

Case report: two cases of mature oocytes found in prepubertal girls during ovarian tissue cryopreservation

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Objective: To report two cases of mature oocytes found in prepubertal girls undergoing ovarian tissue cryopreservation (OTC).

Design: Case report.

Setting: Large tertiary care children's hospital and a private fertility clinic.

Patient(s): An 8-year-old prepubertal girl with β -thalassemia and a 2-year-old girl with sickle cell disease who both underwent OTC before bone marrow transplantations.

Intervention(s): Laparoscopic right oophorectomy was performed in each patient. The ovarian cortical tissue was processed for slow freezing and long-term storage, and all oocytes were subsequently vitrified.

Main Outcome Measure(s): Oocytes found at the time of OTC processing for fertility preservation.

Result(s): After a complete right oophorectomy, one mature metaphase II oocyte was discovered on tissue processing for OTC in each patient. Neither patient has yet returned for use of tissue or oocytes.

Conclusion(s): To our knowledge, this is the first report of mature oocytes found during prepubertal OTC processing. These findings may indicate the need for increased research regarding prepubertal oocyte development and suggest that the technique of examining the media for both mature and immature oocytes at the time of OTC should become more widespread and perhaps recommended in prepubertal patients to optimize fertility preservation methods. (Fertil Steril Rep® 2021;2:296-9. ©2021 by American Society for Reproductive Medicine.)

Key Words: OTC, IVM, prepubertal ovary, oocyte, fertility preservation

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INTRODUCTION

Gonadotoxic therapies such as chemotherapy and radiation therapy can lead to oocyte reduction or depletion and complete loss of reproductive potential (1, 2). As a result, fertility preservation measures are typically

implemented before initiating gonadotoxic therapy. In postpubertal females, fertility preservation is most frequently performed by ovarian hyperstimulation followed by oocyte collection and subsequent freezing of mature eggs or embryos. However, this is not a viable or well-researched

option for prepubertal girls. Prepubertal ovaries are functionally suppressed by low levels of gonadotropin-releasing hormone, which blocks ovarian activity and ovulation from occurring naturally (3). There is one case report that utilizes prepubertal ovarian hyperstimulation to collect mature oocytes; however, this finding is novel and investigational (4).

To date, the only option for fertility preservation in prepubertal girls is ovarian tissue cryopreservation (OTC). In December 2019, the American Society for Reproductive Medicine published a committee opinion on fertility preservation in patients undergoing gonadotoxic therapies stating that

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OTC should be an established medical procedure and no longer considered experimental (5). Ovarian tissue cryopreservation has been used successfully in adult women with over 360 transplantations and 130 live births reported worldwide (6). However, little is known about the prevalence of pregnancy after OTC in prepubertal patients. In the current literature, there are only two case reports of live births where the OTC was performed in a prepubertal or premenarchal girl. One live birth was reported in a patient who underwent OTC at 13 years and 11 months old, before hematopoietic stem cell transplantation for sickle cell disease. Of note, the ovarian tissue for this patient was harvested in the postpubertal but premenarchal period. She returned for autologous tissue transplantation at the age of 24 years, which was followed by a spontaneous pregnancy and live birth 2 years later (7). The second live birth was in a prepubertal patient with beta-thalassemia who underwent OTC at the age of 9 years prior to total body radiation and hematopoietic stem cell transplantation. At the age of 21 years, she returned for autologous tissue transplantation, was monitored for 4 months for spontaneous ovulation, and then underwent in vitro fertilization to expedite the process of pregnancy. The live birth was achieved with one antagonist stimulation cycle and one frozen embryo transfer (8).

An experimental fertility preservation technique that has been coupled with OTC is in vitro maturation (IVM) (9); however, there are only three live births from IVM in adult women reported in the literature (10). In this technique, antral follicles aspirated from the ovarian tissue (11) and immature oocytes found in the media after OTC tissue processing (12) are collected and incubated in maturation medium. Following IVM, metaphase II (MII) oocytes are vitrified for future use with in vitro fertilization (Fig. 1) (12). As of December 2020, a literature search indicates that no MII oocytes have previously been found in unstimulated prepubertal ovaries that were processed for OTC with or without IVM (13, 14). Fouks et al. (12) reported that 3.1% of the oocytes found in premenarchal participants were collected at the MII stage; however, it is significant to note that this study does not describe the distinction between prepubertal and premenarchal, and it is possible that the MII oocytes may have been collected from girls who already entered the primary stages of puberty with activated Hypothalamic-pituitary-ovarian (HPO) axis. Furthermore, the investigators of this study found zero MII oocytes in a subgroup analysis of nine girls between 1 and 5 years old (11).

