Comparison between fluorescent in-situ hybridisation and array comparative genomic hybridisation in preimplantation genetic diagnosis in translocation carriers

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ABSTRACT

Objectives: To compare the pregnancy outcome of the fluorescent in-situ hybridisation and array comparative genomic hybridisation in preimplantation genetic diagnosis of translocation carriers.

Design: Historical cohort.

Setting: A teaching hospital in Hong Kong.

Patients: All preimplantation genetic diagnosis treatment cycles performed for translocation carriers from 2001 to 2013.

Results: Overall, 101 treatment cycles for preimplantation genetic diagnosis in translocation were included: 77 cycles for reciprocal translocation and 24 cycles for Robertsonian translocation. Fluorescent in-situ hybridisation and array comparative genomic hybridisation were used in 78 and 11 cycles, respectively. The ongoing pregnancy rate per initiated cycle after array comparative genomic hybridisation was significantly higher than that after fluorescent in-situ hybridisation in all translocation carriers (36.4% vs 9.0%; P=0.010). The miscarriage rate was comparable with both techniques. The testing method (array comparative genomic hybridisation or fluorescent in-situ hybridisation) was the only significant factor affecting the ongoing pregnancy rate after controlling for the women's age, type of translocation, and clinical information of the preimplantation genetic diagnosis cycles by logistic regression (odds ratio=1.875; P=0.023; 95% confidence interval, 1.090-3.226).

Conclusion: This local retrospective study confirmed that comparative genomic hybridisation is associated with significantly higher pregnancy rates versus fluorescent in-situ hybridisation in translocation carriers. Array comparative genomic hybridisation should be the technique of choice in preimplantation genetic diagnosis cycles in translocation carriers.

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New knowledge added by this study

- Fluorescence in-situ hybridisation (FISH) has been widely used in preimplantation genetic diagnosis (PGD) in translocation carriers. However, array comparative genomic hybridisation (aCGH) has largely replaced FISH since its development due to the advantages of testing all 24 chromosomes and improved pregnancy rates. This is the first study to show the use of aCGH in Hong Kong. Compared with FISH, aCGH was associated with significantly higher rate of ongoing pregnancy in translocation carriers (both reciprocal and Robertsonian translocations).

Implications for clinical practice or policy

- Array CGH should be the technique of choice for PGD in translocation carriers.

Introduction

Since the report of first live-birth after preimplantation genetic diagnosis (PGD) published in 1990,[1] more than 21 000 cycles have been performed worldwide, based on the data from ESHRE (European Society of Human Reproduction and Embryology) PGD consortium in the past two decades.[2] Fluorescent in-situ hybridisation (FISH) has been used for PGD in translocation carriers. This technique uses chromosome-specific DNA probes in metaphase chromosomes or interphase nuclei. For PGD in translocation carriers, the usual approach is to use commercially available centromeric, locus-specific and subtelomeric probes depending on the translocated segments.[3]

應用在染色體易位攜帶者中胚胎植入前遺傳學診斷的熒光原位雜交和基因晶片檢測比較

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目的：在染色體易位攜帶者胚胎植入前遺傳學診斷（PGD）中，比較熒光原位雜交（FISH）和基因晶片檢測（aCGH）的妊娠率。

設計：歷史隊列研究。

安排：香港一所教學醫院。

患者：從2001年至2013年為染色體易位攜帶者PGD的治療週期。

結果：研究了共101個PGD治療週期，包括77個週期相互易位和24個週期羅伯遜易位。FISH和aCGH分別在78和11週期使用。aCGH後每啟動週期的懷孕率比FISH顯著為高（36.4%比9.0%，P=0.010）。流產率則沒有顯著分別。邏輯回歸分析證實FISH或aCGH測試方法是唯一顯著影響懷孕率的因素（比數比=1.875，P=0.023，95%置信區間：1.090-3.226）。

