

Live birth after ovarian tissue autotransplantation following overnight transportation before cryopreservation

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Objective: To describe the first live birth after transplantation of ovarian tissue following overnight transportation of the tissue before freezing.

Design: Technical note.

Setting: University department of obstetrics and gynecology.

Patient(s): A 25-year-old cancer survivor with previous Hodgkin disease and relapse.

Intervention(s): The ovarian tissue was kept cool for >20 hours in a special transport medium and a special cooling device before it was cryopreserved. After premature ovarian failure due to preconditioning chemotherapy for bone marrow transplantation, the cryopreserved ovarian tissue was transplanted orthotopically.

Main Outcome Measure(s): Resumption of ovarian function after transplantation, recovery of fertility, and pregnancy.

Result(s): Ovarian function returned in the patient 3 months after transplantation, as shown by follicle development and estrogen production. During the fifth menstrual cycle, mild stimulation with FSH was initiated in accordance with a low-dose protocol. When ultrasonography revealed a follicle 18–20 mm in size in the ovarian graft, hCG was added and the patient had sexual intercourse at the optimal time point. On day 14 of the luteal phase, hCG concentration and vaginal echography confirmed a viable intrauterine pregnancy, which resulted in a healthy live birth.

Conclusion(s): Overnight transportation of ovarian tissue appears to be possible in combination with appropriate transportation logistics. However, further investigations are needed before this procedure can be offered as a chance for women to preserve fertility independently of direct access to a tissue-processing bank. (Fertil Steril® 2012;97:387–90. ©2012 by American Society for Reproductive Medicine.)

Key Words: Fertility preservation, ovarian tissue cryopreservation, ovarian tissue transplantation, orthotopic, pregnancy

As a result of improvements in oncologic treatment, most young cancer patients are now achieving prolonged survival. The 5-year survival rate for all cancers combined is currently >64% in women (1). However, loss of ovarian function and therefore fertility is one of the most common long-term adverse effects affecting premenopausal patients

treated with chemotherapy and/or radiation therapy. Conditioning therapy for bone marrow transplantation (BMT) is considered to be the most gonadotoxic form of treatment, leading to premature ovarian failure in >80% of cases even when performed during childhood (2).

For these young patients, ensuring their reproductive capacity after oncologic treatment has become a major

concern, because it is directly related to their quality of life. A number of strategies have therefore been developed in recent years to enable these patients to have children using their own gametes. When radiotherapy alone is administered, it is possible to place the ovaries outside of the radiation field. When chemotherapy can be postponed, it is possible to use ovarian stimulation to obtain oocytes, which can be frozen in either a fertilized or an unfertilized state (3, 4).

Cryopreservation of ovarian tissue is another, very promising, alternative (5). Ovarian tissue can be extracted laparoscopically without significantly delaying gonadotoxic therapy. The tissue can be cryopreserved at centers specializing in reproductive medicine and can be transplanted into the pelvic cavity (or to a heterotopic site for oocyte

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retrieval and in vitro fertilization) if the women experience premature ovarian failure. Donneze et al. (6) reported the first live birth after autotransplantation of cryopreserved ovarian tissue in humans. Orthotopic reimplantation has so far led to the birth of 17 healthy babies (7–9).

Herein, we report a live birth after ovarian transplantation in a survivor of Hodgkin lymphoma. This is the first live birth after ovarian tissue transplantation in which the tissue was transported chilled on ice overnight before freezing.

CASE REPORT

A 25-year-old woman was diagnosed with nodular sclerosing Hodgkin lymphoma, clinical stage IIA. The patient received six courses of ABVD chemotherapy—adriamycin (doxorubicin), bleomycin, vincristine, and dacarbazine—and mediastinal radiotherapy to a dose of 30 Gy. Because the chemotherapy-associated amenorrhea rate with the ABVD regimen is very low, fertility preservation was not carried out up to this point (10). Remission was achieved, and the patient reported regular menstrual cycles of ~28 days.

However, the disease recurred after 2 years. Intensive chemotherapy consisting of two courses of DEXA-BEAM—dexamethasone, BCNU (carmustine), etoposide, Ara-C (cytarabine), and melphalan—was administered before autologous BMT was performed. Before the start of this treatment, about two-thirds of the ovarian cortex was removed laparoscopically from both ovaries. A histologic reference sample showed numerous primordial and primary follicles.

