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ORIGINAL ARTICLE

Live birth after single euploid frozen embryo transfer in a 39-year-old woman with high-grade mosaic Turner syndrome

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ABSTRACT

Objective: To describe the reproductive and obstetric outcomes of an intracytoplasmic sperm injection cycle with preimplantation genetic testing for aneuploidy in an advanced reproductive-age woman with high-grade mosaic Turner syndrome.

Methods: Case report of a 39-year-old woman diagnosed with mosaic Turner Syndrome 45,X[90]/46,XX[10] karyotype who underwent *in vitro* fertilization treatment with blastocyst trophectoderm biopsy for preimplantation genetic testing using next-generation sequencing.

Result(s): Two of the four blastocysts biopsied were euploid. The patient achieved ongoing pregnancy after the first single euploid frozen embryo transfer, followed by the birth of a healthy child.

Conclusion: Autologous intracytoplasmic sperm injection cycles can be considered in a select group of advanced reproductive-age women diagnosed with high-grade mosaic Turner syndrome.

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Intracytoplasmic sperm injections; fertility preservation; Turner syndrome; chromosome aberrations; preimplantation diagnosis

Introduction

Turner syndrome is the most common disorder of sex chromosomes in women, occurring in approximately 1:2000 female phenotype newborns [1]. Cytogenetically, the Turner syndrome is characterized by sex chromosome monosomy (45,X). This karyotype is found in 50–60% of the cases. The other cases are mosaics with a 45,X cell line accompanied by others with two or more X chromosomes or with structural anomalies. Such structural anomalies of the X chromosome (isochromosomes of the long arm, dicentric chromosomes, deletion of the short arm or ring chromosomes) are present in approximately 30% of the cases, inhomogeneous karyotypes or in mosaics that include a 45,X cell line [2]. Finally, around 5% are accounted by patients with structural abnormalities of the Y chromosome (isochromosomes of the long arm and dicentric chromosomes) and mosaics that include a cell line accompanied by others with at least one Y chromosome, whether complete or not [3,4].

The broad clinical spectrum of Turner syndrome ranges from a classic appearance with many somatic stigmata, including short stature, webbed neck, and enlarged chest, to women without noticeable physical changes [5]. For this reason, Turner syndrome can be diagnosed at various stages of a woman's life.

The main reproductive effect of Turner syndrome is primary ovarian failure [6,7]. Although menarche occurs spontaneously in 15 to 30% of girls with Turner syndrome, only a few women with Turner syndrome can become pregnant with their oocytes, as most will have premature ovarian failure before trying to conceive. The prevalence of natural pregnancy in these women is only 2–7% [6–10].

Increased rates of miscarriage, fetal abnormalities, intrauterine growth restriction, low birth weight, and prematurity have been reported in women with Turner syndrome who became pregnant with their oocytes [11,12]. The higher risk of adverse obstetric outcomes can be attributed to fetal genetic

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abnormalities and an unfavorable uterine environment [8,13,14]. Several studies found that women with monosomy X or structural abnormalities of the X chromosome may produce gametes with the sex chromosome abnormalities, resulting in affected embryos and consequent miscarriage or offspring with Turner syndrome [15]. Several groups consider intracytoplasmic sperm injection (ICSI) treatment with preimplantation genetic testing for embryo selection for women with Turner syndrome who have preserved gonadal function and desire to become pregnant, enhancing pregnancy rates and decreasing the incidence of miscarriage [5].

We report the case of a 39-year-old woman diagnosed with high-grade mosaic Turner syndrome who had a live birth after a single euploid frozen embryo transfer in a cycle of ICSI with preimplantation genetic testing for aneuploidy (PGT-A).

Case report

Written informed consent for publication of her details was obtained from the patient. A 39-year-old G0P0 woman presented to our clinic with a recent diagnosis of high-grade mosaic Turner syndrome after karyotyping for preconception counseling purposes. Her husband was 35 years old, healthy, with no relevant findings in his medical history. The semen analysis was normal. The couple had not been diagnosed with infertility and had no children to date. They mentioned the desire for two-child family planning in the initial consultation.

