IVF and ICSI outcomes of female patients with X chromosome mosaicism: A 5-year retrospective cohort study

Shanshan Liang
Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine
https://orcid.org/0000-0002-6787-4998
Jianzhi Yang
Shanghai First Maternity and Infant Hospital
Haixia Wu
Shanghai First Maternity and Infant Hospital
Shiyi Xiong
Shanghai First Maternity and Infant Hospital
Ming Guo
Shanghai First Maternity and Infant Hospital
Xiaoming Teng (tengxiaoming@hotmail.com)
Shanghai First Maternity and Infant Hospital, 2699 West Gaoke Road, Shanghai, China

Research

Keywords: X chromosome, mosaicism, IVF/ICSI, outcome

Posted Date: May 12th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-27374/v1

Abstract

Background

This study focused on the assisted reproductive treatment (ART) outcomes of female patients with X chromosome mosaicism (XM), who underwent their first IVF/ICSI and day 2 or day3 fresh embryo transfer, and the possible impacts of the different mosaic types.

Results

78 couples with XM female and normal male were included as the X group. 78 couples with normal karyotype were included as the control group. Subgroup X1 included 41 45,X/46,XX cases, Subgroup X2 included 23 47,XXX/46,XX cases, and Subgroup X3 included 13 45,X/47,XXX/46,XX cases. With similar female age and similar body mass index (BMI), the X group had higher total gonadotropin (Gn) dosage than the control group (1800 IU VS 1612 IU). In subgroup analysis, the follical number during oocyte retrieval was less in subgroup X1 than that in X2 or X3. The fertilization rate was lower in subgroup X1 than that in subgroup X2. The utilization rate was higher in subgroup X2 than that in subgroup X3. The implantation rate, clinical pregnancy rate, and miscarriage rate before 12 weeks' gestation were similar in all groups.

Conclusions

Female with 45,X cell line may face higher Gn dosage, less follical number during oocyte retrieval and fewer embryos. But female with X chromosome mosaicism may have similar clinical pregnancy rate and miscarriage rate after fresh embryo transfer.

Introduction

Karyotyping is widely used in the diagnosis of chromosome diseases. Aneuploidy, structure variation and mosaicism can be detected[1, 2]. Double X chromosomes are essential for female[3-5]. Karyotype of infertile female patient could be 46,XX , 45,X , 47,XXX or mosaic type of these[6]. Some cases need to undertake IVF or ICSI to get pregnancy[7]. Whether X chromosome mosaicism (XM) affect the IVF/ICSI outcome was unclear[7, 8]. This study included 78 XM cases. Their history and embryo results of the first IVF or ICSI cycle were collected. The pregnancy outcomes were followed till 12 weeks pregnancy.

Results

Karyotype details

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

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

Subgroup X2 included 23 cases with 47,XXX/46,XX female;

and Subgroup X3 included 13 cases with 45,X/47,XXX/46,XX female.

Table 1 Karyotype of female with X chromosome mosaicism (X group)

Group	Karyotype	case number
X1	45,X/46,XX	41
X2	47,XXX/46,XX	23
X3	45,X/47,XXX/46,XX	13
	45,X/47,XXX/48,XXXX/46,XX	1
X group	all above	78

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

Baseline comparison and stimulation data

The X group and the control group had similar female age, primary/secondary infertility ratio, median of infertility duration, insemination method, height, BMI, basic FSH, ovarian stimulation protocol, times of gestation and times of pregnancy loss before IVF/ICSI treatment (table 2).

Table 2 Baseline of X group and control group

Index	X group (n=78)	Control group (n=78)	P
Female age(ys)	34.0(33.0,38.0)	33.0(30.0,38.0)	0.154
Primary/secondary infertility	42/36	37/42	0.423
Median of infertility duration(ys)	2.00(2.00,4.00)	3.00(2.00,4.00)	0.195
Ovarian stimulation protocol			0.592
insemination			0.712
height(cm)	160(158,165)	162(158,165)	0.948
BMI	21.84±3.02	22.51±3.00	0.861
bFSH	7.10(5.90,9.17)	6.51(5.42,8.32)	0.089
Times of gestation			0.868
Times of pregnancy loss			0.195

Although Gn days and E_2 level on trigger day were similar, follical number during oocyte retrieval were also similar, Gn dosage was higher in X group than that in control group (table 4).

The basic information was similar between subgroup X1, X2 and X3(table 3).

