CASE REPORT

Live birth after ovarian tissue autograft in a patient with sickle cell disease treated by allogeneic bone marrow transplantation

Christophe Roux, M.D., Ph.D.,[a,e] *Clotilde Amiot, M.D., Ph.D.,*[a] *Germain Agnani, M.D.,*[b] *Yves Aubard, M.D., Ph.D.,*[c] *Pierre-Simon Rohrlich, M.D., Ph.D.,*[d,e] *and Pascal Piver, M.D.*[c]

[a] Service de Génétique Histologie Biologie du Développement et de la Reproduction (CECOS Franche-Comté Bourgogne), Centre Hospitalier Universitaire, Université de Franche-Comté, Besançon; [b] Service de Gynécologie-Obstétrique, Besançon, [c] Service de Gynécologie-Obstétrique, hôpital de la Mère et de l'Enfant, Limoges, and [d] Services d'Hématologie Clinique et d'Hémato-Onco-Pédiatrie, Centre Hospitalier Universitaire, Besançon; and [e] Inserm, UMR 645, Institut Fédératif de Recherche (IFR) 133, Besançon, France

Objective: To report the first case of restoration of ovarian activity and live birth after cryopreserved ovarian tissue autograft in a patient without cancer treated by allogeneic bone marrow transplantation.
Design: Case report.
Setting: University hospital.
Patient(s): One woman with homozygous sickle cell anemia.
Intervention(s): An orthotopic autotransplantation of ovarian cortical strips was performed after freeze-thawing.
Main Outcome Measure(s): Cryopreservation of ovarian tissue, bone marrow transplantation, ovarian autograft, and restoration of ovarian function.
Result(s): In autumn 2005, biopsy samples of ovarian tissue were cryopreserved before chemotherapy followed by bone marrow transplantation. In spring 2008, because the patient had been menopausal for 2.5 years as a result of the conditioning therapy, an orthotopic autotransplantation of thawed ovarian cortex was performed. The patient conceived spontaneously in a natural cycle in autumn 2008, and delivered a healthy female child in June 2009.
Conclusion(s): Cryopreservation of ovarian tissue with subsequent autotransplantation is an emerging procedure for preserving the fertility of young patients with a high risk of premature ovarian failure (POF) resulting from gonadotoxic treatment. This case opens up new perspectives in cases of nonmalignant diseases. (Fertil Steril® 2010;93:2413.e15–e19. ©2010 by American Society for Reproductive Medicine.)

Key Words: Ovarian tissue, cryopreservation, autotransplantation, live birth, sickle cell disease

Life expectancy of patients with malignancy has greatly increased with advances in diagnosis and treatment regimens (radiotherapy or chemotherapy) (1). However, women receiving such treatments for cancer or other nonmalignant diseases are likely to experience premature ovarian failure (POF) (2). Consequently, an important quality of life issue for these women and their families is the ability to conceive using their own oocytes. This has led to an increased focus on preserving female fertility through ovarian tissue cryopreservation and assisted reproduction (ART). In 2004, Donnez et al. (3) reported the first live birth after transplantation of cryopreserved ovarian cortex in a woman treated for Hodgkin's lymphoma. In cases of nonmalignant diseases for women where alternative options are not available, ovarian tissue cryopreservation, undertaken before cytotoxic therapy, could be a means of preserving fertility without delaying the initiation of the treatment.

Sickle cell disease is a hereditary anemia for which the only current curative option is allogeneic bone marrow transplantation (BMT). In 2006, Donnez et al. (4) described the first case of restoration of ovarian function after orthotopic transplantation of cryopreserved ovarian tissue in a woman treated by BMT for sickle cell anemia but without live birth.

The present study reports the reestablishment of ovarian function with live birth after a two-step orthotopic transplantation of cryopreserved ovarian tissue in a patient treated by allogeneic BMT for sickle cell anemia.

