm

2.8 Biopsy plate cover

2.9 KSOM

2.10 70% Alcohol

2.11 96-well cell culture plate

2.12 0.1% gelatin solution

2.13 Jm8 + dLIF media

2.14 Media reservoirs

2.15 Sterile glass pipettes

2.16 200µl filter pipette tips

2.17 Digital Timer

2.18 2% Distel

2.19 DPBS

2.20 2% gelatin

2.21 Dispo-safe jar

3.0 Procedure

3.1 General Information

3.1.1 Strict aseptic technique should be adhered to at all times when preparing plates.

3.1.2 Ensure only one blastocyst is plated per well.

3.1.3 If the blastocyst plating day falls on a Saturday, embryos at the morula stage can be plated a day earlier. If the plating day falls on a Sunday, the hatched blastocysts can be plated a day later.

3.1.4 A new row should be started for each stock.

3.2 Culturing Embryos

3.2.1 On a 60mm embryo culture dish write the date on the lid of the dish along with the IVF No. and stock.

3.2.2 Make sure the dish, silicone fluid and KSOM have all passed a QC test prior to use.

3.2.3 Place a 100µl drop of KSOM media in the centre and overlay with silicone fluid.

3.2.4 Culture dishes must be placed in the incubator for a minimum of 15 mins before putting the embryos into culture, allowing time for them to equilibrate.

3.2.5 Keeping the dishes on a 37°C heated stage, move the embryos into the KSOM drop.

3.2.6 Place the culture dish back in the incubator and leave for the required number of days (see Appendix 1 and 2).

3.3 Preparation of 0.1% gelatin solution

3.3.1 Ensure a deep clean of the LAF cabinet has been carried out. Carry out an additional wipe down of cabinet with 2% Distel followed by 70% alcohol prior to proceeding.

3.3.2 Wipe supplies, except serological pipette, into the cabinet with 70% alcohol.

3.3.3 Place a bottle of 2% gelatin in a 37°C water bath for 10-15 minutes to liquefy.

3.3.4 In the LAF cabinet, pipette 25ml warmed gelatin into 500ml DPBS. Mix the solution by inverting the bottle several times. Label the bottle with "0.1% gelatin solution" and date prepared. The expiry period is 1 month. Prior to each use, check the gelatin solution for bacterial growth and ensure the expiry period has not passed.

3.4 Preparation of cell culture plates

3.4.1 Ensure a deep clean of the LAF cabinet has been carried out. Carry out an additional wipe down of cabinet with 2% Distel followed by 70% alcohol prior to proceeding.

3.4.2 Wipe supplies into the cabinet with 70% alcohol – except the timer, serological pipettes and 96-well plates.

3.4.3 Switch on the pump for the aspirator and wipe down the aspirator with 70% alcohol and place inside the LAF cabinet.

3.4.4 Take a 96-well plate with intact outer packaging to the edge of the hood, open pack and place the plate into the hood.

3.4.5 Open the outer packaging of a media reservoir and place 0.1% gelatin solution in the reservoir using a 10ml serological pipette (use a volume of 6ml solution per plate).

3.4.6 Use a multichannel pipette to aliquot 50µl of 0.1% gelatin into each well using 200µl filter tips. Ensure filters in tips have not dropped, reverse pipette, work from left to right covering the occupied wells with plate lid to maintain good aseptic technique.

3.4.7 Start a timer for 5 minutes and do not disturb the culture plate.

3.4.8 After 5 mins, remove excess gel from every well using an autoclaved glass pipette and aspirator by tilting the plate slightly forward and placing the aspirator to the bottom of the well. The aspirator will puncture a hole in the remaining gel layer so aspirate close to the bottom edge when you tilt plate towards you slightly. Continue in a logical manner until gel is removed from every well.

3.4.9 Place JM8 + dLIF media in a fresh media reservoir using a 10ml serological pipette (use a volume of 12ml media per plate).

3.4.10 Use a multichannel pipette to aliquot 100µl into each well using 200µl filter tips. Reverse pipette, dispensing

against the wall of the well and work from left to right, covering occupied wells with the plate lid.

3.4.11 Aspirate excess media and gelatin solution from both reservoirs using a glass pipette and aspirator.

3.4.12 Write the date made on the lid, avoiding covering any wells, and place cell culture plates in incubator to equilibrate for at least 30 minutes before use.

3.4.13 Discard any contaminated supplies in clinical waste bin, pipette tips into dispo safe jar & glass pipettes and used serological pipettes into glass bin.

3.4.14 Run 70% alcohol through the aspirator to clean whilst pressing button and wipe down LAF cabinet with 2% Distel followed by 70% alcohol.

3.5 Plating Blastocysts

3.5.1 Two cell embryos are to be cultured in KSOM until they reach the blastocyst stage (See Appendix 3).

3.5.2 The day the embryos are expected to reach blastocyst stage is the day they should be plated. Embryos can also be plated at the morula or hatching stage.

3.5.3 If the embryos do not develop to blastocyst/morula stage or are dead/fragmented (See Appendix 4), do not plate.

3.5.4 Remove the culture dish containing the blastocysts from the incubator and place on a 37°C heated stage which is situated on the stage of the dissecting microscope.

3.5.5 Remove a plate from the incubator and place on a 37°C heated stage which is kept to the side of the dissecting microscope.

