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ARTICLE

Concordance of PGT for aneuploidies between blastocyst biopsies and spent blastocyst culture medium

BIOGRAPHY

Professor Chang Long Xu, PhD, is Director of the IVF-LAB of the Reproductive Medical Center of Nanning Second People's Hospital. He did a postdoctoral fellowship at McGill University on assisted reproductive technology and has expertise in the fields of semen analysis, oocyte and embryo vitrification, intracytoplasmic sperm injection, embryo biopsy, etc.

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KEY MESSAGE

Non-invasive PGT-A of spent blastocyst culture medium enables precise, reliable assessment of embryo chromosome status. However, its performance may not be superior to invasive PGT of trophectoderm biopsy under current circumstances.

ABSTRACT

Research question: Non-invasive preimplantation genetic testing for aneuploidies (niPGT-A) avoids the possible detrimental impact of invasive PGT-A on embryo development and clinical outcomes. Does cell-free DNA (cfDNA) from spent blastocyst culture medium (BCM) reflect embryonic chromosome status better than trophectoderm (TE) biopsy?

Design: In this study, 35 donated embryos were used for research and the BCM, TE biopsy, inner cell mass (ICM) and residual blastocyst (RB) were individually picked up from these embryos. Whole genome amplification (WGA) was performed and amplified DNA was subject to next-generation sequencing. Chromosome status concordance was compared among the groups of samples.

Results: The WGA success rates were 97.0% (TE biopsy), 100% (ICM), 97.0% (RB) and 88.6% (BCM). Using ICM as the gold standard, the chromosomal ploidy concordance rates for BCM, TE biopsy and RB were 58.33% (14/24), 68.75% (22/32) and 78.57% (22/28); the diagnostic concordance rates were 83.33% (20/24), 87.50% (28/32) and 92.86% (26/28); and the sex concordance rates were 92.31% (24/26), 100% (32/32) and 100% (28/28), respectively. Considering RB the gold standard, the chromosome ploidy concordance rates for BCM and TE biopsy were 61.90% (13/21) and 81.48% (22/27); the diagnostic concordance rates were 71.43% (15/21) and 88.89% (24/27); and the sex concordance rates were 91.30% (21/23) and 100% (27/27), respectively.

Conclusions: The results of niPGT-A of cfDNA of spent BCM are comparable to those of invasive PGT-A of TE biopsies. Modifications of embryo culture conditions and testing methods will help reduce maternal DNA contamination and improve the reliability of niPGT-A.

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KEYWORDS

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Trophectoderm biopsy

INTRODUCTION

Preimplantation genetic testing (PGT) for embryo chromosome abnormalities dramatically improved the clinical outcomes of the first embryos transferred for IVF (*Cimadomo et al., 2020*). As a widely used invasive sampling method for PGT, the trophectoderm (TE) biopsy has a few drawbacks that need to be addressed. A skilled embryologist must harvest four to eight cells from the TE using special equipment, which may harm the viability and developmental potential of the embryo to be transferred (*Makhijani et al., 2021*; *Tiegs et al., 2021*; *Zhang et al., 2019*). Moreover, a TE biopsy does not always accurately reflect the embryonic status when mosaicism exists (*Popovic et al., 2020*).

Because of concerns regarding the safety and accuracy of invasive biopsies, there is a move to replace them with non-invasive preimplantation genetic testing for aneuploidies (niPGT-A). Cell-free DNA (cfDNA) is present in the spent blastocyst culture medium (BCM) and successful niPGT-A was achieved for the first time using two high-quality spent BCM samples of cfDNA (*Stigliani et al., 2013*).

Several studies have assessed the clinical utility of niPGT-A by comparing its results with a TE biopsy as the gold standard. Not surprisingly, these studies have drawn contradictory conclusions. It appears that niPGT-A has similar or comparable performance compared with TE biopsy (*Chen et al., 2021*; *Rubio et al., 2019*, *2020*; *Shitara et al., 2021*; *Xu et al., 2016*). The problem is that success rate of niPGT-A for DNA amplification (63.0%) is low and there is high chromosome discordance (40.4%) (*Hanson et al., 2021*). Therefore, it remains to be determined conclusively whether niPGT-A is superior to the TE biopsy.

