Human Reproduction, Vol.36, No.9, pp. 2612–2621, 2021
Advance Access Publication on July 27, 2021 doi:10.1093/humrep/deab177

human reproduction

ORIGINAL ARTICLE *Reproductive genetics*

Preimplantation genetic testing is not a preferred recommendation for patients with X chromosome abnormalities

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Submitted on February 12, 2021; resubmitted on June 22, 2021; editorial decision on June 30, 2021

STUDY QUESTION: Should women with X chromosome abnormalities (XCAs) be recommended to have embryos selected by both morphological and cytogenetic assessment through preimplantation genetic testing (PGT) rather than morphological assessment only in conventional IVF/ICSI treatment?

SUMMARY ANSWER: PGT is not a preferred recommendation for women with XCAs in the absence of other PGT indications.

WHAT IS KNOWN ALREADY: XCAs are the most frequent sort of chromosomal aberrations in infertile women. Patients with a complete or partial absence of one X chromosome, diagnosed as Turner Syndrome (TS), demonstrate low spontaneous pregnancy rates (5–7%) and high miscarriage rates (22.8–30.8%), as well as high chances of birth defects (20%). PGT is known to improve pregnancy rates and decrease the incidence of miscarriage in couples with chromosomal aberrations such as Robertsonian and reciprocal translocations and Klinefelter Syndrome.

STUDY DESIGN, SIZE, DURATION: A retrospective cohort study was conducted with 394 women with XCAs and undergoing their first oocyte retrieval and first embryo transfer cycle from June 2011 to August 2019 in the Reproductive Hospital Affiliated to Shandong University.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Pregnancy outcomes were compared between the conventional IVF/ICSI group (n = 284) and the PGT group (n = 110) in the first fresh or frozen embryo transfer cycle for each woman with XCAs. Three platforms were applied in PGT: fluorescence *in situ* hybridisation (FISH, n = 34), array comparative genomic hybridisation (aCGH, n = 24) and next-generation sequencing (NGS, n = 51). The embryo aneuploidy rate and distribution of embryonic chromosomal aberrations revealed by aCGH or NGS were analysed and stratified by maternal age and type of XCAs to assess the effect of maternal XCAs on embryo karyotypes.

MAIN RESULT AND THE ROLE OF CHANCE: The live birth rate (LBR) per embryo transfer was similar between the PGT group and IVF/ICSI group both in the first cycle of fresh or frozen embryo transfer respectively (39.13% in PGT_{FISH} vs 42.58% in IVF/ICSI, P_{adj}=0.558; 66.67% in PGT_{FISH} vs 52.08% in $PGT_{aCGH/NGS}$ vs 53.06% in IVF/ICSI, P_{adj}=0.756), as was the clinical pregnancy rate (60.87% in PGT_{FISH} vs 50.97% in IVF/ICSI, P_{adj} =0.672; 88.89% in PGT_{FISH} vs 58.33% in $PGT_{aCGH/NGS}$ vs 69.39% in IVF/ICSI, P_{adj} =0.480) and the pregnancy loss rate (35.71% in PGT_{FISH} vs 16.46% in IVF/ICSI, P_{adj} =0.136; 12.50% in PGT_{FISH} vs 10.71% in $PGT_{aCGH/NGS}$ vs 23.53% in IVF/ICSI, P_{adj} =0.352). The rates of maternal and neonatal complications were also comparable between the PGT and IVF/ICSI groups with fresh and frozen transfers respectively (10.00% vs 8.85%, P = 1.000; 21.74% vs 14.55%, P = 0.272). Intriguingly, the distribution of embryonic chromosome abnormalities was more frequent on autosomes 22 (20.39%), 21 (18.45%) and 16 (17.47%), compared with the X chromosome (8.73%).

[†]These authors contributed equally to this article.

LIMITATIONS, REASONS FOR CAUTION: Selection bias is an inherent drawback of a retrospective study. First, our participants hosted 4.84% X chromosome mosaicism with few typical somatic anomalies of TS. Second, the incidences of history of recurrent miscarriage and abnormal offspring in the PGT group were higher than in IVF/ICSI group although binary logistic regression analysis was performed to attenuate the modifying effect of confounding factors. Third, FISH performed in this study only used X/Y probes and lacked the reference of autosome, which might have resulted in misdiagnosis and bias. Finally, intrinsic disadvantages could not be totally avoided due to the retrospective nature of this study.

WIDER IMPLICATION OF THE FINDINGS: In the current study, comparable pregnancy outcomes were revealed among a large cohort of women with XCAs undergoing their first cycles of PGT or conventional IVF/ICSI treatment. Moreover, the X chromosome abnormality was illustrated to cause no higher frequency of aberrations in embryos. Our data provided perspectives for genetic and reproductive counselling to XCAs individuals and their families.

STUDY FUNDING/COMPETING INTEREST(S): This work was supported by National Research and Development Plan (2016YFC1000604 and 2017YFC1001100), the National Natural Science Foundation of China (81701406), Shandong Science Fund for Distinguished Young Scholars (JQ201720), Taishan Scholars Program for Young Experts of Shandong Province (tsqn20161069) and Projects of Medical and Health Technology Development Program in Shandong Province (202005010520, 202005010523 and 2016WS0368). There is no conflict of interest to declare.

TRIAL REGISTRATION NUMBER: N/A.

