Title

Pregnancy outcomes in female patients with X chromosome mosaicism after the first IVF/ICSI treatment: A 6-year retrospective cohort study

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Running title

X mosaicism female's outcomes after IVF/ICSI

Abstract

Objective: The present study focused on the clinical pregnancy and cumulative live birth rate (CLBR) of female patients with X chromosome mosaicism (XM) after the first in-vitro-fertilization (IVF)/intracytoplasmic sperm injection (ICSI) treatment, and the possible impact of different mosaic subtypes.

Design: Retrospective cohort study

Setting: Single Center study

Population: Infertility couples

Methods: In total, 76 couples with XM female partners and normal male partners were included (2014–2019) as the X group, with another 76 couples with normal karyotype included as the control group. Subgroup X1 included 41 45,X/46,XX cases, Subgroup X2 included 22 47,XXX/46,XX cases, and Subgroup X3 included 12 45,X/47,XXX/46,XX cases.

Main Outcome Measures: The ovarian stimulation, embryo results and pregnancy outcomes were analyzed.

Results: The X group presented similar CLBR but required a higher total gonadotropin (Gn) dosage than the control group (1800 IU vs. 1612 IU). Following subgroup analysis, the number of follicles during oocyte retrieval and average number of fertilized oocytes was lower in subgroup X1 than in X3. The clinical pregnancy rate and CLBR were similar in all groups.

Conclusion: Females with XM may present a similar CLBR until one year after oocyte retrieval, but may require a higher total Gn dosage. Females with 45,X cells

may recover fewer follicles during oocyte retrieval, resulting in fewer embryos. A higher 45,X cell ratio (over 5%) may lead to a lower CBLR.

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Keywords

X chromosome mosaicism, IVF/ICSI outcome, cumulative live birth rate

Tweetable abstract

Females with X chromosome mosaicism may present a similar cumulative live birth rate, but may require a higher total Gn dosage. More than 5% 45,X cell may affect CBLR.

Introduction

Karyotyping is widely used in the diagnosis of chromosomal diseases, allowing the detection of aneuploidy, structural variation, and mosaicism [1, 2]. Double X chromosomes are essential for female development [3-5].

A total or partial absence of one X chromosome will affect female health throughout life [6], affecting multiple organ systems with varying severity of clinical manifestations [7-11]. The specific comorbidities for women with Turner syndrome, including infertility, cardiac malformations, bone dysgenesis, and autoimmune diseases, may depend on a complex relationship between genes and transcriptional and epigenetic factors affecting gene expression across the genome [12]. Some manifestations could be modulated by medical treatment [9, 10], with no effective treatment for the diminished ovarian reserve (DOR). In the other direction, most women presenting 47,XXX mosaicism are reportedly fertile. Moreover, there may be a slightly increased risk for premature ovarian failure (POF).

For female somatic cells, one X chromosome is derived from the oocyte and the other from the sperm. XM could be caused by post-zygotic non-disjunction during the cleavage/blastula stage [13, 14]. Typically, the individual presents 45,X/47,XXX or 45,X/47,XXX/46,XX mosaicism. If one X chromosome is missed during mitosis, 45,X/46,XX or 47,XXX/46,XX (partial trisomy rescue) could develop. These variations could also be detected in females presenting infertility [15].

Despite undergoing in-vitro-fertilization (IVF) or intracytoplasmic sperm injection (ICSI), the impact of X chromosome mosaicism (XM) on outcomes of

assisted reproductive technology (ART) remains unclear[16, 17]. This study included 76 XM cases (X group) and 76 normal karyotype cases (control group). Their history, as well as embryo results of the first IVF or ICSI cycle, were collected. The cumulative live birth rate (CLBR) was followed until one year after oocyte retrieval

Methods

Study design and experimental subjects

This cohort study was conducted in couples experiencing infertility, who underwent their first IVF or ICSI cycle from June 1, 2014, to September 9, 2019. Cases using donor egg or donor sperm, as well as those with women aged ⩾45 years, were excluded.

The sample size was calculated according to Walters' normal approximation and Fisher's exact conditional test based on previous research data. In the present study, 76 XM cases were enrolled in the X group, in which the karyotype of the female partner was 45,X/46,XX , 47,XXX/46,XX , 45,X/47,XXX/46,XX or 45,X/47,XXX/48,XXXX/46,XX, and the karyotype of all the male partners was 46,XY. Additionally, 76 couples with normal karyotype were selected as the control group by SPSS 22.0 propensity score matching, according to the female age, female body mass index (BMI), insemination method, and ovarian stimulation protocol.

The history, examination results (Tables 1, 2, and 4), embryological data, and pregnancy outcomes (Tables 3 and 5) were analyzed.

