

## Original Study

# Oocyte Cryopreservation for Fertility Preservation in Postpubertal Female Children at Risk for Premature Ovarian Failure Due to Accelerated Follicle Loss in Turner Syndrome or Cancer Treatments

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## ABSTRACT

**Objective:** To preliminarily study the feasibility of oocyte cryopreservation in postpubertal girls aged between 13 and 15 years who were at risk for premature ovarian failure due to the accelerated follicle loss associated with Turner syndrome or cancer treatments.

**Design:** Retrospective cohort and review of literature.

**Setting:** Academic fertility preservation unit.

**Participants:** Three girls diagnosed with Turner syndrome, 1 girl diagnosed with germ-cell tumor, and 1 girl diagnosed with lymphoblastic leukemia.

**Interventions:** Assessment of ovarian reserve, ovarian stimulation, oocyte retrieval, in vitro maturation, and mature oocyte cryopreservation.

**Main Outcome Measure:** Response to ovarian stimulation, number of mature oocytes cryopreserved and complications, if any.

**Results:** Mean anti-müllerian hormone, baseline follicular stimulating hormone, estradiol, and antral follicle counts were  $1.30 \pm 0.39$ ,  $6.08 \pm 2.63$ ,  $41.39 \pm 24.68$ ,  $8.0 \pm 3.2$ ; respectively. In Turner girls the ovarian reserve assessment indicated already diminished ovarian reserve. Ovarian stimulation and oocyte cryopreservation was successfully performed in all female children referred for fertility preservation. A range of 4-11 mature oocytes (mean  $8.1 \pm 3.4$ ) was cryopreserved without any complications. All girls tolerated the procedure well.

**Conclusions:** Oocyte cryopreservation is a feasible technique in selected female children at risk for premature ovarian failure. Further studies would be beneficial to test the success of oocyte cryopreservation in young girls.

**Key Words:** Fertility preservation, Oocyte cryopreservation, Turner syndrome, Germ cell tumor, Lymphocytic leukemia, Ovarian stimulation

## Introduction

Recent advances in assisted reproduction and cryopreservation technologies as well as the growing emphasis on the maintenance of quality of life post cancer led to the development of the field of fertility preservation. Depending on the method used, fertility preservation procedures offer not only the opportunity to preserve fertility but restoration of gonadal function as well. While oocyte and embryo freezing are performed for the purpose of preserving fertility only, an experimental procedure, ovarian tissue freezing and transplantation, can also reverse menopause and restore ovarian function.<sup>1</sup>

Among female children, many conditions can lead to impaired fertility. Fertility preservation should be considered in girls facing gonadotoxic treatments, requiring ovarian surgery or in cases of endocrine or genetic diseases, such as Turner syndrome, that are associated with premature

ovarian failure.<sup>2</sup> However, fertility preservation options are usually limited among children due to their sexual and psychosocial immaturity. Traditionally, ovarian cryopreservation, the most experimental fertility preservation technique, has been the method of choice in prepubertal and postpubertal young girls because no ovarian stimulation is needed. Given that oocyte cryopreservation is no longer considered experimental and that it is currently uncertain how successful ovarian transplantation will be, especially in girls with Turner syndrome, oocyte cryopreservation may be the preferred method of fertility preservation in postpubertal young girls.<sup>3-5</sup>

The purpose of this report is to analyze the feasibility of performing ovarian stimulation and oocyte cryopreservation in postpubertal adolescent girls between the ages of 13 and 15 and characterize this particular subset of the adolescent females who can best benefit from oocyte cryopreservation.

## Materials and Methods

The retrospective cohort study was approved by the Institutional Review Board at New York Medical College. The Data were collected from the medical records of 5

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consecutive cases that underwent oocyte cryopreservation at ages between 13 and 15 years. Baseline characteristics describing the study population are outlined in Table 1. Pre-procedural counseling with parental involvement was initiated with each patient to assess the candidate's physical and psychosocial development, the presumed risk of ovarian failure, as well as to discuss the details of the oocyte cryopreservation procedure. Consensus of understanding among parents and the child as well as the assent of the child were also sought.

