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Case Report

Ovarian tissue cryopreservation after chemotherapy and successful pregnancy after autograft and additional chemotherapy

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Background: Ovarian tissue cryopreservation (OTC) is a valid procedure that may allow to preserve endocrine and reproductive health in girls and young women at high risk of premature ovarian failure. At disease remission, the OTC may be transplanted in the woman, allowing recovery of the production of oocytes and reproductive hormones. *Case*: This report describes the case of a Ewing Sarcoma/Primitive Neuroectodermal Tumor (ES/PNET) survivor who cryopreserved ovarian tissue after the first line of chemotherapy. After completion of treatment the patient experienced premature ovarian failure. Seven years later ovarian tissue autotransplantation was performed and ovarian function recovery was obtained in a few months. The woman had a shoulder ES/PNET recurrence and restarted the chemotherapy. The menstrual cycle reappeared 4 months after the second line of treatment and the patient spontaneously conceived 10 months later giving birth to a healthy girl. *Conclusions*: In malignancies OTC performed even after the start of chemotherapy seems to be effective in preserving fertility and allows more women to become candidates for ovarian function preservation.

Keywords

Ovarian tissue cryopreservation; Ovarian tissue transplantation; Ewing sarcoma; Recurrence; Live birth

1. Introduction

The progress made in cancer diagnosis and the introduction of new therapeutic protocols have improved the survival rates of girls and young women with cancer. However, one of the specific side effects of the antitumor therapy is gonadotoxicity, gonadotoxic and that can severely impact the long term fertility and reproductive health of these patients inducing premature ovarian insufficiency (POI) [1].

Ovarian tissue cryopreservation (OTC) is a promising procedure to preserve endocrine and reproductive function in girls and young women at risk of POI. At disease remission, the OTC may be transplant in the woman, allowing recovery of the production of oocytes and reproductive hormones for reproductive functions. Ovarian tissue cryopreservation programs are becoming increasingly available and the results of OTC transplantation were established in terms of ovarian tissue function recovery (95% of cases), number of full term pregnancies probably more than 200 by now (41.6% live births/total patient data from three major centers: Sheba Medical Center, Israel, Cliniques universitaires Saint Luc, Belgium, and St LouisInfertility Center, USA) [2] and induction of puberty [3].

In this case report we describe the first live birth after ovarian tissue autotransplantation in a Ewing Sarcoma (ES) survivor treated with chemotherapy before OTC and after OTC transplantation for recurrence.

2. Case report

In September 2008, at age 22, the woman underwent lumbosacral computerized tomography (CT) and magnetic resonance imaging (MRI) for neuralgic symptoms in the lumbar and right gluteal region. CT confirmed the presence of solid tissue neoformation that obliterated the vertebral cul de sac. Biopsy of the lesion reported the diagnosis of sacral ES/PNET. Neurosurgery was performed immediately with partial removal of the intrarachial and extrarachial sacral neoplasia (S1–S4). Then, the woman received 6 cycles VIDE (vincristine IV 1.5 mg/m^2 on day 1 and ifosfamide IV 3 g/m^2, doxorubicin IV 20 mg/m^2 and etoposide IV 150 mg/m^2 on

days 1–3) and 5 cycles VAI (vincristine IV 1.5 mg/m^2 and actinomycin IV 0.75 mg/m^2 on day 1 and ifosfamide IV 3 g/m^2 on days 1 and 2).

The young woman asked to preserve the endocrine function and after signed written informed consent, a laparoscopy was performed to collect the ovarian tissue for cryopreservation [4]. Approximately half of each ovary was retrieved and the ovarian tissue was cryopreserved according to Fabbri *et al.* [5]. She subsequently completed further treatments with 3 VAI cycles and local radiotherapy (5580 cGy). The monthly LHRH analogue was administered during all therapies. The woman finished all therapies in September 2009.

In the following months the woman became amenorrheic and both transvaginal sonography (TVS) evaluation (small ovaries—0.4 cc left ovary and 0.5 cc right ovary—without antral follicles) and hormone evaluation (FSH 70.6 mIU/mL, LH 44.7 mIU/mL and E2 $<$12 pg/mL) showed a menopausal status. Thereafter she started hormone replacement therapy (HRT), to counteract menopausal symptoms (hot flashes and osteoporosis).