Table 3 Baseline of subgroups of X group

Index	X1 (n=41)	X2 (n=23)	X3 (n=13)	P
Female age(ys)	35.22±3.97	34.96±3.86	34.00(30.50,36.00)	0.811
Primary/secondary infertility	21/20	11/12	9/4	0.433
Median of infertility duration(ys)	3.00(1.75,4.00)	2.00(2.00,3.00)	2.00(1.50,4.00)	0.827
Ovarian stimulation protocol				0.990
insemination				0.803
height(cm)	161.5±5.7	161.5±5.3	161.4±4.7	0.998
BMI	21.84±2.88	22.40±3.47	20.76±2.60	0.299
bFSH	7.51±2.59	7.32(5.98,8.87)	7.44±4.59	0.570
Times of gestation				0.251
Times of pregnancy loss				0.321

Group X1=45,X/46,XX; Group X2=47,XXX/46,XX Group X3=45,X/47,XXX/46,XX.

Embryological indexes

The X group and the control group had similar outcome during fertilization and embryo development(table 4).

In subgroup analysis, the follical number during oocyte retrieval was significant less in subgroup X1 than that in X2 or X3. The average fertilized oocyte number was significant less in subgroup X1 than that in X3. The fertilization rate was much lower in subgroup X1 than that in subgroup X2. The average cleavage number was much lower in subgroup X1 than that in subgroup X3. The utilization rate was much higher in subgroup X2 than that in subgroup X3(table 5).

Table 4 IVF/ICSI outcomes of X group and control group

Index	X group (n=78)	Control group (n=78)	P
Gn days	9(8,11)	10(8,11)	0.917
Gn dosage	1800(1350,2456)	1612(1200,2025)	0.034*
E2 on trigger day	2100(1079,2836)	1933(887,2845)	0.876
Follical number during oocyte retrieval	11(5,15)	10(5.75,20.25)	0.501
Oocyte number	571	617	
Average oocyte number	7.32±5.49	6.00(2.00,11.00)	0.756
Average fertilized oocyte number	4(2.25,6)	4(2,9)	0.483
Fertilization rate	401/571(70.23)	452/617(73.26)	0.246
Average normal fertilized oocyte number	3(1,4)	3(2,5)	0.291
Normal fertilization rate	267/571(46.76)	323/617(52.35)	0.054
Average cleavage number	4(2,6)	4(2,8)	0.416
Cleavage rate	378/401(94.26)	435/452(96.24)	0.173
Average usable number	2(1,3.75)	2(1,4)	0.770
Utilization rate(%)	208/378(55.03)	217/435(49.89)	0.143
Fresh embryo transfer cycle(%)	40/78(51.28)	39/78(50)	0.873
Average ET number	1(0,2)	2(2,2)	0.770
Implantation rate	24/64(37.5)	27/73(36.99)	0.951
Clinical pregnancy rate	19/40(47.5)	21/39(53.85)	0.573
miscarriage rate before 12 weeks' gestation	3/19(15.79)	4/21(19.05)	0.787

* P<0.05, the difference was considered statistically significant. Fisher’s exact test was used.

Pregnancy outcomes

Fresh embryo transfer rate, average ET number, implantation rate, clinical pregnancy rate, miscarriage rate before 12 weeks' gestation were all similar between X group and control group, and also similar between subgroup X1, X2 and X3(table 4,5).

Table 5 IVF/ICSI outcomes of subgroups of X group

Index	X1 (n=41)	X2 (n=23)	X3 (n=13)	P
Gn days	9.00(8.00,11.00)	9.00(8.00,12.25)	9.38±1.76	0.935
Gn dosage	2065±1140	1838(1341,2419)	1803±608	0.798
E2 on trigger day	2032±1428	2080±1223	2709(1908,2907)	0.311
Follical number during oocyte retrieval	8.68±4.83	13.35±8.87	16.08±9.44	0.002#
Oocyte number	238	208	124	
Average oocyte number	5(3,9)	9.04±7.38	9.54±5.11	0.044
Average fertilized oocyte number	3.0(2.0,5.0)	5.0(3.0,10.0)	6.0(4.5,10.5)	0.010#
Fertilization rate	151/238(63.45)	158/208(75.96)	91/124(73.39)	0.011#
Average normal fertilized oocyte number	2.00(1.00,4.00)	3.00(1.75,8.00)	4.31±2.98	0.165
Normal fertilization rate	112/238(47.06)	98/208(47.12)	56/124(45.16)	0.930
Average cleavage number	3(2,5)	4(3,10)	5(4,10)	0.016#
Cleavage rate	146/151(96.69)	144/158(91.14)	87/91(95.60)	0.095
Average usable number	2.0(1.0,3.0)	2.5(2.0,5.0)	2.0(1.5,3.5)	0.205
Utilization rate(%)	92/146(63.01)	79/144(54.86)	36/87(41.38)	0.006#
Fresh embryo transfer cycle (%)	20/41(48.78)	14/23(60.87)	5/13(38.46)	0.806
Average ET number	0(0,2)	1(0,2)	0(0,2)	0.585
Implantation rate	11/34(32.35)	9/21(42.86)	4/8(50)	0.289
Clinical pregnancy rate	9/20(45)	6/14(42.86)	4/5(80)	0.299
miscarriage rate before 12 weeks' gestation	3/9(33.33)	0/6(0)	0/4(0)	0.084