Key words: X chromosome abnormalities / preimplantation genetic testing / pregnancy outcomes / aneuploidy / IVF/ICSI

Introduction

X chromosomes play a pivotal role for female somatic development and ovarian maintenance (Ostan *et al.*, 2016). X chromosome abnormalities (XCAs), including numerical and structural aberrations, are the most common type of chromosomal aberrations in women with reproductive difficulties (Zachaki *et al.*, 2020). Women with a complete or partial absence of one X chromosome are defined as having Turner Syndrome (TS). The chance of spontaneous pregnancy in TS women is merely 5–7%, whereas the consequent miscarriage rate is up to 22.8–30.8% (Bernard *et al.*, 2016; Cadoret *et al.*, 2018; Gravholt *et al.*, 2019). Furthermore, congenital deformations, such as heart malformations, Down's Syndrome and Turner Syndrome, account for even 20% of their conceptions (Tarani *et al.*, 1998). The incidence of mosaic 45, X, such as 45, X/46, XX and 45, X/46, XX/47, XXX, reaches 14% in infertile females (Zachaki *et al.*, 2020) but its influence on pregnancy outcomes and neonatal complications remains uncertain.

With the rapid development of ART, preimplantation genetic testing (PGT) is widely accepted to enhance pregnancy rates and decrease the incidence of miscarriage, particularly in couples with chromosomal aberrations, such as Robertsonian and reciprocal translocations and Klinefelter Syndrome (Group *et al.*, 2020). Recently, the live birth rate (LBR) per transfer was revealed to be 22.5% for TS individuals after PGT ($n = 56$), indicating that PGT could be considered as a promising solution for TS patients (Giles *et al.*, 2020). However, due to the insufficient sample sizes in limited studies without controls for comparison, no consensus has been reached about the necessity and superiority of PGT for XCAs, especially mosaic TS.

In this study, in total 394 women with XCAs were enrolled to compare the pregnancy outcomes from their first cycles of PGT or conventional IVF/ICSI treatments. The embryo aneuploidy rate and distribution of embryonic chromosomal aberrations revealed by PGT were also analysed and stratified by maternal age and type of XCAs to assess the effect of XCAs on embryo karyotypes.

Materials and methods

Ethics approval

The study was approved by the Ethics Committee of Reproductive Medicine of Shandong University. Written informed consent was obtained from each participant.

Study design and participants

This is a single-centre retrospective cohort study. Cytogenetic analysis was conducted on peripheral blood samples by G-banding according to standard laboratory protocols. Initially 20 metaphases of peripheral blood were counted. Once abnormalities were detected, the number of metaphases was enlarged to 100. At least three cells with 45, X or two cells with 47, XXX or other numerical XCAs were regarded as a cell line. The level of karyotype mosaicism was defined as the percentage of aneuploidy out of metaphases counted in total. In total 394 women with XCAs were recruited, and underwent their first oocyte retrieval cycle and first embryo transfer cycle, from June 2011 to August 2019, in the Reproductive Hospital Affiliated to Shandong University in China. With full understanding of the effect of their karyotype, age and reproductive history, as well as the benefits and potential risks of PGT after one-to-one genetic counselling provided by professional genetic counsellors, couples chose to undergo PGT with embryos selected by both morphology and cytogenetic outcomes or IVF/ICSI treatment with embryos assessed only by morphological parameters. Eventually, 284 participants underwent conventional IVF/ICSI cycles and 110 women had IVF/ICSI with PGT. Three platforms were applied in PGT, including fluorescence in-situ hybridisation (FISH), array comparative genomic hybridisation (aCGH) and next-generation sequencing (NGS). Subgroup analysis was performed according to female ages ($\leq$30, 31–37 and $\geq$38 years old) (La Marca *et al.*, 2011; Ke *et al.*, 2020). The aneuploidy rate and abnormal chromosome distribution of embryos were also analysed.

Methods

The ovarian stimulation protocols were based on physicians' experiences and previous studies (Chen et al., 2016; Wei et al., 2019). For patients in IVF/ICSI group, one or more embryos were transferred on Day 3 or Day 5 after oocyte retrieval, while supernumerary embryos were frozen on Day 5 or 6 according to embryo development. For patients receiving FISH, embryo biopsy was performed on Day 3 with specific probes detecting the X chromosome, and the properly developed embryos with normal X chromosome were transferred or vitrified on Day 5 or 6. Trophectoderm biopsy was performed on Day 5 or 6 for aCGH or NGS and all euploid embryos were frozen.

Assisted reproductive outcomes

Live birth, defined as the delivery of at least one live infant after 28 weeks of gestation, was the primary outcome. Secondary outcomes included clinical pregnancy (presence of gestational sac in the uterine cavity), early pregnancy loss (miscarriage within the first 12 weeks of gestation), late pregnancy loss (miscarriage between 13 and 28 weeks of gestation) and maternal and neonatal complications (including gestational hypertension, birth defects and so on). Birth defects were evaluated according to the International Classification of Diseases, 10th edition (ICD-10) (Yu et al., 2018; Ke et al., 2020).

Embryo biopsy outcomes

The protocols of embryo biopsy were concretely depicted previously (Wu et al., 2014; Sachdeva et al., 2017; Jiang et al., 2018). For embryos biopsied for FISH, two signals were considered as normal while a single signal or more than two signals for each chromosome pair tested were considered abnormal. A report of 'undetermined' inferred that the sample failed to yield explanatory outcomes. For embryos analysed with the use of aCGH or NGS, results were reported as 'euploidy', indicating that the embryo was eligible for transfer, or 'aneuploidy', indicating that monosomy, trisomy or other chromosomal abnormalities were detected. Mosaicism was ascertained for mixed euploidy/aneuploidy cells.

