Journal of Assisted Reproduction and Genetics (2019) 36:1023–1028
https://doi.org/10.1007/s10815-019-01445-6

FERTILITY PRESERVATION

Successful embryo cryopreservation immediately following a full-term delivery in a woman with newly diagnosed Ewing's sarcoma

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Received: 14 February 2019 / Accepted: 27 March 2019 / Published online: 5 April 2019

Introduction

Ewing's sarcoma is an aggressive, small blue round cell malignancy, affecting one per million people in the USA [1]. Albeit extremely rare, the incidence is much higher in children and young adults, a population who often have not completed their child-bearing. In fact, it accounts for approximately 10% of malignant bone cancers in children and young adults, and 3% of all childhood malignancies [1]. Unfortunately, 25% of patients who present with a clinically apparent localized tumor may also already have signs of occult metastatic disease [2]. Most commonly, metastases are found in the lungs, bones, and bone marrow [2].

While surgery remains a fundamental treatment strategy, the introduction of new chemotherapy agents and the combination of chemo and local radiotherapy (conformational RT and intensity-modulated RT) have led to increased 5-year survival rates from less than 15% to 65–70% for patients with localized disease and to 25–30% for those with metastatic disease [1]. Unfortunately, the most common chemotherapy agents used in the treatment of Ewing's sarcoma are highly gonadotoxic alkylating agents, including cyclophosphamide and ifosfamide [3]. These drugs, through cell cycle non-specific mechanisms, can lead to permanent damage of single- and double-stranded DNA of both quiescent and rapidly dividing cells in the ovary, potentially resulting in post-treatment amenorrhea and infertility [3]. Given these cancers tend to affect a young population, oocyte or embryo cryopreservation techniques can represent an opportunity to achieve pregnancy post-treatment and to profoundly impact cancer survivors' future quality of life [4].

One of the biggest challenges in oncofertility is the limited amount of time available for ovarian stimulation and oocyte/embryo cryopreservation between cancer diagnosis and treatment. Oncologists and reproductive endocrinologists often have to balance the patient's desire to preserve future fertility with the need to promptly start cancer treatment. There is evidence that ovaries can be successfully stimulated at any point during a woman's menstrual cycle [5]. However, there are no guidelines or reported cases of successful controlled ovarian stimulation immediately following a term delivery, which presents additional challenges. Difficulty monitoring, the profound pregnancy-induced ovarian suppression, and the effect of serum hCG on premature follicle luteinization and final oocyte maturation are some of the obstacles to overcome in the postpartum period. Here, we report a case of ovarian stimulation started a few days after a 37-week delivery in a woman newly diagnosed with Ewing's sarcoma, which resulted in the vitrification of six good-quality embryos.

Case report

A 27-year-old, gravida 2, para 2, presented to an academic reproductive medicine clinic for fertility preservation counseling less than 48 h after a normal spontaneous vaginal delivery. Two months earlier, she had noticed mild swelling in her left leg. At that time, Doppler ultrasound of the lower extremities to rule out a blood clot and a left leg X-ray were performed and were both negative. She then presented again to her local emergency department with worsening leg pain and swelling. MRI revealed a "marrow replacing lesion in the proximal tibial shaft with

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extraosseous soft tissue extension. The extraosseous soft tissue component measures 6.5 x 6.7 x 12.7 cm."

The patient was referred to Orthopedic Oncology at our institution and a core needle biopsy revealed pathology consistent with Ewing's sarcoma. She then presented to Medical Oncology at approximately 36 weeks of gestation, and staging scans and systemic therapy were recommended as soon as possible. To promptly complete cancer staging and start treatment, her labor was medically induced at 37 weeks of gestation, leading to an uncomplicated vaginal delivery. Postpartum, a PET scan was remarkable for metastatic disease within the lungs and mediastinal lymph nodes. A bone marrow biopsy showed no evidence of marrow involvement. She was recommended to enroll in Children's Oncology Group study AEWS1221, a randomized phase III trial evaluating the addition of IGF-1R monoclonal antibody Ganitumab to standard multi-agent chemotherapy with vincristine, doxorubicin, cyclophosphamide, ifosfamide, and etoposide [6].

Simultaneously, the patient decided to pursue embryo cryopreservation prior to starting chemotherapy initiation. During her consultation with Reproductive Endocrinology on postpartum day 2, she reported no other significant medical, surgical, or gynecological history, and no history of infertility. A trans-vaginal and trans-abdominal ultrasound performed same day demonstrated an enlarged postpartum uterus and non-visualized ovaries (Fig. 1); thus, we were unable to obtain a baseline antral follicle count. Anti-mullerian hormone (AMH) was drawn and resulted 5 days later at 0.8 ng/mL and serum human chorionic gonadotropin (hCG) was 633 mIU/mL.

