Chromosomal characteristics at cleavage and blastocyst stages from the same embryos

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Abstract

Purpose To investigate chromosomal characteristics in the same embryos at cleavage and blastocyst stage.

Methods Six PGD/PGS cycles were retrospective studied, including five chromosomal translocation PGD cycles and one recurrent abortion PGS cycle. The cleavage embryos were biopsied one blastomere at day 3, followed by blastocyst culture. Trophectoderm cell biopsy was performed when embryos developed into the blastocyst stage. Whole genome amplification and 24-chromosomal analysis by CGH/SNP microarray was performed on each blastomere and trophectoderm cells.

Results After PGD/PGS, only one couple had no euploid embryos to transfer. Five couples delivered a healthy, balanced-karyotype baby. A total of 18 embryos had both blastomere and trophectoderm cell reliable results available. Of the 18 embryos, eleven embryos contained identical chromosomes from cleavage stage to blastocyst stage and six embryos contained almost identical chromosomes. Only one embryo presented opposite chromosomal results for the cleavage and

blastocyst stage, with abnormal chromosomes in the blastomere but normal chromosomes in trophectoderm cells.

Conclusions Most embryos maintain chromosomal stability during embryonic development. Compared to cleavage stage, blastocyst stage may provide more reliable aneuploidy results.

Keywords Comparative genomic hybridization · Single nucleotide polymorphism · Embryo development · Chromosome

Capsule Most embryos maintain chromosomal stability during embryonic development. Compared to cleavage stage, blastocyst stage may provide more reliable aneuploidy results.

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Introduction

Chromosomes are located within the nucleus and are responsible for carrying the genetic information that dictates growth and development of embryos [1]. It is difficult to obtain a comprehensive overview of chromosomes within in vitro cultured embryos because they have only a few cells. Recent innovations in single cell whole genome microarray technology have enabled 24-chromosomal analysis of preimplantation embryos. With whole genome amplification, comparative genomic hybridization (CGH) and single nucleotide polymorphism (SNP) microarrays can examine aneuploidy involving any of the 24 chromosomes (22 autosomes and X and Y sex chromosomes). CGH investigates whole chromosome copy number ploidy, whereas SNP detect polymorphic variation across entire chromosomes [2, 3].

Numerous studies have been performed on human preimplantation embryos from oocytes to blastocysts. Polar bodies are the by-products of oocytes, but can only predict aneuploidy of maternal origin [4]. Oocytes show variable aneuploidy rates ranging from 22 to 72 % [5]. Several studies have reported a mosaicism rate at the cleavage stage ranging between 30 and 85 % [6–8]. Day 4 embryos have also been studied, with

Table 1 General information and pregnant results of the patients

No.	Indication	Number of embryos tested	Number of embryos with no blastomere result	Number of embryos with no trophectoderm result	Number of embryos with no results from both biopsies	Number of the balance embryos	Pregnancy result
Patient 1	46,XY,t(3;15)	4	0	0	0	2+1 ^a	delivered
Patient 2	46,XY,t(8;14)	7	2	1	0	2 ^b	delivered
Patient 3	PGS	2	1	0	0	1	delivered
Patient 4	46,XY,t(1;15)	6	1	1	0	2	delivered
Patient 5	46,XY,t(11;16)	3	1	1	0	0	no embryo to transfer
Patient 6	46,XY,t(3;21)	4	0	0	0	1	delivered

^a This patient had 2 balance embryos (blastomere and trophectoderm cells' results were balance) and 1 embryo which blastomere's result was abnormal but trophectoderm cells' result was balance. After transferred the 2 balance embryos, the patient was not pregnant. With the consent of the couple, the third embryo was transferred and the patient delivered a translocation balance baby

^b One embryo had both blastomere and trophectoderm cells' results were balance. The other embryo had only trophectoderm cells' results was balance, it had no blastomere result

extensive abnormalities observed [9]. When embryos develop to the blastocyst stage they still show chromosomal abnormalities and mosaicism, although the proportion decreases [10].

Only a few studies have performed sequential chromosomal analysis on polar bodies, blastomeres, and trophoblast cells [11]. The results suggest that the polar body stage is the least accurate whereas the blastocyst stage may provide the most reliable aneuploidy results. However, this study only focused on the research rather than clinical application.

Here, we applied CGH and SNP microarrays to investigate chromosomes of the same embryos at the cleavage and

blastocyst stage. Diagnosed normal/balance embryos were transferred and followed until the babies were born.

