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Chromosome constitution of equal-sized three-cell embryos using next-generation sequencing technology

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Abstract

Purpose To study the chromosome constitution of equal-sized three-cell embryo.

Methods We determined the chromosome constitution of 105 blastomeres from 35 embryos using multiple annealing and looping-based amplification cycles (MALBAC) together with NGS sequencing technology. Chromosomal copy number variation (CNV) analysis was successfully performed in 27 embryos. We also analyzed radius, perimeter, area, and volume of each blastomere to explore the possibility of selecting the normal embryos.

Results Majority of the embryos (77.8%, 21/27) studied were mosaic or aneuploid, and only 22.2% (6/27) had normal chromosome numbers. The aneuploid chromosomes spread across all chromosomes and the most frequent aneuploidies were for chromosomes 1, 16, and 18 followed by 13, 19, and 21. Statistical analyses showed no significant difference between euploid and aneuploid embryos regarding radius, perimeter, area, and volume of their blastomeres.

Conclusions Our results showed that majority of the equal-sized three-cell embryos were chromosomally abnormal and could not be distinguished by morphology observation, so they should be given lower priority at selection for transfer.

Keywords Assisted reproduction · Chromosome constitution · Equal-sized three-cell embryos · Next-generation sequencing · Aneuploidy

Introduction

Embryo morphology is the most common criterion used method in assisted reproduction for selection of embryos for transfer and cryopreservation. Consensus for embryo assessment parameters has been reached [1]. Cytokinetic irregularity is one of the assessment parameters. Trichotomic mitosis is an abnormal phenomenon and is considered to be a non-stage-specific cleavage pattern [2]. Time-lapse observations confirm the occurrence of the phenomenon in embryos and cancer cells with a frequency ranging from 1.7 to 12.2% [3–7].

In the zygote stage, trichotomic mitosis produces three-cell embryos, which often have equal-sized blastomeres, regular in symmetry, and without cellular fragments. On the other hand, blastomeres of human embryos are known to cleave asynchronously and can produce a three-cell embryo with one blastomere still undergoing mitosis. In this latter case, the blastomeres are not of equal size as the one in mitosis should be larger than the others. Three-cell embryos with equal-sized blastomeres have lower blastocyst formation rate and a trend of lower implantation rate [3]. The reason for the reduced performance of equal-sized three-cell embryos is not fully known. One study focuses on direct unequal cleavages (DUC), which is a special kind of trichotomic mitosis that include abrupt cleavage of one blastomere into three daughter blastomeres or an interval of cell cycles less than five hours. It reports that the euploid rate in D3 biopsied embryos with DUC-1, namely the direct unequal cleavage at first cleavage, is only 13.3% and the rate of complex abnormality error turns out to be 83.3% [8]. There is one conference report showing association of these embryos with chromosomal abnormalities; 36 of 37 embryos derived by direct cleavage of zygote into more than two cells had > 50% aneuploid blastomeres as analyzed by FISH on 5 chromosome [9]. The lack of studies on these embryos might be due to their transient existence making them easy to be

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missed in conventional microscopy [7]. Another reason is the difficulty in analyzing all the chromosomes in a single cell, which has now been solved by the recent advances on single-cell comprehensive chromosome screening by microarray and next-generation sequencing (NGS) [10–12].

The aim of this report was to explore the chromosome constitution of all blastomeres in equal-sized three-cell embryos using multiple annealing and looping-based amplification cycles (MALBAC) together with NGS sequencing.

Materials and methods

Sample collection

The Institutional Review Board of Chinese PLA General Hospital (S2016-106-01) approved this study. All the recruited patients signed a written consent. This study was carried out from Dec 2016 to Aug 2017. The etiology of infertility fell into tubal factor (32%), endometriosis (4%), ovulatory factor (16%), male factor (28%), unexplained factor (12%), and combination factor (8%). Controlled ovarian hyperstimulation and oocyte retrieval were undertaken as described [13]. Oocytes were inseminated with conventional insemination or ICSI followed by culturing in G-1medium (Vitrolife AB, Göteborg, Sweden). Fertilization was assessed after 16–18 h by the presence of two pronuclei under an inverted microscope with × 200 magnification (Nikon Ti-U, Japan). A total of 35 embryos, each consisting of three equal size blastomeres, were collected at 42–44 h post fertilization. They were freed from their zona pellucida in acidified Tyrode's medium (Sigma-Aldrich, USA) and seggregated into 105 individual cells by mechanical means followed by washing in PBS (Sigma-Aldrich, USA) three times.

