Preimplantation genetic diagnosis and screening by array comparative genomic hybridisation: experience of more than 100 cases in a single centre

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ABSTRACT

Introduction: Preimplantation genetic screening has been proposed to improve the in-vitro fertilisation outcome by screening for aneuploid embryos or blastocysts. This study aimed to report the outcome of 133 cycles of preimplantation genetic diagnosis and screening by array comparative genomic hybridisation.

Methods: This study of case series was conducted in a tertiary assisted reproductive centre in Hong Kong. Patients who underwent preimplantation genetic diagnosis for chromosomal abnormalities or preimplantation genetic screening between 1 April 2012 and 30 June 2015 were included. They underwent in-vitro fertilisation and intracytoplasmic sperm injection. An embryo biopsy was performed on day-3 embryos and the blastomere was subject to array comparative genomic hybridisation. Embryos with normal copy numbers were replaced. The ongoing pregnancy rate, implantation rate, and miscarriage rate were studied.

Results: During the study period, 133 cycles of preimplantation genetic diagnosis for chromosomal abnormalities or preimplantation genetic screening were initiated in 94 patients. Overall, 112 cycles proceeded to embryo biopsy and 65 cycles had embryo transfer. The ongoing pregnancy rate per transfer cycle after preimplantation genetic screening was 50.0% and that after preimplantation genetic diagnosis was 34.9%. The implantation rates after preimplantation genetic screening and diagnosis were 45.7% and 41.1%, respectively and the miscarriage rates were 8.3% and 28.6%, respectively. There were 26 frozen-thawed embryo transfer cycles, in which vitrified and biopsied genetically transferrable embryos were replaced, resulting in an ongoing pregnancy rate of 36.4% in the screening group and 60.0% in the diagnosis group.

Conclusions: The clinical outcomes of preimplantation genetic diagnosis and screening using comparative genomic hybridisation in our unit were comparable to those reported internationally. Genetically transferrable embryos replaced in a natural cycle may improve the ongoing pregnancy rate and implantation rate when compared with transfer in a stimulated cycle.

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New knowledge added by this study

- Array comparative genomic hybridisation is a reliable method for preimplantation genetic diagnosis for translocation/inversion carriers, and for patients with mosaic sex chromosome aneuploidy. Replacement of vitrified embryos after warming in a natural cycle may improve the ongoing pregnancy rate and implantation rate.

Implications for clinical practice or policy

- Preimplantation genetic diagnosis by array comparative genomic hybridisation shall be offered as an alternative to prenatal diagnosis for translocation/inversion carriers, and for patients with mosaic sex chromosome aneuploidy. The results of this local case series provide information, such as the anticipated percentage of genetically transferrable embryos and the expected ongoing pregnancy rate, which is useful for patient counselling before preimplantation genetic diagnosis or screening.

Introduction

Preimplantation genetic diagnosis (PGD) is an alternative to prenatal diagnosis for detection of chromosomal abnormalities in translocation or inversion carrier couples. In the past 13 years, more than 6000 cycles of PGD for chromosomal abnormalities have been performed.[1] Fluorescence in-situ hybridisation (FISH) was first used in PGD for translocation carriers.[2] Due to its technical limitations however,[3-5] it has been replaced by array comparative genomic hybridisation (aCGH) in many centres. In our centre, we have previously shown that

基因晶片檢測作胚胎植入前遺傳診斷和染色體篩查：單一中心內超過100個病例的經驗分享

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引言：過往曾有建議提出以篩選非整倍體胚胎或囊胚作染色體篩查來改善體外受精的結果。本研究回顧以基因晶片檢測進行胚胎植入前遺傳學診斷/染色體篩查（PGD/PGS）的133個週期的結果。

方法：本病例系列研究於香港一所生育轉介中心內進行。研究對象均於2012年4月1日至2015年6月30日期間在香港大學瑪麗醫院輔助生育中心以基因晶片檢測進行PGD/PGS。她們全都接受體外受精（IVF）和卵胞漿內單精子注射（ICSI），於胚胎第3天時進行活檢和基因晶片檢測。有正常拷貝數的胚胎會移植回母體。研究並找出持續妊娠率、著床率及流產率。