Materials and methods

This is a retrospective study, and was ethically approved by the Institutional Review Board of Peking University 3rd Hospital, China. We obtained written informed consent from every participant before the treatment. From August to September 2012, six couples undergoing preimplantation genetic

Table 2 The CGH/SNP results of the 18 embryos

Patient No.	Indication	Embryo No	Blastomere's result	Trophectoderm cells' result
Patient 1	46,XY,t(3;15)	1	-2, -11, -21, +7, +10, +12, +13, +15	balance
		2	-3q	-3q
		3	balance	balance
		4	balance	balance
Patient 2	46,XY,t(8;14)	5	-Y,+11	+19
		6	balance	balance
		7	-13, -16, -20, -21, +1, +5, +8, +15	+8
		8	-14, -16, -17, -19, +3, +8, +20,	-14, +8
Patient 3	PGS	9	balance	balance
Patient 4	46,XY,t(1;15)	10	-1, -20	+10
		11	balance	balance
		12	balance	balance
		13	-1p	-1p
Patient 5	46,XY,t(11;16)	14	+11q, +15, +17, +19, +20, +22	-16q, +11q
Patient 6	46,XY,t(3;21)	15	-3	-3
		16	-3, -17, -18, -19, -20, -21, +7, +9, +11, +13, +15, +22	+7, +18, +21
		17	balance	balance
		18	+3	+3

Fig. 1 The embryo contained identical chromosomes at both the cleavage stage and blastocyst stage. (upper part: CGH result for blastomere: del(1)(pter-p22); bottom part: SNP result for trophoctoderm cells: del(1)(pter-p22))

Fig. 2 The chromosomal abnormalities in the trophoctoderm cells were less distinct than those in the blastomere. (upper part: CGH result for blastomere; bottom part: SNP result for trophoctoderm cells)

◀ **Fig. 3** The opposite chromosomal results at the cleavage stage and blastocyst stage. (*upper part*: CGH result for blastomere: abnormal; *middle part*: CGH result for trophoctoderm cells: balance; *bottom part*: CGH result for blastomere DNA amplification again: abnormal)

diagnosis (PGD) or preimplantation genetic screening (PGS) agreed to further array tests. Five had PGD performed on chromosomal translocations in one individual of the couple, whereas the remaining couple had a history of recurrent abortions and PGS was being performed.

All the oocytes had been inseminated by intracytoplasmic sperm injection. One blastomere was biopsied from each day 3 embryo that had more than five blastomeres and less than 50 % debris, and blastocyst culture performed. When the embryos developed into blastocysts, biopsy was then performed on trophoctoderm cells. Biopsied blastomeres were subjected to whole genome amplification and then 24-chromosomal analysis by CGH microarrays. Biopsied trophoctoderm cells were subjected to whole genome amplification and then 24-chromosomal analysis by CGH or SNP microarrays.

CGH was performed on 24Sure + chips (Bluegenome, UK). The SurePlex DNA amplification system was used for whole genome amplification. Samples and control DNA (8 µl of each) were labeled with Cy3 and Cy5 fluorophores. Labeling time was approximately 3 h, with DNA resuspended in dextsulphate hybridization buffer and hybridized overnight under cover slides. After washing, vacuum centrifuge was used to dry microarray slides, followed by laser scanning. Blue Fuse software was used to analyze microarray data for chromatin loss/gain across all 24 chromosomes.

SNP was performed using Human CytoSNP-12V2.1 chips (Illumina, USA). The Qiagen REPLI-g Mini Kit system (Qiagen, Germany) was used for whole genome amplification. Tagging and hybridization of amplified DNA were performed according to the manufacturer's protocols. Arrays were scanned on a HiScanSQ (Illumina). Data analysis was performed using GenomeStudio (standard settings) (Illumina).

All biopsied blastocysts were vitrified. Only blastocysts with normal/balance results were thawed and transferred to the uterus. Women that became pregnant were followed up until delivery. All fetuses were karyotyped by amniocentesis.

Results

Blastomere biopsy and blastocyst culture were performed on a total of 58 day 3 embryos, resulting in 26 blastocysts. Thus, the rate of blastocyst formation was 44.83 % (26/58). After PGD/PGS, only one couple had no euploid embryo to transfer. Five couples delivered a healthy, balanced-karyotype baby (as determined by fetal karyotype analysis) (Table 1).

There were 18 embryos with both blastomere and trophoctoderm-cell reliable results available. The other eight embryos had only blastomere or trophoctoderm cell results, or the results were too noisy to be diagnosed. Therefore, subsequent analyses were performed on the 18 embryos with both sets of results. Table 2 shows the CGH/SNP results of the 18 embryos.

Eleven embryos contained identical chromosomes at both cleavage and blastocyst stage, including seven normal and four abnormal embryos. Figure 1 shows results for blastomere del(1)(pter-p22) from the same embryo at the cleavage and blastocyst stage. Six embryos contained almost identical chromosomes at cleavage and blastocyst stage; however, they all had abnormal chromosomes. These embryos had less distinct chromosomal abnormalities in trophoctoderm cells than in the blastomere (Fig. 2).