Morphology assessment of the blastomeres

The radius, perimeter, area, and volume of each blastomere were measured after disaggregation of the embryos into single cells using the software of a laser micromanipulator system (Research Instruments, UK).

Whole genome amplification and DNA sequencing

MALBAC whole genome amplification protocol was used according to the manufacturer's standard protocol (Yikon Genomics Inc., China). Each cell was transferred carefully into 5 μL of the Lysis Reaction Mix in a PCR tube. Then the cell was lysed by heating and sequential amplification. Following purification of the products and library preparation, the amplified genome of each sample was sequenced, yielding at least ~ 0.005× sequencing depth (1.5 Mb/3 Gb) on an Illumina HIseq 2500 platform with single read 41.

Chromosome copy number analysis

Chromosomal copy number variation (CNV) analyses were performed as previously described [14]. Read numbers were counted and displayed with a bin size of 10 kb along the whole genome. Copy number gain from two to three copies resulted in a 50% increase in read counts in a genome segment, while copy number loss from two to one resulted in a 50% decrease in read counts in a genome segment. After GC correction, mapped reads were normalized to relative read numbers at 30–35 reads per 10-kb bin for CNV analysis. Relative read numbers in test and control bins were compared to calculate CNV. CNV Z-score analysis using the *Z* test was performed as described previously [15]. CNV analysis was conducted using a set of proprietary Perl scripts, and CNV visualization was processed with a proprietary R script. In this study, data from 10-kb sequencing bins were used for generation of the chromosome CNV plots.

Statistical analysis

The SPSS 19.0 (IBM Inc., USA) statistical package was used to analyze the radius, perimeter, area, and volume of each blastomere. Independent sample *T* test was used to determine whether these parameters differed between groups.

Results

The median age at oocyte retrieval was 32 (range 24–43) and the average oocyte retrieval number was 18.5 ± 9.6. Of the 449 normal fertilized embryos, 35 equal-sized three-cell embryos accounted for 7.79% (35/449). As for the general incidence, there were 106 equal-sized three cells embryos out of 7526 embryos and the frequency is 1.41%. At the same time, we observed 1110 unequal-sized three-cell embryos with the frequency of 14.75%.

NGS results of the embryo chromosome composition

NGS was performed on 105 blastomeres from 35 embryos with 92 of them successfully amplified and analyzed. Thirteen blastomeres gave no result due to amplification failure, poor-quality results or being large anuclear fragment (13/105, 12.4%), including 6 blastomeres of two embryos and 5 blastomeres of five embryos as well as 2 blastomeres in one embryo. The results of these cases were considered to be inconclusive and were excluded in the analyses. All blastomeres of 27 embryos were analyzed (Table 1, genotype of 81 blastomeres from 27 embryos). The data indicated that only 22.2% (6/27) of these equal-sized three-cell embryos were euploid in all the 3 blastomeres (group A). The rest (21/27, 77.8%)