Group X1=45,X/46,XX; Group X2=47,XXX/46,XX Group X3=45,X/47,XXX/46,XX.

# P<0.0167, the difference was considered statistically significant. Fisher's exact test was used.

Discussion

Possible origin of X chromosome mosaicism

Normal female has two copies of X chromosome in all cells. One X chromosome is from the oocyte and the other is from the sperm. XM could be caused by meiosis and/or mitosis error during gamete genesis or in cleavage/ blastula stage[9, 10].The individual usually become 45,X/47,XXX or 45,X/47,XXX/46,XX. If one X chromosome was missed in mitosis, 45,X/46,XX or 47,XXX/46,XX (partial trisomy rescue)could be detected.

Also, previous study indicated that low-grade mosaicism for X aneuploidy was an age-related phenomenon not related to reproductive issues such as recurrent abortions[11]. But these cases usually have one X chromosome loss and become 45,X/46,XX.

One observational study indicated that the time of blood sampling in relation to the menstrual cycle can influence lymphocyte X chromosome mosaicism. In the case group (containing 28 women with X mosaicism and recurrent pregnancy loss), the mean value of aneuploid cells in the follicular and luteal phase samples was 10.0 and 6.3 % respectively and in the control group, it was 2.8 and 1.0 % ($P < 0.0001$). Estrogens are known to selectively influence cell proliferation. Physiological variations of blood hormone concentration might play a role in regulating the level of X chromosome aneuploidy[12].

In this study, we found 4 types of mosaicism, and the percentage of 46,XX cells in peripheral blood lymphocytes was no less than 78%. The menstrual cycle condition of blood sampling was not recorded. Anti-Mullerian hormone (AMH) was not tested. The percentage of every cell line in various tissues were difficult to reveal. The mosaicism was not confirmed by fluorescent in situ hybridization (FISH) or comparative genome hybridization (CMA) of blood lymphocytes or sample from other tissue.

Clinical history of X chromosome mosaicism

A total or partial absence of one X chromosome will affect female health in the whole life[13]. It affects multiple organ systems and the severity of clinical manifestations varies[14-18]. Some manifestations could be adjusted by medical treatment[16, 17]. Some female without symptoms or with mild abnormalities could get married and become infertile.

In this study, all patients in X group had spontaneous and complete puberty. The height ranged from 150 to 172 cm. The age ranged from 27 to 48 years old. One patient had cardiac malformations (interventricular septal defect) and had the operation in her childhood. One patient had uterus duplex and cervix duplex. Bone mineral density was not tested. Spontaneous gestation and miscarriage history seemed similar with control group.

During ovarian stimulation, more Gn were needed for X group. The follical number during oocyte retrieval was significant less in subgroup X1 than that in X2 or X3. The average fertilized oocyte number was significant less in subgroup X1 than that in X3. These results indicated that XM, especially 45,X cell line may affect follicular development and oocyte genesis.

The fertilization rate was lower in subgroup X1 than that in subgroup X2. The mechanism was unclear.

Pregnancy outcome after fresh embryo transfer seemed similar in X group and control group. In X group, one patient had preeclampsia during pregnancy, one patient had premature delivery. No congenital abnormality was found in the resulting babies. We failed to collect the karyotype information of the babies or the fetal tissue from spontaneous miscarriage. The cumulative live birth rate was not analyzed.

Genetic consult

Infertile female with mosaic X chromosome was not rare. The chromosome abnormality may affect the health and outcome of assistant reproductive treatment. Genetic consult should be offered to the couple.