結論：此本地回顧研究證實，在染色體易位攜帶者PGD治療週期使用aCGH後的懷孕率顯著高於FISH，因此以後在染色體易位攜帶者PGD治療週期中，不應再使用FISH而應選擇使用aCGH。

However, FISH itself carries technical difficulties of fixation and spreading of nucleus, with the reported error rate of 7% to 10%.[4-6] Another problem is that in translocation carriers, there is interchromosomal effect so that the proportion of embryos having aneuploidies is higher than those without translocations.[7] Segmental loss or gain is also a frequent event in human embryos.[8,9] Fluorescent in-situ hybridisation would not be able to detect these chromosomal abnormalities, which could be the cause of low success rates of PGD in translocation carriers as most of these embryos would result in implantation failure or miscarriages.[10]

With the development of comparative genomic hybridisation (CGH), it is possible to detect abnormalities in all 24 chromosomes and its application on single blastomere biopsy was first reported in 1996.[11] Comparative genomic hybridisation is a DNA-based technique, employing comparative hybridisation of differentially labelled DNA samples to normal metaphase chromosome on a microscope slide.[4] The ratio of fluorescence reveals the gain or loss of the tested samples. However, the turnover time is about 4 days, which does not fit into the strict time frame of treatment for PGD, and cryopreservation of embryos is mandatory, unless polar body biopsy is used.[12] Array CGH (aCGH), employing DNA probes affixed directly to a microscope slide, solves this problem as the turnover time is about a day, which makes fresh transfer after blastomere biopsy or trophectoderm biopsy possible.[13] It has been demonstrated that using aCGH in translocation carriers is beneficial.[9]

Our centre used the FISH technique for translocation carriers since our team developed the technique of PGD in 2001 which resulted in the first live-birth in Hong Kong.[14] We acquired the platform of aCGH in April 2012. This retrospective analysis aimed to compare the pregnancy outcomes using FISH and aCGH for the treatment cycles of PGD in translocation carriers.

Methods

Study population

Data from all treatment cycles performed for PGD in the Department of Obstetrics and Gynaecology, Queen Mary Hospital/The University of Hong Kong from 2001 till 2013 June were retrieved. Only PGD cycles in translocation carriers were included in the present study, which was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster.

Treatment regimen

The details of the long protocol of ovarian stimulation regimen, gamete handling, cryopreservation of embryos, and frozen embryo transfer have been previously described.[15] The details of PGD have also been previously described.[16] In short, embryo biopsy was performed on day 3 at 6-to-8-cell stage. Two blastomeres were tested from 2001 to 2005 and one blastomere was routinely tested from 2006 onwards. The blastomere was fixed for FISH analysis. Commercially available FISH probes were chosen to flank the break point. For aCGH, the blastomere was transferred into a polymerase chain reaction tube and whole genome amplification was performed (SurePlex; BlueGnome, Cambridge, UK). Array CGH was performed using 24sure V3 (BlueGnome) for Robertsonian translocation carrier or 24sure+ (BlueGnome) for reciprocal translocation carrier. All results were interpreted separately by two laboratory staff.

Outcome measures

The primary outcome measures of the study were clinical and ongoing pregnancy rates. Clinical pregnancies were defined by the presence of one or more gestation sacs or the histological confirmation of gestational product in case of early pregnancy failures. Ongoing pregnancies were those pregnancies beyond 8 to 10 weeks of gestation, at which stage the patients were referred for antenatal care. The secondary outcome measures were miscarriage rate and cancellation rate. Cancellation rate was defined as the percentage of treatment cycles with no embryo transfer after oocyte retrieval.

Statistical analysis

The Kolmogorov-Smirnov test was used to test

the normal distribution of continuous variables. Results of continuous variables were expressed as mean ± standard deviation if normally distributed, and median (range) if not normally distributed. Statistical comparison was carried out by Student's *t* test, Mann-Whitney *U* test, and/or Wilcoxon signed rank test for continuous variables and Chi squared test or Fisher's exact test for categorical variables, as appropriate. Statistical analysis was performed using the Statistical Package for the Social Sciences (Windows version 20.0; SPSS Inc, Chicago [IL], US). The two-tailed value of $P<0.05$ was considered statistically significant. Binary logistic regression using enter method was used to calculate the prediction of the pregnancy rate in PGD cycles.