Oocyte utilisation rate

The oocyte utilisation rate was calculated to evaluate how efficiently the oocytes were utilised in generating usable embryos, with the definition of the proportion of embryos suitable for transfer or for cryopreservation among the retrieved oocytes (Meniru and Craft, 1997; Patrizio and Sakkas, 2009; Yovich et al., 2016). In the IVF/ICSI group, transferable embryos were only based on morphological criteria (Puissant et al., 1987; Gardner et al., 2000) while both morphological scores and PGT outcomes were taken into consideration in PGT group.

X Chromosome loss and ageing

Since X chromosome loss is reported to be age-related (Russell et al., 2007), the incidences of low (<10%) and high (≥10%) levels of mosaicism were compared among different age groups among 182 patients with 45, X/46, XX and the correlation between X chromosome loss level and age was also evaluated.

Statistical analysis

The Statistical Package for the Social Sciences (version 22.0, IBM Corp, Armonk, NY, USA) was utilised for data analysis. Continuous data in normal distribution were presented as mean± SD and compared by Student's *t* test while those in non-normal distribution were presented as median (interquartile ranges, IQR) and compared with Mann–Whitney *U* test. Categorical variables were demonstrated as counts (percentages) and processed by Chi-square or Fisher exact test. To adjust for the influences of confounding factors, binary logistic regression was taken into consideration and Kendall's tau-b analysis was used to analyse the correlation between age and X chromosome loss level. A P value of < 0.05 was regarded as statistically significant.

Results

Baseline characteristics

The current study comprised 394 women with XCAs, in which the median height was 161.0 (157.5–165.0) centimetres and median age was 33 (29–38) years old. In total, X numerical abnormalities accounted for 94.42% (n = 372) and X structural abnormalities accounted for 5.58% (n = 22) of women. The percentage of mosaicism (mos) was 4.84% (3.00–7.00%). The three most common karyotypes were mos 45, X/46, XX (n = 182, 46.19%), mos 46, XX/47, XXX (n = 71, 18.02%) and mos 45, X/46, XX/47, XXX (n = 65, 16.50%), while there were only three cases (0.76%) of complete 45, X showing no obvious somatic anomalies although two of them had remarkably shorter stature (133 and 141 centimetres separately). Nevertheless, no other typical physical deformation or mental retardation was found in this cohort. The prevalence and distribution of XCAs among the 394 women are summarised in Table I.

Reproductive characteristics and endocrine profiles

Of the 394 cases, 110 had IVF/ICSI with PGT and 284 had conventional IVF/ICSI. In the PGT group tested with FISH, 23 patients tested with FISH underwent fresh embryo transfers (ET) while 9 received frozen embryo transfers (FET) because of ovarian hyperstimulation syndrome (OHSS) risk, hydrosalpinx, endometrial factor and other reasons. There were 48 patients tested with aCGH/NGS who received FET. In the whole PGT group, 30 cancelled transfer because of no euploid embryos available. Among the 284 patients in IVF/ICSI group, 155 received fresh ET, 49 underwent FET and 80 cancelled transfer as no good quality embryos were available (Fig. 1). The cancellation rate was not significantly different, i.e. 27.27% (30/110) and 28.17% (80/284), between the PGT and conventional IVF/ICSI groups, respectively ($P = 0.859$).

There were 135 (47.54%) women presenting with primary infertility in IVF/ICSI group, a significantly higher proportion compared with the PGT group (36 women, 32.73%, $P = 0.008$). As expected, the incidences of recurrent miscarriage and history of abnormal offspring in the PGT group were remarkably higher than that in IVF/ICSI group (21.82% vs 4.93%, $P = 0.000$; 7.27% vs 2.11%, $P = 0.029$, respectively). Yet the history of healthy offspring in the two groups were similar (24.55% vs 29.23%, $P = 0.353$). The age, body mass index (BMI), basic

Table I Prevalence and distribution of XCAs among the 394 enrolled women.

Numerical aberration (372/394, 94.42%)	N	Structural aberration (22/394, 5.58%)	N
45, X	3	**Terminal deletion**	**17**
47, XXX	39	46, X, del(X)(q24)	2
mos 45, X/46, XX	182	46, X, del(X)(q25)	2
mos 45, X/47, XXX	6	46, X, del(X)(p22.1)	2
mos 46, XX/47, XXX	71	46, X, del(X)(p22.2)	2
mos 46, XX/48, XXXX	1	46, X, del(X)(p10)	1
mos 45, X/46, XX/47, XXX	65	46, X, del(X)(p21)	1
mos 45, X/46, XX/49, XXXXX	1	46, X, del(X)(p11.2)	1
mos 46, XX/47, XXX/48, XXXX	1	46, X, del(X)(p11.3)	1
mos 45, X/46, XX/47, XXX/48, XXXX	2	mos 45, X[11]/46, X, del(X)(p11)[89]	1
mos 45, X/46, XX/47, XXX/49, XXXXX	1	mos 45, X[24]/46, X, del(X)(p21)[76]	1
		mos 45, X[88]/46, X, del(X)(p11.4)[12]	1
		mos 46, X, del(X)(p21)[38]/46, XX[62]	1
		mos 46, X, del(X)(p21)[83]/46, XX[17]	1
		Isochromosome	**3**
		46, X, i(X)(p10)	2
		46, X, i(X)(q10)	1
		Complex rearrangement	**1**
		46, X,? der(X)t(X; X)(p21; q23)	
		Duplication	**1**
		46, X, dup(X)(q23q28),dup(8)(q24.3)	

XCAs, X chromosome abnormalities.

