Pregnancies and live births after 20 transplantations of cryopreserved ovarian tissue in a single center

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Objective: To report the results of 20 orthotopic retransplantations of cryopreserved ovarian tissue after cancer treatment.
Design: Retrospective analysis.
Setting: Tertiary gynecology department.
Patient(s): Twenty patients with malignant disease: 11 with hematological malignancies (55%), four with breast cancer (20%), three with anal cancer (15%), and two with ovarian cancer (10%); the mean age before oncological treatment was 30.5 years.
Intervention(s): Ovarian tissue was removed from patients in various centers in Germany in 2005–2009. All patients received chemotherapy and/or radiotherapy. Afterward, 17 patients had complete premature ovarian insufficiency, while three still showed some ovarian activity. Overnight transportation of tissue before freezing was necessary in eight cases. Cryopreservation followed slow freezing protocols in all cases. Retransplantation was performed at Erlangen University Hospital 3.75 years after extraction, on average. Thawed tissue was transplanted into a peritoneal pouch in the broad ligament region, below the tube, in 16 cases. Fragments were sutured both onto the remaining ovary and into a peritoneal pouch in four cases.
Main Outcome Measure(s): Restoration of ovarian activity, pregnancy, birth.
Result(s): Ovarian activity resumed in all patients except one. Seven patients conceived, with one miscarriage and four ongoing pregnancies. Four patients delivered healthy babies. One pregnancy and live birth after oocyte donation need to be considered separately.
Conclusion(s): These data clearly demonstrate that preserving fertility by cryopreserving ovarian tissue is a successful and safe clinical option that can be considered for selected cancer patients. (Fertil Steril® 2015;103:462–8. ©2015 by American Society for Reproductive Medicine.)
Key Words: Fertility preservation, cryopreserved ovarian tissue, transplantation, live birth, pregnancy

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Modern treatments for oncological diseases have significantly increased survival rates in cancer patients, but chemotherapy and radiotherapy often lead to sterility due to destruction of the ovarian reserve. It is therefore important to advise patients who require gonadotoxic treatment on the options that are available for preserving their fertility. The ability to have children of one's own is an important aspect of quality of life (1). A number of strategies have therefore been developed in recent years to enable these patients to have children using their own gametes (2). The individual patient's specific situation needs to be taken into account when fertility-preserving measures are being selected. Several options are currently available for preserving fertility, and cryopreservation of oocytes (3) and cryopreservation of embryos (4) are well-established procedures. The choice of which procedure is used depends on various parameters: the type and timing of gonadotoxic therapies, the type of cancer (with possible involvement of the ovaries), the patient's age, and her partner status. However, cryopreservation of ovarian tissue is a promising method for fertility preservation because it not only restores fertility after retransplantation but also is able to avoid hormone insufficiency (5).

Ovarian tissue can be easily extracted laparoscopically, regardless of the current phase of the menstrual

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cycle, without significantly delaying chemotherapy or radiotherapy. Patients who are younger than 35 years are particularly suitable, as the ovaries contain large numbers of oocytes in these patients and the chances of successful retransplantation are therefore greater. The ovarian tissue can be cryopreserved at specialized centers for reproductive medicine, and—depending on the oncological therapy required—can be retransplanted after a certain time if the patient has treatment-induced premature ovarian failure. Cryopreservation of ovarian tissue before oncological treatment is a promising method, since a large number of follicles survive the freeze-thaw procedure (6). Donnez et al. reported the first live birth after autotransplantation of human ovarian tissue in 2004 (7). In Germany, the first live birth after retransplantation of cryopreserved ovarian tissue was reported in 2012 (8). Using this technique, more than 25 live births have been reported worldwide to date (9). It can be expected that in the near future, more and more oncology patients who have survived cancer and have been cured of their disease will wish to undergo reimplantation of ovarian tissue.

The purpose of the present review is to report on and discuss the techniques used and the results in 20 orthotopic retransplantations of cryopreserved tissue in a single center.