Table 1 Genotype of 81 blastomeres from 27 embryos

No.	Group	Conventional IVF or ICSI	Embryo no.	Copy number results	Genotype
1	A	IVF	1-1	Euploid	46,XX
2			1-2	Euploid	46,XX
3			1-3	Euploid	46,XX
4		ICSI	2-1	Euploid	46,XX
5			2-2	Euploid	46,XX
6			2-3	Euploid	46,XX
7		ICSI	3-1	Euploid	46,XX
8			3-2	Euploid	46,XX
9			3-3	Euploid	46,XX
10		IVF	4-1	Euploid	46,XX
11			4-2	Euploid	46,XX
12			4-3	Euploid	46,XX
13		IVF	5-1	Euploid	46,XY
14			5-2	Euploid	46,XY
15			5-3	Euploid	46,XY
16		IVF	6-1	Euploid	46,XX
17			6-2	Euploid	46,XX
18			6-3	Euploid	46,XX
19	B	ICSI	7-1	Euploid	46,XY
20			7-2	Euploid	46,XY
21			7-3	Aneuploid	57,XY,+1(×3),+3(×3),+4(×3),+5(×3),+7(×3),+9(×3),+10(×3),+11(×3),+13(×3),+17(×3),+19(×3)
22		ICSI	8-1	Aneuploid	44,XX,-5(×1),-9q(×1),-15(×1),-18q(×1),+19q(×3)
23			8-2	Euploid	46,XX
24			8-3	Euploid	46,XX
25		ICSI	9-1	Aneuploid	46,XY,-12p(p11→p15,~37M,×1),+12q(q21.1→qter,~59M,×3)
26			9-2	Aneuploid	47,XY,+12(p11.21→q15,~40M,×3),-12q((q21.1→qter,~61M,×1),+19(×3)
27			9-3	Euploid	46,XY
28		IVF	10-1	Euploid	46,XY
29			10-2	Aneuploid	45,XX,-11(×1)
30			10-3	Aneuploid	45,XX,-5q(q33.1→qter,~30M,×1),-11(×1)
31		ICSI	11-1	Aneuploid	46,XX,+1(×3,mos*,~50%),+2(pter→q12.3,~107M,×3,mos*,~50%),+2q(q14.1→q37.1,~116M,×3,mos*,~50%),+13(×3,mos*,~60%),+15(×3,mos*,~50%),+17(p11.2→qter,~50M,×3,mos*,~50%),+18(×3,mos*,~50%)
32			11-2	Aneuploid	32,XX,-1(×0),-2(×0),-13(×0),-15(×0),-17(×0),-18(×0),-22(×0)
33			11-3	Euploid	46,XY
34		IVF	12-1	Euploid	46,XX
35			12-2	Euploid	46,XX
36			12-3	Aneuploid	47,XX,-1p(pter→p32.3,~54M,×1,mos*,~50%),-1q(q24.3→qter,~77M,×1,mos*,~70%),+7p(pter→p11.2,~52M,×3,mos*,~50%),+7q(q11.22→q35,~82M,×3,mos*,~60%),+18(×3)
37	C	ICSI	13-1	Aneuploid	45,XY,-18(×1)
38			13-2	Aneuploid	45,XY,-18(×1)
39			13-3	Aneuploid	45,XY,-18(×1)
40		IVF	14-1	Aneuploid	46,XX,-4(×1),+21(×3)
41			14-2	Aneuploid	46,XX,-4(×1),+21(×3)
42			14-3	Aneuploid	46,XX,-4(×1),+21(×3)

Table 1 (continued)