In the following years the woman remained in complete remission and in 2015 she decided to stop HRT to evaluate ovarian function, which unfortunately confirmed a definitive menopausal status with hot flashes reappearance. So she started HRT again.

In January 2017 the woman requested the tissue transplantation. After oncologists approval pre-transplant evaluations were performed. The presence of ovarian micrometastases was evaluated by Real-Time RT-PCR to identify the specific translocation t11; 22 EWS-FLI1 type 1 in a thawed tissue sample for each ovary. No fusion transcript was shown with molecular analysis. The absence of malignant cells in thawed ovarian tissue was also confirmed by QuantStudio 3D Digital PCRSystem (Thermo Fisher Scientific). TVS showed ovaries without functional signs and high contrast sonography bilateral tubal patency. The husband's spermiogram showed a normozoospermic seminal fluid.

On the day of transplantation (July 2017) four cortical strips (50% of total amount of frozen tissue) were thawed according to our protocol [6] and rapidly transferred to the operating room. Two strips each were sutured in the left and right ovarian pocket, respectively [7]. During laparoscopy some biopsies of the residual ovaries were taken confirming the absence of follicles and extensive fibrosis (Fig. 1).

Two months after the transplantation, the woman reported cervical mucus discharge and blood spotting; TVS revealed follicular development in both ovarian sites: one antral follicle in the left ovary and two in the right. FSH, LH and E2 levels remained at menopausal levels.

In January 2018 the woman had an ES/PNET recurrence localized to the shoulder. She underwent surgical removal of the recurrence and histologic evaluation reported the presence of round small cells with the following immunophenotypes CD99+/FLI1+/ERG–/vimentin+/desmin–/EMA–/pan-CK+/S100–/CD45–/TdT–; in addition, the neoplasm showed partial positivity for CD56, rare expression of synaptophysin and presence of hemorrhage. FISH analysis confirmed the presence of EWSR1 gene rearrangement.

A month later (February 2018) the woman had the first menstrual cycle after the OTC transplantation and FSH, LH and E2 levels were 2.4 mIU/mL, 7.9 mIU/mL and 34.6 pg/mL, respectively with TVS showing two antral follicles and a corpus luteum in the right ovary (volume 2 cc) and five antral follicles in the left ovary (volume 2.2 cc).

In March 2018 the woman restarted chemotherapy with 6 cycles every 21 days of Termozolomide 100 mg/mq for 1–5 days and Irinotecan 50 mg/mq for 1–5 days. In June 2018 restarted local radiotherapy for 25 days, and then performed the last 2 cycles of chemotherapy. Monthly LHRH analogue was also administered during therapies, all treatments ended in August 2018.

In January 2019 the menstrual cycle spontaneously returned and in the following months continued regularly with FSH 33.1 mIU/mL, LH 13.7 mIU/mL and E2 43.6pg/mL, respectively. In November 2019, TVS reported a 14 mm follicle in the right ovary (2.9 cc) and a 9 mm follicle in the left ones (1.4 cc). A month later, the βHCG test was positive and TVS confirmed a viable intra-uterine pregnancy with fetal heartbeat. The pregnancy progressed to a natural birth of a healthy girl weighing 3550 g and 50 cm in length at 41 weeks + 3 days of gestational age.

The timeline of clinical events are reported in Fig. 2.

3. Discussion

This case report describes the first live birth after ovarian tissue autograft in a ES survivor treated with chemo/radiotherapy before OTC and after OTC transplantation for recurrence. Recommendations regarding OTC in patients already undergoing cancer therapy are conflicting. Usually OTC is performed before chemotherapy to preserve the highest quality of tissue. In effect it was observed that chemotherapy is able to increase the rate of nuclear abnormalities in granulosa cells and oocyte vacuolization and also to generate vascular alterations and ovarian fibrosis [8, 9], depending on the type and dosage of chemotherapy and on the patients' age at the time of treatment [10].

Since OTC commonly precedes the start of chemotherapy, the possibility that this tissue may be infiltrated by neoplastic cells should be considered and require the exclusion of neoplastic contamination before OTC. Modern sequencing techniques have considerably increased the sensitivity in diagnosing tissue infiltration by neoplastic cells. In addition, the dramatic repercussions on the quality of life of these young women who decide to undergo OTC and discover their ovarian tissue contaminated by neoplastic cells must also be considered.