The cfDNA in spent BCM comes from the apoptosis of cells in both the TE and inner cell mass (ICM), with variable percentages of contaminating maternal DNA (*Bolton et al., 2016*; *Chen et al., 2021*; *Hardy, 1999*). Theoretically, it is a better indicator of the whole embryo than TE biopsy if the maternal DNA contamination is low. Accordingly, niPGT-A should have higher concordance with the PGT-A of the ICM or residual blastocyst (RB) (blastocyst with most of the ICM removed) than TE biopsy. Previous studies have been unable to validate this because they have either used donated blastocysts that vitrify on days 5/6, which means that cfDNA would accumulate to day 6/7; they have used donated blastocysts diagnosed with aneuploidy; or they have used only ICM or a mixture of ICM with TE (*Chen et al., 2021*; *Huang et al., 2019*; *Li et al., 2021*; *Rubio et al., 2019*, *2020*).

In this study, a TE biopsy, the ICM, RB and spent BCM of each of the 35 donated embryos were picked up separately for study (FIGURE 1) and the results of chromosome status testing compared among these groups.

MATERIALS AND METHODS

Study subjects and ethical approval

A total of 35 embryos (26 day 3 embryos + 9 day 5 blastocysts) from 23 patients (aged 26–42 years) were used for this study at the Reproductive Center of the Second People's Hospital of Nanning from January 2020 to January 2022. This study was approved by the Ethical Committee of the Reproductive Center (approval no. Y2020065; 17 November 2020) and all enrolled participants provided written informed consent.

Sample collection

Intracytoplasmic sperm injection (ICSI) was performed according to standard protocols. In total, 87 donated day 3 embryos were warmed and individually cultured in G-1 medium (Vitrolife, Sweden) supplemented with 10% Serum Substitute Supplement (SSS) (Irvine Scientific, USA). The embryos were carefully washed three times and individually cultured in 15 µl microdroplets of BCM (COOK, Australia) on day 4. Embryos were allowed to grow until high-quality expanded blastocysts (better than 4BC/4CB) developed (26 of the 87 embryos) according to Gardner's morphological assessment criteria (*Gardner et al., 2000*). At the same time, another nine donated day 5 blastocysts were thawed and individually cultured in 15 µl microdroplets of BCM for 24 h. Biopsies were performed on day 5 (n = 21) or day 6 (n = 5 + 9). The spent BCM was collected after the embryo was transferred into a separate biopsy dish. The biopsies were performed in a separate biopsy dish rather than in the culture medium. Embryos were washed three times to remove contamination before sampling. Five to ten cells were firstly picked up from the TE using the biopsy needle. The ICM was then picked up using a separate needle. The TE biopsy and ICM, as well as the RB, were separately transferred into RNase/DNase-free polymerase chain reaction (PCR) tubes and stored at –80°C for the following analysis.

WGA and next-generation sequencing

TE biopsy, ICM, RB and BCM samples were separately processed in lysis buffer and subjected to WGA using the PicoPLEX WGA Kit (TAKARA, Japan). TE biopsy, ICM and RB amplifications were performed using a standard PicoPLEX WGA method according to the manufacturer's instructions. BCM amplifications were performed using the same PicoPLEX WGA method with minor modification (BCM-WGA). Because the volume of BCM sample for amplification was large (10 µl), the reaction systems of each step of the BCM-WGA method were doubled compared with the standard PicoPLEX WGA method. In addition, the BCM-WGA method added three more PCR cycles (17 cycles versus 14 cycles) in the second-round amplification to increase the final DNA yield. The BCM-WGA method was strictly tested before being used for BCM amplification to prevent amplification bias. The Ion Plus Fragment Library Kit (Thermo Fisher Scientific, USA) was applied for library construction. The Qubit dsDNA HS Assay Kit (Life Technologies, USA) was used to measure the DNA concentration of WGA products and sequencing libraries with the Qubit 3.0 fluorometer (Life Technologies). Next-generation sequencing was performed using the DA8600 platform (Basecare, China). Sequencing data were processed using Ion Reporter™ Software (Thermo Fisher Scientific, USA).