No.	Group	Conventional IVF or ICSI	Embryo no.	Copy number results	Genotype
43		ICSI	15-1	Aneuploid	46,XX,-11(×1),+19(×3)
44			15-2	Aneuploid	46,XX,-11(×1),+19(×3)
45			15-3	Aneuploid	46,XX,-11(×1),+19(×3)
46		IVF	16-1	Aneuploid	47,XX,+16(×3)
47			16-2	Aneuploid	47,XX,+16(×3)
48			16-3	Aneuploid	47,XX,+16(×3)
49	D	ICSI	17-1	Aneuploid	38,-X(×0),-Y(×0),-14(×0),-15(×0),-18(×0)
50			17-2	Aneuploid	39,XX,-1(×1),-8(×1),-9(×1),-16(×1),-17(×1),-19(×1),-20(×1)
51			17-3	Aneuploid	57,XXX,+X(×3),+1(×3),+8(×3),+9(×3),+14(×3),+15(×3),+16(×3),+17(×3),+18(×3),+19(×3),+20(×3)
52		ICSI	18-1	Aneuploid	35,X-X,-1(×1),-3(×1),-7(×0),-8(×1),-12(×1),-13(×1),-14(×1),-16(×1),-17(×1),-21(×1)
53			18-2	Aneuploid	35,X-X,-1(×1),-3(×1),-7(×0),-8(×1),-12(×1),-13(×1),-14(×1),-16(×1),-17(×1),-21(×1)
54			18-3	Aneuploid	54,XX,+1(×3),+3(×3),+7(×3),+8(×3),+12(×3),-13(×1),+14(×3),+16(×3),+17(×3),+21(×3)
55	E	IVF	19-1	Aneuploid	37,Y,-X(×0),-5(×1),-8(×0),-12(×1),+13(×3),-17(×1),-18(×1),-19(×1),-21(×1),-22(×1)
56			19-2	Aneuploid	47,XY,-1q(×1),+2p(×3),+6q(×3),-10p(×1),+22(×3)
57			19-3	Aneuploid	46,XY,+1q(×3),+2(×3),-3(×1),+4p(×3),-6q(×1),+7p(×3),+8(×3),-9(×1),+10p(×3),+12(×3),-13(×1),+19(×3),-20(×1)
58		IVF	20-1	Aneuploid	47,XX,+16(×3)
59			20-2	Aneuploid	47,XX,+16(×3)
60			20-3	Aneuploid	45,XY,-4(×1)
61		ICSI	21-1	Aneuploid	49,XXY,+X(×2),Y(×1),-1(×0),+2(×3),-3p(×1),+5(×3),-6(×1),+13(×3),+20(×3),+21(×3)
62			21-2	Aneuploid	63,XXXY,+X(×3),+2(×4),+5(×4),+10(×3),+11(×3),+12(×3),+13(×3),+15(×3),+16(×3),+17(×3),+18(×3),+19(×3),+20(×3),+21(×4)
63			21-3	Aneuploid	63,XXXY,+X(×3),+2(×4),+5(×4),+10(×3),+11p(×3),+11q(×5),+12(×3),+13(×3),+15(×3),+16(×3),+17(×3),+18(×3),+19(×3),+20(×3),+21(×4)
64		IVF	22-1	Aneuploid	49,XXX,+X(×3),+6(×3),+12(×3),-13(×1),+16p(×3),+17(×3)
65			22-2	Aneuploid	52,Y,-X(×0),+5q(×3),-6(×0),+7(×3),+8(×3),+9(×3),+11(×3),+13(×3),+14(×3),+15(×3),+16(×3),+20(×3)
66			22-3	Aneuploid	44,XY,-7p(×1),-8p(×1),-9(×1),-11(×1),-12q(×1),+13(×3),-14(×1),-15(×1),-16(×1),-17q(×1),+18(×3),+19(×3),-20q(×1)
67		IVF	23-1	Aneuploid	45,XY,+1p(pter→p21.1,~102M,×3),-4(×1),-9(×1,mos*)
68			23-2	Aneuploid	41,XY,-1p(×0),-1q(×1),+2(×3),-3(×1),-4(×1),+5(×3),-6(×1),+7(×3),-9(×1),-10(×0),-11(×1),-12(×1),-14(×0),+16(×4),+18(×3),+19(×3),+21(×3),-22(×0)
69			23-3	Aneuploid	46,XY,+1q(×3),-2(×1),+3(×3),-4(×1),-5(×1),+6(×3),-7(×1),-9(×1),+10(×4),+11(×3),+12(×3),+14(×4),-16(×0),-18(×1),-19(×1),-21(×1),+22(×4)
70		IVF	24-1	Aneuploid	55,YY,-X(×0),+Y(×2),+1(×3),+3(×3),+4(×3),+6(×3),+8(×5),+17(×4),+18(×3),+19(×3),-21(×0)
71			24-2	Aneuploid	45,XX,-Xq(×0),-Y(×0),-1(×0),+4q(×3),+5p(×3),+5q(×5),+9(×3),+12(×3),+13(×3),+15(×3),-17(×0),-18(×0),+20(×3),+21(×3),-22(×0)
72			24-3	Aneuploid	53,XX,X(×2),+1(×3),+2(×3),-4q(×0),-5q(×0),+7(×3),-8(×0),+10(×3),+11(×3),+14(×3),+16(×3),+18(×3),+21(×3)

Table 1 (continued)

