

Ovarian Tissue Cryopreservation Scientific Roundup



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Cryopreservation of Ovarian Tissue

Ovarian tissue freezing has been used clinically for fertility preservation in children, adolescents, and adults with cancer since 2004, following the world's first live birth using the cryopreservation and transplantation of ovarian tissue. It has been accepted in an increasing number of countries and to date, more than 130 children have been born from this procedure worldwide. Nevertheless, ovarian tissue cryopreservation and transplantation is still a relatively new procedure within the area of assisted reproduction technologies.

Cryopreservation is a basic procedure that is used frequently in the daily work within assisted reproduction techniques. It is routinely and successfully applied to sperm samples, spare embryos from in vitro fertilization (IVF) cycles and oocytes.

Ovarian tissue cryopreservation represents a new technique to preserve fertility in women where these normal fertility preservation techniques are not an appropriate option.

Currently, embryo and oocyte cryopreservation are the only fertility preservation techniques considered by the American Society for Reproductive Medicine not to be classified as experimental. Unfortunately there are several reasons why one of these approaches may not be appropriate for some patients:

- Both embryo and oocyte cryopreservation require preparation and stimulation which can take several weeks to complete. This delay is often not possible or appropriate for females requiring urgent therapy
- Not all patients have partners with whom to create embryos for cryopreservation.
- These techniques are not indicated for young and pre-pubertal female patients due to their inability to produce mature eggs

For these patients, the possibility of cryopreservation of ovarian tissue (cortex) has become an urgent and highly-demanded technology

Ovarian tissue cryopreservation does not require ovarian stimulation, and offers a promising option for women at high risk of premature ovarian failure and sterility. Furthermore, transplanting ovarian tissue not only restores fertility but also restores endocrine function.

There are numerous centres around the world currently developing techniques for ovarian tissue cryopreservation, many of which are using slow freezing in a controlled rate freezer. In this scientific round-up, we feature two of these. The first focuses on the work from a combined team from Singapore's Sincere IVF and Gleneagles Hospital and the second outlines the approach used by Belgium's Universite Catholique de Louvain.

Planer would like to thank the authors of both techniques for their kind permission to use their documented procedures.

Ovarian Tissue Cryopreservation Scientific Roundup



Cryopreservation of ovarian tissue

The technique used by the combined team from Singapore's Sincere IVF and Gleneagles Hospital

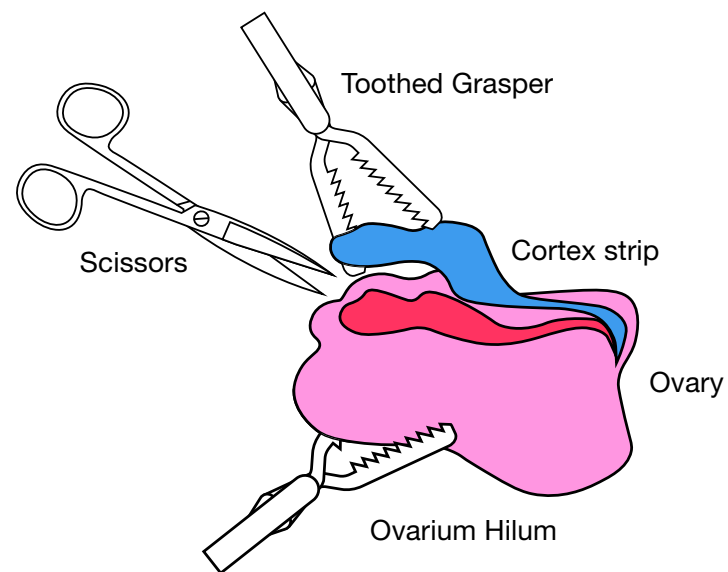
Acknowledgements:

Planer would like to thank the authors of this paper for their kind permission to use this documented procedure. The following technique is taken from 'Successful oocyte retrieval and fertilisation after transplantation of cryopreserved ovarian tissue: case report'. Written by Chen NQ, Siaw KY, Wong YH, Lim J, Tan JL, LIM WH, Quek SC, Ng SC and published in the Journal for Reproductive Biotechnology Fertility 2019; 8: 1-8

1.

