Fertility preservation by ovarian stimulation and oocyte cryopreservation in a 14-year-old adolescent with Turner syndrome mosaicism and impending premature ovarian failure

Kutluk Oktay, M.D., Kenny A. Rodriguez-Wallberg, M.D., Ph.D., and Gulnaz Sahin, M.D.

Institute for Fertility Preservation, Department of Obstetrics and Gynecology, N.Y. Medical College–Westchester Medical Center, Valhalla, New York

Objective: To report a novel approach to fertility preservation in adolescents with Turner syndrome mosaicism by repeated controlled ovarian stimulation and oocyte cryopreservation.
Design: Case report.
Setting: Academic reproductive medicine center.
Patient(s): Fourteen-year-old adolescent diagnosed with Turner syndrome mosaicism.
Intervention(s): Two cyles of controlled ovarian stimulation and oocyte cryopreservation within 1 year.
Main Outcome Measure(s): Recovery of oocytes after controlled ovarian stimulation and oocyte cryopreservation.
Result(s): Eleven oocytes were retrieved, of which eight were mature and three were immature during the first cycle. One year later, four mature and three immature oocytes were retrieved after a treatment cycle with even higher gonadotropin doses. All oocytes were cryopreserved by vitrification.
Conclusion(s): Controlled ovarian stimulation and oocyte cryopreservation may be an option for fertility preservation in selected adolescents with Turner syndrome mosaicism and impending ovarian failure. (Fertil Steril® 2010;94:753.e15–e19. ©2010 by American Society for Reproductive Medicine.)

Key Words: Fertility preservation, Turner syndrome, Turner mosaicism, cryopreservation, oocytes, controlled ovarian hyperstimulation, gonadotropins, adolescents

Turner syndrome occurs in 1 in 2,500 to 1 in 3,000 live-born girls. Although almost half of them present with monosomy X (45,X), mosaicism for a second, normal 46,XX cell population, occurs in approximately 15% of the cases (1). About one-third of girls with Turner syndrome will have spontaneous puberty onset. However, because of accelerated follicular atresia, only a few of them will reach menarche, and eventually 2% to 5% will have the chance to become pregnant spontaneously later in life (2).

The possibility of preserving fertility in young women and girls with Turner syndrome was raised a decade ago (2, 3). Currently, several fertility preservation programs offer cryopreservation of ovarian tissue to children and adolescents with Turner syndrome as an experimental method to preserve future fertility (4–6), and in some cases in combination with in vitro maturation of oocytes (7).