Six days after delivery, her serum hCG level had decreased to 71 mIU/mL, and the patient was started on 150 of follicle stimulation hormone (FSH, Gonal-F®, EMD Serono, Rockland, MA, USA) and 75 IU of human menopausal gonadotropin (hMG, Menopur®, Ferring Pharmaceuticals, Parsippany, NJ, USA). The patient came into the office for routine monitoring ultrasounds and blood draws every 1–2 days. Gonadotropins were titrated up based on estradiol rise and follicular growth to reach final doses of 225 FSH and 225 hMG (Table 1). On treatment day 9, ganirelix acetate, 250 μg, (GnRH antagonist, Antagon®, EMD Serono, Rockland, MA, USA) was started. Final oocyte maturation was triggered with 250 μg recombinant hCG on treatment day 18 (Ovidrel®, EMD Serono, Rockland, MA, USA). Thirty-six hours after the hCG trigger, 16 oocytes were retrieved trans-vaginally under ultrasound guidance. The oocytes were fertilized with standard in vitro fertilization (IVF). Six blastocysts developed and were vitrified in liquid nitrogen on day 5 post-retrieval (Table 2). Post-retrieval, the patient received 3 days of GnRH antagonist for hormonal downregulation. Her serum hCG level was monitored during the initial phase of her ovarian stimulation and was < 5 mIU/mL by treatment day 9.

Throughout the patient's fertility preservation treatment regimen and after her retrieval, no adverse events were encountered. She initiated cancer treatment 3 days after her oocyte retrieval.

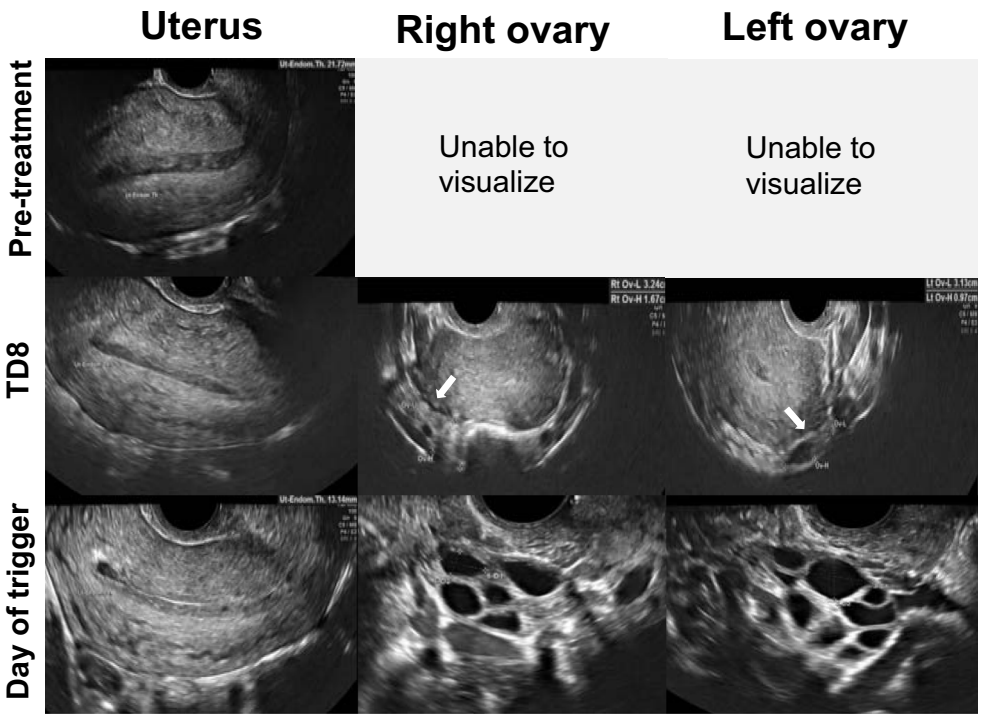

Fig. 1 Ultrasound findings at three time points during the patient's ovarian stimulation cycle. TD, treatment day

Table 1 Ultrasound findings (endometrial stripe thickness, right and left ovarian follicles measurement), estradiol and progesterone levels, and doses of medications administered (FSH, hMG, GnRH antagonist, and r-HCG) during the patient's controlled ovarian stimulation cycle