In the following, we describe two rare cases of mature oocytes found in the ovarian tissue of prepubertal patients undergoing OTC; one was discovered in 2013, and the other was in 2020. During this time period, 32 OTC procedures were performed at our institution; 11 were in prepubertal girls. In 21 postpubertal patients, two patients had one and two MII oocytes, respectively.

CASE ONE

The first patient was a 2-year-old girl with sickle cell disease whose parents, after extensive counseling regarding gonadotoxic risk and the benefits of OTC, decided for her to undergo

fertility preservation before starting chemotherapy and total body radiation for a peripheral blood stem cell transplant (PBSCT). Her treatment plan was to receive total body irradiation of 3 Gy, which put her in the significantly increased risk category per the oncofertility Pediatric Initiative Network risk stratification system (15), which was used since this case occurred in 2020. The patient was prepubertal, with no history of premature breast budding, pubic hair, excessive hair growth, or body odor. Her past treatment for sickle cell disease included hydroxyurea treatment that started 21 months before OTC.

The patient underwent a laparoscopic complete right oophorectomy at the time of central venous line and femoral line placement by the interventional radiology team. In our practice, the right ovary is typically removed because it is easier to visualize, unimpeded by the location of the rectum and sigmoid colon, and is known to have a higher incidence of ovarian torsion compared with the left side (16). In addition, complaints of right lower quadrant pain in the future will simplify the differential diagnosis of appendicitis. The patient's surgery was uncomplicated. The excised ovary was subsequently submerged in 4 °C ART 8040 and 8050 holding media produced by CooperSurgical F&G and transported to a local private fertility clinic for processing and cryopreservation. The ovarian cortical tissue was separated from the medullary tissue, processed as described by the Northwestern Oncofertility Consortium (17), and then cryopreserved using modifications of the techniques described by Gosden et al. (18). Once the medulla was separated from the cortex, the cortical tissue was cut into multiple strips measuring 1 cm × 0.5 cm with a thickness of approximately 1 mm and cryopreserved by a slow freezing technique in multiple cryovials and then transferred to a long-term cryostorage bank. It is a routine procedure at our center to send a small ovarian biopsy sample back to the hospital for pathological examination, which was normal in this patient.

During the ovarian tissue processing, visible follicles are almost always seen on the ovaries. Those visualized are routinely aspirated with a 21-gauge needle connected to a 3-mL syringe before making the initial incision on the ovary. Aspirates are flushed with the holding media and examined for oocyte-cumulus complexes under a dissecting microscope. If additional follicles are seen when separating the cortex from the medulla, they are aspirated as well. The ovary is processed until the cortex is approximately 1 mm thick. During this process, the ovary is moved to new dishes several times when the media in the dish has too much blood or contains debris. The dishes and media are then inspected using a polished glass pipet under a microscope, and needles are used to pull apart any large pieces of medullary debris. Any oocytes that are found are moved to a dish with drops of 10% Serum Protein Substitute (SPS) in Modified Human Tubal Fluid (mHTF) under oil. Since this is a retrospective case, from the information available, it is not specified whether the MII oocytes were collected from the aspirated follicles or the media during tissue dissection. After all the dishes are analyzed, the oocytes are denuded with hyaluronidase (80 IU/mL) (Irvine Scientific, Santa Ana, CA, USA) and vitrified using Vitrification Kit (Kitazato Corporation). In the patient presented

FIGURE 1

Oocyte maturation. General morphology of immature germinal vesicle and metaphase I oocytes and mature metaphase II oocytes retrieved in vitro. These images are used with permission from Shady Grove Fertility.

Hanfling. Mature oocytes from prepubertal ovaries. *Fertil Steril Rep* 2021.

earlier, one MII oocyte plus four immature germinal vesicle (GV) oocytes were found during OTC processing (Fig. 1). The five oocytes were cryopreserved by vitrification (Table 1).