Micrometastases were observed in ES, even in those newly diagnosed patients who have no measurable metastases with chest CT or Positron Emission Tomography [11–14]. EWSR1 fusion transcripts are characteristic of ES. Using

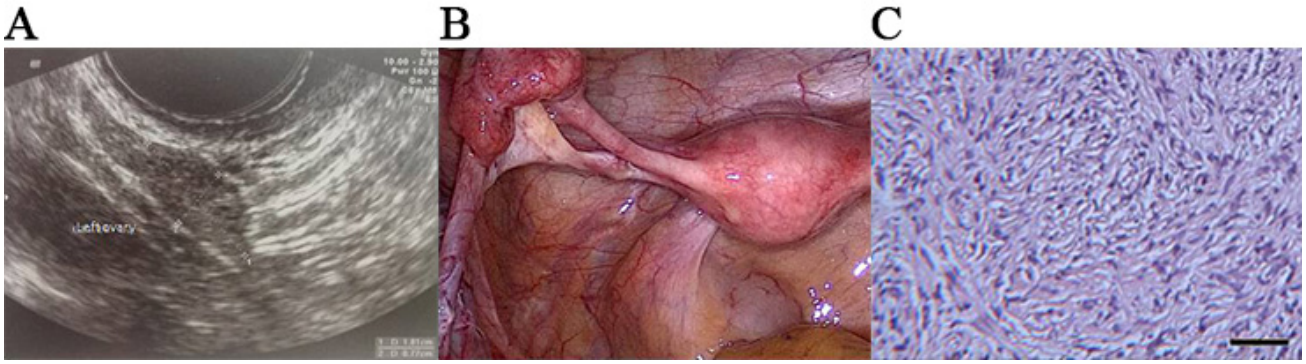

Fig. 1. Appearance of left ovary before reimplantation. (A) Transvaginal sonography evaluation: small left ovary without antral follicles; (B) uterus and left ovary before cryopreserved ovarian tissue reimplantation; (C) histological appearance of stroma with loss of structural organization, fibrotic areas, interstitial edema and absence of follicles (scale bar 50 μm).

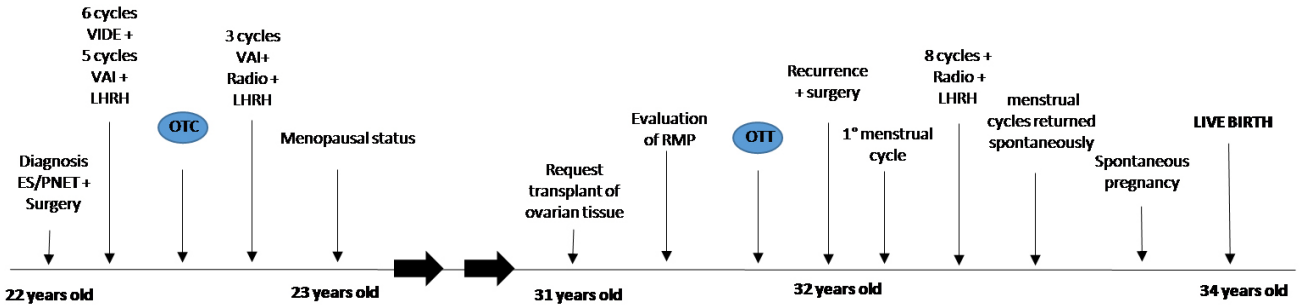

Fig. 2. Timeline of clinical events.

a polymerase chain reaction (PCR) assay to detect occult ES cells in the peripheral blood (PB) and in bone marrow (BM), between 20% and 40% of newly diagnosed ES patients showed to have detectable EWSR1 fusion transcripts in their specimens [11–14]. Moreover, new techniques, such as quantification of ctDNA, may increase the chance of detecting micrometastatic disease in ES.

The data on a potential metastatic infiltration of ovarian tissue by ES cells are conflicting [15–18]. The most comprehensive analysis of the presence of sarcoma cells in ovarian tissue prepared for transplantation included a total of 16 patients, nine of whom had ES [15]. Ovarian tissue was transplanted into immunodeficient mice for 20 weeks and evaluation of the presence of the EWS/FL1 translocation in all cases revealed no signs of malignant cell contamination [15]. However, few cases of metastatic infiltration of ovarian tissue by ES cells are reported in literature: one case of ES recurrence after four and a half years after OTC transplantation [16], one case of ovarian tissue positive for EWS/FL1 translocation on eight patients evaluation [17] and one case of the presence of CD99 positive cells on the surface of ovarian tissue harvested from a ES patient [18].