The X group was further divided into subgroups (Table 1):

Subgroup X1 included 41 cases with 45,X/46,XX female;

Subgroup X2 included 22 cases with 47,XXX/46,XX female;

Subgroup X3 included 12 cases with 45,X/47,XXX/46,XX female.

Karyotyping

The protocol for peripheral blood lymphocyte karyotyping was followed as previously described [18]. The karyotype results were reported according to the International System for Human Cytogenomic Nomenclature 2016 (ISCN 2016). For each patient, no less than 50 metaphase cells were analyzed, with cells utilized from two separate culture bottles. The exact cell numbers for every type of karyotype were reported.

Ovarian stimulation, IVF/ICSI-ET, and follow-up

In the present study, the ovarian stimulation protocol employed has been previously described [19]. The initial gonadotropin dosage (Gn) depended on the female age, basic follicle-stimulating hormone (FSH), antral follicle count (AFC), and BMI. Follicle development was monitored by ultrasound examination and serum estradiol (E2) concentration.

The standard operating procedure and indications of oocyte retrieval, insemination method, embryo evaluation, and fresh embryo transfer (ET) have been previously detailed [18]. Pregnant patients visited the clinic every two weeks until 12 weeks of gestation, while others were contacted by phone call. The delivery data were collected by phone calls or through the inpatient information system. No cases were lost during follow-up in the present study.

Indexes of outcomes

To determine whether XM affects IVF and ICSI outcomes, the following indexes were analyzed: Gn days, Gn dosage, E2 on trigger day, follicular number during oocyte retrieval, average oocyte number, normal fertilization rate, cleavage rate, embryo utilization rate, fresh ET rate, clinical pregnancy rate (CPR), implantation rate, miscarriage rate before 12 weeks of gestation, and CLBR.

Statistical analysis

Statistical analyses were performed using SPSS 22.0 (IBM, Armonk, NY, USA). Differences with a two-sided P value less than 0.05 were considered statistically significant. Data are expressed as mean ± standard error of the mean (SEM). Missing values were ignored directly by default. As appropriate, Student's t-test or the non-parametric test (Mann-Whitney U test) was used to analyze numerical data, while Pearson's Chi-squared test or Fisher's exact test was used to analyze categorical data. The Kruskal-Wallis analysis or LSD one-way analysis of variance by ranks pairwise comparison was used to analyze multiple sets of data. Bonferroni correction was used as needed.

There was no patient involvement or public involvement in the design and conduct of this research.

Results

Baseline comparison

The X and control groups revealed similar female age, primary/secondary infertility ratio, the median duration of infertility, insemination method, height, BMI, basic FSH, ovarian stimulation protocol, times of gestation, and time of pregnancy

loss before IVF/ICSI treatment (Table 2).

The basic information was similar between subgroups X1, X2, and X3 (Table 4).

Ovarian stimulation data

Although Gn days and E2 levels on the trigger day were similar, with similar follicular numbers during oocyte retrieval, the Gn dosage was significantly higher in the X group than in the control group (1800 vs. 1612IU, P=0.028) (Table 3).

Embryological indexes

The X and control groups revealed similar outcomes during fertilization and embryo development (Table 3).

Following subgroup analysis, the number of follicles during oocyte retrieval, the average number of fertilized oocytes, and average cleavage number were significantly lower in subgroup X1 than in X3. The fertilization rate was considerably lower in subgroup X1 than in subgroup X2. The utilization rate was markedly higher in subgroup X1 than in subgroup X3(Table 5).

Pregnancy outcomes

The fresh ET rate, average ET number, implantation rate, CPR, miscarriage rate before 12 weeks of gestation, and CLBR were all similar between the X group and control group and were similar between subgroups X1, X2, and X3. However, the CLBR in X1 seemed lower (26.83%) (Table 3,5).

Based on the ratio of 45,X cell(s), the CLBR was calculated in subgroups (Table 6, one case did not report the ratio). In subgroup X1, the CLBR was 60% (3/5) when 45,X cells were less than 5%, 25.8% (8/31) when 45,X cells were 5%~10%, and

0%(0/4) when 45,X cells reached or exceeded 10%. In subgroup X3, the CLBR was 75%(3/4), 66.6%(2/3), and 25%(1/4). Although the differences were not statistically significant, a higher 45,X cell ratio seemed to result in a lower CLBR (Figure 1).

Discussion

Main findings

The cause of low-grade mosaicism for X aneuploidy

In the X group, the percentage of 46,XX cells ranged between 78.79% and 98.00%. In subgroup X1, the percentage of 45,X cells among the total cells ranged between 3.03% and 17.17%. In subgroup X2, the percentage of 47,XXX cells among the total cells ranged from 2.00 to 10.00%. In subgroup X3, the percentage of 45,X cells among total cells ranged between 2.00% and 12.00%, and the percentage of 47,XXX cells among total cells was 2.00%-10.00%.