CASE TWO

The second patient was an 8-year-old girl with beta-thalassemia who underwent OTC for fertility preservation before a PBSCT. Before the PBSCT, the patient was on hydroxyurea treatment. Her estimated cyclophosphamide equivalent dose was 5.2 mg/m² (calculated from melphalan 140 mg/m²). This case was performed in 2013, prior to the Oncofertility Pediatric Initiative Network Risk Stratification System; however receiving a PBSCT put her at high risk for premature ovarian insufficiency. The patient's family was counseled on the risks, benefits, and alternatives to surgical treatment for fertility preservation. After counseling, the patient assented, and her parents consented to the OTC procedure. Although no hormonal laboratory examinations were performed, the patient was prepubertal based on Tanner stage 1 breast and pubic hair. She underwent an uncomplicated laparoscopic right salpingo-oophorectomy at the same time as a central venous line placement.

After tissue collection, the ovary was transported to the local private fertility clinic in holding media, processed, and cryopreserved the same day. During tissue processing, the ovarian cortex was dissected from the medulla and then cut

into six cortical strips and one strip of the medullary tissue. After tissue dissection, the processing media was examined in the same fashion as described in the previous patient. Subsequently, one mature MII oocyte and eight GV oocytes were isolated from the media and vitrified for preservation (Table 1 and Fig. 1).

DISCUSSION

To our knowledge, this is the first documentation of MII oocytes collected and preserved in prepubertal girls without the use of hyperstimulation or maturation techniques after tissue collection. More research is needed; however, our findings may indicate that MII oocyte retrieval is a possible fertility preservation method in prepubertal patients. Although IVM has been used successfully to mature and cryopreserve MII oocytes from patients as young as 5 years old (19), studies have reported that premenarchal patients have substantially lower maturation rates than postmenarche patients, with even lower rates in prepubertal girls aged <5 years (11, 12). One study has suggested that patients aged <5 years are not suitable for ex vivo oocyte collection and maturation due to a low number of oocytes retrieved during tissue collection (20).

Our findings contradict these reports and encourage more research of both mature and immature oocytes spontaneously discovered in prepubertal patients during OTC. Although we

TABLE 1: CASE SPECIFICATIONS

Case	Age (years)	Relevant comorbidities	Treatment plan	OTC surgery	Oocytes retrieved
1	2	Sickle cell disease	PBSCT	Right oophorectomy	1 MII, 4 GV
2	8	Beta-thalassemia	PBSCT	Right oophorectomy	1 MII, 8 GV

Note: PBSCT = peripheral blood stem cell transplant; MII = metaphase II; GV = germinal vesicle.

Hanfling. Mature oocytes from prepubertal ovaries. *Fertil Steril Rep* 2021.

do not know if the MII oocytes in our findings have the ability to fertilize, grow as an embryo, implant, and result in live birth, this may be a significant opportunity to pursue. It is not routine or required for hospitals and fertility clinics that perform OTC to check the holding media for oocytes or aspirate visual follicles in the tissue. With advances in IVM and our case findings, we suggest that the technique of examining the media at the time of ovarian tissue preparation for OTC should become more widespread and perhaps recommended in prepubertal patients to optimize fertility preservation methods.

Furthermore, it is important to point out that both of these cases had a nonmalignant hematologic disorder requiring bone marrow transplant, so it is possible that these populations, in contrast to patients with cancer, may have premature follicular development as a result of their chronic disease or pre-PBSCT treatment. Both cases also received hydroxyurea before OTC; therefore, it is possible that there is an association between hydroxyurea and in vivo oocyte maturation in prepubertal girls.

Additionally, research in adult patients has demonstrated that vitrifying MII oocytes yields better oocyte survival rates and higher embryo-transplant success than vitrifying immature oocytes (21). Finding MII oocytes in prepubertal patients may result in improved fertility outcomes; unlike immature GV oocytes that have to undergo extensive in vitro processing to mature to the MII phase, the naturally retrieved MII oocytes can be vitrified more rapidly, which may preserve the survival of cells.

Finally, while OTC is no longer considered an experimental technique and has been proven to be successful, researchers have pointed to the invasiveness of the procedure (22) and advised the necessity for future research into OTC for young patients (23). Although OTC is currently the “standard of care” for prepubertal girls, it is imperative to continue fertility preservation research in the prepubertal population to open more possibilities in the future.

In conclusion, this case report describes the unusual finding of mature oocytes collected from the media during OTC processing of two prepubertal girls. Although more research is needed in prepubertal ovarian physiology, we believe that awareness of these findings may prompt increased research regarding the prevalence of prepubertal MII oocytes and the ability for these mature oocytes to be used for future fertility preservation.

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