Data analysis

Generally, data analysis used the same method as described previously with necessary modifications (*Gui et al., 2019*). Sequencing data of the TE biopsy, ICM and RB were analysed using a regular PGT-A analysis algorithm (algorithm-PGT-A); sequencing data of spent BCM were analysed using a modified algorithm (algorithm-BCM), which was developed based on the algorithm-PGT-A. As the BCM sample had a limited volume and highly degraded genomic DNA, this makes the coefficient of variation of

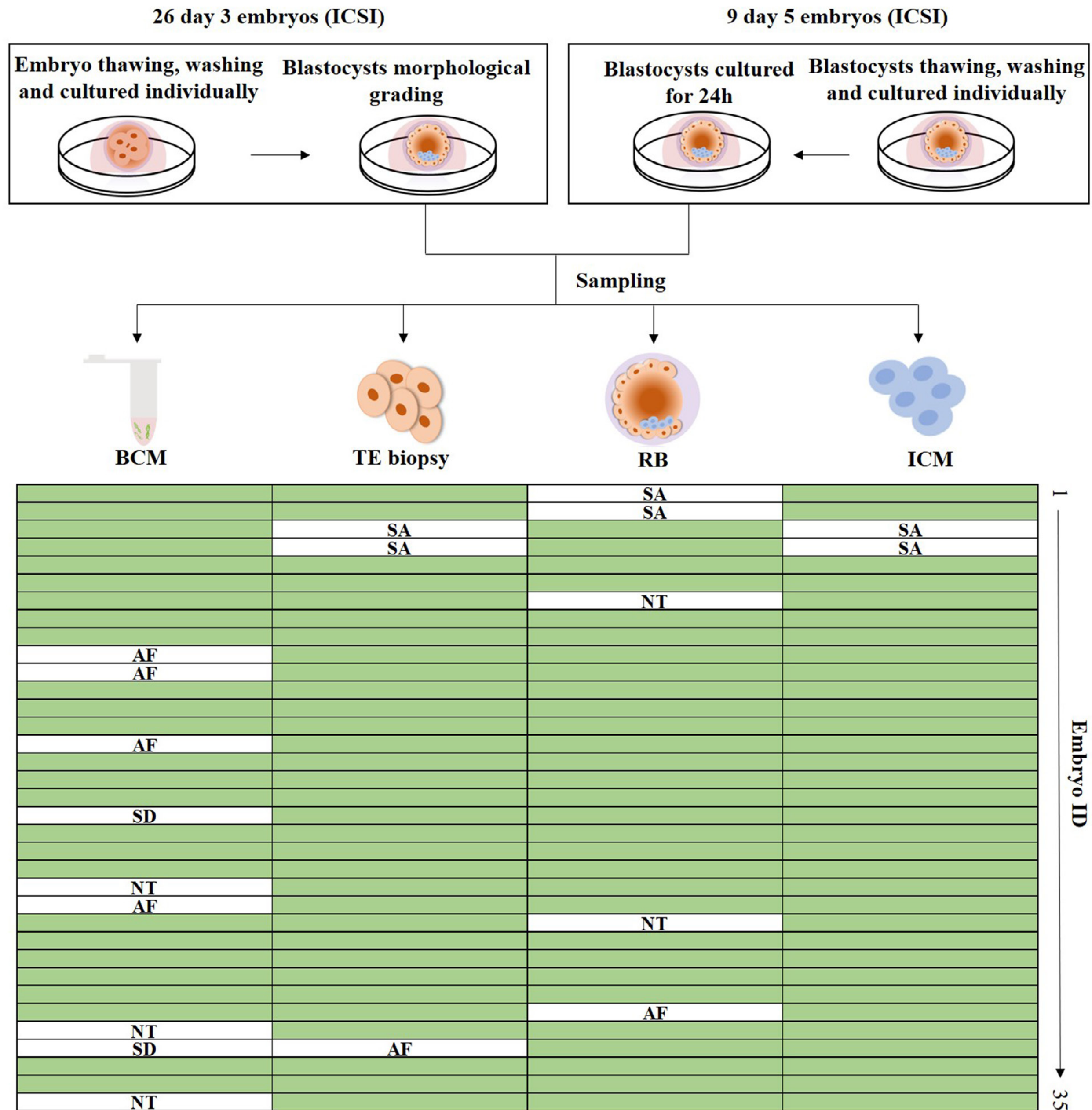

FIGURE 1 Schematic diagram showing the study design and genetic testing results of each of the 35 donated embryos. Retrieved oocytes were fertilized using standard ICSI protocols. Twenty-six cleaved embryos were thawed, washed (separately three times) and cultured individually on day 4. The embryos were graded morphologically on day 5/6, and high-quality blastocysts (superior to 4CB/4BC grade) were collected for sampling. Another nine day 5 blastocysts were thawed, washed and cultured individually for 24 h for sampling. The ICM, TE biopsy, RB and corresponding spent BCM of the 35 blastocysts were picked up individually and stored at –80°C for subsequent analysis. The table at the bottom shows the genetic testing results for BCM, TE biopsy, RB and ICM (the four columns) of each blastocyst (each row; sample ID are listed on the right) in green (informative results) or white (non-informative results). Samples gave non-informative results mainly attributed to four conditions: amplification failure (AF), sex discordant (SD), sample absent (SA) and noisy trace (NT). BCM = blastocyst culture medium; ICM = inner cell mass; ICSI = intracytoplasmic sperm injection; RB = residual blastocyst; TE = trophectoderm.

the BCM profile larger than that of TE biopsy, RB and ICM. In order to provide more reliable genetic testing profiles, optimized parameters (Chen et al., 2020) were used and the algorithm-BCM was achieved. Data analysis was conducted using the Euclidean distance and circular binary segmentation (ED-CBS) analysis system. Each sample achieved at least 1 million sequencing reads. High-quality clean sequencing data were achieved by removal of sequencing adaptors and filtering of low-quality reads. Sequence alignment was performed using human genome hg19 as a reference. Cleaning of PCR-derived duplication and correction of GC bias were performed subsequently. Average read density of reference samples was used for data normalization. The chromosome window was set to 1 Mb with a sliding window of 500 kb. A circular binary segmentation algorithm was used for copy number variation (CNV) calling. For algorithm-PGT-A, the mosaic was defined when the mosaicism of identified chromosome abnormalities (deletion or duplication, >10 Mb) was between