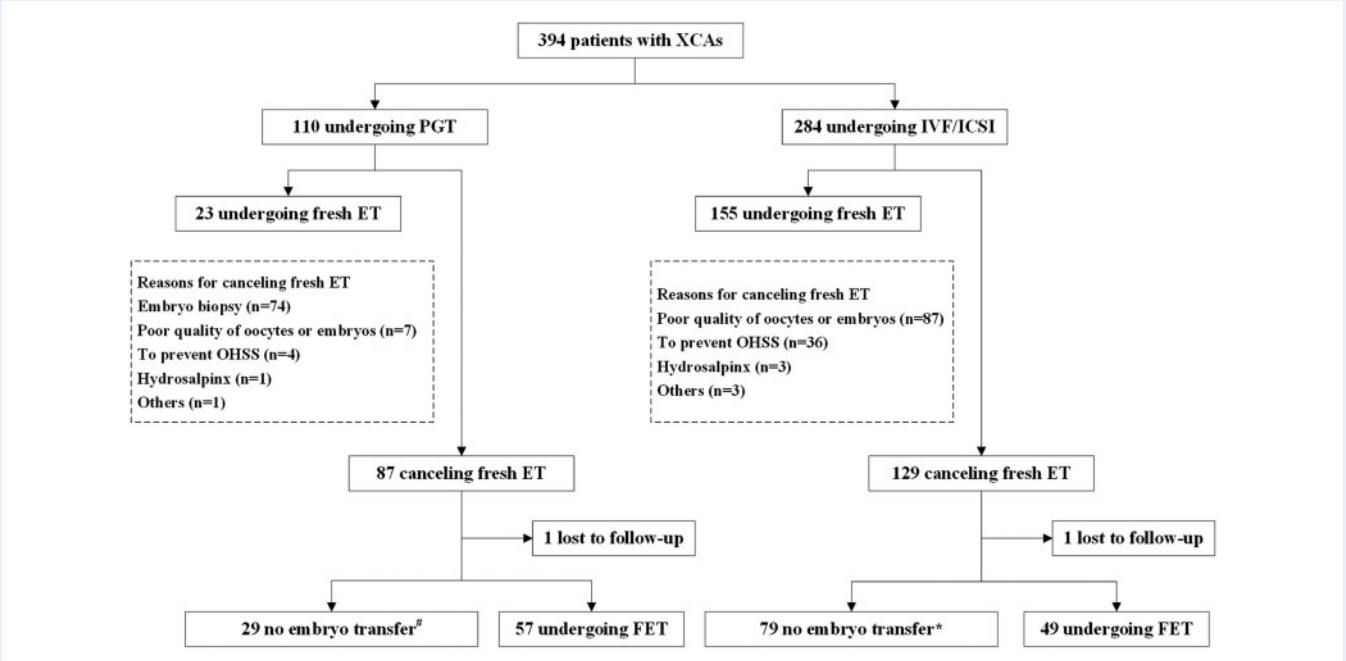


Figure 1. Flow chart of study enrolment and outcomes. XCAs, X chromosome abnormalities; PGT, preimplantation genetic testing; IVF/ICSI, in-vitro fertilisation / intracytoplasmic sperm injection; ET, embryo transfer; FET, frozen embryo transfer. # Including 25 without normal signals and 4 without good-quality embryos. * Because of no good-quality embryos.

follicle stimulating hormone (FSH) levels and antral follicle count (AFC) were all comparable between the two groups (Table II).

Pregnancy outcomes

First, the pregnancy outcomes between PGT_{FISH} and IVF/ICSI groups in the first fresh ET cycles were compared. The LBRs per embryo transfer were comparable between the two groups (PGT_{FISH} 39.13% vs IVF/ICSI 42.58%. P_{adj} =0.558) after adjusted by potential confounding factors, including age, rate of primary infertility, recurrent miscarriage, history of abnormal offspring, anti-Müllerian hormone (AMH) level, AFC as well as timing and number of embryos transferred. The clinical pregnancy rates and pregnancy loss rates were also similar between PGT_{FISH} group and IVF/ICSI group (60.87% vs 50.97%, P_{adj} =0.672 and 35.71% vs 16.46%, P_{adj} =0.136, respectively). Second, the LBRs (66.67% in PGT_{FISH} vs 52.08% in $PGT_{aCGH/NGS}$ vs 53.06% in IVF/ICSI, P_{adj} =0.756) and pregnancy rates per embryo transfer (88.89% in PGT_{FISH} vs 58.33% in $PGT_{aCGH/NGS}$ vs 69.39% in IVF/ICSI, P_{adj}=0.480) and the pregnancy loss rates (12.50% in PGT_{FISH} vs 10.71% in $PGT_{aCGH/NGS}$ vs 23.53% in IVF/ICSI, P_{adj}=0.352) among the PGT groups and IVF/ICSI group in the first FET cycles were also analysed and the results showed no significant differences (Table III).