After BMT, the patient was considered to be disease free, but she became amenorrheic and developed vasomotor symptoms. Cyclical hormone replacement therapy was administered with E₂ and dydrogesterone (Femoston) for a 5-year period. During this time, she had regular menstrual cycles. However, amenorrhea returned when the hormone replacement therapy was stopped, and her hormonal status confirmed premature ovarian failure (antimüllerian hormone 0.1 ng/mL).

Transportation and Cryopreservation Procedure

After laparoscopic removal at Dresden University Hospital, the ovarian tissue was rinsed for blood and cells with 0.9% saline solution in sterile conditions and placed in 30-mL plastic tubes containing a special medium for transporting ovarian tissue, precooled to 4°C (Brahma I; CryoBioSystem). The plastic tubes were placed in a special isolated transportation box (DeltaT) with cooling elements to ensure optimal conditions during transport and to preserve the viability of the tissue by maintaining a stable temperature of 5–8°C for 36 hours. The package was sent by an express overnight transportation service and reached the Reproductive Medicine Laboratory in Bonn within 20 hours.

Immediately after arrival in Bonn, the biopsies were dissected in medium for ovarian tissue manipulation (Brahma II; CryoBioSystem) (11). The biopsy specimens were then cut into pieces measuring ~1 × 2 × 1 mm and equilibrated in a freezing solution containing 1.5 mol/L dimethylsulfoxide (DMSO) in Leibovitz medium. The pieces of ovarian tissue were then frozen in standard cryopreservation containers (1.8 mL

Nunc cryovials) using a slow-cooling protocol with a closed freezing system (Icecube 14S; Sylab) with autoseeding (12).

Thawing and Retransplantation

Five years after complete remission, the patient requested autotransplantation of the cryopreserved ovarian tissue. The tissue was sent in a shipping container cooled with liquid nitrogen at –196°C to the Reproductive Medicine Laboratory in Erlangen.

Thawing was fast in a water bath with warm water. The tissue fragments were released from the protective cryopreservation medium in reverse order with the addition of 0.25 mol/L sucrose. The thawed tissue was transplanted into a 1.5-cm deep pouch of peritoneum in the region of the broad ligament, below the right tube. Six fragments of ovarian tissue 1–2 mm in size were introduced into this pouch, and the pocket was closed with a Vicryl suture (Fig. 1).

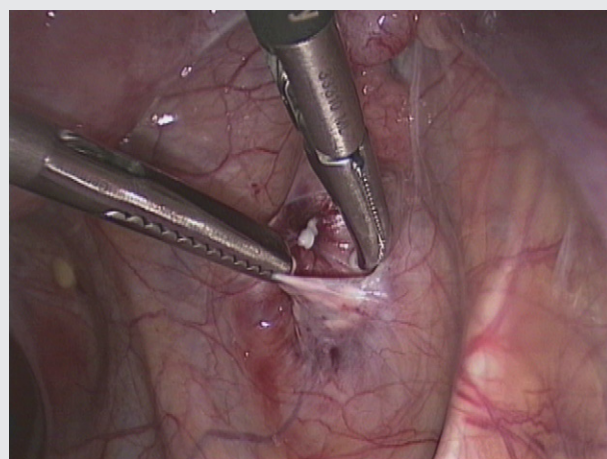
At the same time, chromoperturbation was performed, which showed bilateral tubal patency. Andrologic examination of the partner exhibited normal sperm parameters.

RESULTS

The preoperative FSH and LH levels were high and E₂ levels low. Three months after transplantation, a fall in gonadotropins (LH to 3.9 U/L, FSH to 11.1 U/L) and a rise in E₂ (to 114 pg/mL) was detected. Transvaginal ultrasonography showed an antral follicle in the ovarian graft that had been transplanted into the pelvic wall. The patient afterwards reported her first postoperative menstrual period.

As she urgently desired to have children during the following 4 months, cycle monitoring was performed, and when follicles in the transplanted graft reached 17–18 mm in size, ovulation was triggered with 5,000 U hCG (Predalon). The patient was encouraged to have regular sexual intercourse, but a pregnancy was not achieved. During the fifth menstrual cycle, therefore, mild stimulation with 25 U FSH

FIGURE 1



View of the transplantation site. The frozen/thawed tissue was transplanted into a deep pouch of peritoneum in the region of the broad ligament, below the right tube.

Dittrich. Overnight ovarian tissue transportation. *Fertil Steril* 2012.

