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Ovarian function and spontaneous pregnancy after combined heterotopic and orthotopic cryopreserved ovarian tissue transplantation in a patient previously treated with bone marrow transplantation: Case Report

Isabelle Demeestere[1,2,5], Philippe Simon[2], Frédéric Buxant[2], Valérie Robin[3], Sergio Aguilar Fernandez[4], Julie Centner[1], Anne Delbaere[1,2] and Yvon Englert[1,2]

[1]Research Laboratory on Human Reproduction, [2]Department of Obstetrics and Gynaecology, [3]Department of Haematology and [4]Department of Pathology, French Speaking Free University of Brussels (ULB), Erasme Hospital, Brussels, Belgium

[5]To whom correspondence should be addressed at: Research Laboratory on Human Reproduction, Free University of Brussels (ULB), Campus Erasme (GE-niv 2), 808 Route de Lennik, 1070 Brussels, Belgium. E-mail: idemeest@ulb.ac.be

Cryopreservation of ovarian tissue has been proposed for storing gametes of young patients at high risk of premature ovarian failure. Autotransplantation has recently provided some promising results and is still the unique option to restore ovarian function from cryopreserved ovarian tissue in humans. In this article, we analyse data from the combined orthotopic and heterotopic transplantation of cryopreserved ovarian tissue that restored the ovarian function and fertility. Orthotopic transplantation of cryopreserved ovarian tissue at ovarian and peritoneal sites, together with a heterotopic transplantation at the abdominal subcutaneous site, was performed to restore the ovarian function of a 29-year-old woman previously treated with bone marrow transplantation (BMT) for Hodgkin's disease. Ovarian reserve markers progressively suppress within values 5 months after the transplantation (basal FSH 5 mUI/ml and inhibin B 119 ng/ml). Follicular development was observed at all transplantation sites but was predominant at the ovarian site. Six natural cycles were fully documented and analysed. The patient became spontaneously pregnant following the sixth cycle, but unfortunately she later miscarried. Combined orthotopic and heterotopic transplantations succeeded in the restoration of normal spontaneous cycles. Furthermore, this spontaneous pregnancy confirmed the efficiency of this procedure for restoring human fertility.

Key words: cancer/cryopreservation/ovarian tissue/pregnancy/transplantation

Introduction

Premature ovarian failure is one of the most common long-term adverse effects affecting young patients treated with alkylating agents and/or radiation therapy, usually for malignant diseases (Meirow, 2000). Cryopreservation of ovarian tissue before gonadotoxic treatments is currently proposed as an experimental alternative to oocyte and embryo freezing, in the hope of restoring future fertility (Demeestere *et al*., 2003). Ovarian tissue cryopreservation has the main advantage of allowing storage of a large number of gametes, and it can be rapidly performed, at any period of the cycle, without delaying the oncological treatment. This procedure is also a unique opportunity for selected prepubertal patients to store their gametes before gonadotoxic treatments (Wallace *et al*., 2005). Ovarian tissue cryopreservation can also be proposed to adolescent girls with Turner's syndrome to preserve gametes before ovarian failure (Abir *et al*., 2001; Hreinsson *et al*., 2002).

Options to restore fertility from cryopreserved ovarian tissue include follicular *in vitro* maturation and ovarian tissue autotransplantation. The development of *in vitro* culture systems to support human primordial follicular growth until the ovulatory stage will probably need some additional years because of technical difficulties and the complexity of the folliculogenesis process in humans (Gosden *et al*., 2002). Live offspring resulting from primordial follicles developed entirely *in vitro* have been reported only in mice (Eppig and O'Brien, 1996; O'Brien *et al*., 2003).