## Cases

Case 1 was diagnosed with mosaic Turner syndrome at age 13 with a karyotype of 45,X (27/30)/47,XXX (3/30), and had spontaneous pubertal development and menarche in the same year, though her menstrual cycles were irregular, having only 2 cycles thereafter. The patient did not carry the classic features associated with Turner syndrome and no cardiac or renal anomalies were detected. In the same year, she was referred by her pediatric endocrinologist to our Institute for fertility preservation counseling.

Case 2, previously reported,<sup>5</sup> was diagnosed with mosaic Turner syndrome at the age of 13, subsequent to endocrine evaluation because of repeated fractures due to low impact trauma. Her karyotype was found to be 46,XX (11/20)/45,X (9/20). She was also diagnosed with a duplicated ureter and underwent corrective surgery during childhood. No cardiac anomalies were found. At the time of her presentation to us at age 13, her sexual characteristics were consistent with Tanner stage 4 having reached menarche at age 11. Her cycles were also irregular with a frequency of 21–42 days and lasting 6 days. Before undergoing ovarian stimulation for oocyte cryopreservation, the patient was placed on a 4-week pretreatment of oral contraceptives for timing the procedure. One year after the procedure, the patient wished to undergo a second stimulation cycle to improve her oocyte yield. The menstrual cycles remained irregular and she was placed again on 5 weeks of oral contraceptive treatment prior to initiating ovarian stimulation for the second time.

Case 3 was diagnosed with mosaic Turner syndrome following an evaluation of her short stature at the age of 11. Her karyotype was 46,XX (16/20)/45,X (4/20). She had a normal physical exam and both cardiac and renal anomalies were ruled out. Her initial thyroid evaluation was consistent with Hashimoto syndrome; however, at the time of our evaluation, she was euthyroid and did not require thyroid

hormone replacement. Her menarche was at 12 years of age. At age 13, she was referred to our Institute for fertility preservation counseling.

Case 4 first presented to the emergency room with acute abdominal pain at the age of 15 years. She experienced menarche at the age of 13, with regular menses until 2 months prior the detection of an abdominal mass. Pelvic ultrasonography revealed the presence of a large heterogeneous mass measuring 10.4 × 10.6 × 6.7 cm and appearing to arise from the left adnexa without evidence of torsion. A diagnostic laparoscopy was performed the same day. Intra-operative findings included a ruptured cyst in the left ovary and a nodule in the omentum. A left salpingoophorectomy was performed as well as a partial omentectomy for possible malignancy. The pathology report confirmed the finding of a mixed malignant germ cell tumor in both left ovary and omentum (stage IIIB). The patient was promptly referred to us for discussion of fertility preservation techniques.

Case 5 had a diagnosis of acute lymphoblastic leukemia and she received induction and consolidation treatment starting at age 5 years. Her chemotherapy course included a multidrug regimen (CCG1994 protocol-cytarabine, dexamethasone, vincristine, pegaspargase, mercaptopurine, cyclophosphamide, thioguanine, methotrexate) for 27 months. At age 12, she was re-evaluated by her pediatric endocrinologist. At that time, she had a normal physical exam, breast development which was consistent with Tanner stage 4, and pubic hair growth consistent with Tanner stage 5. She was referred to our institute to discuss possibilities for fertility preservation at the age of 12 with the concern that she might experience early menopause. Her initial anti-müllerian hormone (AMH) measurement was low indicating an inappropriately low ovarian reserve, presumably due to past chemotherapy. At that point the parents and the girl were undecided about fertility preservation and did not return until 2 years later. By this time she was 14 years old and had experienced menarche at age 13 with irregular cycles thereafter. We then performed ovarian stimulation for oocyte cryopreservation.

