Background: Aggressive chemotherapy generally results in the loss of both endocrine and reproductive functions. For some women, however, oocyte, embryo or ovarian tissue cryopreservation were not proposed at the time. For three such women, orthotopic allotransplantation of fresh ovarian tissue from their genetically non-identical sister was performed.

Methods: Three women, aged 20, 15 and 12 years, respectively, underwent chemotherapy and total body irradiation before bone marrow transplantation (BMT), the donor in each case being their HLA-compatible sister. Years later, HLA group analysis revealed complete chimerism, and ovarian allografting was performed, with the ovarian tissue donor being the sister who had already donated bone marrow. No immunosuppressive therapy was administered. No sign of rejection was observed.

Results: Restoration of ovarian function occurred in all three cases, respectively, 6, 3.5 and 3.5 months after transplantation. The timing of the first estradiol peaks and the persistence of ovarian function were probably related to the primordial follicle density of donor ovarian tissue.

Conclusions: Even in the absence of immunosuppressive therapy, ovarian allografting between genetically non-identical sisters allowed restoration of ovarian function in cases where previous BMT from the HLA-compatible sister resulted in full chimerism, avoiding the threat of rejection.

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

Introduction

Aggressive chemotherapy and radiotherapy generally result in the loss of both endocrine and reproductive functions and, in most female patients, these treatments lead to ovarian failure. Cyclophosphamide is the agent most commonly implicated in causing damage to oocytes and granulosa cells in a dose-dependent manner. Total body irradiation (TBI) required before bone marrow transplantation (BMT) and associated chemotherapy constitute the treatment combination presenting the greatest risk of premature ovarian failure (POF) (Sanders et al., 1996; Teinturier et al., 1998; Meirion and Nugent, 2001; Larsen et al., 2003; Wallace et al., 2005a, b; Donnez et al., 2006a, b).

A large retrospective survey of pregnancy outcomes after hematopoietic stem cell transplantation (HSCT) (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). It is thus obvious that high doses of alkylating agents, irradiation and advancing age increase the risk of gonadal damage (Wallace et al., 2005a, b).

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., 2006a, b).

However, when none of these three options was implemented, or was indeed available at the time of treatment, ovarian allotransplantation from the previous bone marrow donor now represents a possibility.

Here, we describe the first three cases of orthotopic allotransplantation of fresh ovarian tissue between two genetically non-identical sisters, for whom immunosuppression was not required.
Methods

Patients

Patient 1

This patient was partially reported in Donnez et al., 2007. In 1990, a 20-year-old woman presenting with β-thalassemia major underwent chemotherapy (busulfan 16 mg/kg and cyclophosphamide 200 mg/kg) and TBI (750 cGy) before BMT, with the donor being her 17-year-old HLA-compatible sister. The patient was successfully treated but, at the time, no procedure was available to preserve her fertility.

She became amenorrheic shortly after initiation of chemotherapy and radiotherapy. Six weeks later, concentrations of follicle-stimulating hormone (FSH) were 108 mIU/ml, luteinizing hormone (LH) 54 mIU/ml and estradiol 10 pg/ml. This ovarian failure profile was confirmed 1 month later and hormone replacement therapy (HRT) was initiated.

The bone marrow donor was 32 years of age at the time of ovarian tissue implantation to her menopausal 35-year-old non-identical sister.

Patient 2

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.

Shortly (2 months) after initiation of chemo- and radiotherapy, the patient showed a typical ovarian failure profile, as confirmed by an FSH level >100 mIU/ml and estradiol <20 pg/ml. HRT was therefore initiated.

The bone marrow donor was 36 years of age at the time of allografting and had two children, whereas her menopausal non-identical sister was 32 years of age.

Patient 3

In 1988, a 12-year-old girl presenting with acute myelogenous leukemia underwent chemotherapy (busulfan 16 mg/kg and cyclophosphamide 200 mg/kg) and TBI (750 cGy) before BMT, with the donor being her 14-year-old HLA-compatible sister. Thereafter, she quickly (after 2 months) experienced POF, evidenced by FSH >50 mIU/ml and estradiol <20 pg/ml.

In subsequent years, the patient was prescribed HRT and underwent two attempts at oocyte donation, but no pregnancy occurred after transfer.

The bone marrow donor, at 34 years of age with two children, wished to donate an ovary to her now 32-year-old menopausal non-identical sister.