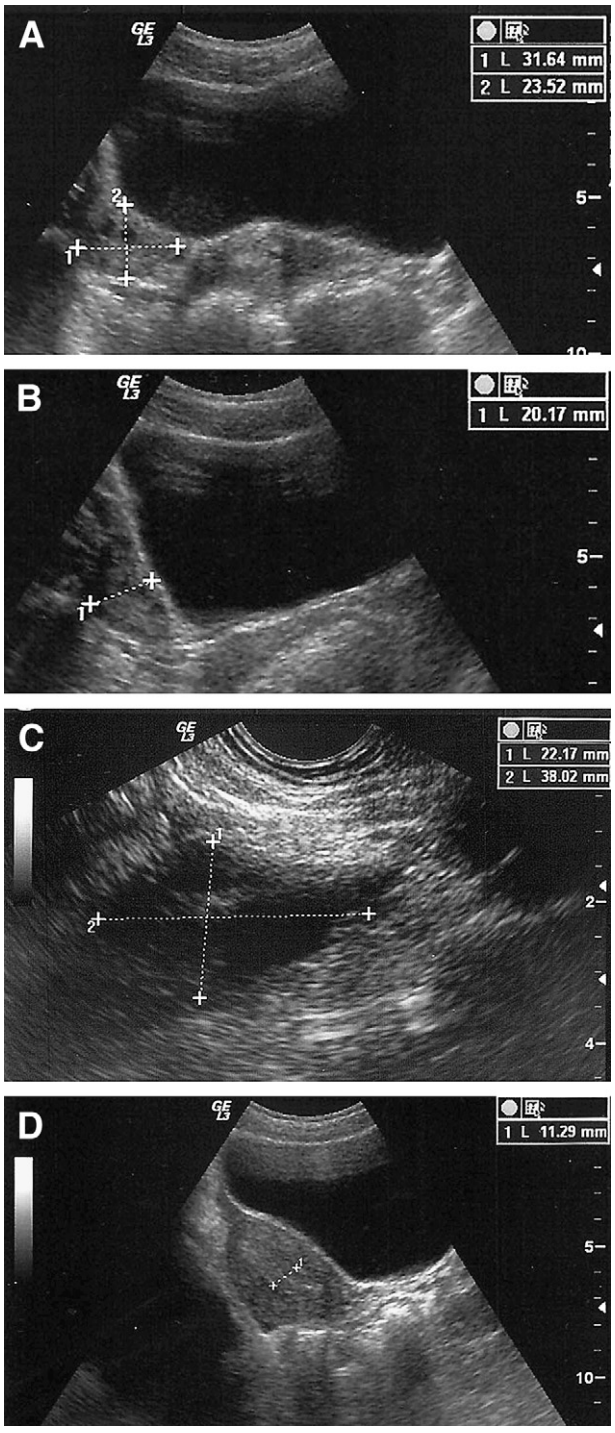
Oocyte cryopreservation is a fertility preservation method that is currently offered to young adult women worldwide. Although the method is still considered experimental, the success rates have risen significantly, approaching to those of standard IVF techniques using fresh oocytes (8, 9). As a result, oocyte cryopreservation is currently regarded as a feasible option for fertility preservation. To increase the yield of oocytes available for cryopreservation, ovarian stimulation and frequent monitoring by ultrasound and hormonal measurements are needed for approximately 2 weeks. Although ultrasound monitoring can be performed both transabdominally and transvaginally, the preferred method of oocyte aspiration is transvaginal, under ultrasound guidance. Because of the need for frequent monitoring and a transvaginal oocyte retrieval, this approach requires both physical and psychologic maturity, and hence, traditionally has not been recommended to young adolescents. However, in the largest series published of children and adolescents with Turner syndrome who had undergone laparoscopic ovarian biopsies for fertility preservation (6), it was noted that when girls reached the age of 13 to 14 years, most of them were able to understand the possibilities and limitations of preserving ovarian tissue and that they took an active part in decision making. Based on this accumulating evidence of fertility preservation procedures in young adolescents, we have initiated oocyte cryopreservation in selected young girls with medical indications. Here we report the management of a 14-year-old girl with Turner syndrome who was referred by her pediatric endocrinologist for possible fertility preservation.

Received November 23, 2009; revised and accepted January 14, 2010; published online February 26, 2010.
K.O. has nothing to disclose; K.A.R-W. has nothing to disclose. G.S. has nothing to disclose.
Supported by research grants from The Swedish Society of Medical Research and The Swedish Society of Medicine.
Reprint requests: Kutluk Oktay, M.D., Department of Obstetrics and Gynecology, New York Medical College–Westchester Medical Center, Munger Pavillion, Room 617, Valhalla, NY 10595 (FAX: 914-594-3441; E-mail: koktay@fertilitypreservation.org).

doi:10.1016/j.fertnstert.2010.01.044

FIGURE 1

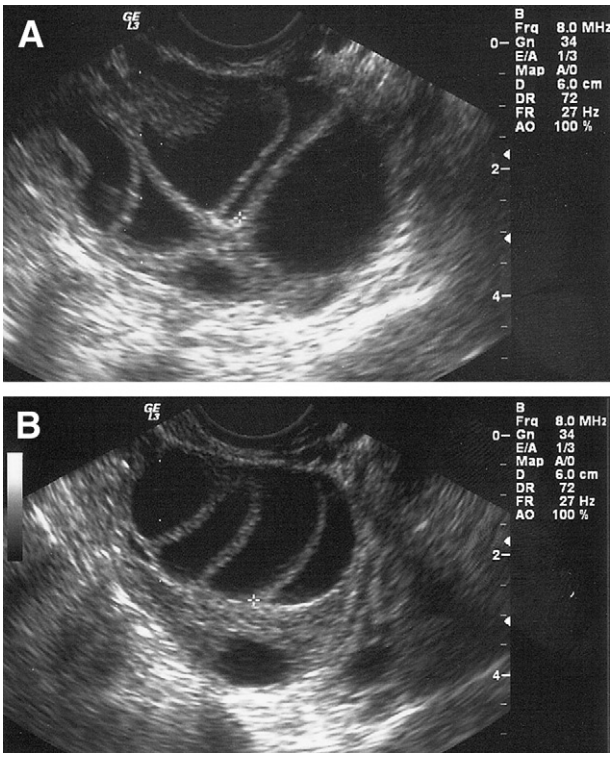
Transabdominal ultrasonography of the uterus and the ovaries 2 weeks after the last menstrual period. Both the right (**A,B**) and left (**C**) ovaries were normal in size and contained a total of 12 antral follicles. A normal-sized uterus had a trilaminar endometrial pattern of 11-mm thickness (**D**).