Results

There were 339 PGD cycles, of which 101 treatment cycles were performed in translocation carriers during the study period: 77 cycles for reciprocal translocation and 24 cycles for Robertsonian translocation. The two techniques, FISH and aCGH, were used in 78 and 11 cycles, respectively (Table 1). The overall cancellation rate was 39.6% (40/101). Four cycles were cancelled due to high risk of ovarian hyperstimulation syndrome; eight cycles due to poor ovarian responses or poor embryo qualities; and 28 cycles due to no normal embryo after PGD with either technique (Table 1). The cancellation rate using FISH technique due to abnormal signals for all embryos was significantly higher than that using aCGH (34.6% vs 9.1%, respectively).

The demographic and clinical data of women who underwent PGD with FISH and aCGH are presented in Table 2. Women in the aCGH group were significantly younger than those in the FISH group, and the serum oestradiol concentration on ovulation trigger day in the aCGH group was significantly higher than that in the FISH group. The total dosage of gonadotropin, the number of follicles larger than or equal to 16 mm, and the number of oocytes retrieved were comparable between the two groups. The demographic and clinical data of cycles for couples with reciprocal and Robertsonian translocation were all comparable (data not shown).

TABLE 1. Information on preimplantation genetic diagnosis cycles

	Reciprocal translocation	Robertsonian translocation	Total
PGD for translocations	38 Couples 77 Cycles	13 Couples 24 Cycles	51 Couples 101 Cycles
Technique used			
FISH	57 Cycles	21 Cycles	78/101 (77.2%)
aCGH	9 Cycles	2 Cycles	11/101 (10.9%)
Cancelled cycles	40/101 (39.6%)		
FISH (no normal embryo)	27/78 (34.6%)		
aCGH (no normal embryo)	1/11 (9.1%)		
Poor ovarian response*	8 (7.9%)		
OHSS risk	4 (4.0%)		

Abbreviations: aCGH = array comparative genomic hybridisation; FISH = fluorescent in-situ hybridisation; OHSS = ovarian hyperstimulation syndrome; PGD = preimplantation genetic diagnosis

* One cycle cancelled after PGD due to no blastocyst development, 2 cycles cancelled due to poor embryo quality not suitable for PGD, and 5 cycles cancelled with cryopreservation of embryos for next cycle of PGD due to small number of embryos available

TABLE 2. Demographic and clinical data of subjects included in treatment cycles for preimplantation genetic diagnosis using fluorescent in-situ hybridisation and array comparative genomic hybridisation

Demographic/clinical data	Mean ± standard deviation (range)		P value
	FISH	aCGH	
Women's age (years)	34.7 ± 3.1 (28-40)	32.1 ± 3.64 (28-38)	0.026
Total dosage of gonadotropin used (IU)	2364 ± 769 (1175-4200)	2281 ± 955 (1200-3825)	0.583
No. of follicles ≥16 mm	8.0 ± 2.7 (2-15)	9.9 ± 4.2 (5-19)	0.213
No. of oocytes	13.3 ± 7.3 (1-42)	16.7 ± 6.4 (9-28)	0.077
Oestradiol concentration on hCG day (pmol/L)	13 686 ± 5744 (831-26 413)	18471 ± 5632 (10 596-30 067)	0.015

Abbreviations: aCGH = array comparative genomic hybridisation; FISH = fluorescent in-situ hybridisation; hCG = human chorionic gonadotropin

