# Successful oocyte retrieval and fertilization after transplantation of cryopreserved ovarian tissue: case report

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**Abstract**
Cryopreservation of ovarian tissue is becoming an established fertility preservation method. Here we report our first cryopreserved ovarian tissue transplantation. A 37-year old woman diagnosed with ovarian cancer underwent bilateral salphingo-oopherectomy. Three slices of morphologically normal ovarian tissue (10mmX15-20mm) were transported to the IVF laboratory at room temperature, and ten pieces (5mmX5mm) of tissue were cryopreserved. Histopathology investigation showed serous borderline tumor in both ovaries with no stromal invasion. Neither chemotherapy nor radiotherapy was performed. Ten months later, the patient underwent heterotopic transplantation of cryopreserved ovarian tissue into a peritoneal pocket. Menstruation commenced two months later after induction with Progyluton. Follicle growth was monitored in her fifth menstruation cycle, and one follicle was observed in the peritoneal pocket. The oocyte was successfully retrieved and fertilized with the husband's frozen-thawed testicular sperm. One day-3 embryo was frozen. No embryo transfer was performed. The results of our first ovarian tissue transplant confirmed previous reports and lent support to the objective ovarian tissue freezing can preserve fertility for cancer patients.

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## Introduction

Since the world's first live birth in 2004 following ovarian tissue cryopreservation and transplantation (Donnez et al., 2004), ovarian tissue freezing has been used clinically for fertility preservation in female children, adolescents, and adults with cancer. It has been accepted in an increasing number of countries and to date, more than 100 children have been born from this procedure worldwide (Andersen et al., 2018). Nevertheless, ovarian tissue cryopreservation and transplantation is still a relatively new procedure within the area of assisted reproduction technologies. The American Society for Reproductive Medicine published the following opinion in 2014: "Ovarian tissue cryopreservation is an experimental technique and should not be offered to women with benign disease or women who wish to delay childbearing. It is a technique that should not be used for a social reason." (Practice Committee, 2014). Here we report the findings of our first case of ovarian tissue transplantation.

## Methods

### *Patient description*

A 37-year-old married nulliparous woman was referred for fertility preservation. She had been healthy until diagnosed with ovarian cancer, and her menstrual cycle was regular. Her 41-year-old husband had azoospermia and testicular sperm frozen after testicular biopsy.

The patient was counselled on the risk of tumor cells being present in the transplanted ovarian tissue. Moreover, the case was discussed at the hospital's Tumor Board, and her case was allowed to proceed for infertility

treatment by transplanting back her remnant ovarian tissue.

The patient was 37 years old when the treatment was initiated, with 20 years of infertility, due to her husband's azoospermia. She decided to push ahead with the treatment when she realized that she was diagnosed with ovarian borderline cancer.

***Extraction, transportation and preparation of ovarian tissue***

Bilateral salphingo-oopherectomy was done laparoscopically on 31/05/2016, in Gleneagles Hospital, Singapore. Histopathology investigation showed serous borderline tumor for both ovaries with no stromal invasion.

Three slices of normal tissue (Fig. 1) from the right ovary were collected in handling medium (GMops, Vitrolife Sweden), and immediately delivered to the laboratory within 10 minutes at room temperature. The rest of the ovarian tissue of both ovaries was sent for histological analysis.

After the medullary tissue was removed from the ovarian pieces, a total of 10 pieces of ovarian cortex tissue of approximately 5 mm in length, 5 mm in width, and about 1 mm in thickness were prepared (Fig.2).

***Cryopreservation procedure***

The slow freezing method of Anderson et al. (Andersen et al., 2008) was employed to freeze the prepared pieces of the ovarian tissue. All ten pieces of ovarian tissue were 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). After equilibration, the tissue pieces were placed into five 1.8 mL cryo-vials pre-filled with 1mL of the freezing solution (2 pieces per vial). 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. 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.

***Thawing procedure***

Two cryo-vials containing four pieces of ovarian tissue were thawed for transplantation on 16 March 2017.

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. The ovarian tissue was transferred to thawing solution 1 for 10 minutes (0.75 mol/L ethylene glycol and 0.25 mol/L sucrose in GMops plus medium); and then transferred to thawing solution 2 for another 10 minutes (0.25 mol/L sucrose in GMops plus medium). The tissue was then transferred to handling medium (GMops, Vitrolife Sweden) for 10 minutes before transfer to the operating theatre for transplantation.