30% and 70%; and aneuploid was defined when the mosaicism of identified chromosome abnormalities (deletion or duplication, >4 Mb) was larger than 70%. For algorithm-BCM, the mosaic was defined when the mosaicism of identified chromosome abnormalities (deletion or duplication, >20 Mb) was between 40% and 70%; and aneuploid was defined when the mosaicism of identified chromosome abnormalities (deletion or duplication, >10 Mb) was larger than 70%.

Chromosome ploidy concordance was defined when the results for the two samples were the same or had an intersection. Chromosome ploidy concordance includes two conditions: (i) full concordance, when the chromosomal status for all the chromosomes in both samples are exactly the same; (ii) partial concordance, when detected chromosomal abnormalities (regardless of the type: aneuploid, CNV or mosaic) have intersections (any abnormality involving the same chromosome, in the absence or presence of additional aneuploidies). For example, the profiles of TE biopsy (47XY;+21;+19[mos, 38%]) and RB (47XY;+21) samples of embryo 21 (TABLE 1) were deemed concordant (partial concordance) according to the chromosome ploidy concordance criterion described above. Mosaic samples (BCM: 40% < mosaicism < 70%; TE biopsy, ICM and RB: 30% < mosaicism < 70%) were deemed aneuploid in chromosome ploidy concordance calculation. Diagnostic concordance was defined when the results from both samples were euploid–euploid or aneuploid–aneuploid. When a sample was distributed into the aneuploid group, the exact chromosome abnormalities were not taken into consideration in diagnostic concordance calculation. Mosaic samples (BCM: 40% < mosaicism < 70%; TE biopsy, ICM and RB: 30% < mosaicism < 70%) were deemed euploid in diagnostic concordance calculation. Sex concordance was defined when the sex chromosomes of the samples were exactly the same.

RESULTS

WGA and genetic testing results

Thirty-five embryos were carefully washed and cfDNA was allowed to accumulate on day 4/5. Samples were selected only after a high-quality blastocyst formed on days 5/6. BCM cfDNA accumulated for 24–48 h until sampling. The successful amplification (purified DNA yield >150 ng) rates of TE biopsy, ICM, RB and BCM were 97.0% (32/33; two samples were absent), 100% (33/33; two samples were absent), 97.0% (32/33; two samples were absent) and 88.6% (31/35), respectively. The DNA yield enabled informative genetic testing results from 91.4% (32/35) of TE biopsies, 94.3% (33/35) of ICM, 85.7% (30/35) of RB and 74.3% (26/35) of BCM (FIGURE 1, TABLE 1, Supplementary Table 1). The non-informative genetic testing was mainly attributed to absent samples, DNA amplification failure, sex discordance or noisy trace (FIGURE 1, TABLE 1, Supplementary Table 1).