Regarding the issue of ovarian tissue transplantation, experiments in animals are encouraging. Normal young animals have been obtained after the transplantation of cryopreserved ovarian tissue or organ transplantation in mice (Candy *et al*., 2000), rats (Wang *et al*., 2002), rabbits (Almodin *et al*., 2004) and sheep (Gosden *et al*., 1994). In humans, the first case of ovarian function restoration after orthotopic transplantation of cryopreserved ovarian cortex was described by Oktay in 2000

(Oktay and Karlikaya, 2000). Different surgical approaches and transplantation sites were investigated (Callejo *et al*., 2001; Radford *et al*., 2001; Leporrier *et al*., 2002; Kim *et al*., 2004; Oktay *et al*., 2004; Donnez *et al*., 2005; Schmidt *et al*., 2005). Despite those attempts, only one spontaneous pregnancy after orthotopic transplantation of cryopreserved ovarian tissue was reported so far (Donnez *et al*., 2004). However, in this case, controversies emerged regarding the possibility of fertility restoration having originated from the remaining native ovary, as the author himself described spontaneous ovulation before the ovarian tissue transplantation (Oktay and Tilly, 2004). Even if the probability of such an event was low, conclusive evidence of the feasibility of spontaneous pregnancy from such a procedure is lacking. Recently, the first pregnancy after orthotopic transplantation of ovarian tissue, oocyte collection and embryo transfer after IVF was reported (Meirow *et al*., 2005). Both the spontaneous and the assisted pregnancies were obtained from cryopreserved ovarian tissue transplanted at the remaining ovarian site. However, heterotopic sites were also investigated in humans, and a transferable embryo was obtained (Oktay *et al*., 2004).

Here, we analysed six fully documented spontaneous cycles in a woman after combined orthotopic and heterotopic cryopreserved ovarian tissue transplantation allowing, for the first time, the comparison of follicular activity at different sites. We also reported a second case of spontaneous pregnancy, providing further evidence of the efficiency of ovarian tissue transplantation for restoring human natural fertility.

Methods

Patient history

The patient was affected by Hodgkin's disease stage IV at age 24. In November 1999, after signed written informed consent, the right ovary was retrieved by laparoscopy for ovarian tissue cryopreservation. One adriamycine, bleomycine, vincristin, dacarbazine (ABVD) cycle was administered before the cryopreservation procedure. The patient then received five more ABVD cycles, but the disease rapidly recurred. Three cycles of etoposide, vinblastine, doxorubicine and prednisolone were administered before performing autologous bone marrow transplantation (BMT) in November 2000. The conditioning regimen for BMT included carmustine, vetoposide and cyclophosphamide. After the BMT, the patient was considered disease-free but never recovered spontaneous cycles. After 2 years of complete amenorrhoea, hormonal substitution (estradiol and Norgestrel) was started. At that time, menopausal status was already confirmed by a hormonal test (FSH 114 mUI/ml, March 2002). Four years after the BMT, she requested the ovarian tissue transplantation to achieve pregnancy. After stopping hormonal substitution, amenorrhea and hormonal status confirmed the premature ovarian failure. Three blood tests were performed before and at the time of the transplantation procedure (inhibin B <15 pg/ml, FSH 69, 33 and 128 mUI/ml in May, September and November 2004, respectively). The decision for autotransplantation was taken after multi-disciplinary assessment (gynaecologist, oncologists and psychologists), and the patient gave written informed consent. The partner's sperm analysis was normal.

Procedure

The procedure for freezing ovarian tissue was previously described (Demeestere *et al*., 2003). The ovary was rapidly transported to the laboratory in Leibovitz L-15 medium (Life Technologies, Merelbeke, Belgium). The ovarian cortex was carefully dissected to obtain 40 small pieces ($5 \times 5 \times 2$ mm), which were then incubated for 30 min in the cryoprotective solution (Leibovitz medium supplemented with 1.5 M dimethylsulphoxide, 0.1 M sucrose, both provided by Sigma Aldrich, Bornem, Belgium and 10% patient's serum at 4°C). Each piece of ovarian cortex was cryopreserved in one 2-ml vial (Simport, Merck Eurolab, Leuven, Belgium), containing 1.4 ml of cryoprotectant solution. The cryopreservation procedure was performed using a programmable Planer freezer (Planner Kryo 360-3.3, Depex, Belgium). The following programme was used: started at 4°C, 2°C/min to –7°C, 10 min of soaking, then manual seeding, 0.3°C/min to –40°C, 10°C/min to –140°C and then plunged into liquid nitrogen.

