CASE REPORT

Infertility

Live birth after allografting of ovarian cortex between genetically non-identical sisters

Jacques Donnez1,*, Jean Squifflet1, Céline Pirard1, Dominique Demylle1, Anne Delbaere2, Laetitia Armenio2, Yvon Englert2, Anne-Céline Cheron3, Pascale Jadoul1, and Marie-Madeleine Dolmans1

1Department of Gynecology, Cliniques Universitaires St. Luc, Université Catholique de Louvain, Avenue Hippocrate 10, B-1200 Brussels, Belgium. "Fertility Clinic, Departement OB/Gyn, Université Libre de Bruxelles (ULB), Erasme Hospital, B-1070 Brussels, Belgium
3Department of Obstetrics, Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, B-1200 Brussels, Belgium

*Correspondence address. Tel.: +32-2-764-95-01; Fax: +32-2-764-95-07; E-mail: jacques.donnez@uclouvain.be

Submitted on January 26, 2011; resubmitted on February 23, 2011; accepted on March 2, 2011

ABSTRACT: Aggressive chemotherapy generally results in the loss of both endocrine and reproductive functions. If the patient has not undergone previous oocyte, embryo or ovarian tissue cryopreservation, orthotopic allotransplantation of fresh ovarian tissue from a genetically non-identical sister may be considered. Here, we describe a case report. The patient, aged 15 years and presenting with homozygous sickle cell anemia, underwent chemotherapy (busulfan, cyclophosphamide) and total body irradiation before bone marrow transplantation, the donor being her HLA-compatible sister. HLA group analysis later revealed complete chimerism. When the patient was 32 years old, ovarian allografting was performed, with the ovarian tissue donor being the same sister who had already donated bone marrow. The goal was to restore ovarian activity and natural fertility. No immunosuppressive therapy was administered. No sign of rejection was observed. Restoration of ovarian function was achieved 3.5 months after transplantation, as proved by the first estradiol peak and follicular development detected by ultrasound. After 9 months of regular ovulatory cycles, IVF was attempted because proximal tubal stenosis (unknown at the time of grafting) could not be repaired by tubal reanastomosis. After stimulation, three oocytes were retrieved. Two embryos were obtained. One embryo was frozen and the other was transferred, resulting in an ongoing pregnancy. The patient delivered a healthy baby girl weighing 3.150 g at 37 2/7 weeks of gestation.

Key words: ovarian tissue / transplantation / BMT / premature ovarian failure / chemotherapy

Introduction

Total body irradiation (TBI), required before bone marrow transplantation (BMT) and associated with chemotherapy, constitutes the treatment combination presenting the greatest risk of premature ovarian failure (POF) (Sanders et al., 1996; Teinturier et al., 1998; Meirow and Nugent, 2001; Larsen et al., 2003; Wallace et al., 2005a; Donnez et al., 2006). Cyclophosphamide is the agent most commonly implicated in causing damage to oocytes and granulosa cells in a dose-dependent manner (Meirov et al., 1999).

A large retrospective survey of pregnancy outcomes after hematopoietic stem cell transplantation (SCT; peripheral blood or BMT) involving 37,362 patients revealed that only 0.6% of patients conceived after autologous or allogeneic SCT (Salooja et al., 2001; Lutchman Singh et al., 2006). The high doses of alkylating agents, irradiation and advancing age increase the risk of gonadal damage (Wallace et al., 2005a).

Several options are available to preserve fertility in patients facing POF, including immature and mature oocyte cryopreservation, embryo cryopreservation and ovarian tissue cryopreservation (Donnez et al., 2006; Jeruss and Woodruff, 2009). However, when none of these three options was implemented or was indeed available at the time of treatment, patients refusing oocyte donation have little chance of ever becoming pregnant.

In this report, we show that ovarian allotransplantation from a previous bone marrow donor now represents a possible alternative. The goal was to restore ovarian activity and natural fertility.

Very recently, restoration of ovarian function was described in three cases after allografting of ovarian cortex between genetically non-identical sisters (Donnez et al., 2007, 2010). We now report the...
first pregnancy and live birth to occur after orthotopic allotransplantation of fresh ovarian tissue between two genetically non-identical sisters in one of these three cases.