Concordance of genetic testing among BCM, TE biopsy, RB and ICM

To determine which samples performed the most accurately, the genetic testing results of BCM, TE biopsy and RB were compared with that of ICM as the gold standard; the chromosomal ploidy concordance rates for BCM, TE biopsy and RB were 58.33% (14/24), 68.75% (22/32) and 78.57% (22/28), respectively (TABLE 2, Supplementary Figures 1 and **2**). The diagnostic concordance rates for BCM, TE biopsy and RB were 83.33% (20/24), 87.50% (28/32) and 92.86% (26/28), respectively (TABLE 2, Supplementary Figures 1 and 2). RB had the highest concordance with ICM using both criteria, as expected.

Concordance of genetic testing among BCM, TE biopsy and RB

The cfDNA in BCM comes from apoptotic TE and ICM cells. Theoretically, genetic testing of BCM should have higher concordance with RB than TE biopsy. To test this hypothesis, RB was considered the gold standard, and the chromosome ploidy concordance rates for BCM and TE biopsy were 61.90% (13/21) and 81.48% (22/27), respectively (TABLE 2, Supplementary Figures 1 and 2). The diagnostic concordance rates for BCM and TE biopsy were 71.43% (15/21) and 88.89% (24/27), respectively (TABLE 2, Supplementary Figures 1 and 2). Unexpectedly, the TE biopsy showed higher concordance with RB than BCM according to both criteria, but not vice versa.

Sex concordance and maternal contamination

Several works have reported that spent BCM contains variable amounts of maternal DNA contamination, which may bias the final results (*Leaver and Wells, 2020*; *Ouchi et al., 2022*; *Rubio et al., 2020*). More than 30% of spent BCM samples have severe (>60%) maternal DNA contamination (*Chen et al., 2021*). A simple method to assess maternal DNA contamination is to compare the sex chromosomes (for male embryos) between the sample and ICM. Considering ICM the gold standard, the respective sex concordance rates for BCM, TE biopsy and RB were 92.3% (24/26), 100% (32/32) and 100% (28/28) (TABLE 2). This clearly demonstrated maternal DNA contamination did exist (BCM profiles of embryos 19 and 32; Supplementary Figure 3) and showed that the BCM was more likely to be affected than TE biopsy.

DISCUSSION

This work, for the first time, systemically compared the genetic testing performance of TE biopsy, ICM, RB and BCM samples selected from the same embryos. Although the samplings were performed on different days (21 embryos on day 5 and 14 embryos on day 6), there was no significant difference in chromosome ploidy between samples of the two groups of embryos. It was found that TE biopsy still performed better than cfDNA from spent BCM according to all three criteria (chromosomal ploidy, diagnostic and sex concordances). Unexpectedly, TE biopsy also had higher testing concordance with RB according to the same criteria than cfDNA of spent BCM. It is speculated that maternal DNA contamination is the main cause of these results, as the highest percentage of sex discordance was detected in BCM. By contrast, no sex discordance was detected in TE biopsies. Even with these problems, there is cause to remain optimistic regarding the potential of niPGT-A.

The concordance definition has important impact on assessing the clinical significance of niPGT-A. Informative genetic testing profiles include four types of results: euploid, chromosome aneuploid, CNV and mosaic. In this study, two concordance definitions were given: (i) the chromosome ploidy concordance holds a strict standard, which takes all chromosomal abnormalities (aneuploid, CNV and mosaic) into consideration in the calculation; (ii) the diagnostic concordance holds a flexible standard,

