Fertility preservation using controlled ovarian hyperstimulation and oocyte cryopreservation in a premenarcheal female with myelodysplastic syndrome

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Objective: To report the first case of fertility preservation in a premenarcheal female by use of controlled ovarian hyperstimulation and oocyte cryopreservation.
Design: Case report.
Setting: Reproductive endocrinology and infertility unit of a tertiary care university-based medical center.
Patient(s): A 13-year-old premenarcheal female with Tanner stage 3 breast development and Tanner stage 1 pubic hair diagnosed with myelodysplastic syndrome, referred by her medical oncologist for fertility preservation before undergoing a potentially sterilizing antineoplastic therapy.
Intervention(s): Evaluation of ovarian reserve, ovarian stimulation, transvaginal oocyte aspiration, in vitro maturation of immature oocytes, and oocyte cryopreservation.
Main Outcome Measure(s): Cryopreservation of mature oocytes.
Result(s): Successful controlled ovarian hyperstimulation allowed for the cryopreservation of 18 mature oocytes before the patient's gonadotoxic treatment. The oocyte retrieval and cryopreservation did not delay the patient's planned chemotherapy.
Conclusion(s): Ovarian stimulation and oocyte cryopreservation can be successfully performed in premenarcheal/peripubertal patients, thus providing a viable alternative to ovarian tissue freezing for fertility preservation in the pediatric population. (Fertil Steril® 2012;98:1225–8. ©2012 by American Society for Reproductive Medicine.)
Key Words: Cancer, fertility preservation, oocyte cryopreservation, pediatric, prepubertal

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In 2012, more than 11,000 children in the United States will be diagnosed with cancer, potentially compromising their future reproductive function and fertility after gonadotoxic treatment (1, 2). Indeed, improvements in cancer therapy have led to a growing cohort of cancer survivors who suffer from premature gonadal failure as a result of their treatment (3). An increasing focus in the field of reproductive medicine has thus been the preservation of fertility for patients facing potential sterility as a consequence of their cancer treatment.

Ovarian tissue freezing is the primary treatment modality currently available to prepubertal/premenarcheal patients facing gonadotoxic chemotherapy or radiation therapy (4). Ovarian tissue cryopreservation has been implemented with increasing frequency for this population, but the efficacy of this practice is far from proven; few pregnancies after ovarian tissue cryopreservation have been reported in the literature, and in several cases the provenance of such pregnancies has remained unclear, as in some cases endogenous ovarian function within remaining ovarian tissue may have returned (5). Moreover, subsequent

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transplantation of tissue harvested before cancer treatment could potentially expose a patient in remission to their original disease (6–9).

Whereas pediatric patients with cancer typically undergo ovarian tissue freezing, most reproductively mature females undergo oocyte or embryo cryopreservation after a period of ovarian stimulation, a technique with substantial and proven efficacy (2). It has long been held, however, that controlled ovarian hyperstimulation would be ineffective in sexually immature patients, and there are no reported cases in the literature of premenarcheal ovarian stimulation (10, 11). We here present a case demonstrating successful controlled ovarian hyperstimulation and oocyte cryopreservation of 18 mature oocytes in a premenarcheal female patient who had not completed pubertal development.

MATERIALS AND METHODS

A 13-year-old patient initially presented to her pediatrician after several episodes of syncope, nausea, and emesis. Her lab work reflected pancytopenia, and she was thus referred to the Memorial Sloan Kettering Cancer Center for bone marrow biopsy. Bone marrow aspirate revealed 1% blasts, an inverted myeloid to erythroid ratio, and dysplastic erythroid cells; a diagnosis of myelodysplastic syndrome was made. She was scheduled for multiagent chemotherapy with busulfan, melphalan, and fludarabine in preparation for subsequent stem cell transplantation. Before initiating treatment, the patient and her parents were referred by their medical oncologist to discuss options for preserving her fertility. Before this referral, the patient had already been scheduled for an oophorectomy with ovarian tissue freezing by the oncology group.

