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Original Article

Performance of preimplantation genetic testing for aneuploidy in IVF cycles for patients with advanced maternal age, repeat implantation failure, and idiopathic recurrent miscarriage

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ABSTRACT

Objective: The primary objective of this study was to investigate whether preimplantation genetic testing for aneuploidy (PGT-A) of blastocysts through array comparative genomic hybridization (aCGH) improves live birth rates (LBR) in IVF cycles for patients with high prevalence of aneuploidy.
Materials and Methods: This study included 1389 blastocysts with aCGH results derived from 296 PGT-A cycles in IVF patients with advanced maternal age (AMA) (n = 87, group A), those with repeated implantation failure (RIF) (n = 82, group B), those with recurrent miscarriage (RM) (n = 82, group C), and oocyte donors (OD) (n = 45, young age, as a control group). Another 61 AMA patients without PGT-A procedures were used as a control group for group A. Vitrification was performed after blastocyst biopsy, and thawed euploid embryos were transferred in a nonstimulated cycle.
Results: For the AMA group, a significant increase in LBRs was found in the PGT-A group compared with the non–PGT-A group (54.1% vs. 32.8%, p = 0.018). Consistent LBRs (54.1%, 51.6%, 55.9%, and 57.1%, respectively, in group A, B, C, and young age group) were obtained for all the indications.
Conclusions: LBRs can be improved using PGT-A of blastocysts with aCGH in IVF cycles for patients with a high rate of aneuploidy, especially for patients with AMA.
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Introduction

The success rate of in vitro fertilization (IVF) has improved significantly in the last 40 years. Currently, numerous IVF stimulation protocols facilitate the application of individually tailored treatments. Moreover, rates of implantation and pregnancy due to IVF have been gradually increasing each year [1]. Aneuploidy rate of in vitro–produced embryos can exceed 60% [2]. Embryonic aneuploidy is the main cause of miscarriage and failure of assisted reproductive technology (ART) [3]. Aneuploidy screening of embryos originating from IVF patients is termed as preimplantation genetic testing for aneuploidy (PGT-A); it enables the examination of numeral and structural chromosomes of embryos before transfer [4]. This technique has been applied to treat patients with an increased risk of aneuploid embryos, such as those with advanced maternal age (AMA) [5–7], repeated implantation failure (RIF) [8–10], and recurrent miscarriage (RM) [11–13].

The combination of PGT-A performed on day 3 cleavage stage embryo biopsy and fluorescence in situ hybridization has been an acceptable procedure for selecting and transferring euploid embryos to improve the reproductive outcome of ART [14]. However, the efficacy of this strategy has been demonstrated to be low: a meta-analysis of nine randomized controlled clinical trials (RCTs) [15] revealed that this strategy did not improve but impaired the

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rate of live birth in women with AMA; this is attributable to the harmful effects of cleavage stage biopsy, the inability to test all chromosomes, and the relatively high rate of mosaicism on day 3 cleavage stage [16]. This has spurred the development of new technologies that overcome the shortcomings of previously validated procedures. Over the last decade, new techniques have been developed and evaluated for comprehensive chromosome screening (CCS), for example, array comparative genomic hybridization (aCGH), quantitative real-time polymerase chain reaction (RT-PCR), single-nucleotide polymorphism array, and next generation sequencing [17,18].

Similar to the cleavage stage, the blastocyst stage may also increase the efficiency of morphological selection as well as the proportion of euploid embryos [19]. In addition, Scott et al. [20] showed that cleavage stage biopsy significantly impairs the implantation potential of human embryos, whereas trophectoderm (TE) biopsy at blastocyst stage does not. However, the time available for performing genetic analysis prior to fresh transfer remains limited, particularly considering the period after blastocyst biopsy. Vitrification has been verified to be efficient for the cryopreservation of blastocysts; moreover, vitrification of biopsied blastocysts would yield the time necessary for genetic analysis. Furthermore, a recent review evidences that frozen embryo transfer decreases the risk of ovarian hyperstimulation syndrome and improves the perinatal outcomes [21].

A recent systematic review concluded that the combined use of PGT-A and CCS following blastocyst biopsy can improve the IVF outcome in good-prognosis patients [22]. Whether these findings apply to poor-prognosis patients remains to be determined.

In younger patients undergoing IVF, high proportions of human embryos were found to be aneuploid [23,24]. Donor oocytes are usually collected from young and fertile women. However, their oocytes are collected after controlled ovarian stimulation by using high dosages of gonadotropins, following which the oocytes are exposed to in vitro environments for manipulation; many aspects of this process, especially the genetic alternations, remain unclear.