The patient was diagnosed with Turner syndrome at 39 years of age. Despite her short stature, the patient had no other physical characteristics that motivated the investigation (i.e. webbed neck, enlarged chest, low-set ears, or short fingers and toes). Her parents were also short; therefore, this finding never motivated an investigation. The patient had spontaneous pubertal development and menarche at 12 years of age. The patient presented menstrual irregularity in the first two years after menarche, probably due to immaturity of the hypothalamic axis, with regular menstrual cycles after that period. She was placed on combined contraceptive pills from adolescence to age 39, stopping the method 3 months prior to the start of ovarian stimulation. G-banding karyotype of peripheral blood lymphocytes with 30 metaphases analyzed demonstrated 45,X[26]/46,XX [4]. This finding was confirmed with a 100-metaphase karyotype demonstrating 45,X[90]/46,XX[10], confirming the finding of high-grade mosaic Turner syndrome (Figure 1). For this reason, we considered treatment with ICSI with PGT-A.

Before starting treatment, the patient underwent an evaluation by a cardiologist as suggested by guidelines for women with Turner syndrome [16]. She underwent an echocardiogram, ultrasound with Doppler velocimetry of the carotid arteries, and an exercise stress test, all of which returned normal results. Ultrasound examination of the entire abdomen with an evaluation of kidneys and urinary tract revealed no abnormalities. Thyroid-stimulating hormone levels were normal, and thyroid antibodies were negative. The patient underwent ovarian reserve tests with an anti-Mullerian hormone level of 1.99 ng/mL and an antral follicle count of 16.

The patient underwent ovarian stimulation using a protocol with highly purified follicle-stimulating hormone (Fostimon®, IBSA, Switzerland) and menotropin (Merional®, IBSA, Switzerland). A gonadotropin-releasing hormone (GnRH) antagonist (Orgalutran®, Schering-Plough, United States of America) was also used daily during the follicular phase, starting when the dominant follicle reached 14 mm in mean diameter. Oocyte maturation was triggered with a GnRH agonist (Gonapeptyl daily®, Ferring, Germany) after 11 days of ovarian stimulation when at least two follicles were $>$ 18–20 mm in mean diameter. The control of ovarian stimulation, assessed through follicular growth, was performed using transvaginal ultrasound. Oocyte retrieval was performed under general anesthesia, guided by transvaginal ultrasound, without complications.

A total of 12 oocytes were retrieved, resulting in 10 mature oocytes. Intracytoplasmic sperm injection was performed using fresh semen collection from the partner, with discontinuous density gradient processing. Seven oocytes showed signs of complete fertilization with two pronuclei and two polar bodies. According to the Istanbul criteria, the blastocysts were analyzed by morphological characteristics [17]. Three day 5 (D5) blastocysts were biopsied and frozen (3BC, 3CC, 4AB), and one day 6 (D6) blastocyst was biopsied and frozen (5 CC). Trophectoderm cells were sent for PGT-A using next-generation sequencing (NGS). Two embryos were euploid (blastocysts D5 3BC and 4AB) and were kept frozen for implantation. Two embryos were aneuploid as a result of autosomal chromosomal aberrations and were discarded. We found trisomy of chromosome 16 in blastocyst D5 3CC and trisomy of chromosomes 16 and 20 in blastocyst D6 5CC.