Chimerism and HLA compatibility

Quantitative chimerism analysis was used to reflect the proportion of recipient and donor genotypes. It is based on the identification of genetic markers characteristic of a given transplant pair. In our study, this genetic fingerprinting was done by PCR amplification of short tandem repeats (for review, see Starzl, 2004; Gineikiene et al., 2009).

HLA group analysis revealed complete chimerism (HLA compatibility) between the sisters in each group, proving that no immunosuppressive treatment would be necessary, even though they were genetically non-identical.

Surgery

Twenty days before surgery, recipient patients were given GnRH agonist and estroprogestogen therapy for a period of 2 months to decrease endogenous FSH levels, as previously suggested in cases of reimplantation of cryopreserved ovarian tissue (Donnez et al., 2006a, b, 2008).

The operating rooms of the gynecology department are two communicating surgical suites (ORI, Storz, Tuttlingen, Germany). The procedures were carried out as follows.

Both atrophic ovaries of the recipient (measuring 1.5 × 1 cm) were decorticated by laparoscopy (n = 1) or minilaparotomy (n = 2) (Fig. 1A). Since the ovaries were atrophic, approximately two-third of the cortical tissue was removed in order to accommodate the graft, and sent for histological analysis.

When the ovaries of the recipients were ready to receive donor ovarian cortex, for Patient 1, one large biopsy measuring 10 × 6 mm was taken from the right donor ovary and immediately sutured laparoscopically to the recipient ovary using 7-0 Prolene (Ethicon, Johnson and Johnson, USA). A second biopsy measuring 6 × 4 mm was taken from the left ovary and also immediately sutured as before (Fig. 1B). This laparoscopic technique has been previously described (Donnez et al., 2007).

From the donor sisters of Patients 2 and 3, a very large biopsy (2 × 2 cm; Fig. 2A) was taken from their left ovary and divided into two parts measuring 2 × 1 cm (Fig. 2B). Each part was immediately sutured to a decorticated recipient ovary.

The fragments were 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 receiv-er medulla was optimal. The minilaparotomy technique used was similar to that recommended by our group (Donnez et al., 2006a, b), and by Silber et al. (2005, 2008).

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

Results

Ovarian biopsy

Recipient ovarian biopsy

Serial sections of the recipient ovarian cortex did not show the presence of any follicles in any of the three cases (Fig. 3).

Donor ovarian biopsy

In case 1, serial sections of the donor ovary revealed the presence of 2 primordial follicles (PF) and 1 primary follicle out of 11 serial sections (0.75 follicles/mm³).

In cases 2 and 3, the donor ovarian biopsy revealed numerous PF, with a density of 10 and 12 PF/mm³, respectively.

Hormone levels

Patient 1

Six months after reimplantation, a first increase in estradiol (65 pg/ml) was observed, concomitant with follicular development (11 mm) (Fig. 4). FSH and LH levels were, respectively, 31.5 and 26.1 mIU/ml. Seven months after reimplantation, a second estradiol peak was observed, reaching almost 100 pg/ml, which was followed by menstrual bleeding. The patient then experienced menstrual ovulatory cycles characterized by estradiol peaks of more than 200 pg/ml, follicular size between 18 and 21 mm and a thickened endometrium. FSH values decreased to 15.2 mIU/ml. Cycle length was between 30 and 48 days.

Patient 2

Three and a half months after reimplantation, restoration of ovarian function was confirmed by vaginal echography (follicle of 20 mm in size, thickened endometrium) and estradiol levels reached between 100 and 200 pg/ml (Fig. 5). FSH levels dropped to values under 10 mIU/ml. Follicular development was observed monthly. One year after transplantation, the graft is still functioning and the patient has regular ovulatory cycles every 28 days.

Patient 3

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 were as high as 180–200 pg/ml (Fig. 6). FSH levels fell to values under <10 mIU/ml and follicular development was noted monthly.

One year after transplantation, the graft is still functioning, as demonstrated by the presence of regular ovulatory cycles.

Discussion

Transplantation of organs like kidneys has already been successfully 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, with the kidney donor being the BMT donor in all cases. The patients did not receive immunosuppressive treatment and there were no signs of rejection. Hamawi et al. (2003) thus concluded that BMT recipients who receive a kidney from their bone marrow donor do not require immunosuppression. From this study, we extrapolated that ovarian allotransplantation between a BMT recipient and donor would also be possible and effective without immunosuppressive therapy.

Our protocol was authorized by the Ethics Committee of the Catholic University of Louvain which, back in 1995, had approved such research protocols, including reimplantation of ovarian tissue to preserve or restore fertility in women treated with high doses of chemotherapy, which could induce ovarian failure.