## Ovarian Stimulation, Oocyte Retrieval, and Cryopreservation

In each case, ovarian stimulation was initiated with recombinant follicle-stimulation hormone (FSH; rFSH, Gonal-F, EMD Serono, Rockland, MA) or highly purified FSH (hFSH, Bravelle, Ferring, Parsippany, NJ). Due to concerns with hypothalamic immaturity, luteinizing hormone

**Table 1**  
Indications for Oocyte Cryopreservation and Ovarian Reserve Assessment

Characteristics	Case 1	Case 2	Case 3	Case 4	Case 5
Age (y)	13	14	13	15	14
Clinical diagnosis	Turner syndrome	Turner syndrome	Turner syndrome	Germ cell tumor	Acute lymphoblastic leukemia
Serum FSH (mIU/ml)	5.7	5.3	5.6	5.6	7.8
Serum LH (mIU/ml)	3.9	9.5	5.3	9.2	8.1
Serum estradiol (ng/ml)	15.1	65.2	33.5	66	28.15
Serum AMH (ng/ml)	1.59	0.9/1.7*	0.76	1.6	0.8/1.3†
Serum Inhibin B (pg/ml)	54.8	<30.0	47.2	-	-
Antral follicle count	6	12	6	11	5

\* Case 2 had AMH levels done at the time of the first ovarian stimulation and one year later, at the time of the second ovarian stimulation.

† Case 5 had AMH levels done at age 12 and repeated 2 years later, just before the stimulation attempt.

**Table 2**  
Outcomes of Oocyte Cryopreservation Cycles

ART	Case 1	Case 2*	Case 3	Case 4	Case 5
Diagnosis	Turner	Turner	Turner	Germ cell tumor	Acute lymphoblastic leukemia
Protocol	Antagonist	Antagonist	Antagonist	Antagonist	Antagonist
Total gonadotropin stimulation dose	2475 IU rFSH + 150 IU rLH	1800 IU rFSH + 450 hMG/ 3750 IU rFSH + 2100 hMG	2025 IU hFSH + 75 IU rLH	1837.5 IU rFSH + 300 IU rLH	1550 IU rFSH + 225 IU rLH
Duration of ovarian stimulation	11	10/14	10	11	12
Peak E2 levels on the day of trigger (ng/ml)	1548	2275/2029	1613	1004	2279
Trigger medication and dose	3300 IU hCG	Lupron 1 mg/Lupron 1 mg	250mcg rhCG	Lupron 1 mg	Lupron 1 mg
Total number of oocytes retrieved	19	11/7	16	8	21
Number of mature oocytes cryopreserved	9 + 1 (IVM)	8/4	7 + 5 (IVM)	4	10 + 1 (IVM)

\* Case 2 underwent 2 oocyte cryopreservation cycles.

supplementation in the form of human menopausal gonadotropins (hMG, Menopur, Ferring) or recombinant LH (rLH, Luveris, EMD Serono, discontinued) was added during the ovarian stimulation. A GnRH antagonist (Cetrotide, Merck Serono or Ganirelix, Organon) also was administered daily during the follicular phase to prevent LH surge when the leading follicle reached 14-mm in diameter. Oocyte maturation was triggered either with recombinant human chorionic gonadotropin (Ovidrel, EMD Serono), highly purified human chorionic gonadotropin (Pregnyl, Organon Netherlands) or Leuprolide acetate (Lupron, TAP Pharmaceuticals, Lake Forest, IL) when at least 2 follicles were > 18-20 mm in the mean diameter. Ovarian response was assessed by the measurement of follicular growth via transabdominal sonography as well as serum estradiol levels. All oocyte retrievals were performed under general anesthesia. Transvaginal oocyte retrieval was performed under ultrasonographic guidance, after parental consent. All retrieved mature oocytes were cryopreserved by MedCult Vitrification Cooling protocol. Additional matured oocytes were obtained through 24-hour in vitro maturation (IVM) of retrieved germinal vesicle stage oocytes.