TABLE 1 GENETIC TESTING RESULTS FOR BCM, TE BIOPSY, RB AND ICM SAMPLES

ID	Age	Grade	BCM	TE biopsy	RB	ICM	Chromosome ploidy concordance	Diagnostics concordance	Sex concordance
1	26	4BB	46XY; del 11(q13.3-q25, 64M)	46XY; -6(mos, 41%); del 11(mos, p15.5-q13.3, 61M, 55.51%); del 11(q13.2-q25, 65M)	SA	46XY; del 11 (mos, p15.5-q13.2, 60M, 59.46%); del 11, q13.2-q25, 66M)	AI/AI/*/AI	A/A/*/A	M/M/*/M
2	26	4BC	46XY	46XY; del 9(mos, q31.1-q34.3, 34M, 31%)	SA	46XY	E/AT/*/E	E/E/*/E	M/M/*/M
3	26	5BC	46XY +X(mos, 57%); -Y(mos, 66%)	SA	46XY	SA	AB/*/E/*	E/*/E/*	M/*/M/*
4	28	5BC	46XX	SA	46XX	SA	E/*/E/*	E/*/E/*	F/*/F/*
5	38	4BC	46XX; +4(mos, 67%)	45XX; -4	46XX; -4(mos, 68%)	46XX; -4(mos, 46%)	AI/AI/AI/AI	E/A/E/E	F/F/F/F
6	38	4BC	46XY; +X(mos, 65%); -Y(mos, 65%)	46XY	46XY; +X(mos, 39%); -Y(mos, 60%); dup 11(p15.5-p15.4, 3.5M)	46XY	AR/E/AR/E	E/E/A/E	M/M/M/M
7	41	4BC	46XY;+12;-14	46XY; dup14(mos, q31.1-q32.3, 30%, 25M); del 14(mos, q11.2-q31.1, 36%)	NT	48XY; +19; +22; dup6 (q27, 4M); del 8(q23.1-q23.3, 8M)	AT/AT/*/AI	A/E/*/A	M/M/*/M
8	36	4BB	46XY; +X(mos, 55%); dup 3 (p24.1-p12.3, 46M)	46XY	46XY	46XY	AB/E/E/E	A/E/E/E	M/M/M/M
9	35	4BC	46XY	46XY	46XY	46XY	E/E/E/E	E/E/E/E	M/M/M/M
10	42	4BC	AF	45XY; -15	45XY; -15	45XY; -15	*/AI/AI/AI	*/A/A/A	*/M/M/M
11	42	6BB	AF	46XX	46XX	46XX	*/E/E/E	*/E/E/E	*/F/F/F
12	42	6BC	47XX;+16	47XX; +16	47XX; +16	47XX; +16	AI/AI/AI/AI	A/A/A/A	F/F/F/F
13	42	5BC	46XX	46XX;	46XX	46XX	E/E/E/E	E/E/E/E	F/F/F/F
14	38	5BB	46XX	46XX	46XX	46XX	E/E/E/E	E/E/E/E	F/F/F/F
15	32	6BB	AF	46XX	46XX	46XX	*/E/E/E	*/E/E/E	*/F/F/F
16	39	4BB	46XX; del 1(q21.1-q24.2, 21M); del 1(mos, q24.2-q44, 77 M, 62%)	46XX	46XX	46XX; +19(mos, 42%)	AB/E/E/AI	A/E/E/E	F/F/F/F
17	39	4BC	46XY;Choatic	46XY	46XY	46XY	AB/E/E/E	A/E/E/E	M/M/M/M
18	39	4BC	45XY;-8	45XY; -8	45XY; -8	45XY; -8;dup1(mos, p36.3-p13.2, 109M, 62%)	AI/AI/AI/AI	A/A/A/A	M/M/M/M
19	39	4BB	SD	46XY;	46XY	46XY; dup 2(mos, p25.3-p21, 44 M, 53.5%)	*/E/E/AI	*/E/E/E	F/M/M/M
20	39	4BC	46XX	46XX; -5(mos,33%); -16(mos, 36%); +17(mos, 33 %)	46XX	46XX;	E/AT/E/E	E/E/E/E	F/F/F/F
21	30	5BC	47XY; +21; dup9(mos, p24.3-p13.2, 37M, 59%)	47XY; +21; +19(mos, 38%)	47XY; +21	47XY; +21; dup10(p15.3-p15.1, 5M)	AI/AI/AI/AI	A/A/A/A	M/M/M/M
22	30	5BC	46XX	46XX	46XX	46XX; dup 1(mos, p36.3-p36.1, 19M, 39%)	E/E/E/AI	E/E/E/E	F/F/F/F
23	30	4BB	NT	46XY	46XY; +19(mos, 52%); dup14(mos,q11.2-q21.1, 20.0M, 53%); dup1(mos, p36.3-q22, 123M, 40%); dup14(mos, q21.2-q31.1, 37M, 48%)	46XY	*/E/AR/E	*/E/E/E	*/M/M/M
24	28	4BB	AF	47XY; +9; -12; +16; -22; +X; dup 20, p13-p11.2, 25M)	46XY; +9; -12; -22; +X; dup 16(p13.3-q24.3, 67M); dup 20(mos, p11.2-q13.3, 36M)	47XY; +9; -12; +16; -22; +X	*/AI/AI/AI	*/A/A/A	*/M/M/M

