# Preparation of Lifor

## 1.0 Equipment

**1.1** Fume hood

**1.2** LAF cabinet

**1.3** Safety glasses

**1.4** 10ml Gilson pipette

**1.5** Balance

**1.6** Weighing Spoons/Spatulas

**1.7** -80°C freezer

**1.8** 10ml & 100ml volumetric flasks

**1.9** Beaker

**1.10** Conical flask

## 2.0 Supplies

**2.1** DMSO

**2.2** Lifor Solution

**2.3** 10ml pipette tips

**2.4** 1.5ml Eppendorf tubes (autoclaved)

**2.5** Gloves

**2.6** Weighing Boats/Paper

**2.7** Purified Water

**2.8** Tissues

**2.9** Quercetin

## 3.0 Procedure

### 3.1 General Information

3.1.1 The Lifor solution is to be made in a deep cleaned LAF cabinet and fume hood, wearing gloves.

3.1.2 The Lifor + Quercetin + DMSO solution, is not sterilised by filtration therefore it is essential that the solution is aliquoted into autoclaved 1.5ml Eppendorfs.

3.1.3 The solution has a shelf life of 12 months in the -80°C freezer.

3.1.4 Rinse weighing spoons and spatulas with purified water before and after use, dry thoroughly with tissues.

### 3.2 Preparing Quercetin at 1mg/ml

3.2.1 In a deep cleaned LAF cabinet pour the contents of a 10ml vial of DMSO into a clean glass beaker. It may be necessary to open an extra 5ml vial throughout the procedure in order to get the required volumes.

3.2.2 Using a 10ml pipette, aspirate 5ml of DMSO from the beaker into a clean conical flask. Parafilm the conical flask and take to a clean fume hood.

3.2.3 Using the balance in the fume hood, weigh out 0.01g of Quercetin. Add the Quercetin to the conical flask containing the 5ml of DMSO. Mix well until all the Quercetin has dissolved.

3.2.4 Cover the conical flask with parafilm or a bung and making sure there is a tight seal, invert 3-4 times to mix. Still covered, take the conical flask to the LAF cabinet where the rest of the procedure is to be carried out.

3.2.5 Transfer the DMSO + Quercetin solution into a 10ml volumetric flask with a 10ml pipette.

3.2.6 Use the remainder of the previously opened DMSO in a beaker to make the solution up to 10ml in the volumetric flask by rinsing the conical flask 2-3 times.

3.2.7 Use parafilm or a bung to cover the opening of the volumetric flask and invert 3-4 times to mix.

## 3.3 Prepare a 10% solution of DMSO and Quercetin at 100µg/ml

3.3.1 Transfer approx. 50ml of Lifor into a 100ml volumetric flask, then transfer the 10ml DMSO + Quercetin solution into the same 100ml volumetric flask.

3.3.2 Make the solution in the volumetric flask up to 100ml using Lifor, rinsing the 10ml volumetric flask 2-3 times.

3.3.3 Use parafilm or a bung to cover the opening of the volumetric flask and invert 3-4 times to mix.

3.3.4 Aliquot 1ml into autoclaved 1.5ml Eppendorf tubes, label with 'Lifor + DMSO + Q', date, and store at -80°C.

## 3.4 Testing the Lifor

3.4.1 Prepare 3 x 60µL dishes of CPA overlayed with silicone fluid. The dishes should be made with tested CPA.

3.4.2 To test the Lifor, dissect the epididymides from a WT male. Place one epididymis into Lifor and sperm freeze the second epididymis immediately. Determine the pre- freeze progressive motility for the sperm frozen male. Place the frozen straws into short term storage until required.

3.4.3 Repeat 3.4.2 for another 2 x males.

3.4.4 Place the epididymides that are in Lifor in a biotube, place the biotube between two cool packs within a

silver box and place in the fridge at 4°C. Hold the epididymides that have been placed in Lifor for 24hr, 48hr and 72hr.

3.4.5 After the various time periods have elapsed, freeze the sperm within the epididymides held in Lifor. Determine the pre- freeze progressive motility and place the frozen straws into short term storage until required.

3.4.6 Once all of the epididymides have been sperm frozen, thaw 1 straw from each group into a warm 90ul MBCD dish, incubate at 37°c for 30 mins and determine post-freeze progressive motility of all males with and without holding in Lifor.

3.4.7 Sperm from both treatments should have similar results.