Pieces of ovarian tissue were thawed on the day of the transplantation procedure following a rapid protocol: vials were placed 2 min at room temperature and then 2 min in water at 25°C. The ovarian tissue was washed stepwise for 5 min each in progressively lower concentrations of cryoprotectant solution (1.5, 1, 0.5 and 0 mol/l). Three fragments of tissue were thawed before the transplantation procedure to check for the absence of malignant cells in the tissue and to test the viability after *in vitro* culture.

The orthotopic transplantation procedure was performed in two steps (Donnez *et al*., 2005). A first laparoscopy was performed to induce a neovascularization process at the transplantation sites. During this first laparoscopy, a window was created in the pelvic peritoneum of the ovarian fossa above the ureter. The left atrophic ovary was incised longitudinally, and a biopsy was taken to confirm the absence of remaining follicles (Figure 1). Tubal permeability was also controlled using blue test. A second laparoscopy was performed 1 week later, and both peritoneal and ovarian sites looked well vascularized. Eighteen fragments of ovarian tissue were thawed and rapidly transferred to the operating room in fresh Leibovitz L-15 medium. Three fragments were placed into the incision in the ovary and nine into the peritoneal pocket (Figure 1). Both sites were secured by one surgical point. The six last fragments were transplanted s.c. above the left abdominal incision used for the trocar. No surgical or post-surgery complications were observed.

The patient received 2 g of cefalozoline i.v. at each surgery and 100 mg per day of acetyl salicylic acid for 8 days following the transplantation procedure.

The Ethical Committee approved all the procedures.

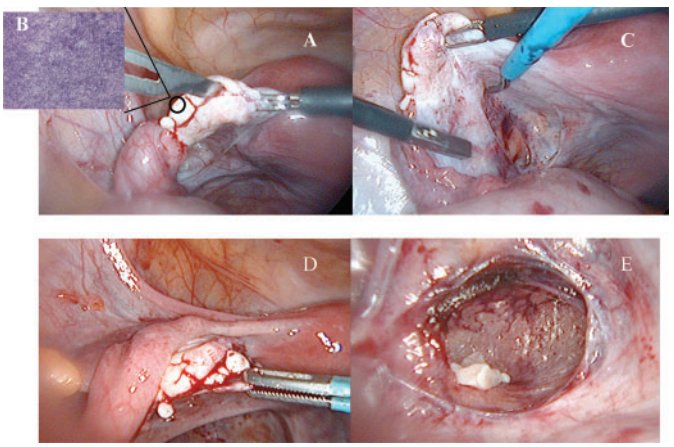

Figure 1. Orthotopic ovarian tissue transplantation sites. First laparoscopy to prepare the transplantation sites: longitudinal incision of the ovarian cortex (**A**) and creation of a peritoneal pocket (**C**). A biopsy of the native ovary confirms the absence of follicles (**B**). Second laparoscopy 1 week later to transplant the cryopreserved ovarian pieces at the ovarian (**D**) and the peritoneal (**E**) sites.

Results

The analysis of the first three thawed fragments confirmed the presence of primordial follicles (12 follicles/mm^2) and the absence of malignant cells. An increased concentration of estradiol (E_2) measured in the culture medium after 15, 25 and 35 days of *in vitro* culture of small pieces ($1 \times 1 \times 3$ mm) of thawed tissue confirmed the viability of the follicles.

Three months after the transplantation procedure, the FSH serum levels started to decrease in parallel with an increase in inhibin B levels (Figure 2). E_2 levels slowly rose to reach a first peak (590 pg/ml) at day 148 after transplantation associated with a first spontaneous ovulation (progesterone 4.6 ng/ml during the luteal phase), as shown in Figure 3. During the following spontaneous cycles, the markers of ovarian reserve reached normal premenopausal values (inhibin B >50 ng/ml and FSH <10 mUI/ml, measured between days 3 and 5 of the cycles). E_2 and LH were measured at different times during the follicular phase until the LH peak, confirming spontaneous ovulation. Progesterone was measured during luteal phase. Five spontaneous ovulations and menstruations following this first one were documented. During the second cycle, the follicular phase was short (5 days) but normalized during the next four cycles (between 10 and 12 days). Normal luteal phases were observed from the second cycle onwards (13–15 days and progesterone values ranging from 9.1 to 16.8 ng/ml). Follicular development was observed at all sites. However, large follicles were predominant at the ovarian site. The follicles that developed at the subcutaneous site never reached sufficient size to be punctured