Oktay. Fertility preservation by ovarian stimulation. Fertil Steril 2010.

FIGURE 2

Transvaginal ultrasonography of the right (**A**) and left (**B**) ovaries at the time of oocyte retrieval during the first COH cycle. From a total of 11 follicles identified, eight had a mature-size of >17 mm.



Oktay. Fertility preservation by ovarian stimulation. Fertil Steril 2010.

METHODS

The Patient

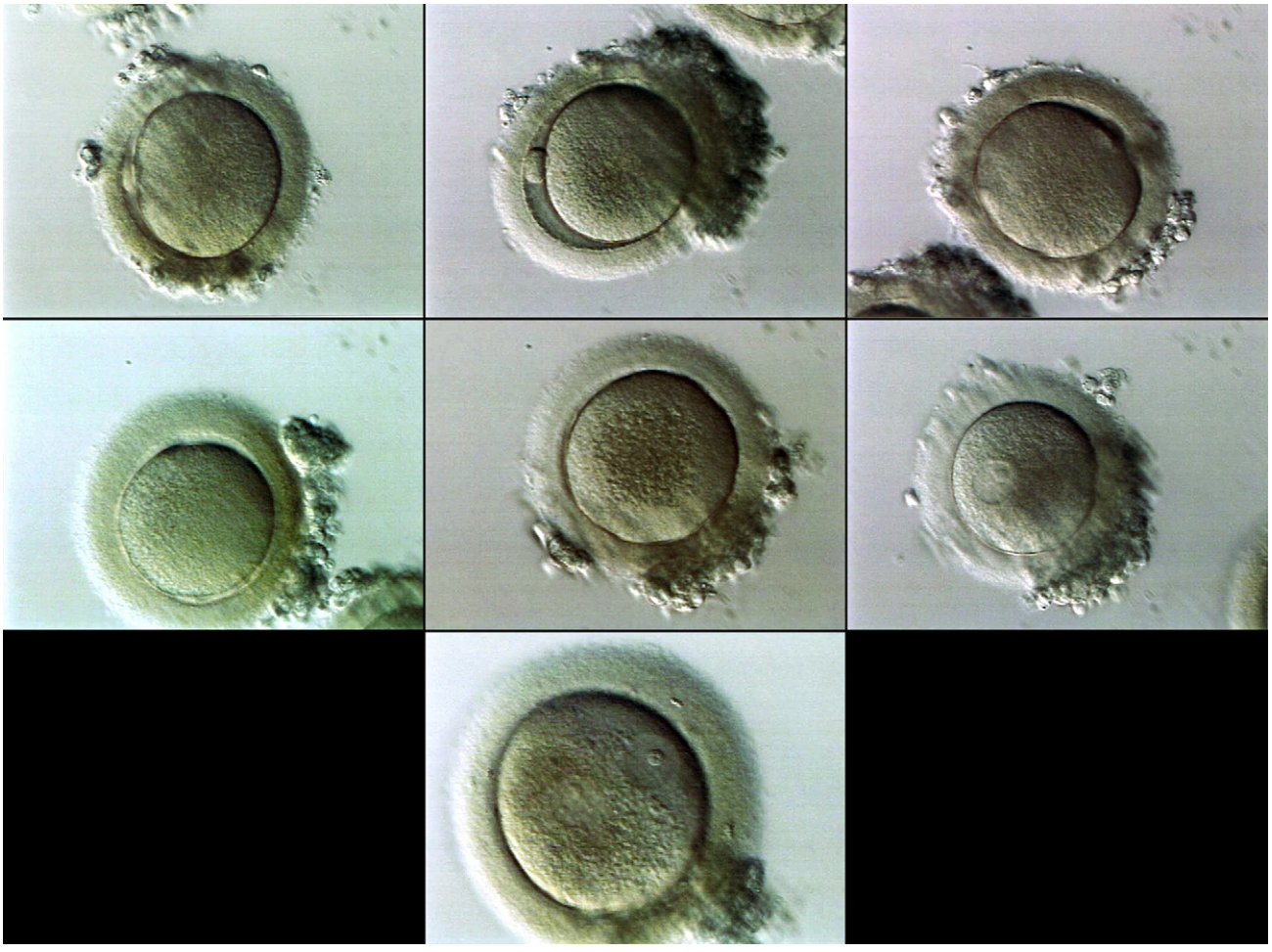
The patient was diagnosed with Turner syndrome at the age of 13. The endocrine investigations were initiated at the age of 12 because of several fractures after low-impact trauma. During early childhood, the patient was diagnosed with duplicated ureter that required surgery. The girl was otherwise healthy, and had presented with normal spontaneous pubertal development with menarche at 11 years of age. Menstrual periods were irregular, however, varying between 4 to 6 weeks and lasting 6 days. The psychosocial development was appropriate for age.