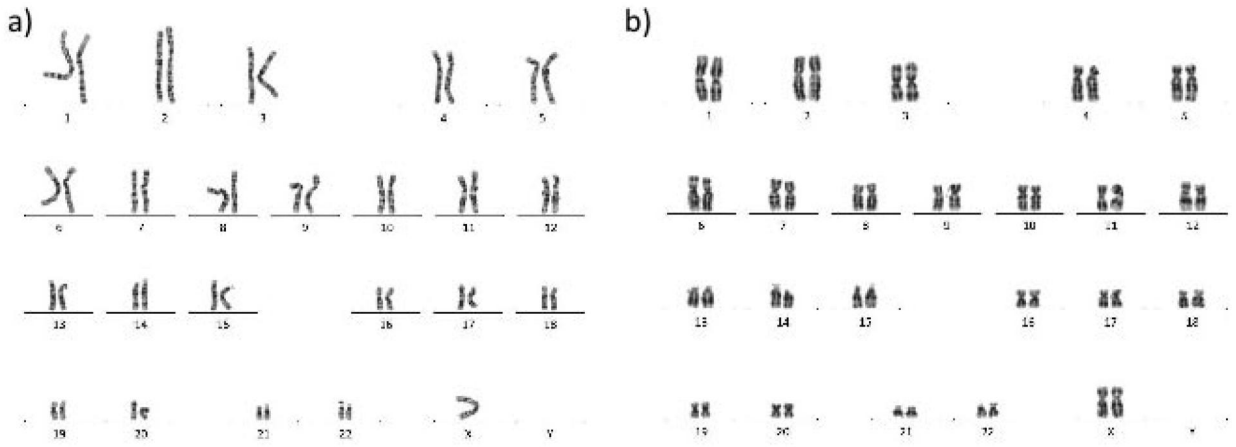

Figure 1. G-banding Karyotype. Mosaic karyotype with 90 metaphases with X chromosome monosomy (a) and 10 normal metaphases (b).

After the oocyte retrieval procedure, artificial endometrial preparation was started in the following menstrual cycle. On the second day of the menstrual cycle, we started using estradiol valerate 8 mg/d (Primogyna®, Bayer, Germany). The patient underwent a transvaginal ultrasound after 11 days of endometrial preparation, revealing an endometrial thickness of 7 mm. Her hormonal profile on this occasion revealed estradiol levels of 392.0 pg/mL and progesterone levels of 0.21 ng/mL. We introduced micronized progesterone vaginally at 1200 mg/d (Utrogestan®, Besins, Belgium), and after 5 days, we performed an uneventful transfer of the 4AB euploid blastocyst. Her hormonal profile on the day of embryo transfer demonstrated estradiol levels of 194.1 pg/mL and progesterone levels of 8.87 ng/mL. The patient achieved pregnancy after the first embryo transfer, diagnosed using a quantitative beta-human chorionic gonadotropin test measured 9 days after the transfer, with a result of 105.6 mIU/mL. We recommend maintaining estrogen therapy until 8 weeks of pregnancy and progesterone therapy until 12 weeks of pregnancy.

The patient presented for her first prenatal consultation at 6 weeks of pregnancy. At that time, the initial obstetric transvaginal ultrasound showed the presence of a single gestational sac with a single live embryo, a fetal heart rate of 121 bpm, and a yolk sac with a mean diameter of 3.4 mm. The initial course of pregnancy was adequate, without complications.

The patient underwent a morphological ultrasound examination in the first trimester, revealing the absence of anomalies in the fetus and a nuchal fold thickness measuring 1.2 mm. The nasal bone was adequately visualized, and the Doppler study indicated a positive a-wave in the ductus venosus. We suggested performing noninvasive prenatal testing; however, the patient refused. The second-trimester ultrasound morphological assessment performed at 21 weeks showed a morphologically normal fetus.

At 26 weeks of pregnancy, the patient underwent a 75-g oral glucose tolerance test that showed a normal result. A fetal echocardiogram revealed normal findings. On that occasion, she underwent another cardiological evaluation, with an echocardiogram and consultation with a cardiologist, without any clinically relevant changes. Fetal vitality tests were performed throughout the prenatal period with a systematic investigation of ultrasound markers (i.e. estimated fetal weight, degree of placental maturation, amniotic fluid index, and Doppler of the uterine, umbilical, and middle cerebral arteries) and cardiotocography.

The patient complained of spontaneous vaginal fluid loss at 36 weeks of pregnancy, and preterm premature rupture of membranes was diagnosed in the maternity emergency room. Due to maternal desire and increased risks related to the cardiovascular system, an uneventful cesarean delivery was performed, resulting in the birth of a male fetus, weighing 2210 grams, height = 46.5 cm, Apgar scores 8/10.

Discussion

We described the case of a patient of advanced reproductive age diagnosed with high-grade mosaic Turner syndrome who achieved live birth after a cycle of ICSI with PGT-A using NGS.