## Results

The total gonadotropin dosage, type of trigger, and cryopreservation outcomes are detailed in Table 2.

On average,  $13.6 \pm 5.8$  oocytes (range 7-21) were retrieved, of which 51.2% were mature. This resulted in the cryopreservation of a mean number of  $7.0 \pm 2.5$  mature oocytes. Of the immature, 17.5% (7/40) matured after 24 hours of in vitro maturation. This increased the mature oocyte yield to 59.7% and the mean number of mature oocytes cryopreserved to  $8.1 \pm 3.4$ .

All children tolerated the procedure well and there were no complications. No ovarian hyperstimulation syndrome was encountered.

## Discussion

In the present retrospective cohort, we demonstrated that ovarian stimulation and oocyte cryopreservation

could be successfully achieved in postpubertal children at risk for premature ovarian failure and infertility. This retrospective cohort demonstrates that ovarian stimulation and oocyte cryopreservation is a clinically viable option for selected female children who recently experienced menarche.

At present, strategies for fertility preservation in postpubertal young girls who are at risk for premature ovarian failure are limited to experimental methods, such as ovarian tissue cryopreservation alone or in combination with immature oocyte collection from the tissue followed by in vitro maturation and vitrification of mature oocytes.<sup>6,7</sup> Although a large population of primordial follicles can be preserved by cryopreservation of ovarian tissue, significant loss of follicles can occur because of the ischemia encountered after ovarian transplantation.<sup>8</sup> Furthermore, the germ-cell loss is expected to resume after ovarian transplantation in patients at risk for premature ovarian failure for genetic conditions, such as Turner syndrome.<sup>9</sup> Another concern is the theoretical possibility of reintroducing malignant tumor cells in patients diagnosed with childhood cancer.<sup>10</sup> Though it is a very promising fertility preservation method, because of the aforementioned reasons, ovarian freezing still remains experimental.

On the other hand, with improvement in cryopreservation methods over the past decade, mature oocyte vitrification success rates have increased significantly and are comparable to in vitro fertilization-intracytoplasmic sperm injection (IVF-ICSI) cycles using fresh eggs in young women.<sup>11,12</sup> Furthermore, although the data are limited, no increases in chromosomal abnormalities, birth defects, and adverse obstetric and neonatal outcomes have been reported in the children born from cryopreserved oocytes when compared to those born after the use of fresh oocytes and IVF-ICSI or general population.<sup>13</sup>

As a result of the foregoing recent information, the American Society for Reproductive Medicine<sup>14</sup> as well as American Society of Clinical Oncology<sup>15</sup> have recognized oocyte cryopreservation as an established fertility preservation technique.

Mosaic Turner syndrome was the medical indication for oocyte cryopreservation in 3 of the 5 girls in this study. Most

girls with Turner syndrome undergo ovarian failure at a very early age. In fact, only approximately 40%–50% of girls with Turner syndrome, nearly exclusively of mosaic type, will have at least some pubertal development, about 10% may undergo menarche and only 2%–10% achieve spontaneous pregnancy. This suggests that the majority of females with Turner syndrome will be infertile due to premature ovarian failure.<sup>16</sup> Historically, oocyte donation was the only infertility treatment offered to women with Turner syndrome.<sup>17</sup> However, this is not an acceptable option to all women.

Because the rate of oocyte depletion may vary after birth in patients with Turner syndrome, it may be prudent to offer oocyte cryopreservation for these patients as soon as they initiate pubertal development.<sup>18</sup> Borgstrom et al examined predictors for the presence of follicles and concluded that follicles were more likely to be found in girls with a mosaic karyotype, a low FSH level, a high antimüllerian hormone level, spontaneous onset of puberty, and spontaneous menarche.<sup>19</sup> Consistent with these reports, 2 of 3 Turner cases in our study showed AMH levels within normal levels (>1.1 ng/mL), but diminished for their age. The third patient had already diminished AMH levels. There may be variability in AMH levels, however, as our cases 2 and 5 showed; we find that antral follicle counts should also be taken account and can be more reliable in some cases. In addition, patients with Turner syndrome are candidates for preimplantation genetic diagnosis for karyotyping because of the increased risk of fetal chromosomal abnormalities.<sup>20</sup> Having undergone oocyte cryopreservation, preimplantation genetic diagnosis can be performed in the future when the child is an adult and attempting pregnancy via IVF.