結果：研究期間94位婦女在PGD/PGS過程中進行了133個週期的超排卵，其中112例成功進行胚胎活檢，65例進行了胚胎移植。在進行PGS或PGD後，持續妊娠率分別為每移植週期50.0%及34.9%，著床率分別為45.7%及41.1%，流產率分別為8.3%及28.6%。基因晶片檢測診斷為正常的胚胎，凍存後進行了26次凍融胚胎移植週期，經過PGS及PGD的持續妊娠率分別為36.4%及60.0%。

結論：本院使用基因晶片檢測進行PGD/PGS的臨床結果可媲美其他國家。在自然週期進行凍融胚胎移植有可能增加持續妊娠率及著床率。

the use of aCGH for PGD in translocation carriers results in a significantly higher rate of ongoing pregnancy than PGD by FISH.[6]

Aneuploidy is the most common abnormality found in embryos derived from in-vitro fertilisation (IVF), and leads to poor outcomes.[7-13] Morphological assessment of embryos or blastocysts alone, however, cannot negate the potential risk of replacing aneuploid embryos or blastocysts.[14] Preimplantation genetic screening (PGS) has been proposed to improve the IVF outcomes by screening for aneuploid embryos or blastocysts. More than 26 000 PGS cycles have been performed worldwide.[1] The aCGH technique enables us to screen all 24 chromosomes within 24 hours and makes fresh transfer possible after blastomere biopsy or trophectoderm biopsy.[15] A randomised study has shown that PGS by aCGH plus selection by morphology of blastocysts can significantly improve the ongoing pregnancy rate in patients with good prognosis when compared with selection of blastocysts by morphology alone.[16] Another randomised study also showed an improvement in the implantation rate after PGS by aCGH in addition to morphological assessment of embryos.[17] We report here the clinical outcome of 133 cycles of PGD/PGS by aCGH in a local unit.

Methods

Study population

Data from all treatment cycles performed for PGD and PGS in the Department of Obstetrics and Gynaecology, Queen Mary Hospital/The University of Hong Kong from 1 April 2012 to 30 June 2015 were retrieved. This study was done in accordance with the principles outlined in the Declaration of Helsinki. Patient consent has been obtained. Data were stored in a database and coded for indication. Indications for PGS were defined as: (1) advanced maternal age (AMA) group for patients aged >38 years; (2) recurrent miscarriage (RM) group with patients having at least two clinical miscarriages and negative investigations for RM; (3) repeated implantation failure group (RIF) with those who failed to get pregnant after three embryo transfer cycles with at least six good-quality embryos replaced; and (4) optional PGS group included those with normal karyotype but who had experienced a previous pregnancy with abnormal karyotype, and those who opted for PGS when performing PGD for monogenetic disease.

Indications for PGD by aCGH were divided as follows: (1) mosaic were those with mosaic sex chromosome abnormalities on karyotyping, including mosaic Klinefelter's or mosaic Turner's syndromes; (2) Robertsonian translocation; (3) reciprocal translocation; (4) inversion; and (5) double translocations.

Treatment regimen

The details of the ovarian stimulation regimen, gamete handling, and frozen-thawed embryo transfer (FET) have been previously described.[18] Surplus good-quality blastocysts with no aneuploidy/unbalanced chromosome detected were vitrified by the CVM Vitrification System (CryoLogic, Victoria, Australia). If the patient did not get pregnant in the stimulated cycle, the vitrified blastocysts were warmed and replaced in subsequent FET cycles. The details of biopsy and PGD/ PGS by aCGH have been described elsewhere.[6,19] In brief, a single blastomere was removed from good-quality day-3 embryos (6-to-8 cell stage) and the blastomere underwent whole-genome amplification (SurePlex; BlueGnome, Cambridge, United Kingdom). Array CGH was performed using 24sure+ (BlueGnome) on reciprocal translocation and inversion cases while other cases were tested by 24sure V3 (BlueGnome) according to the manufacturer's protocol. All results were interpreted independently by two laboratory staff, usually with a high concordant rate (>95%). Discrepancies were resolved through consensus.

Results

Between 1 April 2012 and 30 June 2015, 94 couples underwent 133 cycles of ovarian stimulation for PGD for chromosomal abnormalities, or PGS with indications listed in Table 1. The most frequent indication for PGD/PGS was reciprocal

translocation (35.3%) followed by RM (27.1%) and Robertsonian translocation (16.5%). The median age of the women was 36.5 (range, 25-44) years. Embryo biopsy was performed in 112 cycles. The mean number of embryos biopsied per retrieval cycle was 5.6 (740/133), with 99.2% of biopsies resulting in a conclusive diagnosis, of which only 25.8% (191/740) were genetically transferrable. The whole-genome amplification failed in all the samples with inconclusive diagnosis.