**Laparoscopic ovarian biopsy
for cryopreservation**

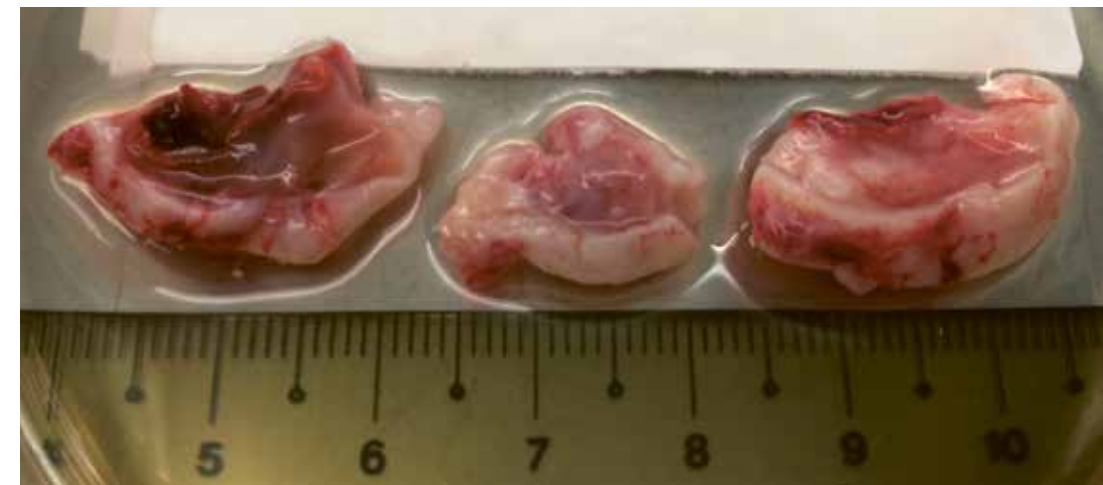
- I. Access the peritoneal cavity laparoscopically in the usual manner.
- II. Identify both ovaries to confirm that they are free of adhesions and to identify where the dominant follicle(s) are, if any
- III. Start with the more easily accessible ovary, by grasping the lateral apex with a toothed-grasper. Make an incision with a laparoscopic scissors over the outer cortex near the grasper, then grasp the cortex flap with another grasper. Use the scissors to cut the sub-cortex close to the cortex in order to lengthen the flap across the length of the ovary to the other apex near the ovarian vessels. Do not use cautery for hemostasis until the flap is completely removed from the ovary, in order to preserve as much pre-antral follicles as possible. When completely separated, the avascular flap should be about 2-3 x 0.5 cm, with the thickness being the cortex only. Remove the flap atraumatically through the side port, but if larger, it can be removed through the subumbilical port. The strip can be put into a sterile container with N/S and handed over to the embryologist, who should be on standby in the OT (the strip should be processed immediately after collection). The raw surface of the ovary can now be point-cauterized to secure hemostasis.
- IV. Repeat the process for the other ovary.



2.

Prepare tissue before freezing

- I. Transfer to laboratory. Ovarian tissue (Fig. 1) were collected in handling medium (GMops, Vitrolife Sweden), and immediately delivered to the laboratory within 30 minutes at room temperature, or transferred to laboratory in few hours with ice.
- II. After the medullary tissue was removed from the ovarian pieces, ovarian cortex pieces of approximately 5 mm in length, 5 mm in width, and about 1 mm in thickness were prepared (Fig.2).

**Fig.1** Ovarian Tissue**Fig.2** Ovarian tissue preparation before freezing. A) & B) Removing medulla, C) Cut into small pieces about 5 mm X 5 mm.