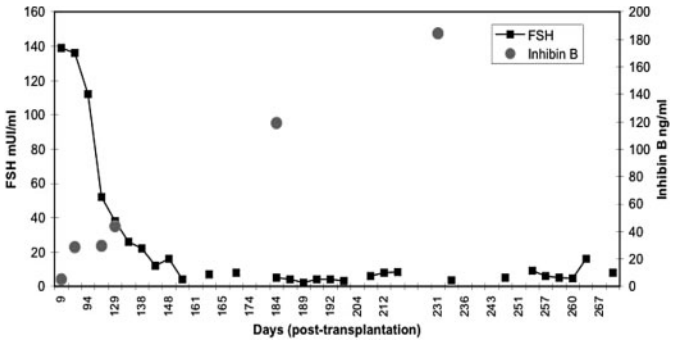

Figure 2. FSH and inhibin B levels after ovarian tissue transplantation.

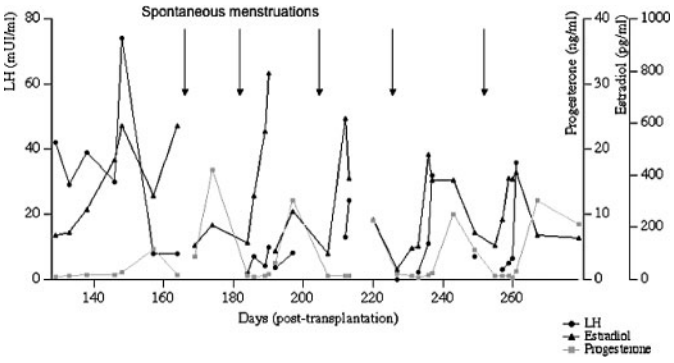

Figure 3. LH, progesterone and estradiol (E_2) levels after ovarian tissue transplantation.

at the time of ovulation (maximum 13 mm). Only one dominant follicle was observed at the peritoneal site. In August 2005, the patient had her sixth LH peak at day 11 of the cycle (E_2 410 pg/ml). Two follicles were observed by vaginal ultrasound at the ovarian site (19.5 mm and 10 mm) and one follicle at the subcutaneous site (11 mm). The endometrium measured 7.7 mm (Figure 4). Thirteen days later, the patient reported vaginal bleeding. However, 5 days later, the blood test revealed a detectable human HCG. The ultrasound confirmed the presence of an intrauterine viable pregnancy (Figure 4). Unfortunately, the patient experienced a miscarriage at 7 weeks of gestation because of aneuploidy. The cytogenetic analysis of the abortion product showed a 70 XXY,+10 chromosomal constitution in all cells (triploidy associated with a tetrasomy of chromosome 10).

Discussion

To our knowledge, this report is the first combined heterotopic and orthotopic cryopreserved ovarian tissue transplantation in a woman. Follicular development was observed in all transplanted sites. However, interestingly, dominant follicles developed more frequently at the ovarian site compared to the peritoneal and the subcutaneous sites. Previous studies showed that the transplantation sites and the host could influence subsequent follicular development (Hernandez-Fonseca *et al.*, 2004). Human xenograft experiments in mice suggested the importance of peritoneal milieu for the neovascularization process (Nisolle *et al.*, 2000). Our results suggest that the local environment and/or the vascularization in the ovary could favour the restoration of ovarian function from transplanted thawed ovarian tissue. However, the possibility of ovarian tissue transplantation in the remaining native ovary was limited because of the small size of the atrophic organ. In a recent study, ovarian volume of patients with premature ovarian failure following oncological treatment ranged from 0.3 to 1.3 cm^3 (Schmidt *et al.*, 2005). In this case, we were able to place only three pieces of tissue at the ovarian site. Considering the massive loss of the primordial follicle population by the ischaemic process after transplantation, the remaining pool of functional follicles from these three pieces is likely to be limited. So, despite the fact that peritoneal and subcutaneous sites do not seem to be optimal, they could allow an increase of the transplanted follicular population and consequently an improvement in the quality of the cycles. Six months after transplantation, basal markers of the ovarian reserve (FSH, inhibin B measured between day 3 and day 5 of natural cycles) returned to levels within normal premenopausal values. These results contrast with the persistence of elevated basal FSH values previously reported in humans and sheep after cryopreserved human ovarian tissue transplantation (Campbell *et al.*, 2000; Donnez *et al.*, 2004, 2005). The difference could be explained by the young age of the patient and the large number of tissue fragments transplanted. It also confirms the efficiency of this procedure for restoring normal ovarian function. However, a short follicular phase and a high E_2 level, as reported in older women, were observed during the first cycle in our case, suggesting a restoration of the ovarian function from a limited follicular