***Ovarian tissues autotransplantation***

Laparoscopic autotransplantation was performed on 16/03/2017 (10 months after cryopreservation). The 4 thawed pieces of ovarian tissue were transplanted into a 1.5 cm deep pouch of peritoneum in the region of the broad ligament, below the fallopian tube and the pocket was closed without suturing (Fig.3).

## Results

Histopathology results showed serous borderline tumour in both ovaries with no stromal invasion. No chemotherapy or radiotherapy was performed.

Menstruation started on 04/05/2017 after induction with Progyluton (sequential etradiol-norgestrel; Bayer, Weimar, Germany). Her subsequent two menstrual cycles started on 04/06/2017 and 05/07/2017 following Progyluton. She was asked to undergo ovarian stimulation in her August cycle, which was be the spontaneous cycle without Progyluton. However following menses on 03/08/2017 she was unable to undergo treatment because of her work schedule. Hence ovarian stimulation was performed in September 2017. DUOSTIM (double stimulation in the same ovarian cycle) was planned for the patient in view of her poor folliculogenesis. However, after the first oocyte collection, the patient had to return to her home country due to a medical emergency in her immediate family. Consequently her second stimulation cycle was aborted resulting in the cancellation of the planned embryo transfer.

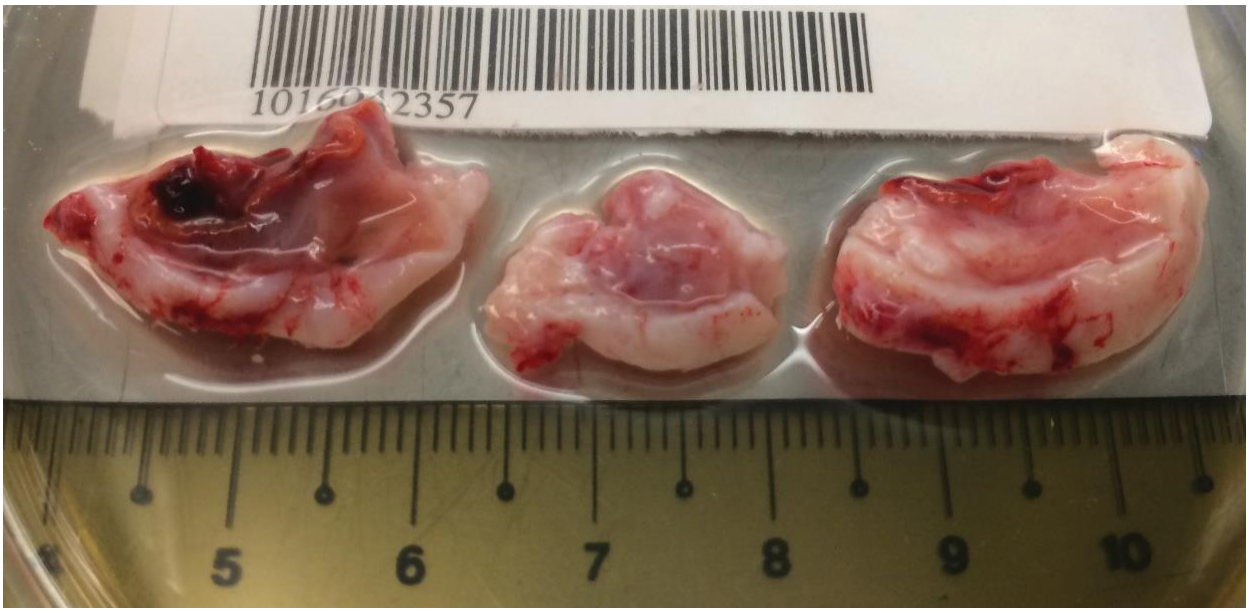


**Figure 1: Ovarian Tissue**

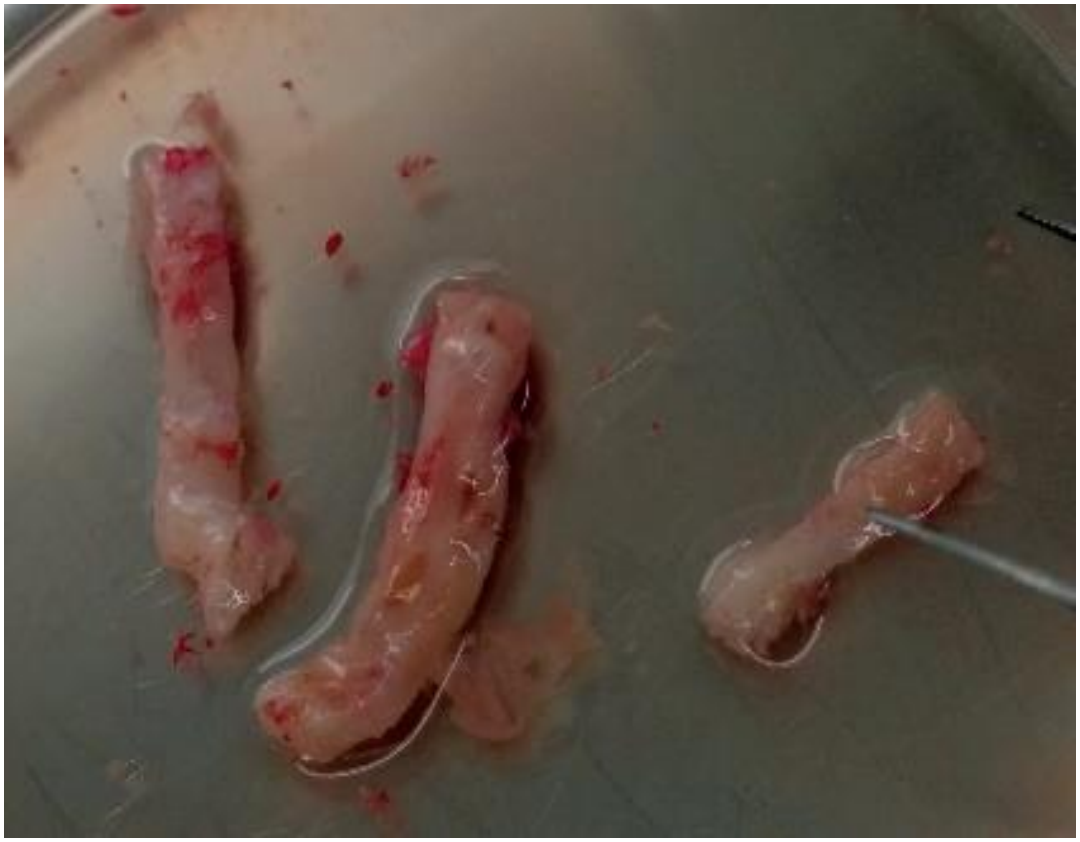

**Figure 2A. Ovarian tissue preparation before freezing**.
Removing medulla

**Figure 2B. Ovarian tissue preparation before freezing**.
Removing medulla

