

Culturing Embryos

1.0 Equipment

- 1.1** Embryo Handling Device
- 1.2** Scissors
- 1.3** Dissecting microscope
- 1.4** Electronic timers
- 1.5** Oxygen monitor
- 1.6** Metal rod
- 1.7** 1000ul pipette
- 1.8** 100ul pipette
- 1.9** Powerpette
- 1.10** Safety Glasses
- 1.11** 1L dewar for liquid nitrogen

2.0 Supplies

- 2.1** Tissues
- 2.2** Liquid nitrogen
- 2.3** 60mm embryo culture dishes
- 2.4** M2 media
- 2.5** KSOM media
- 2.6** Silicone fluid
- 2.7** 100ul – 1000ul pipette tips
- 2.8** 1-200ul pipette tips
- 2.9** 10mL strippette

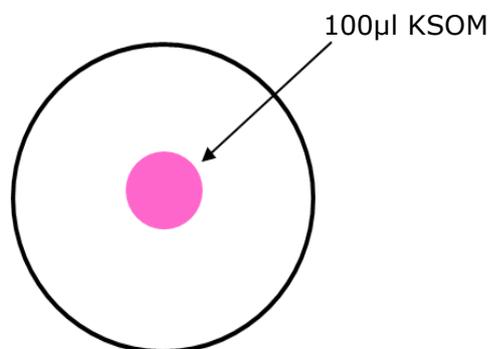
3.0 Procedure

3.1 Preparing Dishes

- 3.1.1 Place a 100µl drop of KSOM media in the centre of a 60mm embryo culture dish (3004) and overlay with silicone fluid (Picture 1).

NOTE: Make sure the dish is from a tested batch. If the culture test is for the purpose of batch testing dishes, always use a tested dish as a control.

Picture 1



- 3.1.2 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 Identifying embryos and straws for culturing

- 3.2.1 For oil and dish tests fresh embryos generated from IVF control dishes can be used for the culture. If there aren't any IVF's scheduled, frozen embryos from a tested FZ session can be used.
- 3.2.2 The embryos used for the test and control dishes have to be from the same cross. If not enough fresh IVF control embryos are produced, then frozen embryos from the same cross will have to be used (e.g. C57BL6N Tac USA x C57BL6N Tac USA).
- 3.2.3 If selecting straws to test an FZ session, identify one straw from each untested session.

- 3.2.4 When selecting straws for oil or dish tests identify straws that are available to be thawed from a proven freeze session.

3.3 Thawing embryos

- 3.3.1 Thaw embryos using the appropriate thawing protocol (controlled rate/vitrification). Aim for ~20 embryos per test. Ensure safety glasses are worn.

NOTE: Make sure the embryos are from a tested batch. If the culture test is for the purpose of testing an embryo freezing session, always use tested embryos as a control.

3.4 Culturing Embryos

- 3.4.1 Once the culture dishes have been equilibrated, the embryos can be placed into culture. Move the embryos to the required culture drop.

NOTE: With the exception of cultures to test freezing sessions, pool embryos together then move them into required culture drops.

NOTE: For embryos thawed to test freezing session, keep the embryos from each straw separate and move into different culture drops.

- 3.4.2 Dishes for an **oil culture test** should be labelled with the date, number of embryos per drop and the oil number.
- 3.4.3 Dishes for an **embryo freeze session test** should be labelled with the straw identifier, date, number of embryos per drop and the freeze session number.
- 3.4.4 Dishes for a **dish batch test** should be labelled with the date, number of embryos per drop and the dish batch number.
- 3.4.5 See Appendix 1 for a summary of the controls and variables for each test.

3.5 Checking Embryo Development Daily

3.5.1 Complete the culture test form each day to record the development of the embryos (Picture 2).

Picture 2

| <u>Culture Test</u> | | | | | | |
|--|--------------|---------------|----------|------------------|----------|----------|
| Date: | | | | | | |
| Purpose: Dish Test/Oil Test/Media Test/Other: | | | | | | |
| Dish Batch: | | Oil #: | | KSOM Ref: | | |
| Dish | Test Details | 0 Hours | 24 Hours | 48 Hours | 72 Hours | 96 Hours |
| 1 | | | | | | |
| 2 | | | | | | |
| 3 | | | | | | |
| 4 | | | | | | |
| 5 | | | | | | |

3.5.2 Only count the number of good cells that are at the correct stage of development or beyond (See Appendix 2).

3.5.3 Allow 24 hours to lapse before checking the cultures each day to allow the embryos sufficient time to reach the next developmental stage.

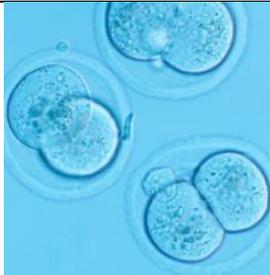
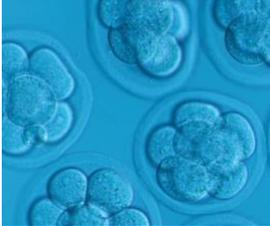
4.0 Appendix 1

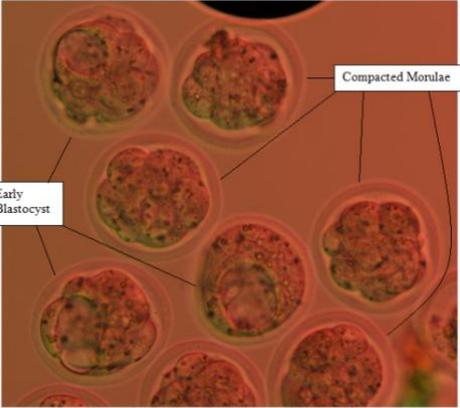
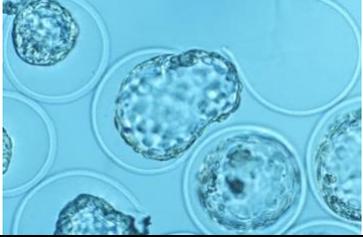
Table to show tested/untested variables when setting up culture tests.

| | | | |
|-----------|----------|-----------------|-----------------------|
| Variables | Oil Test | Dish Batch Test | Freezing Session Test |
|-----------|----------|-----------------|-----------------------|

| | | | |
|--------------|--|---|--|
| Oil | UNTESTED | TESTED | TESTED |
| Dish batch | TESTED | UNTESTED | TESTED |
| Embryos | IVF CONTROL/FZ TESTED | IVF CONTROL/FZ TESTED | UNTESTED |
| Media | TESTED | TESTED | TESTED |
| Pool embryos | YES | YES | NO |
| Control | TESTED OIL BATCH | TESTED DISH BATCH | TESTED EMBRYO FREEZE SESSION |
| Label dish | Date # of embs/drop Oil # | Date # of embs/drop Dish Batch # | Date # of embs/drop FZ/#### |

5.0 Appendix 2

| Cell Development Stage | |
|------------------------|---|
| 2 cell |  |
| 4 cell |  |
| 8 cell |  |

| | |
|----------------------------|--|
| <p>Morula</p> |  <p>Micrograph showing several morulae and early blastocysts. Labels include 'Early Blastocyst' and 'Compacted Morulae'.</p> |
| <p>Blastocyst</p> |  <p>Micrograph showing several blastocysts.</p> |
| <p>Hatching blastocyst</p> |  <p>Micrograph showing several hatching blastocysts.</p> |