Overall, PGD/PGS was cancelled in 21 (15.8%) cycles after ovarian stimulation due to poor response (19 cycles), failed fertilisation (1 cycle), or no sperm found in the testicular biopsy (1 cycle). In case of poor response (<4 good-quality embryos on day 3), cleavage-stage embryos were frozen/vitrified, subsequently thawed/warmed, and pooled with fresh embryos from the following stimulation cycle for diagnosis. Fresh embryo transfer was cancelled in 47 (42.0%) cycles after biopsy due to unavailability of genetically transferrable embryo (31 cycles), high serum progesterone level on the day of human chorionic gonadotropin (>5 nmol/L; 10 cycles), risk of ovarian hyperstimulation (2 cycles), delayed assay (3 cycles), or patient request (1 cycle). Overall, 65 PGD/PGS cycles proceeded to embryo transfer in the stimulated cycles with one or two blastocysts replaced on day 5 (mean, 1.4). As shown in Table 2,

TABLE 1. Indications for PGD and PGS

	No. (% of total) of cases	Median age (years)	% Of transferrable embryos*	OPR per ET (%)
PGS				
Recurrent miscarriage	36 (27.1)	37.5	30.7	52.9
Repeated implantation failure	8 (6.0)	40.5	25.4	33.3
Advanced maternal age	4 (3.0)	42.5	0.0	0.0
Optional PGS†	4 (3.0)	34.5	46.9	50.0
PGD				
Mosaic Turner's / Klinefelter's syndrome	8 (6.0)	37.5	32.7	80.0
Robertsonian translocation	22 (16.5)	33.0	31.8	25.0
Reciprocal translocation	47 (35.3)	34.5	17.5	30.4
Inversion	3 (2.3)	31.0	36.4	33.3
Double translocation	1 (0.8)	40.0	0.0	0.0
Overall	**133**	**36.5**	**25.8**	**40.0**

Abbreviations: ET = embryo transfer; OPR = ongoing pregnancy rate; PGD = preimplantation genetic diagnosis; PGS = preimplantation genetic screening

* Genetically transferrable embryos

† Couples with normal karyotypes but opted for PGS due to previous pregnancy with abnormal karyotype or PGD on monogenetic disease

TABLE 2. Results of PGS / PGD by aCGH in stimulated and frozen-thawed embryo transfer cycles

	Stimulated ET		Frozen-thawed ET	
	PGS	PGD	PGS	PGD
No. of cycles started	52	81	-	-
No. of aCGH performed	43	69	-	-
No. of transfer	22	43	11	15
Mean No. of embryos replaced	1.6	1.3	1.1	1.2
Pregnancy rate per transfer	54.5% (12/22)	48.8% (21/43)	36.4% (4/11)	66.7% (10/15)
Ongoing pregnancy rate per transfer	50.0% (11/22)	34.9% (15/43)	36.4% (4/11)	60.0% (9/15)
Miscarriage rate	8.3% (1/12)	28.6% (6/21)	0% (0/4)	10% (1/10)
Implantation rate	45.7%	41.1%	41.6%	50.0%

Abbreviations: aCGH = array comparative genomic hybridisation; ET = embryo transfer; PGD = preimplantation genetic diagnosis which includes the cases with indications of mosaic Turner's syndrome, mosaic Klinefelter's syndrome, Robertsonian translocation, reciprocal translocation, and inversion; PGS = preimplantation genetic screening, which includes the cases with indications of recurrent miscarriage, repeated implantation failure, advanced maternal age, or optional PGS (simultaneous PGD on monogenetic disease + PGS, or patient with previous pregnancy with abnormal karyotype)

the result of aCGH was further subdivided into two categories (PGS and PGD) based on indications. The ongoing pregnancy rates (pregnancy beyond 8-10 weeks of gestation) of PGS and PGD were 50.0% (11/22) and 34.9% (15/43), respectively.