(continued on next page)

TABLE 1 *(continued)*

ID	Age	Grade	BCM	TE biopsy	RB	ICM	Chromosome ploidy concordance	Diagnostics concordance	Sex concordance
25	31	6BC	46XX; +7(mos, 41%); +7(mos,43%)	46XX; dup7(mos, p11.2-q11.23, 14M)	NT	46XX	AT/AT/*/E	E/E/*/E	F/F/*/F
26	32	5BC	47XY; +3; -13; +17; +4(mos, 67%)	46XY	46XY	46XY	AB/E/E/E	A/E/E/E	M/M/M/M
27	32	5BC	46XY	46XY	46XY	46XY	E/E/E/E	E/E/E/E	M/M/M/M
28	31	6BC	46XY; +5(mos, 41%); dup 7 (p22.3-q21.1, 77M); del 7 (q21.1-q36.3, 68 M); del 9(mos, p24.3-q33.1, 86M)	46XY	46XY; del 7(q21.1-q36.3, 68M)	46XY; del 7(q21.1-q36.3, 67M)	AI/E/AI/AI	A/E/A/A	M/M/M/M
29	31	6BB	46XY; -14(mos,42%); +19(mos,48%)	46XY	46XY	46XY	AB/E/E/E	E/E/E/E	M/M/M/M
30	27	5BC	46XX	46XX	AF	46XX	E/E/*/E	E/E/*/E	F/F/*/F
31	27	4BC	NT	46XY	46XY	46XY	*/E/E/E	*/E/E/E	*/M/M/M
32	27	4BB	SD	AF	46XY	46XY	*/*/E/E	*/*/E/E	F/*/M/M
33	35	4BB	46XY; Choatic	46XY	46XY	46XY; Choatic	AI/E/E/AI	A/E/E/A	M/M/M/M
34	35	5BB	46XY; +X(mos, 52%)	46XY; del 4(mos, p16.3-p14, 36M, 48%)	46XY	46XY	AB/AT/E/E	E/E/E/E	M/M/M/M
35	39	4BB	NT	46XY	46XY	46XY	*/E/E/E	*/E/E/E	*/M/M/M

* Samples gave non-informative results, including four conditions: amplification failure (AF); sex discordant (SD); sample absent (SA); noisy trace (NT).BCM = (spent) blastocyst culture medium; TE = trophectoderm; RB = residual blastocyst; ICM = inner cell mass; CPC = chromosome ploidy concordance; DC = diagnostic concordance; SC = sex concordance; del = deletion; dup = duplication; mos = mosaic; AF = amplification failure; SD = sex discordant; SA = sample absent; NT = noisy trace; A = aneuploid; E = euploid; M = male; F = female.

which takes the aneuploid and CNV into consideration but ignores all mosaic. Guidance from the Preimplantation Genetic Diagnosis International Society (PGDIS) (*Leigh et al., 2022*) holds the opinion that transfer of mosaic embryos is acceptable in clinic and the risk of giving birth to unhealthy babies is low. Given this, all the mosaic was deemed euploid in the diagnostic concordance calculation.