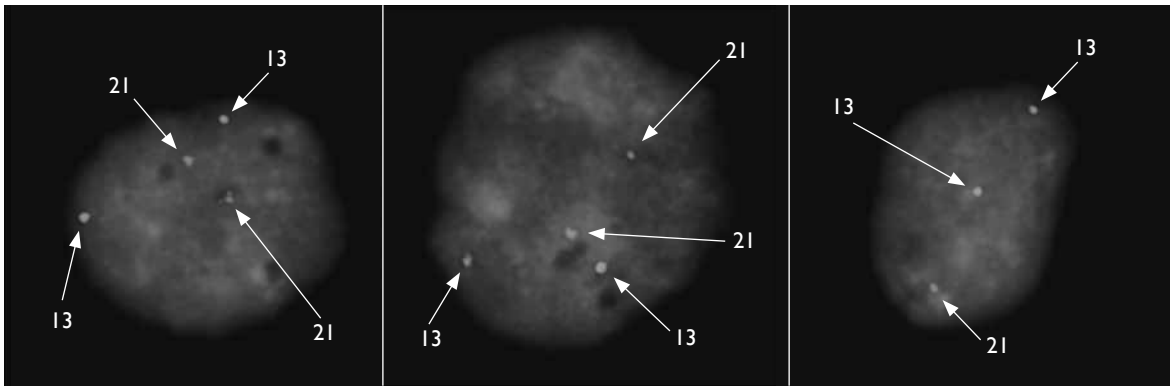
The pregnancy rates per cycle and per transfer were all significantly higher in cycles performed using aCGH. The miscarriage rates were similar between the two groups (Table 3). A subgroup analysis of cycles performed from 2006 to 2013 showed similar results in all the above comparisons with significantly higher clinical and ongoing pregnancy rates per initiated cycle and per transfer in cycles using aCGH than those using FISH, but with comparable miscarriage rates (data not shown). Figures 1 and 2 show PGD results with FISH and aCGH, respectively.

Logistic regression revealed that the method of testing (FISH or aCGH) was the only factor that significantly affected the ongoing pregnancy rate; age of the women, the type of translocation, or other clinical information including number of oocytes retrieved, the gonadotropin dosage used, and the oestradiol concentration on the day of human chorionic gonadotropin administration did not affect the outcome. The method of testing remained a significant factor after controlling for the age of

TABLE 3. Pregnancy rates

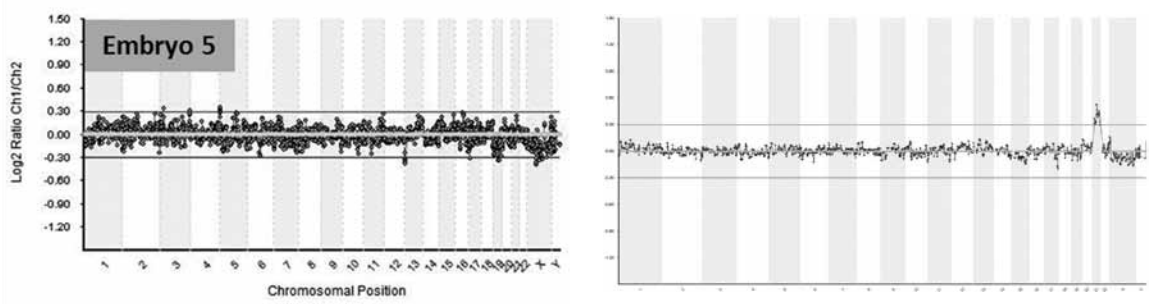
	FISH	aCGH	P value
Clinical pregnancy rate/initiated cycle	11/78 (14.1%)	6/11 (54.5%)	0.001
Ongoing pregnancy rate/initiated cycle	7/78 (9.0%)	4/11 (36.4%)	0.010
Clinical pregnancy rate/embryo transfer	11/51 (21.6%)	6/10 (60.0%)	0.013
Ongoing pregnancy rate/embryo transfer	7/51 (13.7%)	4/10 (40.0%)	0.048
Miscarriage rate	4/11 (36.4%)	2/6 (33.3%)	0.901

Abbreviations: aCGH = array comparative genomic hybridisation; FISH = fluorescent in-situ hybridization



Embryo #20 Normal | Embryo #21 Normal | Embryo #27 Monosomy 21

FIG 1. Preimplantation genetic diagnosis by fluorescent in-situ hybridisation



No aneuploidy detected on aCGH (46,XY) | 47,XY,+21 (Abnormal embryo with trisomy 21)

FIG 2. Results of array comparative genomic hybridisation (aCGH)

women and type of translocation (Table 4).