A previous study has indicated that low-grade mosaicism for X chromosomal aneuploidy is an age-related phenomenon not associated with reproductive issues such as recurrent abortions [20], and these cases usually demonstrate a loss of one X chromosome, presenting 45, X/46,XX. One observational study has suggested that the time of blood sampling in relation to the menstrual cycle can influence lymphocyte XM. Physiological variations in blood hormone concentration, especially estrogen, might play a role in regulating the level of X chromosome aneuploidy[21].

In this study, we identified four types of mosaicism. Overall, 35 cases presented 47,XXX, with one 48,XXXX case. These cases could not be explained by previously

established rationales. The possibility of multiple anomalies occurring during leukocytogenesis or karyotyping is limited. Post-zygotic non-disjunction that causes X chromosome aneuploidy in daughter cells should be considered. It remains unclear whether this situation occurred randomly or owing to defects in gene functions.

The history and treatment of XM infertile female

All patients in the X group underwent spontaneous and complete puberty, with no secondary amenorrhea. Their age ranged from 27 to 44 years, and height from 150 to 172 cm. One patient presented cardiac malformations (interventricular septal defect) and underwent surgery during childhood. One patient presented a duplex uterus and duplex cervix. Spontaneous gestation and miscarriage histories were similar to those in the control group.

During ovarian stimulation, a higher Gn dosage was required in the X group. The number of follicles during oocyte retrieval was significantly lower in subgroup X1 than in X3. The average number of fertilized oocytes was significantly lower in subgroup X1 than in X3. Even with a higher Gn dosage, the number of follicles and oocytes were lower in subgroup X1. These results indicated that XM, especially the 45,X cell line, may affect follicular development and oogenesis.

The pregnancy outcomes in XM infertile female

The CLBR was similar between the X and control groups. This result indicated that XM may not affect embryo implantation and fetal development. However, subgroup X1 showed a tendency toward lower CLBR than other subgroups, and a higher 45,X cell ratio seemed to lead to lower CLBR. The 45,X cell line may affect

oogenesis and lead to fewer eggs, and in turn, fewer babies.

In the X group, one patient experienced preeclampsia during pregnancy, and another underwent premature delivery. No congenital abnormalities were detected in the resulting babies.

The genetic consultation of XM infertile female

Reportedly, infertile females with XM are not rare. Chromosomal abnormalities may affect the health and outcomes of assisted reproductive treatment. Genetic counselling should be offered to couples experiencing these chromosomal anomalies.

In patients with Turner syndrome, risks during pregnancy include aortic disorders, hepatic disease, thyroid disease, type 2 diabetes, and cesarean section delivery [3]. XM may cause various effects according to different karyotypes. The risk of POF and cardiac aortic disorders should be mentioned. Preimplantation genetic testing should be considered in cases of advanced maternal age, recurrent spontaneous abortion, or recurrent implantation failure, and prenatal diagnosis is highly recommended. Non-invasive prenatal testing should not be recommended owing to maternal mosaicism [20-22].

Strengths and limitations

The main strengths of our study are the relatively large study group, with follow-up until one year after oocyte retrieval. Subgroup analyses were conducted based on different mosaic cell types and ratios.

Mosaicism was not confirmed by fluorescent in situ hybridization (FISH) or comparative genome hybridization (CMA) of blood lymphocytes or samples from

other tissues. During blood sampling, the menstrual cycle phase was not recorded. Bone mineral density was not determined. Levels of the anti-Mullerian hormone (AMH) were not investigated. We failed to collect karyotype information of the babies or fetal tissue from spontaneous miscarriage.

Interpretation

Our findings may have clinical implications. During ovarian stimulation, a higher Gn dosage was required for XM female. The 45,X cell line may affect oogenesis and lead to fewer eggs and fewer babies. Genetic counselling should be offered to XM patients.

Conclusion

In summary, females with XM may present similar CPR and CLBR one year after oocyte retrieval. However, females with XM may require a higher Gn dosage during IVF/ICSI treatment. Females with 45,X/46,XX may encounter fewer follicles during oocyte retrieval, and in turn fewer embryos. Patients with XM associated infertility should receive genetic counseling before undergoing ART. A large cohort with long-term follow-up of the mothers and resultant babies should be undertaken.

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Disclosure of Interests

The authors declare no potential conflicts of interest with respect to the research,

authorship, and/or publication of this article.

Contribution to Authorship

XT planned the study. SL conducted the survey and submitted the study. XT and SL are responsible for the overall content as guarantors. JY regulated ovarian stimulation. HW did statistical work. SX conducted genetic counseling. MG rechecked karyotype. All authors read and approved the final manuscript.

Ethical Approval

This study was approved on July 5, 2019, by the Ethics Committee of Shanghai First Maternity and Infant Hospital (approval no. KS1965).

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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