Obtaining high-quality WGA products is a prerequisite to achieving interpretable niPGT-A results. The cfDNA in the BCM is continually being generated and degrading. The amount of cfDNA present in BCM is affected by the genetic composition of the embryo, length of culture and sample collection time. The reported success rates of BCM amplification range from 62.7% to 95% but the studies reporting these data were not designed to match a clinical setting (*Hanson et al., 2021*; *Shitara et al., 2021*; *Yeung et al., 2019*). To enable the accumulation of cfDNA,

TABLE 2 CONCORDANCE RATES OF GENETIC TESTING RESULTS AMONG BCM, TE BIOPSY, RB AND ICM SAMPLES

Parameter	BCM vs ICM	TE biopsy vs ICM	RB vs ICM	TE biopsy vs RB	BCM vs RB
Chromosomal ploidy concordance	58.33 (14/24)	68.75 (22/32)	78.57 (22/28)	81.48 (22/27)	61.90 (13/21)
FPR	50.00 (7/14)	21.05 (4/19)	11.76 (2/17)	11.11 (2/18)	53.33 (8/15)
FNR	30.00 (3/10)	46.15 (6/13)	36.36 (4/11)	33.33 (3/9)	0.00 (0/6)
Sensitivity	70.00 (7/10)	53.85 (7/13)	63.64 (7/11)	66.67 (6/9)	100.00 (6/6)
Specificity	50.00 (7/14)	78.95 (15/19)	88.24 (15/17)	88.89 (16/18)	46.67 (7/15)
Diagnostics concordance	83.33 (20/24)	87.50 (28/32)	92.86 (26/28)	88.89 (24/27)	71.43 (15/21)
FPR	23.53 (4/17)	4.35 (1/23)	4.55 (1/22)	5.00 (1/20)	31.25 (5/16)
FNR	0.00 (0/7)	33.33 (3/9)	16.67 (1/6)	28.57 (2/7)	20.00 (1/5)
Sensitivity	100.00 (7/7)	66.67 (6/9)	83.33 (5/6)	71.43 (5/7)	80.00 (4/5)
Specificity	76.47 (13/17)	95.65 (22/23)	95.45 (21/22)	95.00 (19/20)	68.75 (11/16)
Sex concordance	92.31 (24/26)	100.00 (32/32)	100.00 (28/28)	100.00 (27/27)	91.30 (21/23)

Data are presented as % (*n*).

Note: The genetic testing result of the ICM/RB sample was considered the gold standard respectively when calculating the FPR, FNR, sensitivity and specificity of other samples.

BCM = (spent) blastocyst culture medium; ICM = inner cell mass; TE = trophectoderm; RB = residual blastocyst; FPR = false-positive rate; FNR = false-negative rate.

collecting BCM after a prolonged culture may hurt the developmental potential of embryos, which is unacceptable clinically (*Hernandez-Nieto et al., 2019*; *Kuznyetsov et al., 2018*). In this study, the embryo culture conditions were modified by reducing the culture medium volume to 15 µl. The PicoPLEX WGA method was also modified to make it suitable for BCM amplification. The modified BCM-WGA method underwent a rigorous testing process before being used for BCM amplification, to prevent possible amplification bias. With this, it was possible to successfully amplify 88.6% of BCM samples.

Maternal DNA contamination negatively affects niPGT-A results. cfDNA from spermoblasts and cumulus cells in the BCM can lead to contamination (*Ottolini et al., 2015*). To avoid this, the embryos from patients who had undergone intracytoplasmic sperm injection were washed carefully and the culture medium was changed on day 4. However, at least two samples (BCM of embryos 19 and 32) showed severe maternal contamination (TABLE 1, Supplementary Figure 3). Further optimization of the embryo culture conditions and testing methods is needed to minimize maternal contamination in clinical application.

There are some study caveats to mention. In total 96 donated embryos (87 slow frozen day 3 embryos plus nine day 5 blastocysts) were used in this study. Because of the low blastocyst development rate of the day 3 embryos (26/87, 29.9%), 35 blastocysts were finally obtained, making the sample size of this study relatively small. In addition, the RB samples used here had variable percentages of ICM cell contamination. As ICM cells are firmly packed into the blastocyst, it was very difficult to separate the ICM from the RB. The RB samples were in fact combinations of TE and ICM cells. Although previous studies have reported that cfDNA in BCM is mainly derived from the TE and ICM, niPGT-A of BCM should theoretically have higher concordance with PGT-A of RB than PGT-A of TE biopsy. However, the current data did not support this hypothesis due to maternal DNA contamination (Supplementary Figure 3).

In conclusion, niPGT-A of BCM enables precise, reliable assessment of embryo chromosome status, although the performance may not be superior to invasive PGT of TE biopsy under current circumstances. Further studies are needed to modify the current niPGT-A testing process to minimize maternal DNA contamination and improve the reliability.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.rbmo.2022.10.001.

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