CASE REPORT

The patient was born in 1985 and suffered from homozygous sickle cell anemia. She underwent splenectomy and cholecystectomy when she was five and then repeated transfusions. She presented

Received October 7, 2009; revised November 24, 2009; accepted December 7, 2009; published online February 1, 2010.
C.R. has nothing to disclose. C.A. has nothing to disclose. G.A. has nothing to disclose. Y.A. has nothing to disclose. P.-S.R. has nothing to disclose. P.P. has nothing to disclose.
The protocol for cryopreservation of ovarian tissue from women treated by high doses of chemotherapy was funded by the Clinical Research Hospital Program of Limoges (General Hospital Management French Labor and Social Affairs Ministry). This work was supported by grants from the Ligue Contre le Cancer (Doubs, France).
Reprint requests: Christophe Roux, M.D., Ph.D., Hospital, Biologie de la Reproduction, Place saint Jacques, 25030 Besançon, France (FAX: 33-3-81-21-86-68; E-mail: christophe.roux@univ-fcomte.fr).

doi:10.1016/j.fertnstert.2009.12.022

with cerebrovascular stroke in spring 2005 and underwent monthly exchange transfusions. The BMT with an HLA-identical sibling donor was offered to the patient in autumn 2005.

Because the patient had no immediate plans to have children and treatment for her sickle cell anemia could not be delayed, the only option for preserving her fertility was autopreservation of ovarian tissue. The patient was included in a multicentric protocol for cryopreservation of ovarian tissue funded by the Clinical Research Hospital Program of Limoges and approved by the clinical ethics committee of Besançon University Hospital on June 5, 2002. Right oophorectomy was performed by laparoscopy under general anesthesia in October 2005 before BMT (Fig. 1A). Eight ovarian cortical fragments (1 cm/0.5 cm) were cryopreserved in cryovials containing freezing solution (1.5 mol/L dimethyl sulfoxide [DMSO] and 0.1 mol/L sucrose in Leibovitz L-15 medium supplemented with 10% decomplemented patient serum), according to a protocol using a slow cooling with manual seeding (5, 6). After freezing, the vials were individually conditioned in Ccryoflex® and stored in liquid nitrogen.

The quality of ovarian tissue was assessed before and after freezing/thawing by histology and by trypan blue staining of isolated follicles (6).

After ovarian cryopreservation, the patient received a conditioning regimen with busulfan (12.8 mg/kg total dose), cyclophosphamide (200 mg/kg total dose) (7), and then 2.4×10^8 nucleated cells/kg, from her HLA genoidentical brother, who was heterozygous for hemoglobin S. Despite receiving graft-versus-host disease prophylaxis, the patient developed grade II acute graft-versus-host disease with keratitis and skin rash (8), followed by limited chronic graft-versus-host disease. A stable 100% donor chimerism was obtained at day 60.

After BMT, the patient presented with clinical and biological POF. Hormone replacement therapy (HT) with oestro-progestogens was started 6 months after BMT and was maintained until ovarian function restoration.

Eighteen months after BMT, the patient wanted to become pregnant. An assessment of gonadotropin levels showed FSH at 98 UI/L and LH at 32 UI/L. Ultrasound evaluation of the remaining ovary revealed no follicle. After receiving a positive recommendation from the local ethics committee on October 24, 2007, transplantation was carried out in April 2008, 29 months after BMT.

Two weeks before the ovarian autograft, a cortical fragment was thawed for long-term viability and histologic assessments of the cortex, and for microbiological monitoring of the freezing solution. Viability of the tissue, based on the percentage of trypan blue unstained isolated primordial and primary follicles after freezing/thawing, was 73%, compared with 100% before cryopreservation. Histology of the ovarian thawed strip revealed the presence of well-preserved follicles (Fig. 1B).

Ovarian cortical strips were thawed according to the previously modified technique (9). After quickly thawing the vials, the strips were washed in decreased solutions of DMSO 1.5 M (5 minutes), 1 M (5 minutes), 0.5 M (10 minutes), and in a solution of 0.05 mol/L sucrose in Leibovitz L-15 medium supplemented with 10% decomplemented patient serum. The strips were then rinsed and transferred to the operating theatre for the graft in medium containing 20% serum only.