Figure 3 shows the results of aCGH in embryos produced from reciprocal translocation carrier. Array CGH can detect segmental changes in translocated chromosomes (embryo 18) and other chromosomes (embryo 3). It can also detect whole chromosome aneuploidy (embryo 16). Embryo 7 was replaced and resulted in an ongoing pregnancy.

Discussion

The present study showed that PGD using aCGH was associated with significantly higher pregnancy rates (both per initiated cycle or per embryo transfer) versus FISH. The testing method, ie using aCGH or FISH, was the only significant factor affecting the ongoing pregnancy rate in logistic regression.

Couples carrying balanced reciprocal or Robertsonian translocations are well-known to produce a high percentage of unbalanced gametes and embryos,[17] resulting in high miscarriage rates and a variable chance of unbalanced offspring with multiple congenital anomalies and mental retardation.[18] The high percentage of unbalanced

TABLE 4. Logistic regression of variables associated with ongoing pregnancy rate for PGD in translocation carriers

	Logistic regression				Multivariate regression			
	B	OR	P value	95% CI	B	OR	P value	95% CI
Age (years)	-0.086	0.917	0.384	0.756-1.114	0.040	1.041	0.772	0.795-1.362
Type of translocation	0.162	1.176	0.084	0.978-1.414	0.096	1.199	0.096	0.969-1.483
Method of PGD (FISH or aCGH)	0.586	1.796	0.018	1.107-2.916	0.745	1.875	0.023	1.090-3.226
No. of oocytes retrieved	0.028	1.028	0.512	0.946-1.118	0.066	1.069	0.302	0.942-1.212
Total gonadotropin dosage	-0.070	0.933	0.138	0.851-1.023	-0.031	0.969	0.616	0.858-1.095
Oestradiol concentration on hCG day	-0.013	0.987	0.779	0.898-1.084	-0.083	0.920	0.296	0.788-1.075

Abbreviations: aCGH = array comparative genomic hybridisation; CI = confidence interval; FISH = fluorescent in-situ hybridisation; hCG = human chorionic gonadotropin; OR = odds ratio; PGD = preimplantation genetic diagnosis

