

## Pregnancy and live birth after autotransplantation of frozen-thawed ovarian tissue in a patient with metastatic disease undergoing chemotherapy and hematopoietic stem cell transplantation

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**Objective:** To report a live birth after orthotopic transplantation of cryopreserved ovarian tissue.

**Design:** Case report.

**Setting:** Department of Gynecology, Cliniques Universitaires Saint-Luc, Brussels.

**Patient(s):** Woman with metastatic cancer who had her ovarian tissue cryopreserved in 2001 before undergoing chemotherapy and hematopoietic stem cell transplantation, resulting in premature ovarian failure.

**Intervention(s):** Orthotopic reimplantation of ovarian cortex performed 7 years after cryopreservation.

**Main Outcome Measure(s):** Restoration of ovarian activity.

**Result(s):** Restoration of ovarian activity was observed 3.5 months after reimplantation, and ongoing pregnancy was diagnosed 9 months after grafting. The patient delivered a healthy baby weighing 2.830 kg.

**Conclusion(s):** Our patient represents the thirteenth live birth to occur after orthotopic reimplantation of cryopreserved tissue, but the first in a woman treated for metastatic disease. (Fertil Steril® 2011;95:1787.e1–e4. ©2011 by American Society for Reproductive Medicine.)

**Key Words:** Cryopreservation, fertility preservation, orthotopic transplantation, ovary, pregnancy

Premature ovarian failure (POF) can occur naturally at an early age or be due to iatrogenic agents. Indeed, ovaries are very sensitive to cytotoxic treatment, especially to radiation and alkylating agents, which are classified as high risk for gonadal dysfunction. Cyclophosphamide is the agent most commonly implicated in causing damage to oocytes and granulosa cells in a dose-dependent manner (1, 2).

A large retrospective survey of pregnancy outcomes after hematopoietic stem cell transplantation (peripheral blood or bone marrow transplantation [BMT]) involving 37,362 patients revealed that only 0.6% of patients conceived after autologous or allogeneic stem cell transplantation (3). It is thus obvious that high doses of alkylating agents, irradiation, and advancing age increase the risk of gonadal damage (2).

Several options are currently available to preserve fertility in cancer patients and allow them to conceive when they have overcome

their disease: embryo cryopreservation, oocyte cryopreservation, and ovarian tissue cryopreservation (4). Although freezing of immature and mature oocytes as well as advancing technological developments in in vitro follicular growth are promising techniques, cryopreservation of ovarian tissue is the only option available for prepubertal girls and woman who cannot delay the start of chemotherapy (4). The main aim of this strategy is to reimplant ovarian cortical tissue once treatment is completed and the patient is disease free (4).

Donnez et al. (5) reported the first live birth after autotransplantation of cryopreserved ovarian tissue in humans. Orthotopic reimplantation has so far led to the birth of 12 healthy babies in 11 women (5–14). Here we report the thirteenth live birth. This is also the first case of successful autotransplantation of frozen-thawed ovarian tissue in a patient with metastatic disease, resulting in the birth of a healthy baby.

### MATERIALS AND METHODS

#### Patients

In 2001, at the age of 17 years, the patient presented with a neuroendocrine tumor of the right orbit. She underwent surgery and radiotherapy. Because of the presence of chest metastasis, she received chemotherapy (etoposide, ifosfamide, vincristine, and doxorubicin) before hematopoietic stem cell transplantation. Before chemotherapy, ovarian tissue was cryopreserved. Two large biopsy samples

