

Supplementary information.**Standard operation procedure (SOP) for the vitrification of human ovarian tissue****Tissue preparation:**

Consumables and solutions needed:

Oocyte -wash medium containing heparin, Flushing Medium, MediCult, Jyllinge, Denmark,

Product No: 1076

A test tube, 50ml BD Worldwide, USA, Product No: 352070

A 60x15mm Petri dish- Nunc150270, Copenhagen, Denmark Product No: 42350

A scalpel blade size 15, Fisher Scientific, Göteborg, Sweden, Product No: 136015515

Needles Gauge 22, B BRAUN Melsungen AG, Denmark

Take the ovarian tissue aseptically from the operation into a sterile 50 ml test tube containing 20 ml, 4°C oocyte wash medium supplemented with Heparin. Avoid squeezing the tissue.

Transfer the tissue immediately to a laminar flow hood into the laboratory

Remove medullar tissue from the cortical layer using a scalpel, while keeping the tissue in place by a needle.

Cut the cortical ovarian tissue into small fragments about $1 \times 1.5 \times 5 \text{ mm}^3$

Vitrification solution (VS):

Consumables and solutions needed:

Hank's balanced salt solution (HBSS) 10x liquid, Invitrogen AB, Paisley, Scotland, Product No: 14065049

Dimethyl sulphoxide, Sigma-Aldrich, Sweden, Product No: D2650

1, 2- Propanediol, Sigma-Aldrich, Sweden, Product No: 134368

Ethylene glycol, Sigma-Aldrich, Sweden, Product No: E9129

Polyvinylpyrrolidone, Mol Wt 10000, Sigma-Aldrich, Sweden, Product No: P-2307

Acrodisc Syringe Filter 0.20 μ m, VWR, Stockholm Sweden, Product No: PALL 4612

Human serum albumin (100 mg/ml), Vitrolife, Göteborg, Sweden, Product No: 10064

Preparation of solutions:

Add 1 ml of HBSS 10x to 9 ml sterile water to get a dilution of HBSS1x.

Rinse medium

Add 1 ml of (100 mg/ml) human serum albumin (10%) to 9 ml HBSS 1x

VS: 1

Add 0.25 ml of each cryoprotectant, Dimethyl sulphoxide (0.4 M), Propanediol (0.3 M) and Ethylene glycol (0.4 M) to a test tube containing 8.25 ml HBSS1x and 1 ml (100 mg/ml) human serum albumin (10%) to make a total volume of 10ml.

VS: 2

Add 0.5 ml of each cryoprotectant, Dimethyl sulphoxide (0.8M), Propanediol (0.6M) and Ethylene glycol (0.8M) to a test tube containing 7.5 ml HBSS1x and 1 ml (100 mg/ml) human serum albumin (10%) to make a total volume of 10ml.

VS: 3

Add 1 ml of each cryoprotectant, Dimethyl sulphoxide (1.6M), Propanediol (1.2M) and Ethylene glycol (1.6M) to a test tube containing 6 ml HBSS1x and 1ml (100 mg/ml) human serum albumin (10%) to make total volume of 10ml. Then add 1 g Polyvinylpyrrolidone (10%w/v) to the solution.

Filter all solutions through sterile filter 0.2 μ m.

Vitrification procedure

Consumables and solutions needed:

Sterile 4-well dish Nunc144444, Copenhagen, Denmark, Product No: 42010.

Cryotube 1.8ml, Copenhagen, Denmark, Nunc Product No: 377267

- Prepare a sterile 4-well dish, and pipette 1 ml of Rinse medium, VS: 1, VS: 2 and VS: 3 into respective well.
- Pre- equilibrate the dish at room temperature for 30 minutes.
- Incubate the tissue fragment in Rinse medium for 5 minutes at room temperature.
- Transfer the tissue into VS: 1 and incubate for 5 minutes at room temperature.
- Transfer the tissue into VS: 2 and incubate for 10 minutes at room temperature.
- Transfer the tissue into VS: 3 and incubate for 10 minutes at 4°C.

- Carefully move the tissue into a cryotube, close the lid and quickly dip the cryotube into liquid nitrogen meanwhile gently stirring. The lid should not be immersed in liquid nitrogen, to avoid leaking of nitrogen inside the vial.

Warming/ thawing solution (TS):

Consumables and solutions needed:

Hank's balanced salt solution (HBSS) 10x liquid, Invitrogen AB, Paisley, Scotland, Product No: 14065049

Sucrose, Sigma- Aldrich, Sweden, Product No: S1888-500G

Acrodisc Syringe Filter (0.2 μ m) VWR, Stockholm Sweden, Product No: PALL 4612

Human serum albumin (100 mg/ml), Vitrolife, Göteborg, Sweden, Product No: 10064

Preparation of solution:

Add 1 ml of HBSS 10x to 9 ml sterile water to get a dilution of HBSS1x.

TS: 1

Add 1.71 g Sucrose (0.5M) in 10 ml HBSS 1x.

TS: 2

Add 0.86 g Sucrose (0.25M) in 10 ml HBSS 1x.

TS: 3

Add 0.43 g Sucrose (0.125M) in 10 ml HBSS 1x.

TS: 4 Rinse medium

Add 1 ml of (100 mg/ml) human serum albumin (10%) to 9 ml HBSS 1x.

Filter all solutions through sterile filter 0.2 μ m.

Warming/ thawing procedure

Consumables and solutions needed:

Sterile center well dishes 60x 15mm, Becton Dickinson, USA, product No: 353653

- Prepare 4 sterile center well dishes, and pipette 1 ml of TS: 1, TS: 2, TS: 3 and TS: 4.
- Pre- equilibrate the solutions at room temperature for 30 minutes.
- Remove cryotube out of liquid nitrogen; hold in air for 30 second and thereafter place the cryotube in a 25°C water bath until melted.
- Open the lid and transfer the tissue fragment into the first pre-warmed warming solution TS: 1 for 2 minutes.
- Transfer the tissue into TS: 2, TS: 3 and TS: 4 for 5 minutes incubation in each solution.
- Transfer the warmed/ thawed tissue in a dish containing pre- equilibrated culture media at 37°C and 5 % CO₂.

Keep it in incubator until use.