Embryo biopsy results

Of 110 women receiving PGT, 3 failed to have oocytes retrieved, 34 underwent FISH, 24 used aCGH and 51 had NGS (while 2 patients underwent NGS after FISH). In total, 207 embryos were tested by FISH and 220 embryos were analysed by aCGH or NGS, respectively.

After aCGH or NGS analysis, 43.18% (95/220) embryos were euploid, 46.82% (103/220) were aneuploid, 9.09% (20/220) were mosaic, while two embryos failed to have DNA amplification. The subgroup analysis according to different karyotypes in the women showed that the rates of both euploid and aneuploid embryos were comparable for all types of XCAs (Table IV).

To evaluate the impact of maternal age, patients were stratified into three subgroups: ≤30, 31–37 and ≥38 years old. The euploidy rate was only 22.86% for women older than 38 years old, significantly lower than that for younger patients (57.69% in ≤30 years, P = 0.000; 50.00% in 31–37 years, P = 0.000) (Supplementary Table SI).

To further investigate whether maternal XCAs affects the distribution of embryonic karyotypes, the frequencies of abnormal chromosomes were counted. Interestingly, aberrations occurred most commonly in autosomes 22 (20.39%), 21 (18.45%) and 16 (17.47%), while fewer were in the X chromosome (8.73%) (Fig. 2).

Oocyte utilisation rates

To evaluate the efficiency of retrieved oocytes to generate usable embryos i.e. suitable for transfer or cryopreservation, the oocyte utilisation rate was calculated. A higher rate was observed in IVF/ICSI group compared with PGT group (30.77% vs 22.22%, P = 0.031, Supplementary Table SII).

Maternal and neonatal outcomes

The rates of caesarean section and maternal complications, including the incidence of gestational diabetes, gestational hypertension and preterm rupture of membrane, were equivalent in patients with or without PGT (70.00% vs 58.70%, P = 0.219 and 10.00% vs 8.85%, P = 1.000, respectively, Supplementary Table SIII). The rates of neonatal complications, consisting of the rates of low birth weight and birth defect, were also comparable between the two groups (21.74% vs 14.55%, P = 0.272, Supplementary Table SIII). Additionally, the offspring gender constitution proportions between these two groups were similar. Female offspring accounted for 56.52% in the

Table II Baseline characteristics of patients with XCAs undergoing IVF/ICSI with PGT or conventional IVF/ICSI.

Characteristics	PGT (N = 110)	IVF/ICSI (N = 284)	*P* value
Age, years	33.84 ± 5.26	33.00 (29–38)	0.653
BMI, kg/m^2	24.29 ± 3.72	23.93 (21.40-26.39)	0.645
Primary infertility, n (%)	36 (32.73)	135 (47.54)	0.008*
Recurrent miscarriage, n (%)	24 (21.82)	14 (4.93)	0.000*
History of abnormal offspring, n (%)	8 (7.27)	6 (2.11)	0.029*
History of healthy offspring, n (%)	27 (24.55)	83 (29.23)	0.353
FSH, IU/l	7.03 (5.62–8.65)	6.89 (5.78–8.94)	0.506
LH, IU/l	4.58 (3.35–6.16)	4.42 (3.38–5.89)	0.922
E2, pg/ml	36.30 (26.55-47.50)	33.50 (23.00-45.80)	0.298
T, ng/dl	21.96 (13.07-30.71)	19.86 (13.84-29.44)	0.573
TSH, μIU/ml	2.27 (1.50–3.00)	1.93 (1.44–2.80)	0.149
AMH, ng/ml	1.97 (0.87–3.76)	1.90 (0.87–3.85)	0.661
Bilateral AFC, n	12 (7–15)	10 (6–15)	0.177

XCAs, X chromosome abnormalities; PGT, preimplantation genetic testing; IVF/ICSI, in vitro fertilization/intracytoplasmic sperm injection. BMI, body mass index; FSH, follicle stimulating hormone. LH, luteinizing hormone; E2, oestradiol; T, testosterone; TSH, thyroid stimulating hormone; AMH, anti-Müllerian hormone; AFC, antral follicle count.
*A significant difference was observed.

Table III Pregnancy outcomes of fresh and frozen embryo transfer in patients with XCAs after FISH, aCGH/NGS or conventional IVF/ICSI.

Characteristics	Fresh embryo transfer				Frozen embryo transfer				
	PGT_{FISH} (N = 23)	IVF/ICSI (N = 155)	*P* value	*P*-adj[#]	PGT_{FISH} (N = 9)	$PGT_{aCGH/NGS}$ (N = 48)	IVF/ICSI (N = 49)	*P* value	P_{adj}[¶]
Timing of embryos transferred, n (%)			0.000*					–	
D2	0	6 (3.87)			0	0	0		
D3	0	118 (76.13)			0	0	0		
D4	17 (73.91)	3 (1.94)			0	0	0		
D5	6 (26.09)	28 (18.06)			9 (100.00)	48 (100.00)	49 (100.00)		
No. of embryos transferred, n (%)			1.000					0.019*	
1	7 (30.43)	48 (30.97)			7 (77.78)	48 (100.00)	48 (97.96)		
2	16 (69.57)	106 (68.39)			2 (22.22)	0	1 (2.04)		
3	0	1 (0.64)			0	0	0		
Live birth rate, n (%)	9 (39.13)	66 (42.58)	0.755	0.558	6 (66.67)	25 (52.08)	26 (53.06)	0.738	0.756
Singleton	5 (21.74)	48 (30.97)	0.366	0.413	5 (55.56)	24 (50.00)	26 (53.06)	0.958	0.885
Twins	4 (17.39)	18 (11.61)	0.655	0.719	1 (11.11)	1 (2.08)	0	0.084	1.000
Clinical pregnancy rate, n (%)	14 (60.87)	79 (50.97)	0.375	0.672	8 (88.89) [δ]	28 (58.33)	34 (69.39)	0.164	0.480
Pregnancy loss rate	5 (35.71)	13 (16.46)	0.189	0.136	1 (12.50)	3 (10.71)	8 (23.53)	0.405	0.352
Among clinical pregnancies, n (%)									
Early	5 (35.71)	13 (16.46)	0.189	0.136	1 (12.50)	3 (10.71)	6 (17.65)	0.793	0.558
Late	0	0	–	–	0	0	2 (5.88)	0.606	1.000