There were 26 cycles of FET in a natural cycle in which one or two biopsied and vitrified blastocysts were replaced (mean, 1.2), resulting in a pregnancy rate of 36.4% (4/11) in the PGS group and 66.7% (10/15) in the PGD group. Ongoing pregnancy rates in the PGS and PGD group were 36.4% (4/11) and 60.0% (9/15), respectively (Table 2). The miscarriage rates in the stimulated embryo transfer cycles and FET cycles were 21.2% (7/33) and 7.1% (1/14), respectively. The differences in ongoing pregnancy rate and miscarriage rate between stimulated embryo transfer and FET cycle were not statistically significant. All pregnant women following PGD for chromosomal abnormalities were referred to the Prenatal Counselling and Diagnosis team at Tsan Yuk Hospital for counselling and confirmation of the PGD result by prenatal diagnosis or postnatal cord blood karyotyping. Based on the available results of the confirmation tests, no misdiagnosis was found in this small series.

Discussion

The 13th data report of the ESHRE PGD Consortium includes a total of 1071 oocyte retrieval cycles for chromosomal abnormalities and 2979 oocyte retrieval cycles for PGS, resulting in a delivery rate of 21%-25% per transfer and an implantation rate of 22%-26%.[1] The ongoing pregnancy rate and implantation rate of the present series are 34.9%-50.0% and 41.1%-45.7%, respectively.

As shown in Table 1, the percentage of transferrable embryos varies among different indications for PGD/PGS. In cases of PGD for chromosomal abnormalities, as expected, the lowest percentage of genetically transferrable embryos was found in the reciprocal translocation group (17.5%), followed by the Robertsonian translocation group (31.8%) and the mosaic Turner's / Klinefelter's syndrome group (32.7%). These data are in line with those of the ESHRE PGD consortium,[1] of which the corresponding percentages are 16.6%, 33.5%, and 36.8%, respectively. The high proportion of unbalanced gametes can be explained by the segregation modes and behaviour of the translocated chromosomes during meiosis.[20]

In the PGS group (RM, RIF, AMA, and optional PGS), the overall percentage of genetically transferrable embryos was 27.5% (69/251), similar to that of the ESHRE PGD consortium (30%). It is noteworthy that there were no transferrable embryos in all four cases of AMA (median age, 42.5 years). It is well known that chromosomal aneuploidy increases exponentially with increasing maternal age.[21,22] Therefore, patients with advanced age should be counselled accordingly before the initiation of PGS cycles.

The cancellation rate for PGD/PGS after initiation of stimulation was 15.8% (21/133) and the reason for cancellation in the great majority of cases was poor ovarian response (19/21). Furthermore, for those cases proceeding to biopsy, 42.0% (31/47) did not have an embryo transfer, mainly due to no normal/balanced embryos available. When a low percentage of normal/balanced embryos is expected, patients can consider pooling embryos from several stimulation cycles and perform PGD/PGD in a single batch. Such 'batching' can increase the chance of having normal/balanced embryos and allow selection of the best-quality genetically transferrable embryos for replacement in the PGD/PGS cycle, instead of having multiple cycles with no embryo transfer.

There were 26 cycles of vitrified-warmed blastocyst transfer (11 cycles after PGS and 15 cycles after PGD) performed during a natural cycle. The ongoing pregnancy rate per transfer in these natural cycles after PGD appeared to be higher than those with transfer in a stimulated cycle, while the miscarriage rate of transfer in the natural cycle was lower than that of transfer in a stimulated cycle. Such findings did not reach statistical significance due to the small number of cases, however. Some reports have suggested that transfer of embryos in a natural cycle may result in a higher pregnancy and implantation rate than in a stimulated cycle due to the better receptivity of the endometrium without gonadotropin stimulation.[23-26]

The limitation of the present study was the small number of cases for each indication of PGS. Moreover, it was not a randomised controlled trial. The usefulness of PGS by aCGH in these cases needs to be confirmed in a large randomised controlled trial. It is noteworthy that aCGH cannot detect mutation and/or small chromosomal aberrations (<10 Mb for Robertsonian translocation, mosaic sex chromosome aneuploidy and PGS; <5 Mb for reciprocal translocation and inversion). False results can be attributed to mosaicism of embryos, although no misdiagnosis was found in the present study.

Conclusions

The clinical outcomes of PGD and PGS in our unit were comparable to those reported internationally. A genetically transferrable embryo after PGD that is replaced during a natural cycle may improve the ongoing pregnancy rate and implantation rate when compared with transfer during a stimulated cycle.

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Declaration

All authors have disclosed no conflicts of interest.

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