**Methods**

**Patient**

In 1992, a 15-year-old girl presenting with homozygous sickle cell anemia underwent chemotherapy (busulfan 16 mg/kg and cyclophosphamide 200 mg/kg) and TBI (750 cGy) before BMT, with the donor being her 19-year-old HLA-compatible sister. Her brother died from the same disease.

Shortly (2 months) after initiation of chemo- and radiotherapy, the patient showed a typical ovarian failure profile, as proved by an FSH level >100 mIU/ml and estradiol <20 pg/ml. Hormone replacement therapy (HRT) was therefore initiated in 1994 with triphasic HRT consisting of estradiol and norethisterone, but the patient switched for personal reasons to ethinylestradiol 25 μg/day associated with dydrogesterone 10 days/month. On ultrasound, both ovaries appeared atrophic, each measuring 14 × 8 mm, without any follicular development.

At the time of grafting (December 2009), the patient (aged 32 years) was not yet sexually active and was planning to get married 2 months later. For cultural and religious reasons, the patient preferred to undergo allografting rather than oocyte donation. Indeed, she wanted to be responsible for her own follicular maturation and genesis of the pregnancy. Her husband fully supported her in this. Moreover, the procedure allowed her not to reveal her menopausal status to her family and family-in-law, for whom infertility is an important cultural concern.

The donor was 36 years of age and had given birth to two children. She preferred to undergo ovarian tissue biopsy and donation by means of one-day laparoscopy, particularly because she was living in another country.

**Chimerism and HLA compatibility**

HLA group analysis revealed complete chimerism (HLA compatibility) between the sisters, proving that no immunosuppressive treatment would be necessary, even though they were genetically non-identical. Chimerism was confirmed by molecular biology using the short tandem repeat technique (Donnez et al., 2010).

**Ethics**

Our protocol was authorized by the Ethics Committee of the Catholic University of Louvain which, back in 1995, had approved research protocols including reimplantation of ovarian tissue to preserve or restore fertility in women treated with procedures at risk of iatrogenic ovarian failure.

**Surgery**

Twenty days before surgery, the recipient patient was given GnRH agonist and estro-progestogen therapy for a period of 2 months to decrease endogenous FSH levels, as recommended in cases of reimplantation of cryopreserved ovarian tissue (Donnez et al., 2006, 2010). The technique used in the present case has been previously described (Donnez et al., 2010).

When the ovaries of the recipient were ready to receive donor ovarian cortex, a very large biopsy (2 × 2 cm; Fig. 1) was taken from the left ovary of the donor sister, taking care not to remove medullary tissue, and divided into two parts measuring 2 × 1 cm (Fig. 2A and B). Each part was immediately sutured to the decorticated recipient ovaries.

The ovarian pieces were thus sutured to the recipient ovarian medulla as soon as they were recovered. No medium or ice was used. The time interval between cortex removal and the start of suturing was <1 min, and both sutures were achieved within 30 min of the fragment being excised. The edges of the cortical fragments were sutured to the decorticated edges, so that contact between the donor cortex and receiver medulla was optimal.

A small biopsy (4 × 1 mm) was also taken from the donor ovary for histological analysis to evaluate ovarian reserve.

**Results**

**Ovarian biopsy**

Serial sections of the recipient ovarian cortex did not show the presence of any follicles. Conversely, the donor ovarian biopsy revealed numerous primordial follicles, with a density of 10 mm⁻³.

**Ovarian activity**

Three and a half months after reimplantation, restoration of ovarian function was demonstrated by vaginal echography (follicle of 20 mm in size, thickened endometrium) and estradiol levels as high as 80–200 pg/ml. FSH levels fell to values <10 mIU/ml, and follicular development was noted monthly (Fig. 3). Sperm parameters were normal and the patient showed regular ovulatory cycles for 9 months. As she failed to become pregnant, hysterography was performed, revealing proximal tubal occlusion. Since the patient was a virgin at the time, we never considered the possibility of tubal occlusion, and hysterography was not performed prior to transplantation. It was then decided to carry out a laparoscopy with the goal of checking tubal patency by cervical injection of methylene blue and proceeding with tubal reanastomosis if tubal blockage was established. On laparoscopy (13 months after reimplantation), follicles and corpus luteum were visible in the grafted ovarian tissue (Fig. 4). Since tubal occlusion was indeed confirmed, tubal reanastomosis was laparoscopically attempted. Tubal section was performed at different levels, but stenosis was confirmed throughout, making microsurgical anastomosis impossible. We hypothesized that this tubal stenosis was due to the radiotherapy that the patient underwent at the age of 15 years, before maturation of the Müllerian system was complete (Wallace et al., 2005a).
**In vitro fertilization**