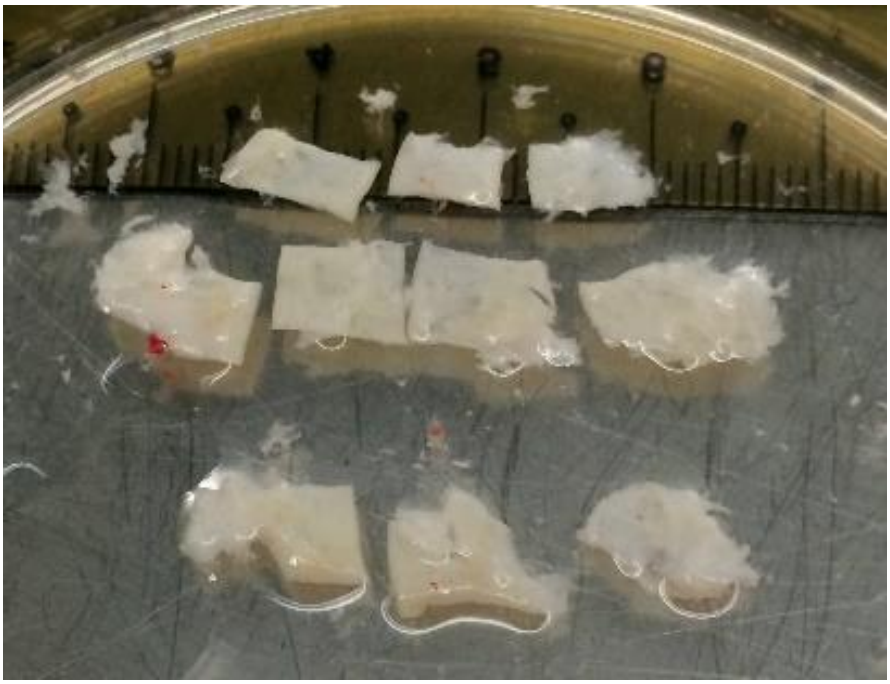

**Figure 2C. Ovarian tissue preparation before freezing**.
Cut into small pieces about 5mmX5mm.

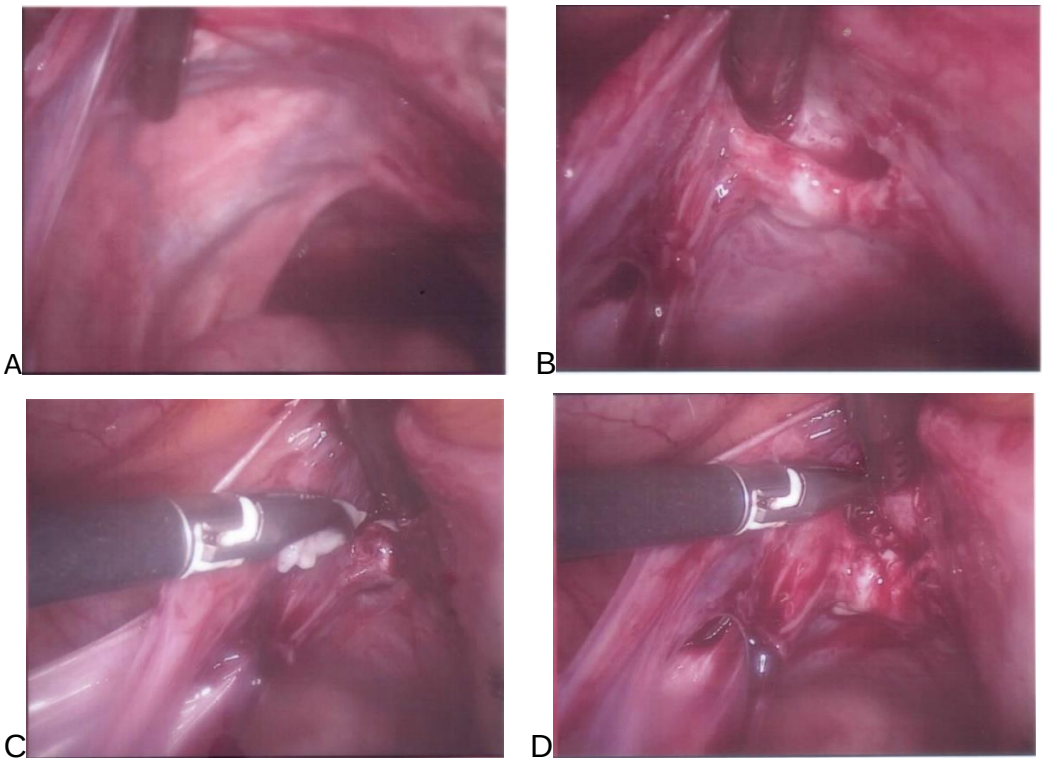

**Figure 3. Laparoscopic ovarian tissue transplantation**.

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

**Table 1. Stimulation and hormone activity in the transplanted tissue.**

| Cycle | Date | E2 pmol/L | LH IU/L | FSH IU/L | P4 nmol/L | Follicle mm | Stimulation |
|---|---|---|---|---|---|---|---|
| Day 0 | 04/09/2017 | 276 | 9.80 | 8.5 | 1.78 | | |
| Day 2 | 06/09/2017 | | | | | | Pergovaris x 2 daily |
| Day 7 | 11/09/2017 | 603.2 | 7.24 | | | 13.0 | Cetrotide 1 daily |
| Day 9 | 13/09/2017 | 807.9 | 8.31 | | | 15.0 | |
| Day11 | 15/09/2017 | 952.9 | 6.64 | | | 18.0 | Ovidrel 250 ug |
| Day13 | 17/09/2017 | | | | | | Oocyte Retrieval 35 hrs |