XCA, X chromosome abnormalities; PGT, preimplantation genetic testing; FISH, fluorescence in-situ hybridisation; aCGH, array comparative genomic hybridisation; NGS, next-generation sequencing; IVF/ICSI, in-vitro fertilisation / intracytoplasmic sperm injection.
[#]Adjusted by age, primary infertility, history of recurrent miscarriage, history of abnormal offspring, AMH, AFC, timing of embryos transferred and number of embryos transferred.
[¶]Adjusted by age, primary infertility, history of recurrent miscarriage, history of abnormal offspring, AMH, AFC and number of embryos transferred.
*A significant difference was observed.
[δ]One case suffered stillbirth.

PGT group and 41.82% in the IVF/ICSI group ($P = 0.093$, Supplementary Table SIV).

X Chromosome loss and ageing

There was no significant difference in X chromosome loss level among the various age groups ($P = 0.730$, Supplementary Table SV), nor was there a correlation between age and percentage of X chromosome loss (correlation coefficient = 0.018, $P = 0.738$).

Discussion

To the best of our knowledge, this is the first study to compare the pregnancy outcomes after PGT or conventional IVF/ICSI treatment in patients with XCAs and to explore the necessity of PGT for these women.

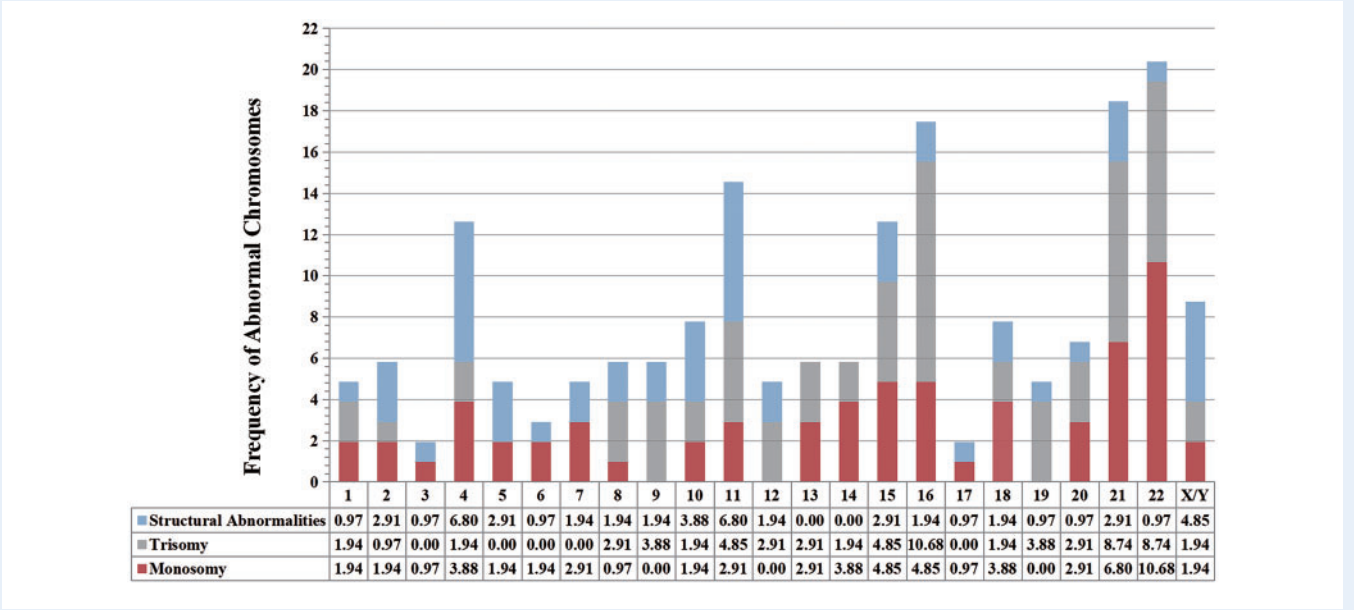
Herein, comparable rates of live birth, miscarriage and maternal and neonatal complications were observed for women with XCAs whether using embryos selected by both morphological and cytogenetic parameters, through PGT, or using embryos assessed by morphological parameters only in conventional IVF/ICSI. Furthermore, in embryos from women carrying XCAs, the X chromosome displayed no higher frequency of malsegregations compared with the autosomal chromosomes. Due to the complete or partial absence of one X chromosome, individuals with XCAs usually suffer from subfertility mainly because of an accelerated depletion and/or degradation of oocytes during meiosis (Sutton *et al.*, 2005; Tucker *et al.*, 2016; Gravholt *et al.*, 2017). As such, their LBR was reported to be merely 5.7% in 10 TS cases undergoing 35 conventional IVF/ICSI cycles, similar to the spontaneous pregnancy rate in TS women (Doğer *et al.*, 2015). Recently, a retrospective study involving 56 patients with TS (median age at 38.16 years) revealed that the LBR per embryo transfer after PGT was 22.5% (Giles *et al.*, 2020). In the current study, relatively higher LBRs per transfer were observed in 394 women carrying XCAs compared with previous studies. The LBR per embryo transfer in conventional IVF/ICSI group was 42.58% and 53.06% in fresh and frozen transfer cycles, respectively, which was comparable with that in the PGT group (39.13% with FISH in fresh transfers, 66.67% with FISH and 52.08% with aCGH/NGS in frozen transfers, respectively). A possible explanation might be that the current study included younger participants (median age at 33 years) with lower rates of mosaicism in their karyotypes (4.84% in average), which will result in relatively better ovarian reserve and less adverse influence on pregnancy outcomes.