Before transplantation, Real-Time PCR and digital PCR were performed to look for ovarian tissue contamination. These techniques were equally sensitive and able of detecting 1 pg/μL of fusion transcript [19], but investigations revealed no evidence of pathologic infiltration.

All these observations lead to consider new perspectives in ES management strategy. A standard-dose chemotherapy phase before the cryopreservation procedure may have the effect of *in vivo* purging of any metastatic ovarian infiltration. Since it has been demonstrated that cryopreservation of functional ovarian tissue after chemotherapy is feasible, especially in younger patients, the potential positive effect of pre-cryopreservation chemotherapy deserves to be studied more extensively. Some recent studies reported that patients previously exposed to chemotherapy were able to recover endocrine and reproductive function after OTC transplantation [10, 20]. Meirow *et al.* [20] reported the outcomes of 10 patients with tissue collected after chemotherapy demonstrating cycle and ovarian function resumption after transplantation, and the achievement of 13 pregnancies and seven live-births. They concluded that patients previously exposed to chemotherapy are good candidates for OTC [20]. Similarly, Poirot *et al.* [10] studied the efficacy of OTC transplant in 22 patients treated with low gonadotoxic chemotherapy but before highly gonadotoxic chemotherapy observing cycle resumption in 91% of cases and the birth of at least one child, in 32% of cases. They concluded that post-chemotherapy OTC should no longer be considered as limitation for OTC [10]. Also in our case we evidenced a regular resumption of ovarian function after OTC transplantation, regardless of the time (pre or post OTC) of the chemotherapy administration. To our knowledge, this case report describes for the first time

spontaneous conception in a woman with ES who received chemotherapy before and after OTC transplantation. It is noteworthy that the woman was very young at the time of cryopreservation, so she had a high ovarian reserve which allowed the recovery of ovarian function and to become pregnant, despite a double exposure to chemotherapy.

4. Conclusions

In young women with cancer, OTC performed at any time, even after the start of chemotherapy, seem to be effective in preserving fertility and allows more women to become candidates for OTC. These findings are very important information to be given to women who wish to preserve ovarian function. Further prospective studies are needed to evaluate the ideal timing to perform OTC in ES patients who are candidates for potentially gonadotoxic treatments.

Author contributions

MEA performed oncological treatment of the patient; RF, RV, MM and VM cryopreserved and thawed ovarian tissue; RP and SR performed surgery; LDM and RS performed gynecologic and obstetrics follow-up of the patient; SB performed molecular analysis. All authors contributed to editorial changes in the manuscript. All the authors read and approved the final manuscript.

Ethics approval and consent to participate

All subjects gave their informed consent for inclusion before they participate in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by our Ethics Committee (S. Orsola-Malpighi, No. 74/2001/O).

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Conflict of interest

The authors declare no conflict of interest.

References

[1] Jeong K, Aslan E, Ozkaya E, Sonmezer M, Oktay K. Ovarian cryopreservation. Minerva Medica. 2012; 103: 37–46.

[2] Dolmans M, Donnez J. Fertility preservation in women for medical and social reasons: Oocytes vs ovarian tissue. Best Practice & Research Clinical Obstetrics & Gynaecology. 2021; 70: 63–80.

[3] Donnez J, Dolmans M. Fertility Preservation in Women. The New England Journal of Medicine. 2017; 377: 1657–1665.

[4] Paradisi R, Fabbri R, Magnani V, Battaglia C, Venturoli S. A new simple technique of laparoscopic temporary ovarian suspension in addition to ovarian cryopreservation for women prior to posterior pelvic radiation. Gynecologic Oncology. 2010; 117: 385–386.

[5] Fabbri R, Pasquinelli G, Keane D, Magnani V, Paradisi R, Venturoli S. Optimization of protocols for human ovarian tissue cryopreservation with sucrose, 1,2-propanediol and human serum. Reproductive BioMedicine Online. 2010; 21: 819–828.

