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Case report

Healthy twins delivered after oocyte cryopreservation and bilateral ovariectomy for ovarian cancer

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Abstract

Anti-neoplastic treatments have significantly increased the survival of cancer patients, but female patients risk premature menopause. Oocyte cryopreservation has been proposed as a fertility-saving option. This report describes the first live birth achieved with autologous cryopreserved oocytes in an ovariectomized borderline cancer patient. A patient with a borderline ovarian tumour asked for oocyte cryopreservation after a right adnexectomy. Ovulation induction resulted in the retrieval and cryopreservation of seven mature oocytes. Thirty-nine months after a left ovariectomy, the patient asked for oocyte thawing and embryo transfer. Endometrial growth was induced using hormone replacement treatment. Three of the seven cryopreserved oocytes were thawed; they survived and, after insemination, normal fertilization took place. Three embryos were transferred into the patient's uterus. A twin pregnancy was achieved with the birth of two healthy females. Oocyte cryopreservation may be a reliable option for preserving fertility in young cancer patients who risk premature menopause due to surgery, chemotherapy or radiotherapy.

Keywords: *cancer, chemotherapy, fertility preservation, oocyte cryopreservation, premature menopause*

Introduction

Oncological patients undergoing chemotherapy or radiotherapy risk the loss of their reproductive potential (Longhi and Porcu, 2000). Patients with ovarian tumours risk surgical menopause. Patients undergoing conservative surgical treatment because of borderline ovarian tumours maintain a good chance of spontaneous or medically induced pregnancy, which does not seem to affect the course of the illness negatively (Seracchioli *et al.*, 2001) even though they are prone to recurrence of the disease and possible loss of fertility as a result of surgical menopause. Along with advances in the management of malignancies, the concept of quality of life and the need for fertility preservation has found a place. In the past, the only reproductive alternative for these women was oocyte donation, giving up the opportunity to use their own genetic material, or preventive IVF with embryo cryopreservation exclusively in partnered patients. Recently, oocyte cryopreservation has become a potentially good option for preserving female fertility. Despite early successes (Chen, 1986), this technique used to be unreliable. Methodological improvement achieved by the end of the 1990s made oocyte freezing more efficient in treatments for infertility (Porcu *et al.*, 1997). Following this a fertility-saving programme with oocyte cryopreservation for oncological patients was started in the authors' institution (Porcu *et al.*, 2004). This report describes the first delivery in an ovariectomized oncological woman who conceived using her own frozen oocytes.

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Materials and methods

In 2002, a 26-year-old nulligravid married patient with a borderline ovarian tumour asked to be enrolled in the authors' oocyte cryopreservation fertility-saving programme. She refused embryo cryopreservation because of personal moral concerns. The patient's informed consent and Institutional Ethical Committee permission were obtained. In 2000, in another unit, an abdominal mass had been detected during a routine examination. The patient was diagnosed with a possible ovarian tumour on the basis of sonographic evaluation, which revealed the presence of a large unilocular cyst (20 × 15 × 14 cm) with papillary projections arising from the cyst wall. The serum concentration of tumour marker Ca-125 was 225 IU/l. The patient underwent laparoscopic intervention and an intraoperative histological examination resulting in the diagnosis of papillary serous carcinoma of borderline malignancy, which was confirmed by definitive histology. Conservative surgical treatment with a right adnexectomy and an omentectomy was performed; careful inspection of the contralateral ovary and of peritoneal biopsies and peritoneal washing cytology were also carried out and were all normal. The tumour grade was stage 1A.

Two years later, in 2002, after appropriate gynaecological, oncological and reproductive counselling, ovulation induction was performed as previously reported (Porcu *et al.*, 1994) with a prolonged protocol administration of gonadotrophin-releasing hormone analogue followed by 150 IU per day of recombinant FSH. After 10 days of ovarian stimulation, nine mature follicles developed. Final maturation was induced with 10,000 IU of human chorionic gonadotrophin (HCG) and oocyte retrieval was performed 36 h later. Seven mature eggs were retrieved and cryopreserved with the propanediol–sucrose protocol, as previously reported (Porcu *et al.*, 1997). The oocytes were cryopreserved using a slow freeze–rapid thaw protocol. They were equilibrated in phosphate-buffered saline (PBS) supplemented with 1.5 mol/l 1,2-propanediol (PROH) and 30% Plasmanate for 10 min. After equilibration, the oocytes were transferred into PBS supplemented with 1.5 mol/l PROH, 0.2 mol/l sucrose and 30% Plasmanate, loaded into plastic straws and placed in an automated Kryo III 10/17 biological vertical freezer with the chamber temperature at 20°C. The temperature was slowly reduced to –7°C at a rate of –2°C/min. Ice nucleation was induced manually by seeding at –7°C. After a hold time of 10 min at –7°C, the temperature was gradually reduced to –30°C at a rate of –0.3°C/min and rapidly lowered to –150°C at a rate of –50°C/min. After 10 min of temperature equilibration, the straws were transferred into liquid nitrogen tanks and stored until thawing. For oocyte thawing, the straws were removed from the liquid nitrogen, held at room temperature for 30 s and put into a 30°C water bath for 40 s. The cryoprotectants were removed by stepwise dilution. The contents of the melted straws were expelled in 1.0 mol/l PROH + sucrose 0.2 mol/l solution + 30% Plasmanate and the oocytes were equilibrated for 5 min. The oocytes were then transferred to 0.5 mol/l PROH + sucrose 0.2 mol/l solution + 30% Plasmanate for an additional 5 min and then in a sucrose 0.2 mol/l solution + 30% Plasmanate for 10 min before final dilution in PBS solution + 30% Plasmanate for 20 min (10 min at room temperature and 10 min at 37°C). Finally, the oocytes were transferred to a fresh culture medium at 37°C in a 5% CO_2 atmosphere for 3 h.