MATERIALS AND METHODS

Patient Cohort

Ovarian tissue was removed from 20 premenopausal women with various types of cancer; the patients' mean age was 30.5 years (range, 20–37 years). Three patients had anal cancer, 10 Hodgkin's lymphoma, one non-Hodgkin's lymphoma, four breast cancer, and two ovarian tumors (Table 1). At the time of retransplantation, their average age was 34.2 years. All patients received chemotherapy, including two patients with Hodgkin's lymphoma who received high-dose chemotherapy. The three patients with anal cancer received pelvic radiotherapy. Signs of residual hormone activity were present before retransplantation in only three of the patients. These patients did not wish to await a natural course and requested retransplantation. Values for antimüllerian hormone (AMH) levels after oncological treatment and before retransplantation were obtained in 12 of the women. All AMH levels were below detection limits. Tubal patency was tested in 14 women, and bilateral obstruction of the fallopian tubes was noted in four of them. The study design was approved by the Institutional Review Board (IRB) at Erlangen University Hospital, which had no concerns about it (IRB no. 522_14 Bc).

Extraction of Ovarian Tissue

In the present study, ovarian tissue was extracted from 20 cancer patients in Germany between 2005 and 2009. The tissue was removed in the Department of Gynecology and Obstetrics at Erlangen University Hospital in six of these patients. In the 14 other patients, the tissue was removed in other centers for reproductive medicine in various cities in Germany (Düsseldorf, Dresden, Munich, Hamburg, Würzburg, Cologne, and Essen).

The ovarian tissue was harvested laparoscopically, with no use of electrocoagulation or thermal cutting to avoid damaging the tissue. In the present group of 20 patients, an average of two thirds of one ovary was removed. The tissue samples from each patient were analyzed histologically to prevent transmission of the underlying disease, and the presence and density of primordial follicles were documented. No complications such as bleeding or infections were observed.

Transportation before Freezing

The ovarian tissue was removed, processed for cryopreservation, and stored at the Department of Gynecology and Obstetrics at Erlangen University Hospital in six cases so that no transportation was necessary. In another six cases, the tissue was frozen outside Erlangen, but within 6 hours after tissue collection, and in eight cases overnight transportation of the removed tissue was required.

After laparoscopic removal, the tissues were sent in a special insulated transportation box (Z100037054; delta T Ltd.) at 4°C by same-day courier service or express overnight transportation for cryopreservation to a specialized processing unit (in either Bonn or Erlangen) (10). If the tissues were stored in an external cryobank, the tissue pieces were sent in shipping containers cooled with liquid nitrogen at −196°C to the Reproductive Medicine Laboratory in Erlangen.

Cryopreservation Procedure

Slow freezing methods were used in all cases. Between −6°C and −40°C, the cooling rate was 0.3°C/min. Dimethyl sulfoxide was used as the cryoprotectant in 11 cases; the cryopreservation method used in these cases has been described elsewhere in detail (11). Ethylene glycol was used as the cryoprotectant in six cases (12). In brief, pieces of ovarian tissue (3 × 3 × 1 mm) were equilibrated for 30 minutes in 1.5 mol/L ethylene glycol and 0.1 mol/L sucrose in phosphate-buffered saline in 2 mL standard cryovials (Simport T309-2A) on a tilting table on ice and then loaded into the open freezing system (CTE-920, CTE). The following cooling program was used: −2°C/min to 1°C, −0.5°C/min to −5°C, −0.3°C/min to −9.3°C, 10 minutes of soaking, then −0.3°C/min to −40°C and −10°C/min to −140°C, at which temperature the samples were plunged into liquid nitrogen at −196°C. Propanediol was used as the cryoprotectant in the remaining three cases.

Storage

The tissue was stored in highly specialized cryobanks in Germany that have expertise in cryopreserving ovarian tissue. The cryobanks are members of the FertiPROTEKT network, a collaborative group of centers in Germany, Austria, and Switzerland.

Thawing

Thawing was fast in a warm water bath (37°C). The tissue fragments were released from the protective cryopreservation