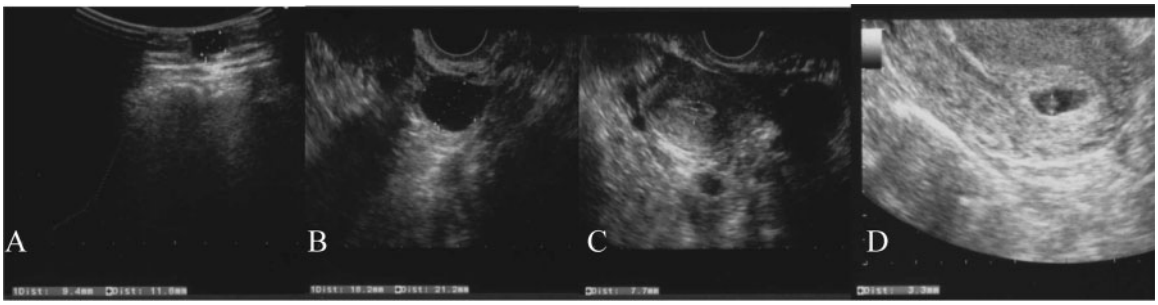

Figure 4. Ultrasound observed at day 11 (LH peak) of the last cycle: subcutaneous follicle (**A**), ovarian follicle (**B**) and endometrium (**C**). Confirmation of an intrauterine pregnancy at day 30 of the luteal phase (**D**).

pool (van Zonneveld *et al*., 2003). Other techniques, such as whole ovarian transplantation with vascular anastomosis, are now being investigated to reduce the ischaemic process and to increase the life expectancy of the ovarian graft (Arav *et al*., 2005).

Our report confirms the possibility of obtaining spontaneous pregnancy after orthotopic ovarian tissue transplantation. Although there is no way of totally ruling out the fact that the pregnancy could have originated from the remaining ovary, this is extremely unlikely in our case. Indeed, the woman was previously treated with BMT for Hodgkin's disease, and the conditioning regimen for BMT induces premature ovarian failure in almost all cases (Meirow, 2000). The amenorrhoea and hormonal status before the transplantation also confirmed premature ovarian failure. No follicles were observed in the biopsy of the remaining native ovary. Moreover, the recovery of ovarian function was observed 4–5 months after transplantation, corresponding to the physiological timing for follicular development from primordial stage (Gougeon, 2005). Finally, follicular development was observed at all the transplant sites, which provides evidence for the restoration of ovarian function from the cryopreserved tissue.

Despite several previous attempts, only two pregnancies have been reported after transplantation of cryopreserved ovarian tissue. This report thus represents a great hope for the patients and confirms the feasibility of spontaneous pregnancy following combined heterotopic and orthotopic transplantation of cryopreserved ovarian tissue. However, regarding our patient's miscarriage, the risk of aneuploidy, which could be related to the procedure, should be considered. Previous experiments using animal models are reassuring, and no abnormalities of the offspring born after ovarian tissue transplantation have been observed so far (Baird *et al*., 1999; Candy *et al*., 2000; Salle *et al*., 2003; Almodin *et al*., 2004). However, a recent study demonstrated abnormal cytoplasmic and nuclear maturation in the oocytes collected after xenograft of human ovarian tissue into mice (Kim *et al*., 2005). The causes are not clearly identified and require further investigations.

In our clinic, 53 patients affected by neoplastic or benign diseases have undergone ovarian tissue cryopreservation since 1999. Age at diagnosis ranged from 4 to 35 years (median 21). Some of them are now cured of the initial disease and want to become pregnant, and the transplantation of cryopreserved ovarian tissue is the only procedure leading to live births in humans under these circumstances. Analysis of the hormonal status of our patient during the first six cycles supports the efficiency of the procedure for restoring normal spontaneous cycles and fertility.

