First reported clinical pregnancy following heterotopic grafting of cryopreserved ovarian tissue in a woman after a bilateral oophorectomy

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ABSTRACT: Ovarian tissue cryopreservation and transplantation is a form of fertility preservation offered to young women at high risk of losing ovarian function after cancer treatment. While there have been successful births resulting from orthotopic site grafts, we report the first case of an ongoing pregnancy from a heterotopic graft in a patient who had previously undergone bilateral oophorectomy for a granulosa cell tumour. Frozen–thawed ovarian tissue was transplanted to the anterior abdominal wall. Subsequent ovarian stimulation and transabdominal ultrasound-guided oocyte retrieval from the grafts resulted in two oocytes. These were fertilized with ICSI and two embryos were transferred. Serial ultrasounds have confirmed an ongoing 26-week intrauterine twin pregnancy. Thus, this first demonstration of a pregnancy from a heterotopic graft site provides unequivocal evidence that cryopreservation preserves complete follicle development and that normal ovarian function can occur at a non-ovarian site. This provides optimism for further efforts to assist women who have had oophorectomy and pelvic surgery or radiotherapy, without an appropriate orthotopic site for grafting.

Key words: fertility preservation / ovarian tissue cryopreservation / heterotopic graft / orthotopic graft / oncology

Introduction

Ovarian tissue cryopreservation is a form of fertility preservation offered to young women at high risk of losing ovarian function after cancer treatment. Given that primordial follicles of the ovary are located in the outer fibrous cortex, a simple graft procedure can be employed with the hope of restoring ovarian function. The advantages include a potentially large source of follicles and the rapidity with which the procedure can be performed. There have been 28 births reported after grafting of ovarian tissue back onto the ovary, broad ligament or adjacent pelvic tissue (orthotopic sites), and it has been shown that follicles present in frozen–thawed ovarian tissue can produce oocytes capable of sustaining new offspring (Donnez et al., 2012); however, this is still considered somewhat experimental, with attrition and poor follicle development hampering success (Demeestere et al., 2009; Martinez et al., 2013). To date, there have been no clinical pregnancies reported after heterotopic grafting, i.e. grafting to a site distant to the ovary (Donnez et al., 2013; Silber, 2012).

Case report

We report an ongoing twin pregnancy, after IVF of oocytes retrieved from cryopreserved ovarian cortex grafted in the anterior abdominal wall.

The patient had a left oophorectomy at 21 years of age, for an early stage granulosa cell tumour, followed 4 years later by right oophorectomy and excision of pelvic wall deposits. She did not require any adjuvant therapy. Prior to the second surgery, and after extensive counselling about the risks of tumour cell transmission in any future grafting procedure, the patient had requested cryopreservation and storage of her ovarian tissue. Histological sections taken from the right ovary at the time of laparotomy were histologically normal and thus ovarian tissue was processed and frozen using the cryoprotectants, propanediol and sucrose, according to previously published protocols (Gook et al., 2005).

The patient was referred 7 years later requesting fertility assistance, at which time she had clinical and biochemical evidence of menopause with an estradiol (E2) level of <10 pmol/l (normal >100 pmol/l), follicle-stimulating hormone (FSH) level of >120 U/l (premenopausal <30 U/l).
and Ca 125 level of 6 U/ml (normal < 30 U/ml). Serial assessments by her oncologist had not revealed any tumour recurrence. The risk of tumour cell transmission via the grafted tissue was again discussed with the patient and her partner.

The couple requested replacement of the frozen ovarian tissue. Following further histological assessment of a sample of frozen—thawed ovarian tissue revealed no tumour cells, a laparoscopic grafting procedure was performed in 2010 with 15 slices of frozen—thawed (Stern et al., 2011) ovarian tissue, 1 mm × 2 mm × 4 mm, grafted into each of the right and left lateral pelvic walls and right and left anterior abdominal walls, just under the peritoneum in all locations (60 slices in total). Four and a half months later, ultrasound and endocrine evidence of ovarian activity was first demonstrated (FSH 11.6 U/l, E2 219 pmol/l). An IVF programme of gentle ovarian stimulation was commenced with recombinant FSH and GnRH antagonist, but despite multiple cycles and episodic follicular development from both lateral pelvic and abdominal graft sites, only three oocytes were retrieved and two embryos were transferred with no pregnancy. Although there was no clinical evidence of menopause and FSH remained < 20 U/l, graft activity became more sporadic.

A further MRI performed as part of oncological surveillance did not reveal any sinister findings and 2 years after the initial grafting procedure, a further laparoscopic graft was performed. Observation of the sites of the previous grafts revealed that the abdominal locations showed significant ovarian development (Fig. 1), and ovarian biopsies from these sites revealed normal ovarian follicles and tissue with no abnormal cells. Thus, 30 slices of cryopreserved-thawed ovarian tissue were grafted into the right and left anterior abdominal walls, adjacent to the previous grafts.