The physical examination revealed no obvious dysmorphic features, a 10th percentile height, 149.1 cm, and a weight of 48.8 kg. Her blood pressure was 130/70 mmHg and heart rate was 76 per minute. However, mild features associated with Turner syndrome such as low posterior hairline, mild neck webbing, multiple nevi, and short fourth metatarsals were later identified. The sexual development of this virginal patient was consistent with Tanner stage 4. The remainder of the physical examination was normal. The initial endocrine investigation revealed normal estradiol, testosterone, gonadotropins, and prolactin for age and Tanner stage, as well as normal thyroid function.

Total 25-Hidroxyvitamin D level was in the lower range, 28 ng/mL, indicating borderline vitamin D deficiency. Calcium, phosphorus, magnesium, parathyroid hormone, bone-specific alkaline phosphatase, and osteocalcin levels were all within normal range. DXA bone densitometry of the total body and spine were within normal limits for age and sex. Scoliosis with normal osseous matrix was demonstrated on roentgenogram, and the patient was recommended to initiate treatment with 1,300 mg/daily calcium and 400 IU/day vitamin D.

FIGURE 3

Aspect of the oocytes retrieved after the second COH cycle. Note that the oocyte in the last row has 2 GVs.



Oktay. Fertility preservation by ovarian stimulation. Fertil Steril 2010.

Cytogenetic analysis was carried out on stimulated lymphocyte cultures. The analysis of 20 cells revealed a normal female karyotype in 11 cells (55%) and an abnormal karyotype 45,X in 9 cells (45%), indicating mosaic Turner syndrome. After the cytogenetic confirmation of the diagnosis of Turner syndrome, the presence of cardiac and renal anomalies was investigated. A full cardiac evaluation with echocardiography was normal, and the renal ultrasound revealed a discrepancy in size between the kidneys but no significant hydronephrosis. Subsequently, the patient was referred by her pediatric endocrinologist to the Institute for Fertility Preservation, to be counseled for fertility preservation options.

Counseling

The patient and her parents received extensive counseling regarding fertility preservation with oocyte freezing and ovarian tissue freezing by means of laparoscopy, the potential risks, and the current available evidence regarding the potential benefits. Additional information was given regarding the need for repeated cardiac evaluations before attempting pregnancy and eventual need for genetic testing in case the patient would become pregnant with her own eggs. Counseling also included information on alternatives to become a parent, should ovarian failure occur, by IVF with oocyte donation or adoption. The patient and the parents expressed their interest in cryopreservation of oocytes as the method of choice and signed the research consent form approved by the institutional review board.