No.	Group	Conventional IVF or ICSI	Embryo no.	Copy number results	Genotype
73		ICSI	25-1	Aneuploid	41,XX,-2(×0),-3(×0),-16(×0),+18(×3)
74			25-2	Aneuploid	31,XX,-1(×1),-4(×1),-5(×1),-6(×1),-7(×1),-8(×1),-9(×1),-10(×1),-12(×1),-13p(×1),-14p(×1),-15(×1),-17(×1),-19(×1),-20(×1),-21(×1),-22(×1)
75			25-3	Aneuploid	45,XX,-13q(q14.3→qter,~61M,×1,mos*),-14q(q21.1→qter,~66M,×1,mos*,~30%),+17(pter→q21.33,~51M,×3,mos*),-18(×1),-X (mos*,~30%)
76		ICSI	26-1	Aneuploid	46,XY,-2q(q22.1→q32.3,~55M,×1,mos*),-5(p15.2→q12.2,~54M,×1,mos*),+7q(q11.21→qter,~94M,×3)
77			26-2	Aneuploid	46,XY,-7q(q11.21→qter,~97M,×1)
78			26-3	Aneuploid	46,XY,-7(p11.2→qter,~104M,×1,mos*,~30%)
79		IVF	27-1	Aneuploid	47,XX,+2(×3),+5(×3,mos*),+6(×3,mos*),-8(pter→q23.1,~110 M,×1,mos*),-9(×1),+10(×3,mos*),+11(×3),-15(×1,mos*),+21(×3,mos*),+22(×3,mos*)
80			27-2	Aneuploid	44,XX,+1p(pter→p34.3,~38M,×3,mos*),+2(p15→q22.1,~148M,×3,mos*),+3(×3,mos*),+8(×3),+9(pter→q22.32,~99M,×3,mos*),-10(×1),-11(×1),+13(×3,mos*),+14(×3),-22(×0)
81			27-3	Aneuploid	42,XX,-2(×1),-3(×1),+7p(pter→p14.1,×4,~43M,mos*),+10(pter→q21.3,~70M,×3,mos*),+11q(q12.3→qter,×3,mos*),-13(×1),-14(×1),+16q(q11.2→qter,~47M,×3),-19(pter→q13.33,~47M,×1),+20p(pter→q11.23,~37M,×3),-21(×1),+22(×3)

were either mosaic or aneuploid; 6 embryos (6/27, 22.2%) were diploid/aneuploidy mosaic with 1 or 2 blastomeres euploid while the other aneuploid (group B); 15 embryos (15/27, 55.6%) were aneuploid in all the blastomeres. The 15 abnormal embryos fell into three groups: group C including 4 aneuploid embryos with blastomeres having the same chromosome composition (4/27, 14.8%), group D including 2 embryos having reciprocal loss/gain of chromosome composition among blastomeres (2/27, 7.4%), and group E including 9 chaotic embryos with different chromosome abnormalities among blastomeres (9/27, 33.3%).

Abnormal chromosome distribution

There were 54 aneuploid blastomeres from 21 mosaic or aneuploid embryos. Aneuploidy screening showed a total of 343 chromosomal abnormalities in these blastomeres. Aneuploidies spread across all chromosomes, and the most frequent aneuploidies were for chromosomes 1, 16, and 18 followed by 13, 19, and 21. Most of the aneuploidy were trisomy (36.7%), followed by monosomy, partial aneuploidies (29.4%), nullisomy (20.7%), tetrosomy (9.0%), and pentasomy (4.1%) (Fig. 1). Twenty-one blastomeres (21/54, 38.9%) had 1–2 chromosome errors, and 33 blastomeres (33/54, 61.1%) were complex aneuploid with more than two chromosome errors.

Morphology analysis between embryos

In order to explore that whether aneuploid embryos can be distinguished from euploid ones by morphology parameters, we analyzed blastomere radius, perimeter, area, and volume between groups (supplement table). Statistics result showed no significant difference between euploid group and aneuploid groups regarding radius, perimeter, area, and volume (group A vs. group C, p value 0.17, 0.17, 0.42, 0.20, respectively; group A vs. group D, p value 0.55, 0.55, 0.21, 0.44, respectively; group A vs. group E, p value 0.11, 0.11, 0.31, 0.11, respectively).