This procedure must, however, still be considered experimental. Although ovarian tissue harvested from lymphoma patients may be safe for autotransplantation, this procedure cannot be proposed to patients with a high risk of reseeding cancer at the time of autotransplantation (Meirow *et al*., 1998; Kim *et al*., 2001). For those patients, alternatives, such as transplantation of isolated primordial follicles or *in vitro* culture systems, must still be developed.

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References

Abir R, Fisch B, Nahum R, Orvieto R, Nitke S and Ben Rafael Z (2001) Turner's syndrome and fertility: current status and possible putative prospects. Hum Reprod Update 7,603–610.

Almodin CG, Minguetti-Camara VC, Meister H, Ferreira JO, Franco RL, Cavalcante AA, Radaelli MR, Bahls AS, Moron AF and Murta CG (2004) Recovery of fertility after grafting of cryopreserved germinative tissue in female rabbits following radiotherapy. Hum Reprod 19,1287–1293.

Arav A, Revel A, Nathan Y, Bor A, Gacitua H, Yavin S, Gavish Z, Uri M and Elami A (2005) Oocyte recovery, embryo development and ovarian function after cryopreservation and transplantation of whole sheep ovary. Hum Reprod 20,3554–3559.

Baird DT, Webb R, Campbell BK, Harkness LM and Gosden RG (1999) Long-term ovarian function in sheep after ovariectomy and transplantation of autografts stored at -196 C. Endocrinology 140,462–471.

Callejo J, Salvador C, Miralles A, Vilaseca S, Lailla JM and Balasch J (2001) Long-term ovarian function evaluation after autografting by implantation with fresh and frozen-thawed human ovarian tissue. J Clin Endocrinol Metab 86,4489–4494.

Campbell BK, Telfer EE, Webb R and Baird DT (2000) Ovarian autografts in sheep as a model for studying folliculogenesis. Mol Cell Endocrinol 163,131–139.

Candy CJ, Wood MJ and Whittingham DG (2000) Restoration of a normal reproductive lifespan after grafting of cryopreserved mouse ovaries. Hum Reprod 15,1300–1304.

Demeestere I, Simon P, Englert Y and Delbaere A (2003) Preliminary experience of ovarian tissue cryopreservation procedure: alternatives, perspectives and feasibility. Reprod Biomed Online 7,572–579.

Donnez J, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, Martinez-Madrid B and van Langendonckt A (2004) Livebirth after orthotopic transplantation of cryopreserved ovarian tissue. Lancet 364,1405–1410.

Donnez J, Dolmans MM, Demylle D, Jadoul P, Pirard C, Squifflet J, Martinez-Madrid B and Van Langendonckt A (2005) Restoration of ovarian function

after orthotopic (intraovarian and periovarian) transplantation of cryopreserved ovarian tissue in a woman treated by bone marrow transplantation for sickle cell anaemia: case report. Hum Reprod 21,183–188.

Eppig JJ and O'Brien MJ (1996) Development in vitro of mouse oocytes from primordial follicles. Biol Reprod 54,197–207.

Gosden RG, Baird DT, Wade JC and Webb R (1994) Restoration of fertility to oophorectomized sheep by ovarian autografts stored at -196 degrees C. Hum Reprod 9,597–603.

Gosden RG, Mullan J, Picton HM, Yin H and Tan SL (2002) Current perspective on primordial follicle cryopreservation and culture for reproductive medicine. Hum Reprod Update 8,105–110.

Gougeon A (2005). Dynamics for Human Follicular Growth: Morphologic, Dynamic and Functional Aspect. In Leung PCK and Adashi EY (eds) *The Ovary*. Elsevier, San Diego, CA, pp. 25–53.

Hernandez-Fonseca H, Bosch P, Sirisathien S, Wininger JD, Massey JB and Brackett BG (2004) Effect of site of transplantation on follicular development of human ovarian tissue transplanted into intact or castrated immunodeficient mice. Fertil Steril 81,888–892.