TD (number)	EMS (mm)	Right follicles (mm)	Left follicles (mm)	E (pg/mL)	P (ng/mL)	hCG (mIU/mL)	FSH (IU)	hMG (IU)	GnRH antagonist (μg)	r-hCG (μg)
18	13.1	17.5, 15.5, 15, 13.5, 12.5	16.5, 16, 15, 13.5, 10.5, 10	2135	1.14	3	225	225	–	250
17	11.5	16, 15.5, 15.5, 14, 13.5, 13.5	15, 13, 11.5, 11, 11	1437 (1.5x)	0.98	–	225	225	250	–
16		–	–	–	–	–	225	225	250	–
15	9.7	15, 13, 12.5, 11	15, 13, 11.5, 11, 11	938 (1.5x)	–	–	225	225	250	–
14	–	–	–	–	–	–	225	225	250	–
13	9.9	11.5, 11, 11, 11, 9.5	13.5, 13.5, 12, 11, 11, 10.5, 10	613 (1.7x)	–	–	225	225	250	
12	–	–	–	–	–	–	225	225	250	–
11	8.4	11, 10.5, 10.5	12, 10.5	354 (2.1x)	–	–	225	225	250	–
10	–	–	–	–	–	–	225	150	250	–
9	8.6	–	–	169	–	4	225	150	250	–
8	8.5	–	–	117 (2x)	–	6	225	150	–	–
7	–	–	–		–		225	150	–	–
6	–	–	–	59 (2.1x)	–	11	225	150	–	–
5	–	–	–	–	–	–	225	150	–	–
4	21	–	–	28	–	21	225	150	–	–
3	–	–	–	–	–	–	150	75	–	–
2	–	–	–	–	–	–	150	75	–	–
1	–	–	–	18	0.4	71	150	75	–	–

TD, treatment day; *EMS*, endometrial stripe measurement; *E*, estradiol level; *P*, progesterone level; *hCG*, human chorionic gonadotropins level; *FSH*, follicle-stimulating hormone dose—Gonal-F®; *hMG*, human menopausal gonadotropin dose—Menopur®; *GnRH antagonist*, ganirelix acetate dose—Antagon®; *r-hCG*, recombinant human chorionic gonadotropin dose—Ovidrel®

Table 2 Embryo development results

Embryo development day	Oocytes/embryos
Day of retrieval	16 oocytes (14 mature oocytes)
Day 1 after retrieval	10x 2PN
Day 3 after retrieval (cleavage stage)	1x 8A, 3x 8B, 1x 7B, 1x 6C, 1x 5C, 1x 4C, 1x 4D, 1x 2D
Day 5 after retrieval (blastocyst stage)	1x 4AB, 1x 4BB, 3x 3BB, 1x 3 BC (all cryopreserved)
	1 non-blast, 1x stage 1, 2x morula (remained in culture)
Day 6 after retrieval (blastocyst stage)	1x non-blast, 1x morula, 1x stage 1, 1x 4CC (all discarded)

PN, pro-nuclei stage embryo

Embryo grading system adopted:

1. Cleavage stage grading:

- First number = number of cells seen in the embryo
- First letter = percentage of embryo fragmentation and cell asymmetry:
  - A (< 10% fragmentation, all cells symmetry)
  - B (10–20% fragmentation, 1–2 cells asymmetry)
  - C (> 25% fragmentation, > 2 cells asymmetry)

2. Blastocyst stage grading:

- First number = grade 1 to 6 from early blastocyst to fully hatched
- First letter = inner cell mass appearance:
  - A (tightly packed and many cells)
  - B (loosely grouped and many cells)
  - C (very few cells)
- Second letter = trophectoderm appearance:
  - A (many cells forming cohesive epithelium)
  - B (many cells forming loose epithelium)
  - C (very few cells)

Informed consent was obtained from the patient described in this case report.

Discussion

The key to a successful cancer treatment plan is based on a multi-disciplinary approach with the goal of treating not only the disease but also the whole person. Fertility preservation counseling and services are a fundamental aspect of cancer treatment and have shown to improve patients' quality of life post-therapy [7]. A critical concern for oncologists and reproductive endocrinologists is whether fertility preservation procedures can be arranged without excessively delaying cancer treatment. Especially in the case of aggressive cancers, such as the one presented here, there is typically a very small window of time before the initiation of chemotherapy to allow for an oocyte or embryo cryopreservation cycle which usually takes approximately 2 weeks. At our center, a comprehensive fertility preservation program, including a patient coordinator, works to mitigate treatment delays by connecting patients to care as quickly as possible. In this particular case, timely referral from the oncology team, efficient care coordination by the patient coordinator, and same day availability of the reproductive endocrinologist were essential to expediting the patient's care.