The specific comorbidities for women with Turner syndrome such as infertility, cardiac malformations, bone dysgenesis, and autoimmune diseases may depend on a complex relationship between genes as well as transcriptional and epigenetic factors affecting gene expression across the genome[19]. Risks during pregnancy include aortic disorders, hepatic disease, thyroid disease, type 2 diabetes, and cesarean section delivery[3]. Mosaic X chromosome may have various effects according to different karyotype. The risk of premature ovarian failure and cardiac disorders should be mentioned.

Non-invasive prenatal testing (NIPT) shouldn't be recommended to them because of the maternal mosaicism[20-22].

Conclusion

Female with mosaic X chromosome (XM) may need more Gn to get similar amount of oocyte. 45,X/46,XX female may face less follical number during oocyte retrieval and fewer embryos. But XM female may have similar clinical pregnancy rate and miscarriage rate after fresh embryo transfer. XM infertile patients should get genetic consult before assistant reproductive treatment. Healthy condition of the mother and baby should be followed.

Methods

Study design and experimental subjects

This cohort study was conducted in infertile couples, who underwent their first IVF or ICSI cycle from June 1, 2014, to September 9, 2019. Every couple was assigned a unique sequence number when they decided to undergo IVF/ICSI treatment. Sample size was calculated according to Walters normal approximation and Fisher's exact conditional test based on previous research data. This study included 78 cases as the

X group. In the X group, the karyotype of 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, the karyotype of all male partners were 46,XY. 78 couples with normal karyotype were selected to be the control group by SPSS 22 propensity score matching, according to the female age, female BMI, the insemination method and the ovarian stimulation protocol. Donor cycles were excluded from this study. The history, examination results (table 1,2 and 4), embryological data and pregnancy outcomes (table 3 and 5) were analyzed.

Karyotyping

The protocol for peripheral blood lymphocytes karyotyping has been described elsewhere [9]. Karyotype and chromosome polymorphisms were reported according to the International System for Human Cytogenomic Nomenclature (ISCN). For each patient, no less than 50 metaphase cells were analyzed, and these cells must come from two culture bottles. The exact cell numbers of every kind of karyotype were reported.

Ovarian stimulation and IVF/ICSI-ET

The ovarian stimulation protocol has been described elsewhere [10]. The initial dosage of gonadotrophin depended on the female partner's age, basal follicle stimulating hormone (FSH), antral follicle count, and BMI. The development of follicles was monitored by ultrasound examination and serum E_2 concentration.

The standard operation procedure and indications of oocyte retrieval, insemination method, embryo evaluation, fresh embryo transfer have been described elsewhere [9]. Pregnant patients visited the clinic every two weeks till 12 weeks' gestation, while others were contacted by phone call with nurses. There was no loss of follow-up case in this study.

Indexes of Outcomes

To explore whether XM affects the IVF and ICSI outcome, indexes were analyzed, such as Gn days, Gn dosage, E_2 on trigger day, follical number during oocyte retrieval, average oocyte number, normal fertilization rates, cleavage rates, embryo utilization rates, fresh embryo transfer rates, clinical pregnancy rates (CPR), implantation rates, miscarriage rate before 12 weeks' gestation.

Statistical analysis

Statistical analyses were performed with SPSS 22 software (IBM, Armonk, NY, USA). A difference with a two-sided P value less than 0.05 was considered statistically significant. Data are expressed as mean ± S.E.M. Missing values were ignored directly by default. Where appropriate, Student's t-test or the non-parametric test (the 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 one-way analysis of variance by ranks Pairwise Comparisons was used to analyze multiple sets of data. Bonferroni correction was used when needed.

Abbreviations

IVF: in vitro fertilization

ICSI: intracytoplasmic sperm injection

XM: X chromosome mosaicism

ART: assisted reproductive treatment

ET: embryo transfer

BMI: body mass index

Gn: gonadotropin

FSH: follicle stimulating hormone

NIPT: non-invasive prenatal testing

SPSS: statistical program for social sciences

ISCN: International System for Human Cytogenomic Nomenclature

CPR: clinical pregnancy rates

Declarations

Ethics approval and consent to participate

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

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by National Key R&D Program of China (2016YFC1000602) in the design of the study and collection of data.

Authors' contributions

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.

Acknowledgements

We thank all the patients, clinicians, embryologists, nurses and colleagues involved in our study.