Gynecologic Evaluation and Pelvic Ultrasound

Since the menarche, the patient had irregular cycles every 10 to 60 days. The last menstrual period was 2 weeks before our gynecologic evaluation. Pelvic examination revealed an intact semiannular hymen. Pelvic ultrasound was performed transabdominally. The right ovary measured 31 × 23 × 20-mm and contained eight antral follicles (Fig. 1A and B) and the left ovary measured 22 × 38 × 13-mm and contained four antral follicles (Fig. 1C) for a total antral follicle count of 12. All follicles were <10-mm in diameter. Uterus measured 60 × 47 × 44-mm and the endometrium had a trilaminar pattern with 11 mm thickness, consistent with follicular phase of the cycle (Fig. 1D).

Ovarian Reserve Testing

The serum levels of FSH (5.3 mIU/mL) and LH (9.5 mIU/mL) were within normal range on the second day of the menstrual cycle. However, serum estradiol levels on day 2 were relatively high, 65.2 pg/mL, serum anti-Mullerian hormone (AMH, DSL Quest Diagnostics Inc., Webster, TX) relatively low, 0.9 ng/mL (reference 0–8 years 0.0–7.1 ng/mL, adult women 0.0–6.9 ng/mL), and serum Inhibin B (DSL Active Inhibin-B ELISA Quest Diagnostics Inc.) was <30 pg/mL (reference: premenopausal: <255 pg/mL, postmenopausal <30 pg/mL), indicating diminished ovarian reserve. Serum progesterone measured 1 week later on cycle day 21 was 1.49 ng/mL, indicating that the patient was anovulatory.

Controlled Ovarian Stimulation Protocol (COH)

Before the COH cycle, the patient was placed on a 4-week pretreatment of combined oral contraceptives (Loestrin). Gonadotropin stimulation began on the second day of the menstrual bleeding off the oral contraceptives with daily subcutaneous injections of 225 IU recombinant FSH (rFSH, follitropin alfa injection, Gonal-F, Serono, Geneva, Switzerland, 900 IU/1.5 mL). A GnRH antagonist Ganirelix acetate (Antagon, Organon Inc., West Orange, NJ, injection 250 μg/0.5 mL) was added daily from the sixth day of stimulation. Because of a fall in serum LH levels, 150 IU human menopausal gonadotropins was added (Menopur, 75 IU/vial Ferring) on the eighth stimulation, whereas rFSH dose was reduced by the same amount.

On day 10, peak estradiol level was 2,275 pg/mL, and the oocyte maturation was triggered with 1 mg Leuprolide acetate (Lupron, TAP Pharmaceuticals Inc., Forrest Lake, IL, injection 1 mg/0.2 mL). The patient underwent transvaginal oocyte retrieval under general anesthesia 36 hours later. Figure 2 shows the appearance of the ovaries by transvaginal ultrasound at the time of retrieval on day 12. Eleven oocytes were retrieved, of which 8 were mature (M-II) and 3 were immature (GV). All oocytes were cryopreserved by using MediCult Vitrification Cooling protocol. The patient recovered promptly after oocyte retrieval and ovarian hyperstimulation syndrome did not occur.

One year later the patient wished to undergo a second stimulation cycle for oocyte cryopreservation. Her periods had been very irregular and infrequent during the previous year. The patient was placed again on 5 weeks pretreatment of combined oral contraceptives and Serum AMH was 1.7 ng/mL at the end of that treatment. Gonadotropin stimulation began on the second day of the menstrual bleeding off the oral contraceptives with 225 IU rFSH + 150 IU of hMG daily. As estradiol levels remained low, rFSH dose was increased to 300 UI on the seventh day and GnRH antagonist was added on the ninth day. On day 14, peak estradiol level was 2,029 pg/ml, and the oocyte maturation was triggered with 1 mg Leuprolide acetate. Four mature and three immature oocytes were retrieved and cryopreserved by vitrification (Figure 3).

DISCUSSION

Historically, the predominant role of the pediatric and reproductive endocrinologists involved in the care of girls and young adolescents with Turner syndrome focused on the achievement of healthy pubertal development and on preparing them to adapt to premature ovarian failure and their implications (10). When ovarian failure occurred, women with Turner syndrome were offered IVF with egg donation (11) or adoption as an option. However, as has been demonstrated in patients suffering of chemotherapy-induced ovarian failure, many of these young patients prefer having a biologic offspring (12).