The purpose of this study was to analyze the clinical outcomes following blastocyst biopsy, vitrification, and aneuploidy assessment by using aCGH in oocyte donation cycles and in IVF cycles for patients with high prevalence of aneuploidy.

Materials and methods

Patient selection

This retrospective study was conducted between November 2012 and January 2015 by using the data of 296 couples undergoing controlled ovarian stimulation for IVF with preimplantation genetic testing for aneuploidy (PGT-A) at the Lee Women's Hospital in Taichung, Taiwan. These couples included infertile patients with AMA (≥38 years) (n = 87), those with RIF (≥3 failed IVF cycles with transfer of good embryos) (n = 82), those with idiopathic RM (≥2 miscarriages of unknown etiology) (n = 82), and oocyte donors (OD) (n = 45, young age, control group). Another 61 AMA patients without PGT-A procedures were used as the control group for the AMA group. Ethical approval (CS-14124) was obtained from the Institutional Review Board of Chung Shan Medical University Hospital.

Blastocyst culture and biopsy

Procedural details of controlled ovarian stimulation, oocyte collection, and insemination have been previously reported [25]. Embryos that reached the blastocyst stage with expanded blastocele were included for biopsy. Zona opening and TE cell retrieval were then performed on day 5 or 6 of preimplantational development. All biopsy procedures were conducted on a heated stage of a Nikon Diaphot 300 Inverted microscope. A Fertilase laser system was used to assist the opening of a 10–20 μm hole in the zona pellucida. After inserting the pipette into the zona, 5–10 TE cells were aspirated into the biopsy pipette followed by laser-assisted separation from the body of the embryo. After biopsy, the blastocyst was moved to a post-biopsy dish and placed into an incubator until vitrification. The biopsied TE cells were immediately placed in RNAse–DNAse-free PCR tubes.

Whole genome amplification (WGA) and array CGH analysis

The biopsied TE cells were collected in 2.5 μL phosphate-buffered saline and amplified using the SurePlex DNA Amplification System (Illumina, Inc., San Diego,CA, USA). WGA amplification resulted in a DNA concentration of 20–40 ng/μL. Samples that were successfully amplified were processed using 24sure V3 microarray (Illumina, Inc.). For array CGH analysis, samples and control DNA were labeled using Cy3 and Cy5 fluorophores. Per manufacturer instructions, 8 μL of amplified DNA or reference DNA were combined with 5 μL primer solution. The combined product was then incubated for 5 min at 94 °C and for 5 min at 4 °C. Then, 12 μL of Cy3 or Cy5 labeling master mix was added to the DNA/primer solution and incubated for 2–4 h at 37 °C. Next, 18 μL of the DNA solution was used for hybridization to the BAC array for 4–16 h at 47 °C. Per the 24sure protocol, the washing steps were performed, and the washed slides were centrifuged at 170 × *g* for 3 min to prepare them for scanning. Fluorescence intensity was detected using a laser scanner (InnoScan 710, Innopsys, Carbonne, France), and the signals were called using the BlueFuseMulti software (BlueGnome, Cambridge, UK) for whole chromosome gain or loss. For the analysis of a diploid cell, the predicted log2 ratio for chromosome gain was +0.58 (log2 3/2) and that for chromosome loss was −1.0 (log2 ½). Results were categorized as euploidy or aneuploidy.

Blastocyst cryopreservation and vitrified embryo transfers

After TE biopsy, blastocysts were cryopreserved through vitrification. The details of the vitrification and thawing protocols were previously reported [26]. Frozen–thawed euploid blastocysts were transferred in hormone replacement cycles for anovulatory women or in natural cycles in ovulatory women. Implantation rate (IR) was defined as the percentage of transferred embryos developing to an implanted gestational sac. Clinical pregnancy rate (PR) per transfer was calculated as the percentage of clinical pregnancies with a fetal heartbeat. Miscarriage rate (MR) was defined as the percentage of clinical pregnancies that were spontaneously miscarried before 20 weeks' gestational age. The live birth rate (LBR) was defined as the number of cycles with a live birth.

Statistics

The PGT-A group and the control group were compared using the chi-square test or the Student's *t-test* to determine statistically significant differences in their basic characteristics and clinical outcomes. All data were analyzed using SPSS 22.0 software package, and differences were considered significant at $P < 0.05$.

Result

This study analyzed 1389 blastocysts with CCS results derived from 296 PGT-A cycles. Embryo transfer was not performed in 61 PGT-A cycles due to all chromosomal abnormalities, including AMA (n = 26), RIF (n = 18), RM (n = 14), and OD (n = 3). In 235 PGT-A

cycles (AMA (n = 61), RIF (n = 64), RM (n = 68), and OD (n = 42)), at least one euploid embryo was available for transfer.