(Puregon) was initiated in accordance with a low-dose protocol. When ultrasonography revealed a follicle 18–20 mm in size in the ovarian graft, hCG was added and the patient had sexual intercourse at the optimal time point. Progesterone (Uterogest) was administered to support the luteal phase. On day 14 of the luteal phase, a positive hCG level was detected, and a clinical pregnancy was later confirmed on vaginal ultrasonography. The pregnancy progressed uneventfully, and the patient delivered a healthy male child weighing 3,360 kg at 38 weeks of gestation by cesarean section.

DISCUSSION

Several studies have demonstrated that follicles are viable after cryopreservation and thawing, and autotransplantation of the frozen/thawed ovarian tissue has now successfully reestablished ovarian function in a number of women, resulting in regular menses and normalization of gonadotropin levels (13–15). To date worldwide, transplantation of frozen/thawed ovarian tissue has yielded 17 live births, and it is expected that in the near future more and more cancer patients who have been cured of their disease will be requesting reimplantation of cryopreserved ovarian tissue (7–9).

Despite these promising results and growing interest in the method, cryopreservation and autotransplantation of ovarian cortex is still a complex procedure that requires experience and validation of the techniques. Many centers do not have the facilities for cryopreserving ovarian tissue. Cryobanking, with transportation of the ovarian cortex before cryopreservation, is therefore used to provide a national cryopreservation service, as in Germany. This makes the option available to patients in regions or countries in which a fertility center with the required facilities and skills is not available. Transportation of ovarian tissue is a particularly useful option for patients with serious illnesses in whom the start of treatment cannot be postponed and who may therefore may not wish to move from one center to another, or may not be able to. Such patients could have ovarian tissue removed safely at a local hospital so that it can be transported to a laboratory that has the required validation and expertise.

The present report describes the first live birth after transplantation of ovarian tissue following overnight transportation of the tissue before freezing. The ovarian tissue was kept cool for >20 hours in a special transport medium and a special cooling device before it was cryopreserved. After thawing and transplantation, the ovarian graft resumed hormone production after 3 months, and resumption of menstrual bleeding was subsequently observed. Eight months after transplantation, a spontaneous pregnancy occurred, resulting in a live birth.

The feasibility of short-term transportation of ovarian tissue before freezing has been proven by research groups in Denmark. Ovarian tissue from four patients was transported for 4–5 hours chilled on ice before cryopreservation and transplantation into nude mice. The ovarian tissue from each of the four patients contained surviving follicles (16). In addition, three children have been born from transplanted frozen/thawed tissue that had been transported for 4–5 hours. These results clearly show that transportation of roughly

isolated ovarian cortex cooled on ice for a period of up to 4 hours allows survival of primordial follicles for cryopreservation and transplantation (17).

Viability of follicles after 20 hours' transportation on ice has been reported by Rosendahl et al. in one case (18). Ovarian cortex from a 6-year old girl was kept at 4°C for 20 hours before freezing and was transplanted into a nude mouse for 4 weeks. On histologic examination, the transplant showed morphologically healthy primordial follicles. Isachenko et al. (19) also indicated that prolonged suprazero temperature exposure of ovarian tissue for 26 hours does not have a negative effect on follicle quality. Ovarian cortical fragments from five patients were transferred in a special modified medium for transport of ovarian tissue (Brama I; CryoBioSystem) and located for exposure at 4°C for different time periods (0, 8, 15, 26, and 36 hours). In vitro culture of the ovarian fragments was performed for 15 days at 37°C in 5% CO₂. Histologic examination showed that exposure of ovarian tissue to suprazero temperatures for 0–26 hours did not inhibit the development of follicles during subsequent in vitro culture (19).

On the basis of these findings, it appears to be possible to extend the period that the tissue can sustain transport to 20 hours. However, there are only limited data to confirm this statement and on the transport conditions required for ovarian tissue in general. There are currently no clear methods for evaluating follicle loss during transportation or the associated effects on the longevity of the ovarian tissue. Except for the present case report, there are no data showing whether the tissue can sustain ovarian function after transplantation.

In conclusion, it has been shown that overnight transportation of ovarian tissue chilled on ice is possible, but it is not yet possible to recommend the procedure in clinical practice. Further research in this field is immediately necessary to clearly guarantee the viability of the ovarian tissue after prolonged cooling and thus to enable centers that would otherwise be unable to offer cryopreservation of ovarian tissue for fertility preservation to collaborate with centralized cryobanks. As in the present case, this method could then provide a good option for women who might otherwise have no access to ovarian cryopreservation.

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