Then we examined the relationship between aneuploid groups (group C vs. group D, p value 0.04, 0.04, 0.04, 0.04, respectively; group C vs. group E, p value 0.91, 0.91, 0.84, 0.78, respectively; group D vs. group E, p value 0.10, 0.10, 0.09, 0.09, respectively). So it is impossible to identify euploid embryos from aneuploid ones by morphology observation. In addition, blastomere 3–3 and 9–3 appeared as multinucleate and chromosome composition were normal. All blastomeres in embryo 15 showed micronuclei and were of same chromosome composition (46, XX,-11(×1), +19(×3)).

Discussion

In normal mitotic cleavage, one cell divides into two equal-sized daughter cells. Whether equal-sized three-cell

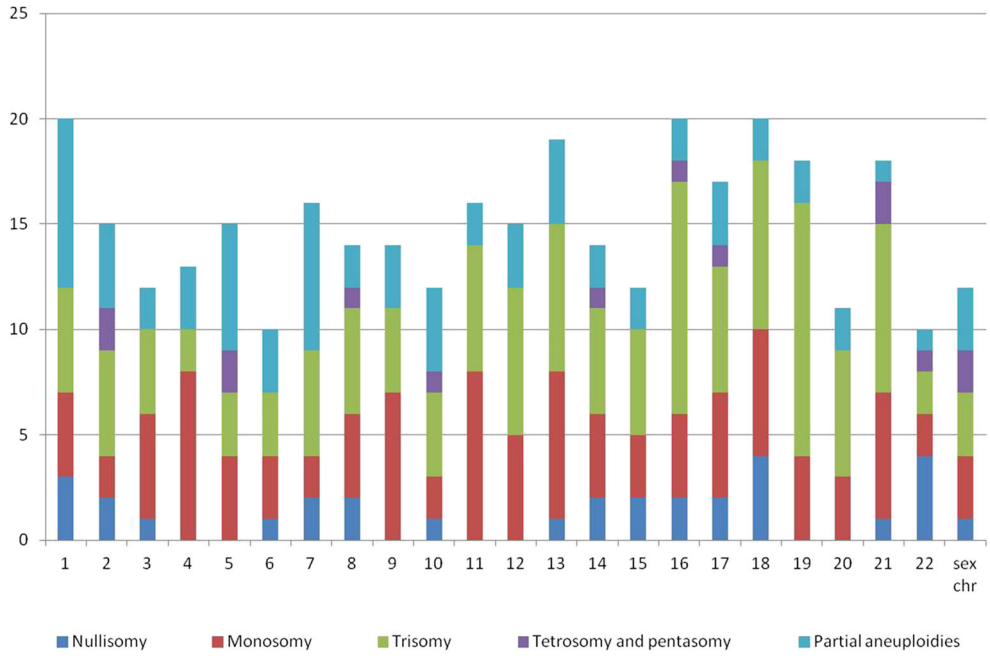

Fig. 1 Abnormal chromosome distribution. Aneuploidy screening on aneuploid blastomeres and aneuploidies spread across all chromosomes

embryos are chromosomally normal remains unclear. Thus, we analyzed the chromosome constitution of each blastomere in these embryos. Our data indicated that vast majority of them (77.8%, 21/27) were mosaic or aneuploid, and only 22.2% (6/27) were normal. Comparing to our PGS data that mosaic and aneuploid rate is 51.9% (94/181) in biopsied blastocysts, the ratio of abnormality in equal-sized three-cell embryos is relatively high. As anomalies arising in early cleavages will affect vast proportion of the blastomeres at later stages of development, equal-sized three-cell embryos should be given low priority for transfer or cryopreservation even if they have good morphology.