3.5.6 Using an EZ Grip and 200µl EZ Tip, pick up some media from the first well of the plate and then pick up a single blastocyst. Expel the whole contents into the first well, including some air to introduce bubbles. This ensures the embryo is no longer in the device and the bubbles act as an indication that an embryo has been placed in that particular well.

3.5.7 Fill the pipette from the next well and repeat step 3.5.6 until all the embryos from that particular stock are plated.

3.5.8 The pipette tip should be changed for each stock being plated.

3.5.9 Once the plating is complete, label the lid with the IVF session number, the stock, and the wells that the embryos of that stock are in (i.e. A1-B12) (ensure this information is not written over the wells). Return plate to the incubator and leave for the required number of days (see Appendix 1 and 2).

3.6 Preparation of Plate for Genotyping

3.6.1 Assess the growth of the cells to make sure they have not died and the plate is worth preparing by looking at them under the microscope.

3.6.2 To prepare the plate for genotyping, use an autoclaved glass pipette and aspirator in the deep cleaned LAF cabinet to remove all of the media from each well that contains embryos. This information is available on the lid of the plate. Place the plate at a slight angle and put the aspirator to the bottom of the well.

3.6.3 Media should not be removed from the wells in which no embryos were placed. A heated stage is not required for this step.

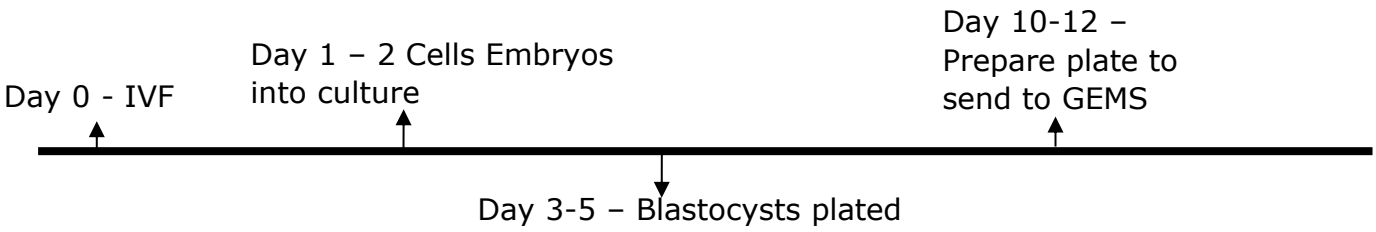
3.6.4 Parafilm over the top of wells (or use a biopsy plate cover) and place the lid on top.

3.6.5 Store the plate in a -20^{o}c freezer.

3.6.6 The plate is now ready to be genotyped.

4.0 Appendix 1

Picture to show the blastocyst genotyping preparation timeline

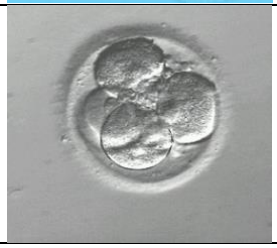


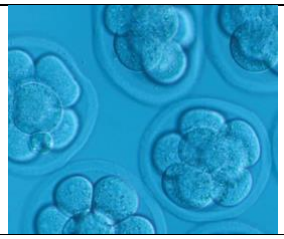
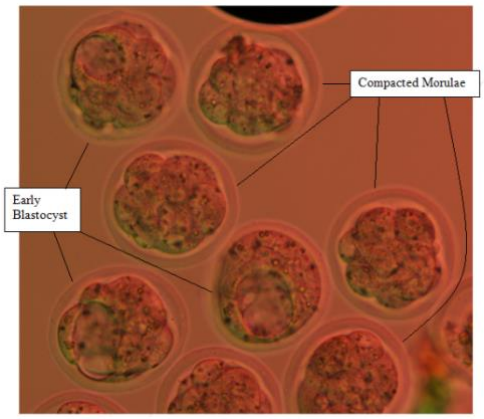
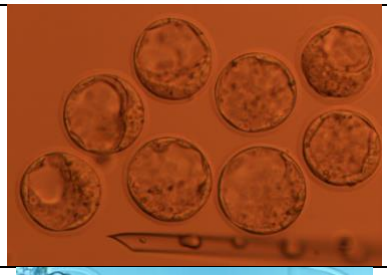
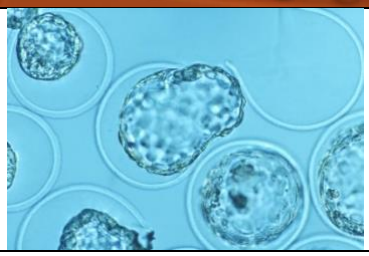
5.0 Appendix 2

Table to show when the embryos should be cultured, plated and prepared.

Day of IVF (Day 0)	Embryos in Culture (Day 1)	Blastocysts Plated (Day 3/4/5)	Plates Prepared for Genotyping (Day 10/11/12)
Monday	Tuesday	Friday	Following Friday
Tuesday	Wednesday	Friday	Following Friday
Wednesday	Thursday	Monday	Following Monday
Thursday	Friday	Monday	Following Monday

6.0 Appendix 3

Cell Development Stage	
2 cell	
4 cell	

8 cell	
Morula	
Blastocyst	
Hatching blastocyst	

7.0 Appendix 4

Degeneration State	
Fragmented	
Dead