Sixteen months after grafting, stimulation was initiated with rec-hFSH (225 mIU) (Serono, Belgium) from Day 2 of the cycle. On Day 7, GnRH antagonist (Cetrotide, Serono, Belgium) was started (0.25 mg/day). Because the patient was hepatitis C-positive, ovarian pick-up (three oocytes) and fertilization were carried out at the Fertility Clinic of Erasme Hospital (Université Libre de Bruxelles), which is fully equipped for gamete management in case of viral diseases (such as human immunodeficiency virus and hepatitis; Gilling-Smith et al., 2005).

Two embryos were obtained. One was transferred on Day 3, resulting in an ongoing intrauterine pregnancy. The other embryo was frozen.

**Pregnancy/delivery**

The triple test conducted at 11 4/7 gestational weeks showed a combined risk of 1/9694 for trisomy 21 and <1/10 000 000 for trisomy 18. Calculation of risk took into account pregnancy-associated plasma...
protein-A and β-hCG levels, age (of the patient’s sister) and nuchal thickness, found to measure 1.2 mm for a cranio-caudal length of 60 mm.

The patient presented to the emergency department at 17 4/7 gestational weeks with metrorrhagia and uterine contractions observed by tocography. Echography revealed a cervical length of 20 mm with the presence of a funnel, while vaginal examination showed a short cervix of 1 cm dilated to 1 cm. As the biological results were reassuring, a McDonald cerclage was performed the next day after administering an indomethacin suppository (Indocin®). Subsequent treatment involved two indomethacin suppositories per day for 48 h and progesterone (3 × 200 mg) (Utrogestan®, Besins International, Brussels, Belgium). The patient was hospitalized for 1 week for observation and then discharged, but required careful monitoring every week.

Echography performed at 19 5/7 gestational weeks showed a normal and complete morphology, biometrics between the 50th and 90th percentiles, and low uterine artery resistance (Doppler). Thereafter, the patient was followed every 3 weeks, delivering a baby girl weighing 3.150 g at 37 2/7 weeks of gestation.

**Discussion**

This paper reports the first pregnancy to occur after allografting of ovarian cortex between genetically non-identical sisters, where the recipient was confirmed to be suffering from ovarian failure due to chemotherapy and TBI before BMT.

Advances in high-dose chemotherapy and radiotherapy treatments have significantly improved cure rates of many young patients affected by certain hematologic malignancies and solid tumors but, unfortunately, sterilization and early menopause are common long-term side effects. Ovaries are very sensitive to cytotoxic treatment, especially to radiation and alkylating agents, which are classified as high risk for gonadal dysfunction (Wallace et al., 2005a,b; Donnez et al., 2006). Cyclophosphamide is the agent most commonly implicated in causing damage to oocytes and granulosa cells in a dose-dependent manner (Meirow et al., 1999). According to Wallace et al. (2005a) the risk of POF is very high after chemotherapy and BMT, as in the case reported here.

Several options now exist to preserve fertility in women who need to undergo aggressive chemotherapy: embryo cryopreservation, oocyte cryopreservation and ovarian tissue cryopreservation (for review, see Donnez et al., 2006; Jeruss and Woodruff, 2009). So far, autologous orthotopic transplantation of cryopreserved ovarian tissue has resulted in 14 live births (Donnez et al., 2004, 2011; Meirow et al., 2005; Demeestere et al., 2007; Silber and Gosden, 2007; Andersen et al., 2008, Piver et al., 2009; Roux et al., 2010; Sánchez-Serrano et al., 2010; Silber et al., 2010), the first pregnancy being reported back in 2004 (Donnez et al., 2004).