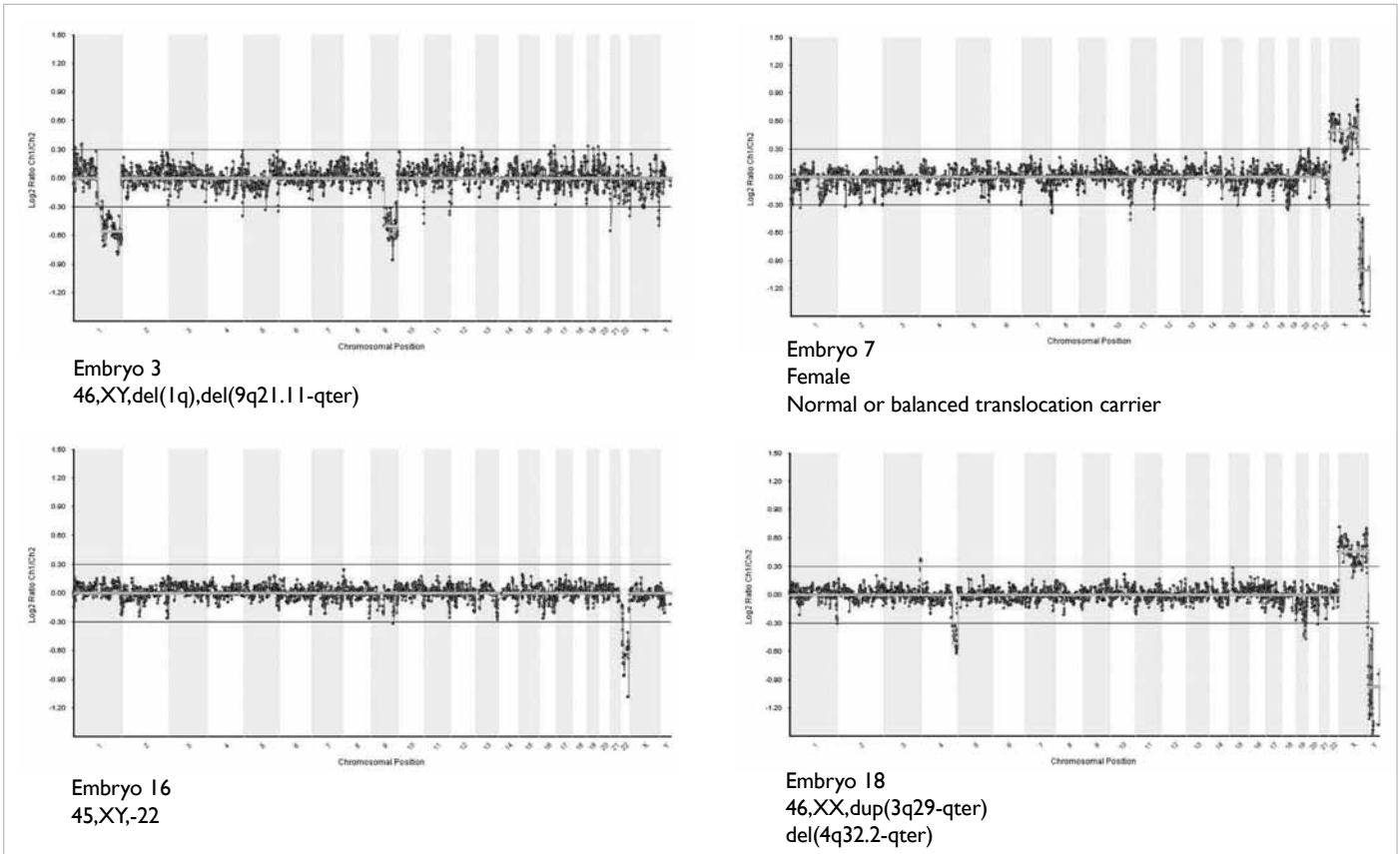


FIG 3. Array comparative genomic hybridisation (CGH) of embryo biopsy

The mother was a reciprocal translocation carrier, 46,XX,t(3;4)(q29;q32). Array CGH could detect segmental changes unrelated to translocation chromosomes (embryo 3), whole chromosome aneuploidy (embryo 16), and unbalanced reciprocal translocation (embryo 18)

gametes can be explained by the segregation modes and behaviours of translocations during meiosis.[19] Not only the direct effect of the translocations on the meiosis, but also the interchromosomal effect exerted by the translocations increases the percentage of aneuploidies in the gametes and embryos of couples carrying translocations.[7,20-22] It further decreases the number of normal/balanced euploid embryos, including those suitable and feasible for transfer. It was reported that only up to 16% of preimplantation embryos were normal/balanced and euploid in translocation carriers.[9]