Extensive evidence has indicated an obvious decline in female fecundity with advanced maternal age, which might be attributed to increased chromosome segregation errors and reduced expression of

Table IV Embryo biopsy outcomes from aCGH or NGS stratified by maternal karyotype.

Characteristics of patients	45, X	47, XXX	45, X/46, XX	45, X/47, XXX	46, XX/47, XXX	45, X/46, XX/47, XXX	Structural abnormalities	Total	*P* value
No. of patients, n	1	11	31	1	10	16	5	75	–
Age, years	29	28 (28-31)$^{\#\alpha\beta\gamma}$	38 (35-41)$^{\#}$	37	38 (34-40.5)$^{\alpha}$	36 (31-40)$^{\beta}$	29 (29-31)	36 (31-39)$^{\gamma}$	0.000*
No. of embryos biopsied, n	1	31	93$^{\delta}$	3	25	53	14	220$^{\delta}$	–
Euploid embryos, n (%)	1 (100.00)	20 (64.52)	33 (35.48)	2 (66.67)	7 (28.00)	25 (47.17)	7 (50.00)	95 (43.18)	0.055
Aneuploid embryos, n (%)	0	9 (29.03)	47 (50.54)	1 (33.33)	16 (64.00)	23 (43.40)	7 (50.00)	103 (46.82)	0.242
Mosaic embryos, n (%)	0	2 (6.45)	11 (11.83)	0	2 (8.00)	5 (9.43)	0	20 (9.09)	0.911

aCGH, array comparative genomic hybridisation; NGS, next-generation sequencing.
*A significant difference was observed.
$^{\#\alpha\beta\gamma}$There is significant difference in age between karyotypes with the same marks.
$^{\delta}$Two embryos with failure in DNA amplification.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X/Y
Structural Abnormalities	0.97	2.91	0.97	6.80	2.91	0.97	1.94	1.94	1.94	3.88	6.80	1.94	0.00	0.00	2.91	1.94	0.97	1.94	0.97	0.97	2.91	0.97	4.85
Trisomy	1.94	0.97	0.00	1.94	0.00	0.00	0.00	2.91	3.88	1.94	4.85	2.91	2.91	1.94	4.85	10.68	0.00	1.94	3.88	2.91	8.74	8.74	1.94
Monosomy	1.94	1.94	0.97	3.88	1.94	1.94	2.91	0.97	0.00	1.94	2.91	0.00	2.91	3.88	4.85	4.85	0.97	3.88	0.00	2.91	6.80	10.68	1.94

Figure 2. Distribution of chromosomal abnormalities of embryos biopsied with aCGH or NGS. aCGH, array comparative genomic hybridisation; NGS, next-generation sequencing. Chromosomal abnormalities of embryos from XCAs appeared most frequently on autosomes 22, 21 and 16.

core spindle assembly checkpoint components as well as mitochondrial dysfunction, thus resulting in fewer euploid embryos (Hassold and Hunt, 2009; Demko *et al.*, 2016; Mikwar *et al.*, 2020). To adjust for the impact of female age, a subgroup analysis was performed among the embryos analysed by aCGH or NGS. As expected, the euploidy rate in this study was 57.69% in patients younger than 30 years, which declined to 50.00% in patients aged 31–37 years, and was only 22.86% in women older than 38 years. Intriguingly, the euploidy rate at different ages in women with XCAs is similar to that in women from the general population (Fragouli *et al.*, 2013; Munne *et al.*, 2017; Fragouli *et al.*, 2019), implying that a more severe impact on embryo quality was exerted by female age, rather than XCAs.

The distribution of abnormal chromosomes uncovered by aCGH or NGS was analysed to explore the effect of different types of XCAs on embryos. Interestingly, comparable rates of euploid embryos were observed among the women with different types of XCAs. Moreover, the X chromosome showed no higher frequency of aberrations in embryos compared with the autosomes. Conversely, embryonic chromosome abnormalities occurred most commonly on autosomes 22, 21 and 16, which was consistent with the distribution of malsegregations of embryos generated by women seeking PGT treatment because of advanced age or history of recurrent miscarriage (Murphy *et al.*, 2019; Liu *et al.*, 2020). This uneven distribution might be attributed to the vulnerability of acrocentric chromosomes 22, 21 and 16 to meiotic errors due to a disturbance in sister-chromatid cohesion and premature chromatid separation, and also to the higher tolerance to faults in above-mentioned autosomes during embryogenesis (Pellestor *et al.*, 2003; Fragouli *et al.*, 2013; Nakhuda *et al.*, 2018; Liu *et al.*, 2020).