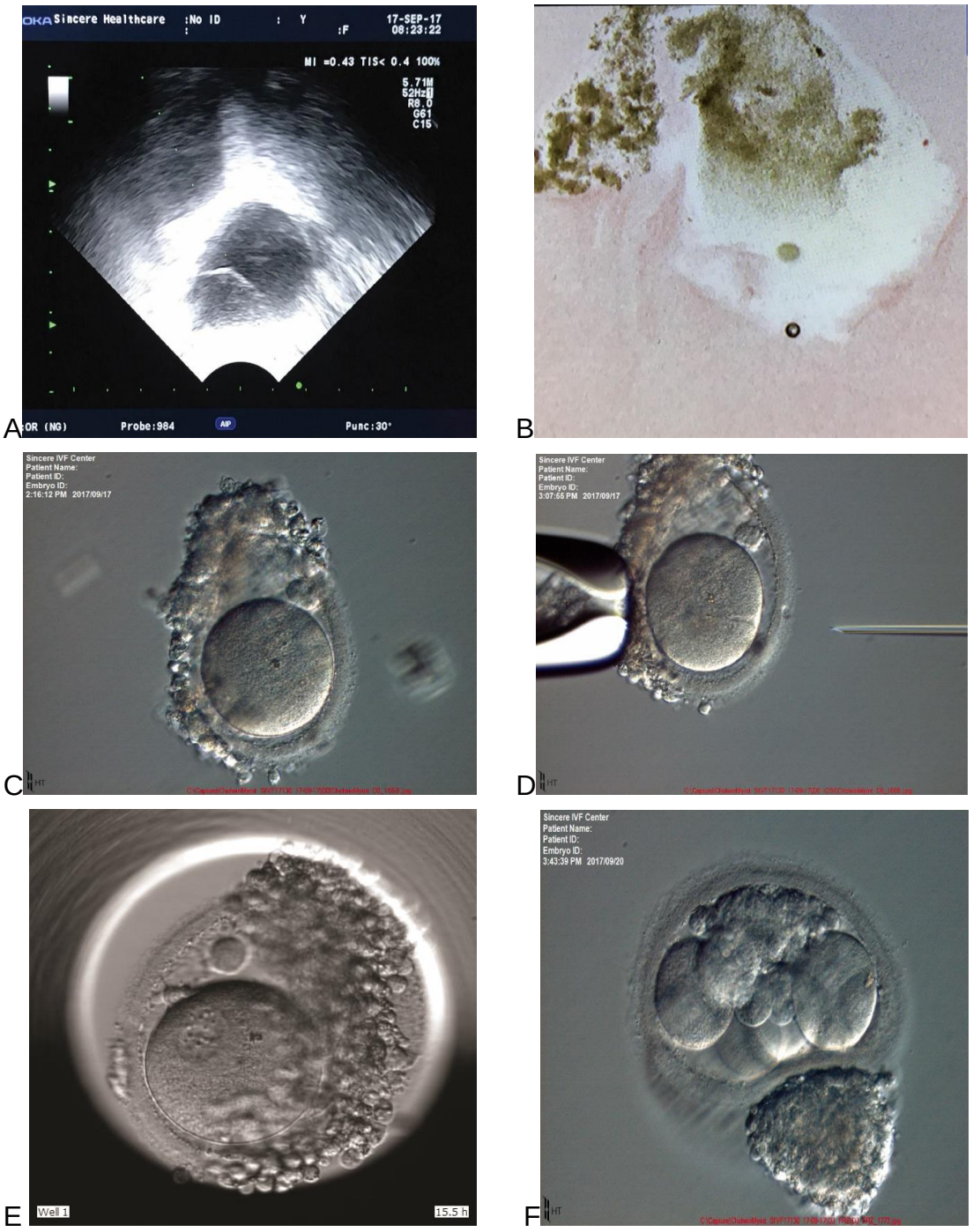


**Figure 4. Grown follicle, collected oocyte, ICSI, and the developing embryo.**
(A) Ultrasound findings of grown follicles. (B) Collected cumulus-oocyte complex. (C) mature oocyte with PB1 after removing cumulus cells. (D) Injection of testicular sperm into oocyte. (E) Fertilized egg with 2 PN and 2 PB. (F) Seven-cell embryo developed after three days of in vitro culture.

During her fifth menstruation cycle that started on 04/09/2017, ovarian stimulation was performed with Pergovaris (150 iu rFSH + 75iu rLH; Merck Serono, Modugno, Italy) 2 ampoules daily from D2, with Cetrotide (Cetrorelix, Halle, Germany) added from D7; follicle growth was monitored by ultrasound (see Table 1). Final maturation was achieved with Ovidrel (choriogonadotropin alfa, 250 ug, 35 hours before the oocyte retrieval; Merck Serono, Modugno, Italy).