Hreinsson JG, Otala M, Fridstrom M, Borgstrom B, Rasmussen C, Lundqvist M, Tuuri T, Simberg N, Mikkola M, Dunkel L et al. (2002) Follicles are found in the ovaries of adolescent girls with Turner's syndrome. J Clin Endocrinol Metab 87,3618–3623.

Kim SS, Radford J, Harris M, Varley J, Rutherford AJ, Lieberman B, Shalet S and Gosden R (2001) Ovarian tissue harvested from lymphoma patients to preserve fertility may be safe for autotransplantation. Hum Reprod 16,2056–2060.

Kim SS, Hwang IT and Lee HC (2004) Heterotopic autotransplantation of cryobanked human ovarian tissue as a strategy to restore ovarian function. Fertil Steril 82,930–932.

Kim SS, Kang HG, Kim NH, Lee HC and Lee HH (2005) Assessment of the integrity of human oocytes retrieved from cryopreserved ovarian tissue after xenotransplantation. Hum Reprod 20,2502–2508.

Leporrier M, Roffe JL, Von Theobald P and Muller G (2002) Autologous transplantation of whole ovaries vs ovarian cortical strips. JAMA 287,44–45.

Meirow D (2000) Reproduction post-chemotherapy in young cancer patients. Mol Cell Endocrinol 169,123–123.

Meirow D, Ben Yehuda D, Prus D, Poliack A, Schenker JG, Rachmilewitz EA and Lewin A (1998) Ovarian tissue banking in patients with Hodgkin's disease: is it safe? Fertil Steril 69,996–998.

Meirow D, Levron J, Eldar-Geva T, Hardan I, Fridman E, Zalel Y, Schiff E and Dor J (2005) Pregnancy after transplantation of cryopreserved ovarian tissue in a patient with ovarian failure after chemotherapy. N Engl J Med 353,318–321.

Nisolle M, Casanas-Roux F, Qu J, Motta P and Donnez J (2000) Histologic and ultrastructural evaluation of fresh and frozen-thawed human ovarian xenografts in nude mice. Fertil Steril 74,122–129.

O'Brien MJ, Pendola JK and Eppig JJ (2003) A revised protocol for in vitro development of mouse oocytes from primordial follicles dramatically improves their developmental competence. Biol Reprod 68,1682–1686.

Oktay K and Karlikaya G (2000) Ovarian function after transplantation of frozen, banked autologous ovarian tissue. N Engl J Med 342,1919.

Oktay K and Tilly J (2004) Livebirth after cryopreserved ovarian tissue autotransplantation. Lancet 364,2091–2092.

Oktay K, Buyuk E, Veeck L, Zaninovic N, Xu K, Takeuchi T, Opsahl M and Rosenwaks Z (2004) Embryo development after heterotopic transplantation of cryopreserved ovarian tissue. Lancet 363,837–840.

Radford JA, Lieberman BA, Brison DR, Smith AR, Critchlow JD, Russell SA, Watson AJ, Clayton JA, Harris M, Gosden RG et al. (2001) Orthotopic reimplantation of cryopreserved ovarian cortical strips after high-dose chemotherapy for Hodgkin's lymphoma. Lancet 357,1172–1175.

Salle B, Demirci B, Franck M, Berthollet C and Lornage J (2003) Long-term follow-up of cryopreserved hemi-ovary autografts in ewes: pregnancies, births, and histologic assessment. Fertil Steril 8,172–177.

Schmidt KL, Yding Andersen C, Loft A, Byskov AG, Ernst E and Nyboe Andersen A (2005) Follow-up of ovarian function post-chemotherapy following ovarian cryopreservation and transplantation. Hum Reprod 20,3539–3546.

Wallace WH, Anderson RA and Irvine DS (2005) Fertility preservation for young patients with cancer: who is at risk and what can be offered? Lancet Oncol 6,209–218.

Wang X, Chen H, Yin H, Kim SS, Lin Tan S and Gosden RG (2002) Fertility after intact ovary transplantation. Nature 415,385.

van Zonneveld P, Scheffer GJ, Broekmans FJ, Blankenstein MA, de Jong FH, Looman CW, Habbema JD and te Velde ER (2003) Do cycle disturbances explain the age-related decline of female fertility? Cycle characteristics of women aged over 40 years compared with a reference population of young women. Hum Reprod 18,495–501.

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