# Fertility preservation and refreezing of transplanted ovarian tissue—a potential new way of managing patients with low risk of malignant cell recurrence

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**Objective:** To report the first successful refreezing of ovarian tissue recovered more than 3 years after transplantation in a woman previously treated for early-stage ovarian cancer.
**Design:** Evaluation of cryopreserved and grafted ovarian tissue.
**Setting:** University hospital.
**Patient(s):** A 23-year-old woman diagnosed with stage 1C ovarian mucinous cystadenocarcinoma.
**Intervention(s):** The patient underwent ovarian tissue cryopreservation for fertility preservation and subsequent heterotopic transplantation for fertility restoration 9 years after freezing. After a successful IVF twin pregnancy, grafted tissue was laparoscopically removed for safety reasons. The recovered tissue was refrozen.
**Main Outcome Measure(s):** Live birth and histologic evaluation of the distribution of pre-antral follicle stages.
**Result(s):** The previously grafted ovarian tissue was successfully refrozen, presenting follicular survival 4 weeks after xenografting. The follicular distribution in the recovered grafts showed a shift toward growing-stage follicles compared with the fresh tissue. The patient subsequently entered menopause, and histologic evaluation revealed a total of five follicles in two remaining grafts which had supported ovarian function a few months earlier.
**Conclusion(s):** This is the second case of delivery following heterotopic grafting as well as the second case of successful transplantation of ovarian tissue from a patient with early-stage ovarian cancer. The recovered grafts showed that a lower number of functional follicles than previously estimated can actually support ovarian function. Removing and refreezing grafted tissue could be a new way of handling not only cancer patients with a risk of malignant cell recurrence, but also certain groups of patients with genetic conditions. (Fertil Steril® 2017;107:1206–13. ©2017 by American Society for Reproductive Medicine.)
**Key Words:** Ovarian cancer, ovarian tissue cryopreservation, follicular distribution post-grafting, refreezing ovarian tissue, fertility preservation

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Ovarian tissue cryopreservation (OTC) for fertility preservation is an emerging technique, which has proven to be successful during the past decade with almost 100 children born after restoration of fertility by transplantation of frozen-thawed ovarian tissue (1). Compared with other well established techniques, such as oocyte and embryo freezing, OTC preserves the actual organ function and thus the production of sex hormones. However, this technique also introduces the risk of transplanting the original disease which may be harbored in the tissue. Preliminary experience with grafted ovarian tissue, however, did not reveal an increased risk of relapse compared with women who had tissue stored for fertility preservation that had not undergone transplantation (2, 3).

In the context of safety, special attention is required for two groups of patients: those in whom the blood-borne nature of the disease could cause an increased risk of relapse upon transplantation (4); and those with diseases in the ovary in whom the disease may be completely absent initially but could potentially develop in the tissue after transplantation. Different centers have already frozen ovarian tissue in a considerable number of cases with early-stage ovarian cancer and borderline ovarian cancers. However, experience with transplanting is very limited. The causes of ovarian cancer are mostly unknown, but genetic mutations such as breast cancer antigens 1 and 2 (BRCA1 and 2) increase the risk considerably (5–7). Nonetheless, OTC and subsequent transplantation was previously performed in two patients diagnosed with early-stage ovarian cancer, and one successful pregnancy was reported without information of relapse (3).

In the present study, the second successful pregnancy after OTC and transplantation in a woman diagnosed with early-stage ovarian cancer is reported. For safety reasons the grafted ovarian tissue was removed after the woman had given birth to healthy twin boys (1). The removed grafted tissue and the special circumstances of this patient yielded an unforeseen and unparalleled insight into the number of follicles and the follicular development stages present in the grafted tissue, including the ability to refreeze ovarian tissue after a period of grafting.

## MATERIALS AND METHODS

### Patient Information

Medical records of the patient were used, and consent from the patient was obtained for publication of the findings. Cryopreservation of ovarian tissue for fertility preservation was approved by the Minister of Health in Denmark and by the Ethical Committee of the municipalities of Copenhagen and Frederiksberg (H-2-2011-044).

### Xenografting

Eight-week-old female immunodeficient mice (NMRI-nu/nu; Taconic) were used in 2010 to rule out the presence of malignant cells in the cryopreserved ovarian tissue before transplantation and in 2015 to evaluate the follicular survival after refreezing of the recovered grafted tissue. Two weeks before transplantation, the mice were ovariectomized. The mice were anesthetized with the use of isoflurane (Baxter) or midazolam (Dormicum; Roche), and buprenorphine and carprofen (Norodyl; Norbrook) were used for analgesia.

In 2010, one piece of frozen-thawed ovarian cortex prepared for clinical use was transplanted to a subcutaneously prepared pocket onto the flank of the mouse for 20 weeks. The skin was visually inspected for skin metastasis, and an autopsy was performed. The human ovarian cortex and selected murine organs (spleen, liver, kidney, heart, lung, and femur) were recovered, visually inspected, and prepared for histologic evaluation. In 2015, two pieces of refrozen ovarian tissue recovered from the transplanted graft were transplanted to subcutaneously prepared pockets onto the flank of the mouse for 4 weeks and subsequently recovered for histologic evaluation. Animal studies were approved by the Danish Animal Experiments Inspectorate (2009/561-1590 and 2015-15-0201-00505).

### Histologic Analysis

Histologic processing was performed with the use of standard methods (8). Follicular survival and development was assessed by evaluating and counting the follicle stages and classified according to Kristensen et al. (2011) (8). Healthy follicles were assessed as such by having organized granulosa cells, dispersed chromatin structures in the oocyte nucleus, and uncondensed oocyte cytoplasm, whereas atretic follicles had unorganized granulosa cells, condensed chromatids in the oocyte nucleus, and/or shrunken oocyte cytoplasm.

### Hormone Measurements

FSH, LH, antimüllerian hormone (AMH), and $E_2$ levels were measured at the Department of Clinical Biochemistry, University Hospital of Aarhus, Denmark, according to in-house standard procedures.

## RESULTS

### Patient Case

In year 2003, at the age of 23 years, the patient contacted the hospital owing to acute abdominal pain; an ultrasound examination revealed what was believed to be a benign ruptured ovarian cyst in the left ovary. The subsequent operation, however, revealed an ovarian tumor, and the patient underwent a unilateral salpingo-oophorectomy and omentectomy. The pathology report confirmed the suspected malignancy, which was diagnosed as a stage 1C ovarian mucinous cysteadenocarcinoma. As a consequence, it was decided to remove the remaining normal-looking right ovary. One-half of the ovary was examined by a pathologist, with no detection of malignancy, whereas the remaining ovarian tissue was prepared for cryopreservation according to our standard procedure (Fig. 1A) (9). A fresh ovarian biopsy was taken for histology on the day of OTC. Subsequently, the patient underwent cancer treatment, receiving four series of paclitaxel and carboplatin.

After having finished her cancer treatment in 2004, the patient was amenorrhoic with elevated gonadotropins and