Several now exist to preserve fertility in women who need to undergo aggressive chemotherapy, allowing them to become mothers when they have overcome their disease: embryo cryopreservation, oocyte cryopreservation and ovarian tissue cryopreservation (for review, see Donnez et al., 2006a, 2008). So far, autologous orthotopic transplantation of cryopreserved ovarian tissue has resulted in 12 live births (Donnez et al., 2004; Meirow et al., 2005; Demeestere et al., 2006; Andersen et al., 2008; Silber et al., 2008; Piver et al., 2009; Sánchez-Serrano et al., 2010). Unfortunately, when the three patients in this report were treated, none of these options could be proposed.

In 2005, Silber published a report on successful ovarian transplantation between monozygotic twins, and the series was further extended (Silber et al., 2005; Silber and Gosden, 2007). In both cases, the grafted tissue functioned without any surgical connection to major blood vessels.

Survival of PF has also been histologically proved in humans after orthotopic grafting of fresh ovarian cortex (Donnez et al., 2005), but follicular density was found to have decreased due to a fall in number of PF.

Here, we report three cases of ovarian transplantation between two genetically non-identical sisters. The three recipient patients

**Figure 4** FSH and estradiol levels in Patient 1.
experienced iatrogenic POF due to chemotherapy and TBI. It is particularly important to stress that the recipients had all received bone marrow from their HLA-compatible sister. HLA group analysis revealed complete chimerism (HLA compatibility) between the two sisters in each case. It was therefore proposed that ovarian tissue be grafted from the sister who had already donated bone marrow to the recipient sister with POF. None of the BMT patients were on any steroid or immunosuppressive therapy.

It should be pointed out that the recipients presented with documented POF, their ovaries were atrophic and serial sections of biopsies failed to demonstrate the presence of any follicles. Indeed, POF was confirmed by very high FSH and LH levels, very low estradiol concentrations and the absence of any follicles in large biopsies removed from the patients’ atrophic ovaries during transplantation.

Successful restoration of ovarian function after implantation of ovarian cortex between genetically different sisters is for the first time reported in this series of three women, although pregnancy has not yet been achieved. It confirms data published earlier as a case report (Donnez et al., 2007). The time interval between implantation of cortical tissue and follicular development was found to vary between 3.5 and 6 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. In their recently published series, the time to first menses after transplantation ranged between 65 and 93 days (Silber and Gosden, 2007). The time interval of 6 months observed in our first case is probably associated with a low follicular reserve in the donor, aged 32 years, and the delay that occurs before PF complete their development. Indeed, the
ovarian reserve of the donor was not optimal in the analyzed biopsy (2 PF and 1 primary follicle out of 11 sections). We know that a small fragment may not be truly representative, and that primary ovarian follicles are located in clusters in the ovary (Qu et al., 2000; Gook et al., 2003; Schmidt et al., 2005). Nevertheless, the observation of an FSH surge between ovulatory cycles and cessation of graft activity now after 3 years is more consistent with a low ovarian reserve in the donor specimen.

In Patients 2 and 3, the follicular density of the donor ovarian specimen was high and recovery of ovarian activity occurred 3.5 months after reimplantation, as observed in the series of Silber and Gosden (2007), who also observed an interval of 3.5 months after reimplantation of fresh ovarian cortex between monozygotic twins.

This interval corresponds to the development of PF 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 (before the fragment is revascularized), will reach the pre-ovulatory stage before the PF. The ischemic period was recently estimated to be between 3 and 5 days by van Eyck et al. (2009, 2010).

In both these patients, cyclic ovarian activity was demonstrated and low levels of FSH (<10 mIU/ml) were systematically recorded.

The fact that serial sections of the recipients’ cortex failed to demonstrate the presence of any PF supports the notion that the origin of steroid secretion was the transplanted tissue. It is extremely unlikely that restoration of ovarian function in the women, who had undergone chemo and/or radiotherapy before BMT, was due to residual follicles in the atrophic native ovary, from which the majority of the cortex was removed.

Our series and studies by Silber et al. (2005; Silber and Gosden, 2007) show the ovarian medulla to be an excellent site for fresh ovarian cortex transplantation.

In conclusion, the present study reports, for the first time, restoration of ovarian activity after ovarian cortex allografting between genetically different sisters, where these sisters were fully HLA-compatible due to previous BMT.

Authors’ roles
J.D. wrote the manuscript and performed surgery with J.S. and P.J.; C.P. was responsible for clinical data collection; M.M.D. followed the patients and participated in the discussion.

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