Furthermore, those who are considering oocyte cryopreservation for Turner syndrome should be counseled regarding the possibility of potential pregnancy complications related to associated cardiac, renal, and other medical conditions. Though the cardiac evaluation of the 3 Turner girls was normal, in many cases, pregnancy maybe contraindicated due to cardiac anomalies. It has been recommended that females with Turner syndrome who are contemplating pregnancy should have a thorough cardiac/hemodynamic evaluation as well as a consultation from a specialist in maternal-fetal medicine.<sup>20</sup>

In our study, 1 girl was diagnosed with germ cell tumor. The treatment of choice for young patients with germ cell tumors is fertility-sparing surgery along with a thorough staging procedure, followed by prompt initiation of adjuvant systemic chemotherapy.<sup>21,22</sup> The early loss of ovarian reserve from the unilateral oophorectomy and adjuvant chemotherapy and the risk of future recurrence in the contra-lateral ovary are among the concerns that lead to the cryopreservation of oocytes in similar cases.

The fifth case was diagnosed with acute lymphocytic leukemia and was treated with multi-agent chemotherapy at the age of 5 years. Studies have shown high rates of cyclic ovarian function after systemic chemotherapy for prepubertal acute lymphoblastic leukemia, with only a 10% premature ovarian failure rate. However, impaired fertility was found in survivors of leukemia.<sup>23</sup> In addition, this

patient was treated with cyclophosphamide, which has clearly been shown to reduce ovarian reserve.<sup>24,25</sup> In fact, the patient's AMH levels indicated diminished ovarian reserve comparable to that of an adult female towards the end of her reproductive life.<sup>26</sup> Therefore, the presence of apparently normal ovarian function at the completion of chemotherapy and menarche should not be interpreted as evidence of lack of gonadal damage from chemotherapy.

In conclusion, oocyte cryopreservation is a feasible technique in selected female children who recently experienced menarche and who are at risk for premature ovarian failure. Larger studies are needed to better describe this young group of female patients and to determine the success of oocyte cryopreservation.