[6] Fabbri R, Vicenti R, Macciocca M, Martino NA, Dell'Aquila ME, Pasquinelli G, *et al.* Morphological, ultrastructural and functional imaging of frozen/thawed and vitrified/warmed human ovarian tissue retrieved from oncological patients. Human Reproduction. 2016; 31: 1838–1849.

[7] Fabbri R, Pasquinelli G, Magnani V, Macciocca M, Vicenti R, Parazza I, *et al.* Autotransplantation of cryopreserved ovarian tissue in oncological patients: recovery of ovarian function. Future Oncology. 2014; 10: 549–561.

[8] Meirow D, Dor J, Kaufman B, Shrim A, Rabinovici J, Schiff E, *et al.* Cortical fibrosis and blood-vessels damage in human ovaries exposed to chemotherapy. Potential mechanisms of ovarian injury. Human Reproduction. 2007; 22: 1626–1633.

[9] Abir R, Ben-Haroush A, Felz C, Okon E, Raanani H, Orvieto R, *et al.* Selection of patients before and after anticancer treatment for ovarian cryopreservation. Human Reproduction. 2008; 23: 869–877.

[10] Poirot C, Fortin A, Lacorte JM, Akakpo JP, Genestie C, Vernant JP, *et al.* Impact of cancer chemotherapy before ovarian cortex cryopreservation on ovarian tissue transplantation. Human Reproduction. 2019; 34: 1083–1094.

[11] Peter M, Magdelenat H, Michon J, Melot T, Oberlin O, Zucker JM, *et al.* Sensitive detection of occult Ewing's cells by the reverse transcriptase-polymerase chain reaction. British Journal of Cancer. 1995; 72: 96–100.

[12] Pfleiderer C, Zoubek A, Gruber B, Kronberger M, Ambros PF, Lion T, *et al.* Detection of tumour cells in peripheral blood and bone marrow from Ewing tumour patients by RT-PCR. International Journal of Cancer. 1995; 64: 135–139.

[13] West DC, Grier HE, Swallow MM, Demetri GD, Granowetter L, Sklar J. Detection of circulating tumor cells in patients with Ewing's sarcoma and peripheral primitive neuroectodermal tumor. Journal of Clinical Oncology. 1997; 15: 583–588.

[14] Avigad S, Cohen IJ, Zilberstein J, Liberzon E, Goshen Y, Ash S, *et al.* The predictive potential of molecular detection in the non-metastatic Ewing family of tumors. Cancer. 2004; 100: 1053–1058.

[15] Greve T, Timmermans Wielenga V, Grauslund M, Sørensen N, Christiansen DB, Rosendahl M, *et al.* Ovarian tissue cryopreserved for fertility preservation from patients with Ewing or other sarcomas appear to have no tumor cell contamination. European Journal of Cancer. 2013; 49: 1932–1938.

[16] Yding Andersen C, Ernst E, Bærentzen S, Birkebæk NH, Clausen N. No malignancy detected in surplus ovarian tissue from a former Ewing sarcoma patient who experienced relapse four years after being grafted with frozen/thawed ovarian tissue. Journal of Assisted Reproduction and Genetics. 2014; 31: 1567–1568.

[17] Abir R, Feinmesser M, Yaniv I, Fisch B, Cohen IJ, Ben-Haroush A, *et al.* Occasional involvement of the ovary in Ewing sarcoma. Human Reproduction. 2010; 25: 1708–1712.

[18] Sørensen SD, Greve T, Timmermans Wielenga V, Wallace WHB, Andersen YC. Safety considerations for transplanting frozen/thawed ovarian tissue to restore fertility in patientswho have recovered from Ewing sarcoma. Future Oncology. 2014; 10: 277–283.

[19] Benini S, Gamberi G, Cocchi S, Garbetta J, Alberti L, Righi A, *et al.* Detection of circulating tumor cells in liquid biopsy from Ewing sarcoma patients. Cancer Management and Research. 2018; 10: 49–60.

[20] Meirow D, Ra'anani H, Shapira M, Brenghausen M, Derech Chaim S, Aviel-Ronen S, *et al.* Transplantations of frozen-thawed ovarian tissue demonstrate high reproductive performance and the need to revise restrictive criteria. Fertility and Sterility. 2016; 106: 467–474.