An evaluation by our team revealed a well-nourished, premenarcheal 13-year-old girl. Physical examination revealed Tanner stage 3 breast development and Tanner stage 1 pattern of pubic hair. She weighed 50 kg and had attained a height of 149 cm. Pelvic examination and single-digit bimanual examination revealed a partially intact hymeneal ring and grossly normal anatomy. Transvaginal sonography was not performed so as to avoid patient discomfort. A transabdominal pelvic sonogram revealed a small uterus approximately equivalent in size to the cervix as well as normal appearing ovaries bilaterally. Antral follicle counts assessed transabdominally revealed five follicles <10 mm on the right ovary and four follicles <10 mm on the left. A hormone assessment demonstrated an antimüllerian hormone (AMH) level of 0.95 ng/mL, an estradiol level of 65 pg/mL, a follicle-stimulating hormone (FSH) level of 5.0 mIU/mL, and a luteinizing hormone (LH) level of 2.9 mIU/mL.

The literature regarding ovarian tissue cryopreservation was discussed with the patient and her parents. Based on the limited data regarding subsequent pregnancies, the patient and her family asked whether any other options might be viable. We reviewed the merits of attempting controlled ovarian hyperstimulation with the goal of cryopreserving oocytes, and felt that this was a potentially viable alternative. After extensive counseling, the patient and her family elected to attempt oocyte cryopreservation, and the patient's hematologist did not feel that her diagnosis precluded the time interval required for stimulation. The patient and her family were referred for psychological counseling and support. Appropriate institutional review board approval was obtained for oocyte cryopreservation.

In light of her impending gonadotoxic treatment, the patient was immediately initiated on 225 IU of human menopausal gonadotropin (Ferring), which was favored over pure FSH given the patient's hypothalamic immaturity. Daily gonadotropin-releasing hormone (GnRH) antagonist (Ganirelix, 0.25 mg; Organon) injections were initiated on the seventh night of stimulation, once the lead follicle had attained a mean diameter of 12 mm. Follicular development was assessed via transabdominal sonography with a full bladder as well as serial measurement of her serum estradiol concentrations. Gonadotropin stimulation was well tolerated by the patient. Recombinant human chorionic gonadotropin (hCG, Ovidrel; EMD Serono) was administered after 9 days of stimulation when the two lead follicles were ≥ 17 mm, with an estradiol concentration of 1,132 pg/mL. Retrieval was scheduled 35 hours after hCG administration.

The day before the planned oocyte aspiration, the patient was admitted for preoperative optimization in light of her pancytopenia. Her complete blood count (CBC) on admission revealed a hematocrit of 19% and platelet count of $15 \times 10^3/\mu$L. She was transfused a total of three units of packed red cells (PRBCs) and one six-pack of platelets. On the morning of the oocyte aspiration, a CBC demonstrated a hematocrit of 35.1% and platelets of $54 \times 10^3/\mu$L. On call to the operating room, she received an additional platelet transfusion, resulting in a preoperative platelet count of $120 \times 10^3/\mu$L. Intravenous aminocaproic acid, 5 grams (Amicar; Xanodyne Pharmaceuticals), was administered just before the procedure.

The patient was brought to the operating room. After an adequate level of intravenous sedation was achieved, the patient was placed in the dorsal lithotomy position and prepped and draped in the usual fashion. A narrow ultrasound transducer (Logiq 400-Pro; General Electric) with an affixed needle guide was inserted vaginally, permitting transvaginal ovarian cyst puncture and aspiration. We obtained 20 oocytes. The postoperative examination revealed excellent hemostasis, and the patient was transferred to the recovery room. Two hours after surgery, the patient's complete blood count was verified to be stable. She was maintained on 5 grams of oral Amicar every 6 hours for the duration of her hospital stay. When she was discharged home the next day, her hematocrit was 29.8%, and her platelet count was $109 \times 10^3/\mu$L.

RESULTS

The retrieved oocytes were cultured in our in-house sequential media at 37°C and 6% CO_2. To assess the oocytes' maturity, the oocytes were stripped of their surrounding cumulus cells with 40 IU of recombinant cummulase. Eight oocytes exhibited mature metaphase 2 (MII) morphology, as evidenced by extrusion of the first polar body, and they were thus separated for freezing. These eight MII oocytes were frozen in pairs 1 hour after cumulus removal (2 hours after retrieval) via vitrification. Oocytes were exposed to ethyl glycol and