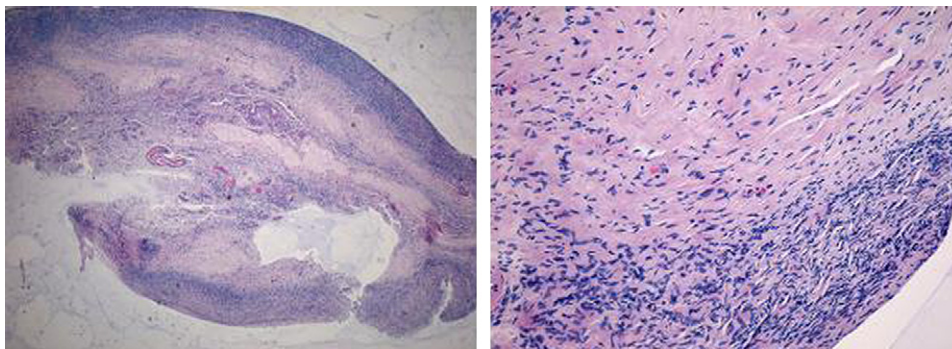
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## FIGURE 1

Several histologic sections of removed ovarian cortex (after decortication) failed to reveal the presence of any follicles.



Donnez. Live birth after ovarian cortex reimplantation. *Fertil Steril* 2011.

(10 × 5 mm) were taken from each ovary, cut into small cubes (2 mm in size), and stored in liquid nitrogen after slow-freezing procedures, as previously described elsewhere (4). Histologic examination of a small cube revealed a very high follicular density (>50 primordial follicles/mm<sup>3</sup>).

The patient then underwent BMT. Soon after, her estradiol (E<sub>2</sub>) (<10 pg/mL) and follicle-stimulating hormone (FSH) (75 mIU/mL) levels were found to be at castrated levels. The patient received hormone replacement therapy for 7 years, until she wished to become pregnant. After stopping hormone replacement therapy, her FSH and luteinizing hormone (LH) values once again returned to castrated levels.

In 2008, the patient expressed a desire to conceive. She underwent reimplantation of her cryopreserved ovarian tissue after decortication of both atrophic ovaries, according to the technique described by Donnez et al. (4, 15). Serial sections of removed cortex failed to demonstrate the presence of any follicles (Fig. 1). The frozen-thawed ovarian pieces were placed on the decorticated

area and covered with Interceed (Johnson & Johnson, New Brunswick, NJ).

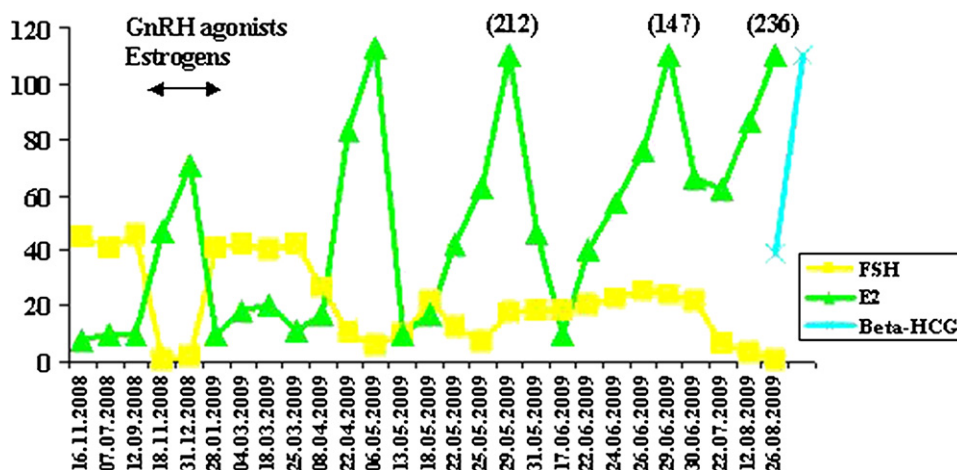
A small piece of frozen-thawed tissue was analyzed and found to contain morphologically normal primordial follicles at a density of 20 PF/mm<sup>3</sup>. Serial sections did not identify any malignant cells.

A decrease in LH and FSH concentrations was induced before reimplantation by administration of gonadotropin-releasing hormone (GnRH) agonist and estroprogestogens to avoid the stimulating effect of high concentrations of gonadotropins on grafted follicles.