***Oocyte retrieval and fertilization In Vitro***

Transvaginal oocyte retrieval was performed on day 13 of the fifth cycle at 6 months after transplantation. One mature oocyte was retrieved from transplanted ovarian tissue and fertilized by intracytoplasmic sperm injection using her husband's frozen thawed testicaular sperm which resulted in a normal 2 PN zygote. One 7-cell cleavage stage embryo was frozen on day-3 (Fig. 4). Embryo transfer was not performed. It will be performed in the future.

While the intent was to procure 2 embryos for transfer; the second cycle being a fresh embryo cycle, this was ultimately not possible due to unforeseen circumstances. This highlights the difficulties of planned cycles, especially in instances of difficult oocyte collection.

## Discussion

Since 2007, 20 women with various forms of cancer have had ovarian tissue cryopreserved in our centre. The mean age was 29.3 years (range, 16–37 years). One sample was discarded due to patient not surviving her cancer treatment. To date, only one transplantation was performed. Worldwide, it is estimated that several thousand women have had ovarian tissue cryopreserved, but the number of transplantations remain low. Typically, the patient needs several years of follow-up after the end of her treatment before receiving transplantation. According to the 2017 report of the ESHRE Working Group on Oocytes in Europe, ovarian tissue cryopreservation was performed in a total of 5529 patients, in 12 of 17 EU countries investigated during the 5 years from 2010 and ovarian tissue transplantation was carried out in 237 patients in 7 countries (Shenfield et al., 2017).

In our first case, after autotransplantation of cryopreserved ovarian tissue, in addition to a regular menstrual cycle, success in collection of one egg and fertilization showed ovarian function thereby demonstrating that cryopreservation of ovarian tissue and subsequent transplantation is a viable option for preserving fertility. Live birth rates of 25%–31% have been reported after ovarian tissue cryopreservation and transplantation (Donnez et al., 2015; Jensen et al., 2015; and Oktay et al., 2016), but it is difficult to obtain a complete picture of this procedure's success rate due to the absence of reports of non-pregnancies in the peer-reviewed literature (Andersen, 2015).

In the present case, after fertilization with husband's testicular sperm, embryo growth was slow and with about 30% fragmentation (day 3: 7-cell grade 2-3; range:1=excellent and 4=poor). This may be due to patient's age. In several countries that offer ovarian tissue cryopreservation, one of the inclusion criteria is the women should be no more than 30 years old. In Denmark, there is no exact maximum age. Rather individual evaluation of the patient's reproductive age can differ from the patient's chronological age. However, ovarian tissue cryopreservation is often not offered to women above 35 years of age due to a diminished ovarian reserve (Jensen et al., 2017). This is because the likelihood of achieving live birth higher as the number of primordial follicles increases, prepubertal/adolescent girls or young women aged up to 30 years with cancer are probably the best candidates for ovarian tissue cryopreservation (Suzuki, 2018).

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 (Dolmans et al., 2013 and Dolmans et al., 2018). It has been reported tumor cells were found in frozen-thawed and xenografted ovarian tissue in 1 of 11 borderline ovarian tumor patients (Masciangelo et al., 2018). In our case, the patient had been diagnosed to have a serous borderline tumor for both ovaries with no definite stromal invasion. After careful consideration of the characteristics

of the cancer, careful discussion with oncologists and full explanation to the patient, ovarian tissue transplantation was successfully performed. To date, the patient has been well and no evidence of relapse has been noted.

## Conclusion

Although cryopreserved ovarian tissue is still regarded as experimental, successful collection of oocytes and fertilization from autotransplanted ovarian tissue show that ovarian tissue freezing can preserve fertility for cancer patients. Goals for the future should include optimizing the outcomes of transplantation, with improved tissue quality, reduced follicle loss, shorter transportation times, and longer periods of functionality in cryopreserved ovarian tissue. Such improvement in technique is important as survival rates for cancer patients in their early reproductive life continue to improve with advances in cancer treatment.

### *In Memoriam*

*This article is dedicated to the memory of the authors' late colleague and friend, Ms Kong Sow Chan, MSc. Her colleagues remember her as a very dedicated embryologist. She was Laboratory Manager from July 2014 to March 2018.*

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