3.

Cryopreservation procedure

After ovarian tissue preparation, a slow freezing method was used.

- I. Equilibration: ovarian tissues are equilibrated at 4° C on a tilting shaker for 30 minutes in freezing solution, containing 1.5 mol/L ethylene glycol and 0.1 mol/L sucrose in GMops plus medium (Vitrolife Sweden).
- II. Loading in to freezing vial: after equilibration, the tissue pieces were placed into 1.8 mL cryo-vials pre-filled with 1mL of the freezing solution (2 pieces per vial).
- III. Freezing: the cryo-vials were then placed into an automated, computer-controlled freezing system (Kryo-360; Planer, UK). The initial cooling rate was -2° C/min to -9° C. After 5 minutes of soaking, manual seeding was performed (fig 3). After keeping the tissue for another 5 minutes, cooling was continued at the rate of -0.3° C/min until -40° C and subsequently with -10° C/min until -140° C, at which temperature the samples were plunged into liquid nitrogen at -196° C, and then transferred to a storage tank.



Fig 3: Manual seeding of sample

4.

Thawing procedure

- I. The cryovials were taken from liquid nitrogen and exposed to room temperature for 5 seconds and then placed in 37° C water for 2 minutes.
- II. The ovarian tissue was transferred to a thawing solution 1 for 10 minutes (0.75 mol/L ethylene glycol and 0.25 mol/L sucrose in GMops plus medium).
- III. Transferred to thawing solution 2 for another 10 minutes (0.25 mol/L sucrose in GMops plus medium).
- IV. The tissue was then transferred to a handling medium (GMops, Vitrolife Sweden), for 10 minutes before transfer to the operating theatre for transplantation.



Fig. 4: Thawing Procedure. A) In Water Bath; B, C) in Thawing Solution 1 and 2.

5.

Ovarian tissues autotransplantation

- I. Create 1.5 cm deep pouch of peritoneum in the region of the broad ligament, below the fallopian tube. Do not use cautery. Be as avascular as possible.
- II. 2-3 pieces of thawed ovarian tissue are transplanted into the pouch.
- III. The pocket is closed without suturing (Fig.5).
- IV. Depending on the vascularity and suitability of the contralateral site, the second site can be similarly prepared.

Subsequent menstruation monitoring, stimulation and oocytes collection

- I. For the first 3 cycles post-replacement, the patient should be on Progyluton (sequential etradiol-norgestrel; Bayer, Weimar, Germany) or similar.
- II. Monitor for subsequent spontaneous menstrual cycles.
- III. If the patient wishes to try for spontaneous pregnancy, she can try but not beyond 3 cycles as the transplanted tissues may not have enough follicles.
- IV. Start the ovarian stimulation for the AR cycle.
- V. When cryopreserved and thawed ovarian tissue is transplanted after remission has been achieved, it is critical to ensure that there is no residual disease in the cryopreserved tissue. If tumor cells have infiltrated the cryopreserved ovarian tissue, there is a possibility of transferring these cells to the patient.