The two-step orthotopic autotransplantation was performed by laparoscopy in April 2008 according to the procedure described by Donnez et al. (3) and modified by Piver et al. (10). The first step aimed to trigger local inflammation and attempted to induce neoangiogenesis. Small ovarian cortical strips were added to facilitate the production of angiogenic factors such as vascular endothelial growth factor (11). Windows were created in the remaining left ovary and in the peritoneum between the right iliac vessels near the fimbria of the right fallopian tube. One thawed strip of ovarian cortex was cut into six fragments. One was sutured in the ovarian incision; five others were deposited in the peritoneal window. A biopsy of the remaining left ovary was carried out for histologic evaluation. Several sections failed to reveal the presence of any follicles (Fig. 1C).

In a second step, 3 days later, three thawed cortical strips were fixed into the left ovary and one into the peritoneal window.

The first signs of ovarian function restoration were a decrease in FSH levels and an increase in anti-Müllerian hormone levels at subnormal values, 9 weeks after ovarian graft (Fig. 1D). Four months after the transplantation, a vaginal echography carried out for abdominal pain revealed follicular development in both transplanted sites. Two follicles were present in the left ovary. Two other follicles were also observed in the graft of the peritoneal window, one resembling an initial corpus luteum (CL). The HT was then stopped and a normalization of FSH levels and a significant level of anti-Müllerian hormone were obtained 19 weeks after the graft (Fig. 1D).

After a first cycle without HT and menstrual bleeding, the following cycle was monitored. Folliculogenesis was confirmed by an increase in 17β-E_2 levels (Fig. 2A). Follicular development was observed sonographically in both ovarian and peritoneal sites (Fig 2B,C). After detecting LH peak, two preovulatory follicles were observed, one on each reimplantation sites, and sexual intercourse was recommended. During the luteal phase, P concentration increased (Fig. 2A) and ultrasonography revealed the presence of a CL on both sites. No luteal phase supplementation by P was administered.

Fourteen days after ovulation, the concentration of hCG was 145 mIU/mL and increased to 6,509 mIU/mL 10 days later. Vaginal ultrasonography at 7 weeks confirmed an intrauterine ongoing pregnancy. The pregnancy resulted in the birth, at 38 weeks of gestation, of a healthy girl, heterozygous for sickle cell anemia, weighing 3,700 g delivered by cesarean section.

DISCUSSION

In recent decades, BMT has been increasingly used for noncancerous diseases, but the high doses of chemotherapy or radiotherapy given before BMT lead to ovarian failure in almost all cases (12). Cyclophosphamide is the most common agent implicated in causing damage to oocytes and granulosa cells (GC) in a dose-dependent manner (13). Complete amenorrhea is reported after a dose of 5, 9, and 20 g/m^2 of cyclophosphamide in women more than 40, 30–40, and 20–30 years of age, respectively (14). When patients receive busulfan and cyclophosphamide, the risk of complete POF is nearly 100% (15, 16). Although an unexplained return of ovarian function and fertility is noted in some patients after total body irradiation (17), only one pregnancy was reported after busulfan/cyclophosphamide conditioning in a retrospective survey (18).

Unlike animals, in which live births are reported after transplantation of whole cryopreserved ovaries (19), births have been observed in humans after ovarian strip autotransplantation for only six women, including four cases of Hodgkin's disease (3, 20–22), one non-Hodgkin's lymphoma (23), and one Ewing sarcoma (20).

Human ovarian tissue shows good survival and function after freezing/thawing, with good preservation of primordial and primary follicles (6), but loss of follicles in the nonvascular cortical strip grafts can occur in proportion to hypoxia and to the time frame before the grafted tissue becomes revascularized (24, 25). Orthotopic sites are preferable to heterotopic sites for ovarian cortical fragment