In the same year (2002), the tumour recurred in the contralateral ovary. Ultrasonography of the left ovary showed three cysts 20, 18 and 17 mm in diameter with intracystic papillary projections. Laparoscopic conservative surgical treatment using cystectomy was carried out in another unit with intensive surgical staging, multiple peritoneal biopsies and cytology. The original histological diagnosis was confirmed and multiple peritoneal biopsies and cytology were negative. The tumour grade was stage 1B. Six months later, ultrasound monitoring revealed the presence of two cysts (20 mm in diameter) with intracystic papillary projections. After further monthly ultrasound monitoring, intracystic papillae appeared hypervascularized at power Doppler evaluation. A laparoscopic left adnexectomy was performed in March 2003. The diagnosis of a borderline papillary serous ovarian tumour was confirmed and multiple peritoneal biopsies and cytology were normal. Gynaecological oncologists conducted a strict follow-up and no recurrence of the disease was detected in the following 39 months. After recovery from the disease, in June 2006, the patient asked for oocyte thawing, fertilization and embryo transfer. Hormonal replacement treatment with 6 mg/day of micronized oestradiol was prescribed for endometrial growth followed by association with 600 mg of micronized progesterone.

Results

After 10 days of therapy with micronized oestradiol, when the endometrial thickness reached 9 mm, 600 mg/day of micronized progesterone were added. This therapy was carried out during the entire luteal phase and up to the eighth week of gestation. Three frozen oocytes were thawed on the first day of progesterone therapy. All of them survived after 4 years of cryopreservation. Oocyte insemination was performed by intracytoplasmic sperm injection resulting in normal fertilization and the development of three embryos (one 4-cell embryo grade 2, one 4-cell embryo grade 3, one 3-cell embryo grade 4) which were transferred into the uterus on day 3 after insemination. After 14 days, the serum βHCG levels were 755 IU/l. Two weeks later, transvaginal ultrasound examination revealed two intrauterine sacs with embryo heart activity. After an uneventful normal pregnancy, at week 38 of gestation, a Caesarean section was performed in another institution resulting in the birth of two healthy females weighing 2100 g and 2400 g.

Discussion

Several preventive tools have been proposed to reduce the reproductive damage deriving from antineoplastic therapies.

Ovarian tissue cryopreservation has been investigated in the past few years as a fertility-saving procedure (Oktay *et al.*, 2004), and one live birth and one pregnancy were reported after the auto-transplantation of ovarian grafts (Donnez *et al.*, 2004; Meirow *et al.*, 2005). However, the risk of reintroducing neoplastic cells with this procedure must be kept in mind. On the other hand, in-vitro maturation of early immature oocytes in tissue is presently unreliable (Gook *et al.*, 2004). For these reasons, in the present study, cryopreservation of the ovarian tissue would not have helped this type of cancer patient. On the contrary, the present report documents that women having a borderline ovarian malignancy can become pregnant and deliver healthy children after a bilateral ovariectomy, after

having previously cryopreserved their own oocytes. The safety of infertility treatments after conservative management of a borderline ovarian tumour has been debated and the currently available data suggest that IVF may be considered for these patients as there is no evidence of any adverse effect of fertility drugs and of pregnancy on the course of the tumour (Fasouliotis *et al.*, 2004). Oocyte cryopreservation may have advantages over ovarian tissue cryopreservation in patients with ovarian cancer or in other types of cancer likely to be reintroduced with ovarian grafts.

Oocyte freezing may also be better than embryo storage owing to ethical and pragmatic considerations. Oocyte storage is free from ethical concerns and might be considered as the ideal strategy to save fertility in cancer patients provided they have at least 2 weeks available for ovarian stimulation and oocyte retrieval before chemotherapy or surgical intervention (Porcu *et al.*, 2004). Yang *et al.* (2007) have recently reported a live birth in a gestational carrier after the transfer of human embryos conceived with cryopreserved oocytes retrieved before chemotherapy/radiotherapy in a patient with Hodgkin's lymphoma. However, surrogate motherhood is not allowed in most countries owing to ethical concerns.

Oocyte cryopreservation has been the object of intensive investigation in the past 10 years, with apparently increasing clinical success (Polak *et al.*, 1998; Porcu *et al.*, 2000; Boldt *et al.*, 2003; Chen *et al.*, 2005; Levi Setti *et al.*, 2006; Borini *et al.*, 2006a,b; La Sala *et al.*, 2006; De Geyter *et al.*, 2007). Oocyte cryopreservation with vitrification seems to be even more promising since Yoon *et al.* (2007) obtained a 43.3% pregnancy rate per embryo transfer, which approaches their results with fresh oocytes.

Gynaecologists, oncologists and general practitioners should be aware of this option for preserving female fertility and should provide oncological patients with the most appropriate and up-to-date information.

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