Fertility preservation is a rapidly evolving discipline extending beyond cancer patients (13). Means to preserve fertility in adolescent patients with Turner syndrome included ovarian tissue cryopreservation alone (4, 6) or in combination with in vitro maturation of immature oocytes from excised ovarian tissue (7). Although a large population of primordial follicles can be preserved in frozen pieces of ovarian cortex for later ovarian transplantation, this experimental method has only been described a decade ago (14) and needs further development (15).

Because oocyte cryopreservation does not require a laparoscopy, as in the case of ovarian tissue freezing, it is generally perceived as less invasive. Furthermore, the advent of vitrification techniques for oocyte cryopreservation has improved the pregnancy rates of oocyte freezing to a level approaching to those obtained in IVF with fresh oocytes (8, 9). Preliminary studies did not show an increase in adverse neonatal outcomes or congenital anomalies (15, 16), and the method is considered feasible in cancer patients (17).

However, the major drawback of oocyte cryopreservation in minors is that it requires them to undergo controlled ovarian stimulation and ooocyte retrieval and at least some degree of sexual and psychologic maturity is required. Thus, a detailed counseling of both the parents and the child is needed.

Because the rate of oocyte depletion may vary after birth in children with Turner syndrome, it may not be easy to determine how early to offer fertility preservation (6). Although our young patient presented with normal levels of FSH on cycle day 2, her AMH levels were similar to those observed in women in their late 30s or early 40s, indicating accelerated depletion of oocyte reserve, prompting us to act immediately. Based on the low AMH level, we stimulated the patient with relatively high doses of gonadotropins for her age during the first cycle. The patient's response was satisfactory by adult standards but less than that of expected from a 14-year-old with normal ovarian reserve. One year later, although she was stimulated with even higher gonadotropin doses for her second treatment cycle, the number of stimulated follicles and oocytes retrieved remained small, indicating a diminished ovarian reserve comparable to that of an older woman of reproductive age.

At this point, it is not possible to determine the probability of pregnancy with the cryopreserved oocytes as some of oocytes will be aneuploidic. Although women with mosaic karyotypes 45,X/46,XX or 45,X/47,XXX are more likely to be fertile (4, 6), spontaneous pregnancies have also been reported in women with nonmosaic Turner syndrome (2). Further on, controlled ovarian stimulation and in vitro fertilization has been reported with success in mosaic Turner with high grade of 45,X cells (18). Considering the improved success of oocyte cryopreservation with vitrification, the patient reported here has a realistic yet undetermined chance of conceiving in the future. The patient will. however, have to undergo either preimplantation genetic diagnosis and/or prenatal genetic evaluation once these oocytes are used for IVF because of an increased risk for aneuploidy in Turner patient's offspring (18–20).

Another important issue of concern for women with Turner who are considering childbearing in the future is the increased risk of maternal complications during pregnancy such as hypertension, diabetes, and Cesarean section because of fetopelvic disproportion (21). More importantly, because of the existence of aortic anomalies in up to 50% of these females (22), serious fatal complications such as aortic dissection and death have been described in Turner patients. Therefore, complete cardiovascular screening with periodic echocardiography should therefore be required. Any significant cardiovascular anomaly should be regarded as a contraindication to carry a pregnancy (23) and the patient should be offered gestational surrogacy.

Women diagnosed with Turner syndrome have rated infertility as a major concern affecting their quality of life (24). Therefore, fertility preservation counseling should be an integral part of the care of these patients. For patients with Turner syndrome, oocyte or ovarian tissue cryopreservation may give them a chance to have their genetic offspring.

REFERENCES

1. Sybert VP, McCauley E. Turner's syndrome. N Engl J Med 2004;351:1227–38.
2. Hovatta O. Pregnancies in women with Turner's syndrome. Ann Med 1999;31:106–10.
3. Abir R, Fisch B, Nahum R, Orvieto R, Nitke S, Ben Rafael Z. Turner's syndrome and fertility: current status and possible putative prospects. Hum Reprod Update 2001;7:603–10.
4. Hreinsson JG, Otala M, Fridström M, Borgström B, Rasmussen C, Lundqvist M, et al. Follicles are found in the ovaries of adolescent girls with Turner' syndrome. J Clin Endocrinol Metab 2002;87:3618–23.