Table 1 lists the clinical outcomes for the different PGT-A indications studied. The maternal age was significantly lower in the OD group than in the AMA, RIF, and RM groups (24.8 vs. 39.6, 35.8, and 34.8; P < 0.001). The rate of all embryos with chromosomal abnormalities was significantly higher in the AMA group than in the young OD group (29.9% vs. 6.7%; P = 0.014). No significant differences were noted among the four groups in their mean number of embryos transferred, IR, PR, MR, and LBR. The LBRs per transfer were 54.1%, 51.6%, 55.9%, and 57.1% in the AMA, RIF, RM, and OD groups respectively.

Regarding AMA, the maternal age was significantly higher in the PGT-A group than the control group (39.6 vs. 38.8; P = 0.003). Similarly, the mean number of embryos transferred per cycle was significantly lower in the PGT-A group than in the control group (1.6 vs. 2.3; P < 0.001). Interestingly, 40 pregnancies and seven miscarriages occurred in the PGT-A group, resulting in an LBR per transfer of 54.1%. By contrast, 30 pregnancies and ten miscarriages occurred in the control group, resulting in an LBR per transfer of 32.8% (P = 0.018) (Table 2).

Discussion

The end goal of PGT-A and ART is to select one or two euploid embryos for transfer in order to maximize the chances of delivering a healthy baby. Aneuploidy rates are extremely high in IVF embryos, especially in patients with AMA [27], RIF [28], and unexplained RM [29]. Because aneuploidy is a leading cause of implantation failure, selection of a euploid embryo has been hypothesized to significantly improve the IR. In this study, the LBRs in the high-aneuploidy groups (AMA, RIF, and RM) might be elevated to as high as those in young age control group (OD) through PGT-A of blastocysts with aCGH.

The PR of IVF decreases with patient age [30,31]. Data from PGT-A suggest that implantation losses are associated with chromosomal abnormalities and that aneuploidy rates increase sharply with AMA [2,32,33]. Therefore, a reliable method for selecting euploid blastocysts is crucial for increasing implantation and decreasing pregnancy losses for patients with AMA. Capalbo et al. [34] demonstrated that blastocyst biopsy with aCGH is a reliable method of detecting euploid embryos for transfer.

Schoolcraft and Katz-Jaffe [6] assessed the outcomes of CCS-based PGT-A versus conventional morphology-based selection in AMA women undergoing single-blastocyst transfer. The ongoing PR of the CCS group was significantly higher than for the morphology-based group (60.0% vs 43.8%, P < 0.05), highlighting the importance of PGT-A in AMA patients. Consistent with this finding, the multicenter study of Harton et al. [35] found that implantation and ongoing PR did not decrease in patients aged 35–42 years subjected to PGT-A–based single embryo transfer (SET). A review by Wu et al. [36] and a recent study by Ubaldi et al. [37] have both emphasized the significance of TE biopsy, vitrification, and CCS for SET in AMA patients. Moreover, Lee et al. [38] showed that the performance of TE biopsy and PGT-A using aCGH could improve the LBR in women aged 40–43 years. The LBR for PGT-A FET cycles was significantly higher compared with no PGT-A FET cycles (45.5% vs 19.0%, P = 0.0028) [38]. Our data demonstrated that although the mean number of embryos transferred was significantly lower in the PGT-A group than in the control group (1.6 vs. 2.3; P < 0.001), a significantly higher LBR was achieved in the PGT-A group than in the control group (54.1% vs. 32.8%, P = 0.018) (Table 2).

Two prospective RCTs performed in RIF patients have shown no significant differences in clinical PR between PGT-A patients (FISH and day 3 biopsy) and control groups [5,10]. However, a clinical study suggested that CCS may help patients with RIF become capable of producing blastocysts and achieve pregnancy [9]. Furthermore, a recent pilot study showed that PGT-A by using aCGH with single euploid blastocyst transfer can improve the rates of clinical pregnancy and implantation (68.3% and 70.5%, respectively) for patients with RIF [8]. Our study showed that PGT-A through array CGH with euploid blastocyst transfer has a LBR of 51.6% and an IR of 45.7% for patients with RIF (Table 1). Our results indicated that endometrial receptivity in addition to embryo a duplicity play a substantial role in RIF patients.

RM is a multifactorial disorder defined by two or more pregnancy losses [39]. Hodes-Wertz et al. [13] found that idiopathic RM is mostly caused by aneuploidy embryos and that PGT-A with aCGH could decrease MR and improve PR. IVF/PGT-A appears to lower the miscarriage risk compared with natural conception; however, the LBR per cycle is variable [12]: a recent study showed that the IVF/PGT-A strategy has a LBR of 53% and a clinical MR of 7%, whereas expectant management had a LBR of 67% and a clinical MR of 24% for patients with unexplained RM [11]. However, the IVF/PGT-A strategy was 100-fold more expensive for a live birth compared with expectant management [11]. Our study also showed that IVF/PGT-A strategy has a LBR of 55.9% and a MR of 7.0% for patients with RM (Table 1). IVF/PGT-A may not be a cost-effective strategy to increase live birth for patients with RM; however, it provides an opportunity to decrease the miscarriage risk in patients who have experienced multiple pregnancy losses and the emotional distress of RM.