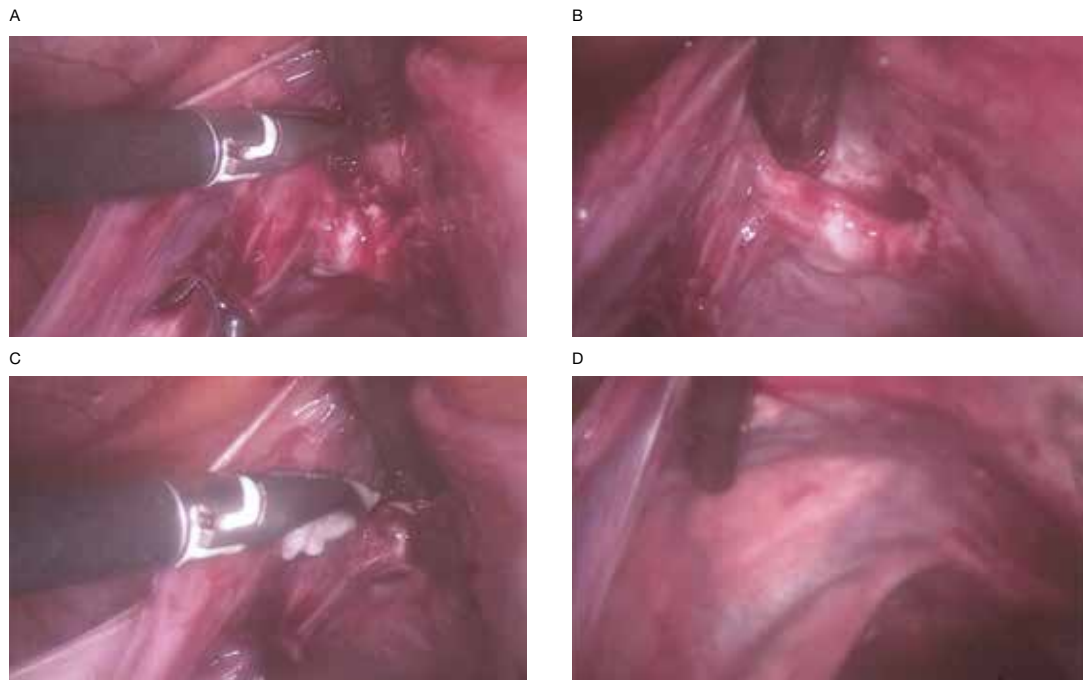


Fig.5: Laparoscopic ovarian tissue transplantation. A) Locating site, B) Opening peritoneal pouch, C) Insertion of the thawed ovarian tissue into the peritoneal pouch, D) Transplantation was done without suturing.

Cryopreservation of ovarian tissue

The technique used by Université Catholique de Louvain

Acknowledgements:

Planer would like to thank the team from UCLouvain for their kind permission to use their documented procedure outlined here.

1.

Laparoscopic ovarian biopsy for cryopreservation

- I. Access the peritoneal cavity laparoscopically in the usual manner.
- II. Identify both ovaries to confirm that they are free of adhesions and to identify where the dominant follicle(s) are, if any
- III. Start with the more easily accessible ovary, by grasping the lateral apex with a toothed-grasper. Make an incision with a laparoscopic scissors over the outer cortex near the grasper, then grasp the cortex flap with another grasper. Use the scissors to cut the sub-cortex close to the cortex in order to lengthen the flap across the length of the ovary to the other apex near the ovarian vessels. Do not use cautery for hemostasis until the flap is completely removed from the ovary, in order to preserve as much pre-antral follicles as possible. When completely separated, the avascular flap should be about 2-3 x 0.5 cm, with the thickness being the cortex only. Remove the flap atraumatically through the side port, but if larger, it can be removed through the subumbilical port. The strip can be put into a sterile container with N/S and handed over to the embryologist, who should be on standby in the OT (the strip should be processed immediately after collection). The raw surface of the ovary can now be point-cauterized to secure hemostasis.
- IV. If necessary, repeat the process for the other ovary.

2.

Prepare tissue before freezing

- I. Transfer to laboratory. Ovarian tissue is collected and transported in Universal IVF medium, and immediately delivered to the laboratory at 4° C.
- II. In the laboratory, the ovarian biopsy is placed in a plastic Petri dish containing Leibovitz's L-15 medium kept at 4° C thanks to a refrigerate plate (Fig. 1).
- III. After measuring the biopsy (Fig. 1), the medullary tissue is removed and the ovarian cortex (around 1 mm thickness) is cut into large (8 x 4 mm; 7 x 4 mm; 7 x 3 mm) and small strips (5 x 5 mm; 4 x 4 mm; 3 x 3 mm).
- IV. Every strip is measured and placed into another dish containing L-15.