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### References

1. Sonmezer M, Oktay K: Fertility preservation in female patients. *Hum Reprod Update* 2004; 10:251
2. Michaeli J, Weintraub M, Gross E, Ginosar Y, Ravitsky V, Eizenman E, Mitrani E, Lebovich M, Laufer N, Kennedy S, Revel A: Fertility preservation in girls. *Obstet Gynecol Int* 2012; 2012:139193, <http://dx.doi.org/10.1155/2012/139193>. Epub 2012 Feb 16. PubMed PMID: 22496695; PubMed Central PMCID: PMC3307018.
3. Rodriguez-Wallberg KA, Oktay K: Fertility preservation medicine: options for young adults and children with cancer. *J Pediatr Hematol Oncol* 2010; 32:390
4. Reichman DE, Davis OK, Zaninovic N, et al: Fertility preservation using controlled ovarian hyperstimulation and oocyte cryopreservation in a premenarcheal female with myelodysplastic syndrome. *Fertil Steril* 2012; 98:1225
5. Oktay K, Rodriguez-Wallberg KA, Sahin G: Fertility preservation by ovarian stimulation and oocyte cryopreservation in a 14-year-old adolescent with Turner syndrome mosaicism and impending premature ovarian failure. *Fertil Steril* 2010; 94:753.e15
6. Huang JY, Tulandi T, Holzer H, et al: Combining ovarian tissue cryobanking with retrieval of immature oocytes followed by in vitro maturation and vitrification: an additional strategy of fertility preservation. *Fertil Steril* 2008; 89:567
7. Oktay K, Newton H, Aubard Y, et al: Cryopreservation of immature human oocytes and ovarian tissue: an emerging technology? *Fertil Steril* 1998; 69:1
8. Newton H, Aubard Y, Rutherford A, et al: Low temperature storage and grafting of human ovarian tissue. *Hum Reprod* 1996; 11:1487
9. Lau NM, Huang JY, MacDonald S, et al: Feasibility of fertility preservation in young females with Turner syndrome. *Reprod Biomed Online* 2009; 18:290
10. Bedaiwy MA, Shahin AY, Falcone T: Reproductive organ transplantation: advances and controversies. *Fertil Steril* 2008; 90:2031
11. Herrero L, Martinez M, Garcia-Velasco JA: Current status of human oocyte and embryo cryopreservation. *Curr Opin Obstet Gynecol* 2011; 23:245
12. Cil AP, Bang H, Oktay K: Age-specific probability of live birth with oocyte cryopreservation: an individual patient data meta-analysis. *Fertil Steril* 2013; 100:492
13. Wennerholm UB, Soderstrom-Anttila V, Bergh C, et al: Children born after cryopreservation of embryos or oocytes: a systematic review of outcome data. *Hum Reprod* 2009; 24:2158
14. Practice Committees of the American Society for Reproductive Medicine/Society for Assisted Reproductive Technology: Mature oocyte cryopreservation: a guideline. *Fertil Steril* 2013; 99:37
15. Loren AW, Mangu PB, Beck LN, et al, American Society of Clinical Oncology: Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. *J Clin Oncol* 2013; 31:2500
16. Hovatta O: Ovarian function and in vitro fertilization (IVF) in Turner syndrome. *Pediatr Endocrinol Rev* 2012; 9(Suppl 2):713
17. Hadnott TN, Gould HN, Gharib AM, et al: Outcomes of spontaneous and assisted pregnancies in Turner syndrome: the U.S. National Institutes of Health experience. *Fertil Steril* 2011; 95:2251

18. Purushothaman R, Lazareva O, Oktay K, et al: Markers of ovarian reserve in young girls with Turner's syndrome. *Fertil Steril* 2010; 94:1557
19. Borgström B, Hreinsson J, Rasmussen C, et al: Fertility preservation in girls with turner syndrome: prognostic signs of the presence of ovarian follicles. *J Clin Endocrinol Metab* 2009; 94:74
20. Karnis MF: Fertility, pregnancy, and medical management of Turner syndrome in the reproductive years. *Fertil Steril* 2012; 98:787
21. Nishio S, Ushijima K, Fukui A, et al: Fertility-preserving treatment for patients with malignant germ cell tumors of the ovary. *J Obstet Gynaecol Res* 2006; 32:416
22. Parkinson CA, Hatcher HM, Ajithkumar TV: Management of malignant ovarian germ cell tumors. *Obstet Gynecol Surv* 2011; 66:507
23. Rossi BV, Missmer S, Correia KF, et al: Ovarian reserve in women treated for acute lymphocytic leukemia or acute myeloid leukemia with chemotherapy, but not stem cell transplantation. *ISRN Oncol* 2012; 2012:956190
24. Oktem O, Oktay K: Quantitative assessment of the impact of chemotherapy on ovarian follicle reserve and stromal function. *Cancer* 2007; 110:2222
25. Oktem O, Oktay K: A novel ovarian xenografting model to characterize the impact of chemotherapy agents on human primordial follicle reserve. *Cancer Res* 2007; 67:10159
26. Lie Fong S, Visser JA, Welt CK, et al: Serum anti-müllerian hormone levels in healthy females: a nomogram ranging from infancy to adulthood. *J Clin Endocrinol Metab* 2012; 97:4650