5. Lau NM, Huang JY, MacDonald S, Elizur S, Gidoni Y, Holzer H, et al. Feasibility of fertility preservation in young females with Turner syndrome. Reprod Biomed Online 2009;18:290–5.
6. Borgstrom B, Hreinsson J, Rasmussen C, Sheikhi M, Fried G, Keros V, et al. Fertility preservation in girls with Turner syndrome: prognostic signs of the presence of ovarian follicles. J Clin Endocrinol Metab 2009;94:74–80.
7. Huang JY, Tulandi T, Holzer H, Lau NM, Macdonald S, Tan SL, et al. Cryopreservation of ovarian tissue and in vitro matured oocytes in a female with mosaic Turner syndrome: Case Report. Hum Reprod 2008;23:336–9.
8. Kuwayama M, Vajta G, Kato O, Leibo SP. Highly efficient vitrification method for cryopreservation of human oocytes. Reprod Biomed Online 2005;11:300–8.
9. Oktay K, Cil AP, Bang H. Efficiency of oocyte cryopreservation: a meta-analysis. Fertil Steril 2006;86:70–80.
10. Bondy CA. Turner syndrome 2008. Horm Res 2009;71(Suppl 1):52–6.
11. Foudila T, Söderström-Anttila V, Hovatta O. Turner's syndrome and pregnancies after oocyte donation. Hum Reprod 1999;14:532–5.
12. Schover LR, Rybicki LA, Martin BA, Bringelsen KA. Having children after cancer. A pilot survey of survivors' attitudes and experiences. Cancer 1999;86: 697–709.
13. Rodriguez-Macias Wallberg, Keros V, Hovatta O. Clinical aspects of fertility preservation in female patients. Pediatr Blood Cancer 2009;53:254–60.
14. Oktay K, Karlikaya G. Ovarian function after transplantation of frozen, banked autologous ovarian tissue. N Engl J Med 2000;342:1919.
15. Oktay K, Oktem O. Ovarian cryopreservation and transplantation for fertility preservation for medical indications: report of an ongoing experience. Fertil Steril 2008. Nov 13 [Epub ahead of print].
16. Chian RC, Huang JY, Tan SL, Lucena E, Saa A, Rojas A, et al. Obstetric and perinatal outcome in 200 infants conceived from vitrified oocytes. Reprod Biomed Online 2008;16:608–10.
17. Lee SJ, Schover LR, Partridge AH, Patrizio P, Wallace WH, Hagerty K, et al. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. J Clin Oncol 2006;24: 2917–31.
18. Manno M, Tomei F, Cervi M, Gaspardo G, Antonini-Canterin F, Nicolosi G. Homologous in vitro fertilization in Turner syndrome: insights from a case report. Fertil Steril 2009;91:1294.e1–4.
19. Tarani L, Lampariello S, Raguso G, Colloridi F, Pucarelli I, Pasquino AM, et al. Pregnancy in patients with Turner's syndrome: six new cases and review of literature. Gynecol Endocrinol 1998;12:83–7.
20. Bryman I, Landin-Wilhelmsen K, Innala E, Simberg N, Sylvén L, Windh M. Pregnancies in Swedish Turner women. Optimizing health care for patients in the 21st century. Proc 5th International Turner Symposium, Naples, Italy, 2000, p D4.
21. Bondy CA, Turner Syndrome Study Group. Care of girls and women with Turner syndrome: a guideline of the Turner Syndrome Study Group. J Clin Endocrinol Metab 2007;92:10–25.
22. Ho VB, Bakalov VK, Cooley M, Van PL, Hood MN, Burklow TR, et al. Major vascular anomalies in Turner syndrome: prevalence and magnetic resonance angiographic features. Circulation 2004;110: 1694–700.
23. Practice Committee of American Society for Reproductive Medicine. Increased maternal cardiovascular mortality associated with pregnancy in women with Turner syndrome. Fertil Steril 2008;90(Suppl):S185–6.
24. Sylvén L, Magnusson C, Hagenfeldt K, von Schoultz B. Life with Turner's syndrome—a psychosocial report from 22 middle-aged women. Acta Endocrinol (Copenh) 1993;129:188–94.