In the past decade, FISH was commonly employed to detect the unbalanced chromosome rearrangement of embryos using probes depending on the translocated segments.[23] Fluorescent in-situ hybridisation is technically challenging, especially with regard to fixation and spreading.[3,5,24] The error rate of FISH was reported to be up to 10%.[5,6,25] As PGD using FISH in translocation carriers only employs fluorescent DNA probes for the translocated segments, aneuploidies and segmental rearrangements which are not related to the translocated segments will be missed.[3] Even in aneuploidy screening, only up to five chromosomes can be tested in one round of FISH, and so, usually up to half of all chromosomes can be tested in repeated rounds. However, repeated rounds were related to the decrease in diagnostic accuracy.[4] Therefore, using FISH would miss a proportion of aneuploidies and abnormal embryos, which may result in misdiagnosis, implantation failure, or miscarriages.[10] This is probably the major reason for the unfavourable results in a systematic review on the use of PGD in translocation carriers[26] and the meta-analysis of preimplantation genetic screening.[27] The cancellation rate, ie no embryo transfer after oocyte retrieval, was higher after FISH than that after aCGH, probably due to technical difficulties.

The development of CGH makes it possible to test for all 24 chromosomes, while the development of aCGH makes it feasible to use the technique in the restricted time frame of PGD. Several groups of investigators have reported success with using aCGH for PGD in translocation carrier couples to improve their reproductive outcomes[9,28]; we have shown similar results in this local study.

Figure 3 shows the result of PGD in a patient with reciprocal translocation. Array CGH detected unbalanced reciprocal translocated segments in embryo 18. It also picked other segmental changes (1q and 9q21.11-qter) not related to translocated chromosomes in embryo 3. It could also detect whole chromosome aneuploidy (monosomy 22) in embryo 16. In FISH, probes flanking the translocation breakpoints are used and, therefore, the abnormalities in embryo 3 and embryo 16 cannot be detected. Furthermore, the average probe density of aCGH used for Robertsonian translocation is 10 Mb while that of one used for reciprocal translocation is 5 Mb. Increase in resolution allows us to easily pick a small abnormality in the embryo. Array CGH offers a more comprehensive way of PGD in translocation carriers and this results in a significant increase in the pregnancy rate compared with FISH.

In our cohort, the age of women for whom aCGH was employed was younger than that of women for whom FISH was employed. This can probably explain the higher oestradiol concentration after ovarian stimulation of in-vitro fertilisation treatment, along with the non-significant, higher number of follicles and oocytes retrieved in the aCGH group. In order to reveal the effect of the testing method on pregnancy rate, we controlled the women's age, type of translocation, and other data of the stimulation including the total dosage of gonadotropin and number of oocytes retrieved in multivariate logistic regression; the testing method remained the only significant factor affecting the ongoing pregnancy rate. This indicates that, after controlling for all the possible confounding factors, PGD cycles using aCGH were associated with a significantly higher ongoing pregnancy rate than those using FISH.

It has been controversial whether PGD can improve the reproductive outcomes compared with natural conception in translocation carriers. A systematic review reported adverse effects on the pregnancy rates after PGD in translocation carriers compared with natural conception.[26] However, all the PGD cycles included in this review were performed with FISH. Moreover, the case reports and case series of PGD included had a small number of subjects; in 16 out of 21 studies, the sample size was only one to three cases. Larger systematic reviews on the use of aCGH in translocation carriers are urgently needed.

This study is retrospective in nature and there may be some confounding factors such as differences in embryo biopsy techniques and culture conditions which were not controlled for and which might have affected the pregnancy outcomes. As we started using aCGH approximately one and a half year ago, the number of cases was smaller than that using FISH. Despite the small sample size, the ongoing pregnancy rate revealed a significant increase after employing aCGH in translocation carriers. This serves to further strengthen our argument in favour of PGD programme using aCGH.

It is well known that two-blastomere biopsy is more detrimental to pregnancy than one-blastomere biopsy.[22] Our team employed two-blastomere biopsy when we first developed our PGD programme. We then switched to one-blastomere biopsy in 2006. Therefore, a subgroup analysis was performed on those cycles between 2006 and 2013. The ongoing

pregnancy rate per initiated cycle remained significantly higher in the group using aCGH than that using FISH.

Conclusion

Use of aCGH can improve the pregnancy outcomes of PGD in translocation carriers compared with FISH. Array CGH should be the technique of choice for PGD in translocation carriers.

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