All in all, there was no apparent influence on karyotypes of blastocysts applied by maternal XCAs and the cytogenetic and molecular reasons warrant further exploration.

Although a similar cancellation rate was observed between the two groups before the first transfer cycle, the oocyte utilisation rate in the conventional IVF/ICSI group significantly outweighed that in the PGT group, indicating that more embryos were available for further transfer. It has been reported that a proportion of the discarded embryos, due to poor morphological evaluation for blastocyst biopsy or cryopreservation, could also result in euploid newborns (Lai *et al.*, 2020). Further embryo loss during PGT process would further reduce the oocyte utilisation rate. In addition, from the perspective of the whole treatment cycle, comparable cumulative live birth rates were observed between in women of advanced maternal age in women with and without PGT in randomised controlled trials (RCTs), although the PGT group had a higher LBR in the first transfer cycle (Rubio *et al.*, 2017). Considering the similar LBRs in the first transfer in our cohort, more desirable cumulative outcomes per started cycle could be anticipated in XCAs individuals with conventional IVF/ICSI treatment because of a higher chance of embryo transfer.

The frequency of female X chromosome loss has been reported to increase with age (Russell *et al.*, 2007), such that less than 10% could be regarded as normal in woman aged 42 or above (for a 30-cell count) or aged 55 or above (for a 60-cell count) without phenotypical abnormalities. Moreover, according to clinical practice guidelines for TS, women older than 50 years with less than 5% 45, X cells are recommended not be diagnosed as TS due to the effects of advanced age but there is no clear limit of the 45, X percentage to define TS in women younger than 50 years old (Gravholt *et al.*, 2017). In the current study, 100 metaphases were counted for detecting mosaicism and there was no significant difference between age and level of X chromosome loss, which might be ascribed to all of our patients being younger, ranging from 22 to 47 years old.

There were a few limitations in this study. First, apart from the two 45, X individuals had apparently short stature, our participants, who hosted 4.84% X chromosome mosaicism, showed no typical somatic anomalies of TS, such as webbed neck, cubitus valgus or severe cardiovascular diseases. Given that X chromosome mosaicism might lead to phenotypical changes from 6% and above of aneuploidy (Homer *et al.*, 2010), no previous study has focused on the level of mosaicism and karyotype constitution of patients with XCAs who have undergone IVF/ICSI and PGT. It is noteworthy that our participants maintained ovarian function to a certain extent with the capability of undergoing ART. Consequently, pregnancy outcomes in women with typical XCAs, especially in those with physical complications, should be explored in the future. Second, the incidences of histories of recurrent miscarriage and abnormal offspring in the women who opted for PGT were significantly higher than in the women in the conventional IVF/ICSI group, which might exert bias amongst the subjects included. Although binary logistic regression analysis was performed to adjust for the confounding factors, further RCTs are required. Third, as is known, in case of analysing X chromosomes with the FISH protocol, it is recommended that the probe set should contain, at least, probes specific for the centromere region of the X and Y chromosomes and one autosome to avoid problems in scoring of diffuse and overlapping signals. However, the FISH performed in this study only used X/Y probes and lacked the reference of autosome, which might have resulted in misdiagnosis and bias. Finally, intrinsic disadvantages could not be totally avoided due to the retrospective nature of this study. However, given the low incidence rate of XCAs in women receiving ART treatment, especially with PGT, we have enrolled the largest cohort of women with XCAs undergoing PGT or conventional IVF/ICSI treatment, to date. Additionally, our results not only indicated the comparable pregnancy outcome in women with XCAs between PGT and conventional IVF/ICSI, but also evaluated the impact of that the abnormal X chromosome contents exerted on embryos, which will provide preliminary data for future RCTs.

In summary, our results illustrate comparable pregnancy outcomes in the first transfer cycle for women with XCAs whether their embryos were selected by both morphological and cytogenetic assessment through PGT or by morphological parameters only in conventional IVF/ICSI treatment. Together with a comprehensive picture of embryo karyotypes and the oocyte utilisation rates, PGT is not suggested to be recommended for women with XCAs in the absence of other PGT indications. Conventional IVF/ICSI treatments may result in better cumulative outcomes due to an increased number of transferable embryos.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Acknowledgements

The authors are grateful to all the patients who participated in this study.

Authors' roles

C.L., Y.D. and Y.Q. were involved in the study concept and design. J.L., H.L. and Y.Z. were responsible for acquisition of data. C.L., Y.D., J.L., H.L. and Y.Z. contributed to the analysis and interpretation of data. C.L., Y.D. and Y.Q. contributed to manuscript drafting. All authors reviewed and approved the final version of this work.

Funding

This work was supported by National Research and Development Plan (2016YFC1000604 and 2017YFC1001100), the National Natural Science Foundation of China (81701406), Shandong Science Fund for Distinguished Young Scholars (JQ201720), Taishan Scholars Program for Young Experts of Shandong Province (tsqn20161069) and Projects of Medical and Health Technology Development Program in Shandong Province (202005010520, 202005010523 and 2016WS0368).

Conflict of interest

None declared.

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