Chromosome aneuploidy is common in embryos following IVF, even in younger women, and is a major factor in IVF failure. It seems

Table 1
Clinical outcomes of PGT-A cycles according to numerous infertility indications.

	AMA	RIF	RM	OD	P-value
Initiated cycles	87	82	82	45	
No ET cycles[a]	26 (29.9%)	18 (22.0%)	14 (17.1%)	3 (6.7%)	0.014
ET cycles	61	64	68	42	
Age (yrs) (Mean ± SD)	39.6 ± 1.7	35.8 ± 4.2	34.8 ± 4.3	24.8 ± 3.0	<0.001
Mean transferred embryos (Mean ± SD)	1.6 ± 0.5	1.7 ± 0.5	1.6 ± 0.5	1.7 ± 0.5	0.509
Implantation rate	56.1% (55/98)	45.7% (48/105)	49.1% (52/106)	52.9% (36/68)	0.485
Pregnancy rate per initiated cycle	46.0% (40/87)	45.1% (37/82)	52.4% (43/82)	66.7% (30/45)	0.090
Pregnancy rate per ET cycle	65.6% (40/61)	57.8% (37/64)	63.2% (43/68)	71.4% (30/42)	0.543
Miscarriage rate	17.5% (7/40)	8.1% (3/37)	7.0% (3/43)	16.7% (5/30)	0.352
Live birth rate per initiated cycle	37.9% (33/87)	40.2% (33/82)	46.3% (38/82)	53.3% (24/45)	0.322
Live birth rate per ET cycle	54.1% (33/61)	51.6% (33/64)	55.9% (38/68)	57.1% (24/42)	0.941

AMA: advanced maternal age; RIF: repeated implantation failure; RM: recurrent miscarriage; OD: oocyte donation.
P value through ANOVA; Chi-square Test or Fisher's exact test as appropriate.
[a] No embryo transfer due to all chromosomal abnormalities.

Table 2
Clinical outcomes of PGT-A and control cycles in AMA patients.

	PGT-A	Control	P-value
Embryo transfer cycles	61	61	
Age (yrs) (Mean ± SD)	39.6 ± 1.7	38.8 ± 1.1	0.003
Mean transferred embryos (Mean ± SD)	1.6 ± 0.5	2.3 ± 0.6	<0.001
Implantation rate	56.1% (55/98)	27.3% (38/139)	<0.001
Pregnancy rate per ET cycle	65.6% (40/61)	49.2% (30/61)	0.067
Miscarriage rate	17.5% (7/40)	33.3% (10/30)	0.126
Live birth rate per ET cycle	54.1% (33/61)	32.8% (20/61)	0.018

unlikely that all embryos will be screened, particularly if it requires invasive and time-intensive biopsy procedures. However, it is becoming increasingly established that testing 24-chromosome copy number in high-risk patients is an integral part of clinical practice. In our study, blastocyst biopsy with array CGH appeared to be associated with increased LBR in AMA patients. However, this strategy has some limitations. First, genetic testing for aneuploidy of blastocysts is time-consuming, requiring several hours to a day, which can result in blastocyst cryopreservation and embryo transfer in the next cycle. Second, the technology is rather expensive; thus, routine use requires careful consideration. A recent systemic review highlighted the lack of data available to evaluate the cost-effectiveness of PGT-A-CCH in clinical practice [40]. Third, most women with AMA experience a decline in oocyte numbers; consequently, it becomes difficult to cultivate embryos up to the blastocyst stage in order to perform PGT-A [41]. Finally, in this retrospective study, we selected only AMA patients without PGT-A as the control group; given the ethical concerns, RIF and RM patients without PGT-A were not included in the analysis. Nonetheless, the clinical PRs and LBRs were lower for RIF and RM patients compared with young age group, respectively, in previous IVF attempts without PGT-A. With the aid of PGT-A, LBRs as high as those in the young age group can be realized.

In conclusion, our study showed that improved LBR can be obtained following blastocyst biopsy, vitrification, and aneuploidy assessment by using aCGH in IVF cycles for patients with a potentially high rate of aneuploidy, especially patients with AMA. However, a large RCT is necessary to affirm our findings.

Conflict of interest

The authors have no conflicts of interest relevant to this article.

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