Three and a half months later, a first E<sub>2</sub> peak was detected, with a concomitant decline in FSH (Fig. 2). Ultrasonography revealed the development of a follicle with each cycle. Later, FSH and LH levels were less than 10 mIU/mL (see Fig. 2). Restoration of consecutive menstrual bleeding was observed, and the patient was encouraged to have regular sexual intercourse. Nine months after grafting, the patient experienced amenorrhea. Her human chorionic gonadotropin (hCG) test was positive, and vaginal ultrasound revealed a viable intrauterine pregnancy. The triple test and nuchal thickness were

## FIGURE 2

Follicle-stimulating hormone (FSH) (mIU/mL) and estradiol (E<sub>2</sub>) (pg/mL) before and after reimplantation.



Donnez. Live birth after ovarian cortex reimplantation. *Fertil Steril* 2011.

normal. After an uneventful pregnancy, in April 2010, she delivered a healthy boy weighing 2.830 kg at 38.5 weeks of gestation. Institutional review board was obtained.

## DISCUSSION

Bone marrow transplantation is commonly used to treat cancerous diseases, but the high doses and/or chemotherapy or radiotherapy required before BMT lead to ovarian failure in almost all cases (2–4). Cyclophosphamide is the agent most often implicated in causing damage to oocytes and granulosa cells in a dose-dependent manner (1).

When patients receive busulfan and cyclophosphamide, the risk of complete POF is practically 100% (2). Although unexplained restoration of ovarian function and fertility is noted in some patients after total body irradiation, only one pregnancy has been reported after busulfan/cyclophosphamide conditioning in a retrospective survey (3).

Human ovarian tissue shows good survival and function after freeze-thawing, with good preservation of both primordial and primary follicles (4). However, loss of follicles can occur in cases of non-vascular grafting of cortical strips, relative to the period of hypoxia and the time frame before the grafted tissue becomes revascularized (16, 17). According to Silber et al. (14) and Kagawa et al. (18), vitrification and reimplantation of very thin slices could minimize this loss. But it should be pointed out that all pregnancies achieved to date were obtained after transplantation of ovarian tissue that had been cryopreserved according to the slow-freezing protocol (4–13).

To date, ovarian tissue has been successfully cryopreserved and transplanted into humans (4–13). Successful fertilization and pregnancy from fresh transplanted ovarian tissue have been described in humans, with the grafted tissue functioning without any surgical connection to major blood vessels (10, 14, 19).

In the present case, it was 3.5 months after reimplantation before a follicle was seen and a rise in  $E_2$  was detected. According to Gougeon (20), the process of folliculogenesis takes approximately 4 months, during which time the oocyte and surrounding somatic cells undergo a series of changes that eventually result in the development of a large antral follicle, capable of producing a mature oocyte. Thus, the appearance of the first follicle originating from the grafted tissue 15 weeks after reimplantation is consistent with the expected time course. The time interval between implantation of cortical tissue and the first  $E_2$  peak is consistent with data previously described in human beings (4, 5, 8, 21), although some variations may be observed. Indeed, in the literature, the delay between transplantation and follicular development was found to range from 8 weeks to 8 months (4, 5, 8, 21). Such a variation could be partially explained by a difference in follicular reserve at the time of cryopreservation, which is age dependent (4, 14, 21). In the present case, the patient was very young, had a high follicular density, and had not received any chemotherapy before cryopreservation.

One of the most important ethical issues is ensuring that the intervention does not harm the patient by dangerously delaying cancer treatment and that no remnant cells are reintroduced by subsequent transplantation (4, 21). If the risk exists, other options must be considered in the future, such as transplantation of isolated follicles (22–26).

To our knowledge, this is the thirteenth live birth obtained after orthotopic reimplantation of frozen-thawed tissue, but the first in a woman treated for metastatic disease. It is time to stop considering the procedure experimental, and we firmly believe that ovarian cortex banking should be offered before chemotherapy in all cases where emergency in vitro fertilization is not possible (26, 27).

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