However, when embryo, oocyte or ovarian tissue cryopreservation procedures are not available or appropriate, allografting can be proposed, even if oocyte donation remains the standard procedure (Donnez et al., 2010). Allografting has the potential to restore, not only ovarian activity, but also natural fertility. Restoration of ovarian function after allografting of the ovarian cortex between genetically different sisters was recently described by Donnez et al. (2010) and the first estradiol peak was detected 3 5–6 months after transplantation, the interval depending on the follicular density.

In the present case, it is particularly important to stress that the recipient had received bone marrow from her HLA-compatible sister. Transplantation of organs like kidneys has already been performed between HLA-compatible sisters who have previously undergone BMT, with one sister acting as donor to the other. Indeed, Hamawi et al. (2003) reported six cases of kidney transplantation after BMT, the kidney donor being the BMT donor in all cases. The patients did not receive immunosuppressive treatment and there was no sign of rejection. Hamawi et al. (2003) thus concluded that BMT recipients who receive a kidney from their bone marrow donor do not require immunosuppression.

Here, HLA group analysis revealed complete chimerism (HLA compatibility) between the two sisters. It was therefore proposed that ovarian tissue be grafted from the sister who had already donated bone marrow to the recipient sister with POF.

The time interval between implantation of cortical tissue and follicular development was found to be 3.5 months. This is consistent with data observed in humans by Silber et al. (2005) who reported the first rise in estradiol 71 days after implantation of fresh tissue. Ovarian allotransplantation between a BMT recipient and donor was thus effective in terms of ovarian activity restoration, without the need for immunosuppressive therapy.

In the present case, the follicular density of the donor ovarian specimen was high and recovery of ovarian activity occurred 3.5 months after reimplantation. This interval corresponds to the development of primordial follicles to the antral follicle stage, but it is also possible that one or two growing follicles, having survived the reimplantation procedure and subsequent ischemic period (estimated to be between 3 and 5 days), could reach the pre-ovulatory stage before the primordial follicle (Van Eysck et al., 2009, 2010).

The fact that serial sections of the recipient’s cortex failed to demonstrate the presence of any primordial follicles and the knowledge that there was not much remaining atrophic native ovarian tissue support the notion that the origin of the oocyte resulting in pregnancy was the transplanted tissue. It is extremely unlikely that restoration of ovarian function in a woman who had undergone both chemo- and radiotherapy before BMT could have been due to residual follicles in an atrophic native ovary, from which the majority of cortex had been removed. Moreover, according to Salooja et al. (2001) and Lutchman Singh et al. (2006), only 0.6% of patients (in a large series including >37 000 subjects) conceived after autologous or allelogeneic SCT.

In conclusion, the present study reports, for the first time, pregnancy after ovarian cortex allografting between genetically different sisters, who were fully HLA-compatible due to previous BMT. Because of tubal occlusion, unknown at the time of the procedure, IVF was mandatory. It may be suggested that oocyte donation from the sister could have been an alternative, but in this instance it was not an option. Indeed, we were not aware of the tubal occlusion. Furthermore, the sister refused to donate oocytes after gonadotrophin stimulation, but was willing to undergo one-day surgery to donate an ovary. It is essential to stress that our goal was to restore ovarian function and natural fertility. In another case, a pregnancy obtained naturally is currently ongoing, constituting a fourth case of ovarian function restoration and proving that the option of allografting should at least be considered and discussed with the patient.
Authors’ roles

J.D. wrote the manuscript and performed surgery with J.S. and P.J., with the help of D.D. (biologist). M.M.D. and P.J. were responsible for clinical data collection, C.P. for monitoring of the IVF attempt, and A.D., L. A. and Y.E. for ovarian pick-up, IVF laboratory procedure and embryo transfer. A.C.C. delivered the baby. M.M.D. followed the patient and participated in the discussion.

Funding

This work was supported by grants from the Fonds National de la Recherche Scientifique de Belgique (grant no 3.4590.08F and 7.4562.08), the Fondation Saint-Luc and the Belgian Federation Against Cancer (non-profit organization), and donations from A. Frère, Ph. de Spoelberch and P. Ferrero.

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