Although ICSI with PGT-A increases pregnancy rates and decreases the incidence of miscarriage, especially in couples with chromosomal abnormalities [5], very little is known about the karyotype of follicles and oocytes in women with Turner syndrome. The

presence of a completely non-mosaic 45,X karyotype in peripheral blood leukocytes does not exclude the coexistence of 45,X/46,XX mosaicism in the ovaries [18]. This information is essential to determine whether attempts at natural pregnancy or autologous cycles of assisted reproduction are a realistic option for this population. It is worth mentioning that a recent study demonstrated that most oocytes from follicles at an early stage of development in women with mosaic Turner syndrome are normal (91%) [19]. Similarly, recent data analysis from several studies generated the hypothesis that the primary cause of the origin of the 45,X or mosaic karyotype is delayed anaphase or other mitotic events, leading to the loss of a sex chromosome at an early conception diploid XX or XY [20].

Data from the literature show that a second sex chromosome is necessary for the fetus to survive, and therefore virtually every liveborn 45,X individual should present more than one cell karyotype line, thus constituting a mosaic. This condition would be necessary for at least some organs, during a certain period of embryogenesis [21]. This hypothesis is based on two main points: the frequency of sex chromosome mosaicism is much higher in liveborn infants with TS than in aborted fetuses; and an estimate that approximately 99% of the embryos with a pure 45,X karyotype die *in utero* [22].

Li et al. evaluated 394 women with X chromosome abnormalities of reproductive age who underwent IVF/ICSI treatment with or without PGT [23]. The authors found similar pregnancy rates between the groups. In patients who underwent PGT, only 8.73% of the analyzed embryos had chromosomal abnormalities of the X chromosome. Most of the chromosomal abnormalities found were in autosomal chromosomes (e.g. 22, 21, and 16). In our case report, the PGT-A showed 50% of the embryos with chromosomal aneuploidy. Similar to what was described by the authors of this study, the two aneuploid embryos had autosomal chromosome abnormalities. Thus, the X chromosome did not present a higher frequency of missegregation than the autosomal chromosomes.

Pregnancy in women with Turner syndrome is associated with higher maternal and fetal risks. The literature reports increased risks of thyroid gland disorders, diabetes, obesity, and hypertensive syndromes, including preeclampsia [16]. An adequate cardiological evaluation should be performed with screening for malformations of the cardiovascular system, such as coarctation of the aorta and bicuspid aortic valve. Measurement of the aortic size index as a risk marker for aortic dissection is essential in the preconception, pregnancy, and postpartum period. Particular attention should be paid to the development of hypertensive syndrome in pregnancy, which can occur in up to 40% of pregnancies. In addition, assessing thyroid hormones and a proper assessment of blood glucose levels is essential to decrease the risk of pregnancy complications.

Our patient had a favorable course throughout the prenatal period. Before starting treatment, preconception tests were requested, following the guidelines for patients diagnosed with Turner syndrome [16]. In addition, possible maternal and fetal risks were extensively discussed with the patient and her husband, and both demonstrated good adherence to all medical recommendations. The patient presented with spontaneous premature rupture of membranes at 36 weeks. Due to maternal desire and the increased risks related to the cardiovascular system, we chose to perform a cesarean delivery with the birth of a healthy child, who progressed well and did not require neonatal intensive care unit admission. The child was discharged to home with the parents.

Conclusion

Our case report demonstrates the possibility of achieving a live birth after performing an autologous cycle of ICSI with PGT-A by NGS in women with high-grade mosaic Turner syndrome and advanced maternal age. In our case, the rate of embryonic aneuploidy was not higher than that of women of the same age group. The aneuploid embryos did not show aberrations of sex chromosomes, only alterations of autosomal chromosomes. Further studies will be needed to determine the actual aneuploidy rate in this selected group of patients. Careful preconception evaluation is necessary, as these patients are at increased risk of underlying disease and adverse obstetric outcomes.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The authors acknowledge that the data supporting the findings of this study are available in the article and its supplementary materials.

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