Seven months after the second graft, a stimulation cycle with recombinant FSH, LH and GnRH antagonist was commenced. On Day 12, two follicles (16 and 18 mm) were observed in the right anterior abdominal wall graft site (Fig. 2). The E2 level was 497 pmol/l and ovulation was triggered 2 days later with 250 μg recombinant hCG. The patient underwent oocyte retrieval from two follicles under transabdominal ultrasound guidance (using a standard straight linear array probe). Two MII oocytes were retrieved. ICSI was performed on both oocytes with subsequent fertilization and two fresh embryos of 8 cells and 5 cells respectively, were transferred 3 days later (Fig. 3).
After an initial positive serum pregnancy test at 4 weeks of gestation, a dichorionic diamniotic twin intrauterine pregnancy was demonstrated on transvaginal ultrasound at 5 weeks and 6 days and was confirmed subsequently at 8, 9 and 12 weeks of gestation (Figs 4 and 5). The pregnancy is ongoing at 26 weeks of gestation at the time of writing this article and the morphology scan at 20 weeks did not reveal any abnormalities.

Discussion
Following cryopreservation, ovarian cortical tissue can be grafted into extra-ovarian heterotopic locations such as abdominal wall, forearm or breast (Oktay et al., 2001; Kim et al., 2004; Rodriguez-Wallberg and Oktay, 2012). Compared with orthotopic transplantation (where tissue is transplanted into the ovarian medulla, broad ligament or ovarian fossa), potential advantages of heterotopic implantation include avoidance of invasive procedures, easy accessibility, increased capacity for cortical slices and feasibility for grafting even if severe pelvic adhesions preclude orthotopic transplantation (Kim, 2012).

Heterotopic sites however may not provide an optimal environment for follicular development due to differences in temperature, paracrine factors and blood supply compared with the intraperitoneal environment (Schmidt et al., 2005; Donnez et al., 2010) and primate models have been used to find suitable locations to overcome some of these challenges (Igarashi et al., 2010). The clinical value of using heterotopic sites has been questioned due to the lack of success compared with orthotopic transplantation, which has resulted in 28 live births worldwide (Silber, 2012). There are 21 cases reported in the literature of ovarian cortex reimplantation where heterotopic graft sites (either alone or in addition to orthotopic sites) have been used (Oktay and Karlikaya, 2000; Callejo et al., 2001; Oktay et al., 2001; Oktay et al., 2003; Kim et al., 2004; Oktay et al., 2004; Schmidt et al., 2005; Walner-Hanssen et al., 2005; Oktay, 2006; Rosendahl et al., 2006; Demeestere et al., 2007; Andersen et al., 2008; Donnez et al., 2010; Oktay and Oktem, 2010; Stern et al., 2011; Kim, 2012). While restoration of endocrine function has been demonstrated consistently (Donnez et al., 2013) and embryo development has occurred, there have only been two biochemical pregnancies thus far reported in the literature, which are certain to have come from oocytes aspirated from heterotopic graft sites (Rosendahl et al., 2006; Stern et al., 2011).

Conclusion
This first demonstration of a twin pregnancy from a heterotopic graft site thus provides optimism for further efforts to assist women who have had oophorectomy and pelvic surgery or radiotherapy, without an appropriate orthotopic site for grafting. This pregnancy provides unequivocal evidence that cryopreservation preserves complete follicle development and that normal ovarian function and pregnancy can both occur at a non-ovarian site.

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Authors’ roles
C.S. is the lead clinician for the ovarian grafting programme at her institution. She was responsible for all aspects of patient care and assisted with the surgical procedures. She coordinated the IVF cycles and surveillance for this patient and made all the clinical decisions. She performed the oocyte retrieval with J.O. C.S. is the corresponding author. D.G. leads the institutional ovarian tissue preservation scientific programme. She performed the ovarian tissue cryopreservation, thawing and preparation.
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for grafting. L.H. contributed to the clinical management of this patient and was the lead surgeon at both grafting procedures. F.A. assisted with the coordination of all medical treatment for this patient and assisted with the writing for this paper. J.O. performed most of the trans-abdominal ultrasounds required for cycle surveillance and management for this patient. She performed the transabdominal oocyte retrieval procedure with assistance from C.S. G.R. assisted with the writing of this paper. T.J. performed the oncological surgery for this patient and provided ongoing oncological surveillance. He referred the patient for fertility management and contributed to the writing of this paper.

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**Conflict of interest**

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**References**