Two kinds of three-cell embryos are observed in our clinic: unequal-sized and equal-sized three-cell embryos. The unequal-sized three-cell embryos account for 14.75% and they usually stem from one asynchronous cleavage of equal-sized two-cell embryos, which resulted in one larger blastomere and two small blastomeres. As for the equal-sized three-cell embryos, trichotomic mitosis was regarded as result of tripolar spindles in triponuclear human zygotes [16]. Time-lapse observation showed that this phenomenon was identified in normal fertilized zygotes [3–6]. According to our results, one incidental observation is that the total number of chromosomes in most of the embryos (19/27), i.e., sum of chromosomes of all the 3 blastomeres is about 3 × 46 (Table 1). It is unlikely to be due to one single cycle of DNA replication but rather one division followed by another division of one of the daughter cells. Rubio et al observed 14% (715/5225) embryos underwent direct cleavage from two to three cells (DC2–3) with abrupt cleavage in less than 5 h [17, 18]. They inspected these DC2–3 embryos at 44 h after insemination and discovered that three equal-sized blastomeres would be atypical for a normal embryo. In our study, we collected embryo at 42–44 h post fertilization that the time point consistent with the previous report. Therefore, these equal-sized blastomeres might probably come from two divisions with an abrupt second cell cycle rather than the expected trichotomic division.

There were six diploid/aneuploid mosaic embryos (group B) with 1 or 2 euploid blastomeres. Accumulating studies show that mosaicism exists in cleavage stage embryos as well as blastocysts from both of young and advanced age women [12, 19–22]. Vanneste et al. indicated mosaicism, uniparental disomies, and frequent segmental deletions in most cleavage stage embryos [12]. Mosaicism occurs in a high percentage of good-quality cleavage stage embryos and mitotic errors contributed significantly to the abnormality [23, 24]. Recent research in glioblastoma cells found that significant amount of daughter cells generated by multipolar mitosis were viable and could complete several rounds of mitosis [5]. Should this apply to preimplantation embryo, the development of the diploid/aneuploid mosaic embryos to blastocysts will give rise to false-negative or false-positive diagnoses in PGS. Indeed Liu et al indicated that the chromosomal content of trophectoderm might not correctly predict that of the inner cell mass and that a high proportion of human blastocysts were genetically abnormal [20]. Norbert Gleicher et al. analyzed 11 embryos previously diagnosed as aneuploid and demonstrated more frequent trophectoderm mosaicism than was reported previously [19]. Transferring of mosaic embryos was associated with significantly poorer

outcomes, and a lower priority for transfer than those that are fully euploid is suggested [25]. Mosaicism is used to explain failures of implantation and live birth after transfer of PGS-screened embryos [22].

Chromosome aneuploidy is a major cause of implantation failure, pregnancy loss, abnormal pregnancy, and live birth following both natural conception and in vitro fertilization [8]. These aneuploidies can arise from gonadal mosaicism, meiotic errors, and mitotic errors following fertilization. Of particular interest, there were four embryos (group C) with the same aneuploidy pattern in their entire sister blastomeres (monosomy 18 for embryo 13, monosomy 4 and trisomy 21 for embryo 14, monosomy 11 and trisomy 19 for embryo 15, and trisomy 16 for embryo 16). It can be deduced that their aneuploidy is derived from anaphase lagging or non-disjunction during two meiotic divisions in oogenesis or spermatogenesis.

There were 2 aneuploid embryos with reciprocal chromosome composition among blastomeres (group D, 2/27). Abnormal spindle along with mitotic non-disjunction may contribute to these observations. Previous studies implied that mosaic embryos with three equal-sized blastomeres at the first mitotic division arose from tripolar spindle, due to the random segregation of chromosomes to the three poles [26, 27]. Tripolar mitosis coupled to incomplete cytokinesis produces multiple trisomies in Wilms tumor in culture [28]. Spindles abnormalities including tripolar and tetrapolar spindles have been reported in both normal developing and arrested human embryos [29]. These abnormalities could result in chromosomal malsegregation. As the microtubules of meiotic spindle are highly sensitive to chemical (hyaluronidase) and physical changes (temperature and pH variations), careful oocyte handling can avoid spindle disturbance and chromosomal malsegregation.

Aside from above part, we measured radius, perimeter, area and volume of each blastomere. As cell shape might change during dissociation process, these parameters may be not equivalent to that of intact three-cell embryo.

Conclusions

Although the sample size is limited and larger studies are required, our findings demonstrated that the vast majority of equal-sized three-cell embryos are chromosomally abnormal and these embryos should be given lower priority at selection for transfer.

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Compliance with ethical standards

The Institutional Review Board of Chinese PLA General Hospital (S2016-106-01) approved this study. All the recruited patients signed a written consent.

Conflict of interest The authors declare that they have no conflict of interest.

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