References

1. Ghosh, S., et al., *Cytogenetic analysis of patients with primary amenorrhea in Eastern India.* J Obstet Gynaecol, 2018. **38**(2): p. 270-275.
2. Berglund, A., et al., *Changes in the cohort composition of turner syndrome and severe non-diagnosis of Klinefelter, 47,XXX and 47,XYY syndrome: a nationwide cohort study.* Orphanet J Rare Dis, 2019. **14**(1): p. 16.
3. Folsom, L.J. and J.S. Fuqua, *Reproductive Issues in Women with Turner Syndrome.* Endocrinol Metab Clin North Am, 2015. **44**(4): p. 723-37.
4. Raznahan, A., et al., *Sex-chromosome dosage effects on gene expression in humans.* Proc Natl Acad Sci U S A, 2018. **115**(28): p. 7398-7403.
5. Sangrithi, M.N. and J.M.A. Turner, *Mammalian X Chromosome Dosage Compensation: Perspectives From the Germ Line.* Bioessays, 2018. **40**(6): p. e1800024.
6. Liu, X.Y., et al., *45,X mosaicism in northeast China: a clinical report and review of the literature.* J Assist Reprod Genet, 2013. **30**(3): p. 407-12.
7. Doger, E., et al., *Reproductive and obstetric outcomes in mosaic Turner's Syndrome: a cross-sectional study and review of the literature.* Reprod Biol Endocrinol, 2015. **13**: p. 59.
8. Giang, H., et al., *Mosaic Turner syndrome shows reduced penetrance in an adult population study.* Prenat Diagn, 2018.
9. Garcia-Mengual, E., et al., *Male infertility: establishing sperm aneuploidy thresholds in the laboratory.* J Assist Reprod Genet, 2019. **36**(3): p. 371-381.
10. Muzinic, V., S. Ramic, and D. Zeljezic, *Chromosome Missegregation and Aneuploidy Induction in Human Peripheral Blood Lymphocytes In vitro by Low Concentrations of Chlorpyrifos, Imidacloprid and alpha-Cypermethrin.* Environ Mol Mutagen, 2019. **60**(1): p. 72-84.
11. Jelsig, A.M., K. Brondum-Nielsen, and A.Z. Tumer, *X-chromosome loss can be an age-related phenomenon in women.* Ugeskr Laeger, 2018. **180**(23).

12. Gersak, K., et al., *Possible influence of menstrual cycle on lymphocyte X chromosome mosaicism.* J Assist Reprod Genet, 2015. **32**(1): p. 111-6.
13. Cameron-Pimblett, A., C. La Rosa, and T.F.J. King, *The Turner syndrome life course project: Karyotype-phenotype analyses across the lifespan.* 2017. **87**(5): p. 532-538.
14. Le Gall, J., et al., *Sex chromosome aneuploidies and copy-number variants: a further explanation for neurodevelopmental prognosis variability?* Eur J Hum Genet, 2017. **25**(8): p. 930-934.
15. Hong, D.S., et al., *Influence of the X-chromosome on neuroanatomy: evidence from Turner and Klinefelter syndromes.* J Neurosci, 2014. **34**(10): p. 3509-16.
16. Oktay, K., et al., *Fertility Preservation in Women with Turner Syndrome: A Comprehensive Review and Practical Guidelines.* J Pediatr Adolesc Gynecol, 2016. **29**(5): p. 409-416.
17. Bucerzan, S., et al., *Clinical and genetic characteristics in a group of 45 patients with Turner syndrome (monocentric study).* Ther Clin Risk Manag, 2017. **13**: p. 613-622.
18. Bois, E., et al., *Otologic disorders in Turner syndrome.* Eur Ann Otorhinolaryngol Head Neck Dis, 2018. **135**(1): p. 21-24.
19. Viuff, M., et al., *Epigenetics and genomics in Turner syndrome.* Am J Med Genet C Semin Med Genet, 2019. **181**(1): p. 68-75.
20. Fleddermann, L., et al., *Current genetic counseling practice in the United States following positive non-invasive prenatal testing for sex chromosome abnormalities.* J Genet Couns, 2019.
21. Suo, F., et al., *Non-invasive prenatal testing in detecting sex chromosome aneuploidy: A large-scale study in Xuzhou area of China.* Clin Chim Acta, 2018. **481**: p. 139-141.
22. Kim, S.C., et al., *Clinically significant maternal X chromosomal copy number variation detected by noninvasive prenatal test.* J Obstet Gynaecol Res, 2019.