Figure 1. Ovarian tissue preparation. (A) measuring the ovarian biopsy and (B) cutting it into strips.

3.

Cryopreservation procedure

After ovarian tissue preparation, a slow freezing method was used.

- I. Cryovials are loaded with 0.8 ml of freezing solution (L-15 + 10% DMSO + 0.4% HSA) at 4° C.
- II. One large or 2-3 small ovarian strips are loaded per cryovial. Then, each cryovial is sealed and placed into an automated, computer-controlled freezing system (Kryo-360; Planer, UK).
- III. Cryovials remain at 0° C for 15 min in the Planer and then the cooling curve starts: from 0° C to -8° C at -2° C/min. After 15 min of soaking, manual seeding is performed (Fig. 2). After keeping the tissue for another 15 min, cooling was continued at the rate of -0.3° C/min until -40° C and subsequently with -30° C/min until -150° C, at which temperature the samples were plunged into liquid nitrogen at -196° C, and then transferred to a storage tank.



Figure 2. Manual seeding of sample.

4.

Thawing procedure

- I. The cryovials were taken from liquid nitrogen and exposed to room temperature for 2 min and then placed in 37° C water for 2 min.
- II. The ovarian tissue is washed 3 times with L-15 (5 min/bath).
- III. The tissue samples are then placed in the Falcon tube containing Universal IVF medium) and transferred to the operating theatre for transplantation.

5.

Ovarian tissues autotransplantation

- I. Create 1.5 cm deep pouch of peritoneum in the region of the broad ligament, below the fallopian tube. Do not use cautery. Be as avascular as possible.
- II. 2-3 pieces of thawed ovarian tissue are transplanted into the pouch.
- III. The pocket is closed without suturing (Fig.3).
- IV. Depending on the vascularity and suitability of the contralateral site, the second site can be similarly prepared.

Cryopreservation of ovarian tissue

Using a controlled rate freezer

Since research began into the possibility of ovarian tissue cryopreservation the Planer Kryo 360 controlled rate freezer has been at the forefront of the development and optimisation of the technique. Centres around the world are using the accurate control and easy programmability of the unit to fine tune their approach and maximise the opportunity of success.

Despite the growing global trend towards a 'vitrify all' strategy within IVF centres, the vast majority of live births, now numbering over 130 worldwide, have been from ovarian tissue that was originally slow frozen in a controlled rate freezer. These global results ensure that controlled rate (or slow freezing) is clinically proven to provide the best opportunity for success for patients who require fertility preservation using cryopreserved ovarian tissue.

Controlled rate freezing allows clinicians and researchers to ensure the repeatability of their results time after time. As it is an automatic process, it also removes the variability between different human operators. Induced nucleation (seeding) of samples is possible with controlled rate freezing, which helps reduce the risk of osmotic stress and cellular damage.

The Planer Kryo 360 controlled rate freezers' 15 sample holder capacity is ideal for ovarian tissue cryopreservation and the 1.7 litre chamber provides the optimal environment for the controlled temperature reduction preventing the formation of lethal intracellular ice, significantly improving the viability yield post thaw.



Ideal for ovarian tissue cryopreservation

Improve viability post-thaw



The Planer Kryo 360 programmable freezer provides the optimal environment for controlled temperature reduction preventing the formation of lethal intracellular ice and significantly improving the viability yield post thaw.

- Repeatability of the results time after time.
- Induced nucleation (seeding) of the samples
- Remove variations between different human operators

To find out more about the Kryo 360 controlled rate freezer and how it can be used for ovarian tissue cryopreservation, please get in touch with your local Planer distributor. A full list of our 80 distributors around the world can be found on the Planer website.

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Planer Limited

110 Windmill Road,
Sunbury-on-Thames
Middlesex, TW16 7HD,
United Kingdom

Tel: +44 (0)1932755 000

Email: enquiries@planer.com

Website: www.planer.com

A Hamilton Thorne Company