It has been previously shown that fertility preservation can be initiated at any time during the menstrual cycle (so called "random start") and as early as the same day that the patient presents to the reproductive endocrinology office [5, 7]. However, to the best of our knowledge, there are no guidelines or reported cases of women who underwent a successful in vitro fertilization (IVF) cycle immediately following a term delivery, which presents additional challenges. The enlarged postpartum uterus can limit the ability to visualize the ovaries, obtain a baseline antral follicle count, and monitor follicular growth, especially during the early stages of the stimulation. Moreover, AMH and other ovarian reserve markers, often used to direct the initial decision of gonadotropin starting doses, are difficult to interpret in the postpartum period due to suppression during pregnancy, and the results of these tests are not always immediately available before initiating the cycle [8]. Furthermore, there is lack of data on the expected response of postpartum ovaries to stimulation and concern over premature luteinization or inability to induce final oocyte maturation in the setting of increased serum hCG levels.

Only two previous case reports of women who underwent IVF after induced first trimester abortion are present in the

literature and have shown conflicting results. Goeckenjan et al. [9] described a successful case of tissue cryopreservation followed by ovarian stimulation in a 37-year-old woman with newly diagnosed breast cancer which was started 8 days after a first trimester pregnancy termination. The cycle leads to the retrieval of 28 oocytes, 26 of which were mature. The patient's hCG levels were initially 5600 mIU/mL then 300 mIU/mL on treatment day 5 and negative at the time of gonadotropin-releasing hormone (GnRH) agonist trigger. Considering her age, AMH level of 1.6 ng/mL, and the successful outcome, the authors further hypothesized a positive hCG effect on supporting follicular recruitment through a luteinizing hormone (LH) androgen-linked priming mechanism.

On the other hand, Cohade et al. [10] started ovarian stimulation in a 31-year-old woman with breast cancer 5 days after an induced abortion which leads to the growth of 6 follicles > 15 mm, with appropriate estradiol and progesterone rise, but unfortunately without retrieval of any oocytes 36 h after GnRH agonist trigger. AMH level was 3.9 ng/mL and antral follicle count was 35. In this case, hCG levels were slightly higher and remained over 100 mIU/mL throughout the entire stimulation which may have induced premature luteinization and prevented the effectiveness of the LH surge, which is fundamental for the final oocyte maturation.

With this knowledge, and considering that after uncomplicated deliveries hCG levels decrease according to a half-life of 24–36 h [8], we chose to postpone the start of this patient's IVF cycle by 4 days (postpartum day 6) until serum hCG reached a level of 71 mIU/mL. We hypothesized that a cutoff of < 100 mIU/mL on the first day of IVF treatment could safely allow for successful ovarian stimulation and effective response to the final hCG trigger without excessively delaying the start of her cancer treatment. Based on the predicted half-life, hCG levels should become negative before late folliculogenesis occurred. Additionally, when choosing the ovulation trigger agent in patients stimulated immediately postpartum, hCG should be strongly considered as some women may have refractory hypophyseal response to GnRh agonist.

Several interesting observations from our case were as follows: (1) the patient's IVF cycle was slightly longer than the average 10–14 days, (2) we started to see follicles > 10 mm on treatment day 11, and (3) her AMH level was slightly lower than expected based on her young age and fertility history. These could suggest that a more aggressive approach and higher doses of gonadotropins are indicated in postpartum patients, especially after a full-term pregnancy, from the beginning of the stimulation to overcome the physiological ovarian suppression induced by pregnancy and to achieve an optimal cycle.

Another conundrum was related to the specifics of the patient's recommended treatment. For the young adult population, particularly with rare cancers, clinical trial participation is critical for optimizing outcomes. As a protection measure, a steadfast study eligibility criterion requires a "negative pregnancy test within 7 days" of treatment enrollment, and exceptions are not made, even in the case of documented exogenous hCG administration for oocyte maturation prior to retrieval. Because the fertility preservation team was aware of this requirement, we were able to run a serum hCG level on the day of trigger, which was negative. Without knowledge of this criterion, standard IVF procedures would have been rendered, and the patient would likely have been ineligible for clinical trial participation when her urine pregnancy test was positive from exogenous hCG administration.

In conclusion, we have reported the successful completion of an embryo cryopreservation cycle immediately after a term delivery in a woman with cancer and provided some recommendations on timing and choice of initial gonadotropin doses. To the best of our knowledge, this is the first report of successful ovarian stimulation in an immediately postpartum woman after a full-term pregnancy. Additionally, this case further reinforces the concept that fertility preservation counseling should be routinely discussed and offered to all patients as part of pre-cancer treatment plan. While there may be an assumption that a multiparous and recently pregnant woman would not desire fertility preservation when facing a new diagnosis of malignancy, patients must be given the opportunity to understand their options and how their decisions may influence their care. With optimal communication between reproductive endocrinology and oncology teams, we can effectively and timely achieve both fertility preservation and optimal oncologic care, even in the face of a recent pregnancy.

Compliance with ethical standards

Informed consent was obtained from the patient described in this case report.

Conflict of interest The authors declare that they have no conflict of interest.

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