Additionally, only one embryo showed abnormal chromosome results at cleavage stage, yet was normal at blastocyst stage. The amplified product of this blastomere was re-run on 24Sure aCGH (Fig. 3). Interestingly, this patient had two balanced embryos (blastomere and trophoctoderm cell results were both balanced), but despite transferring these balanced embryos, the patient did not fall pregnant. With the couple's consent, the third embryo (with abnormal blastomere but balanced trophoctoderm cell results) was transferred, and the patient delivered a healthy baby carrying a chromosomal balanced translocation (as determined by amniocentesis).

Discussion

Early embryonic development directly affects implantation. During the dynamic process of embryonic development, chromosomal changes ultimately determine survival of the embryo. A complete overview of embryonic chromosomes is difficult because of the number of in vitro cultured embryonic cells is generally small. However, recent technical developments in single cell amplification and microarray analysis make it possible to test chromosomes during the early embryonic stages [12–14].

Currently available clinical microarray applications include CGH and SNP. CGH uses imaging techniques to quantitatively analyze copy number changes in chromosomes across the whole genome, by comparing with chromosomes from normal controls. SNP refers to single nucleotide DNA changes with a population frequency of at least 1 % (otherwise it is considered a point mutation). CGH and SNP can detect subtle chromosomal changes such as duplication or deletion of 3 Mb or longer. Moreover, microarray technology does not need cell culture, which greatly decreases diagnosis time, and when combined with single cell whole genome amplification can detect chromosomal changes in a single cell or small number

of cells. Therefore, comprehensive chromosomal analysis of individual cells at different embryonic stages is possible.

During in vitro culture, the proportion of chromosomal abnormalities in cleavage stage embryos is high [6–8], therefore many embryos degenerate or stop developing altogether. To understand how chromosomes behave up to the blastocyst stage, and if a mosaic or chaotic division will occur, we investigated chromosomes from 18 embryos at cleavage and blastocyst stage, recording their dynamic processes at these stages. We found that 94.44 % (17 embryos) contained identical or almost identical chromosomes at two different developmental stages. Of the seventeen embryos, eleven embryos contained identical chromosomes at both cleavage and blastocyst stage, including seven normal and four abnormal embryos. This suggested that most blastocysts maintain chromosomal stability during early embryonic development. Six embryos contained almost identical chromosomes at cleavage and blastocyst stage; however, they all had abnormal chromosomes.

PGS is performed for infertile patients undergoing IVF, with the aim of increasing IVF pregnancy and delivery rates. Indications for PGS include advanced maternal age, repeated implantation failure, severe male infertility, and couples with normal karyotypes who have experienced recurrent miscarriages [15]. Because of a high percentage of mosaicism in cleavage embryos, PGS on blastocyst biopsies is recommended. Moreover, array CGH, SNP, or next generation sequencing can detect 23 pairs of chromosomes and may replace fluorescence in-situ hybridization. In our study, five couples where one of the partners is a translocation carrier and one recurrent abortion couple were included. After PGD/PGS, only one couple had no euploid embryo to transfer. Five couples delivered a healthy, balanced-karyotype baby (as determined by fetal karyotype analysis).

In the present study, one embryo was diagnosed abnormal at cleavage stage yet was normal at blastocyst stage by trophectoderm cell diagnosis. This embryo was transferred and the patient delivered a healthy baby carrying a chromosomal balanced translocation (tested by amniocentesis). There may be two reasons of this embryo, one is mosaicism [6–10], the other is self-correction during embryo development. Several reasons have been proposed for aneuploidy self-correction during later developmental stages, including primary misdiagnosis, aneuploidy allocation in the trophectoderm, cell growth advantage of diploid cells in mosaic embryos, lagging of aneuploid cell division, extrusion or duplication of an aneuploid chromosome, and abundance of DNA repair gene products [16]. We also observed that part of the blastomere failed to incorporate into the blastocyst, which may represent embryonic cells under self-correction or self-elimination. Moreover, six embryos contained almost identical chromosomes at cleavage and blastocyst stage and these embryos had less distinct chromosomal abnormalities in trophectoderm cells than in the blastomere. Self-correction during embryonic

development has been confirmed by other investigators [17–19].

In recent years, single cell whole genome amplification combined with CGH and SNP has successfully been used in PGD and PGS for assisted reproduction. At present, blastocyst stage is the optimal time to perform biopsies for preimplantation genetic testing [10]. Trophectoderm biopsies do less harm to development of the embryo [20], while blastocyst biopsy can obtain multiple trophectoderm cells, resulting in an increased number of DNA copies in the biopsy specimen, and therefore more accurate results compared with a single blastomere cell [21]. In our study, we also found that there were less noisy results at blastocyst stage than that at cleavage stage.

Of course, there are limitations to our study. We obtained 26 blastocysts but only 18 embryos had both blastomere and trophectoderm cell reliable results, and the other eight embryos had only one set of results or one set of results were too noisy to be diagnosed. In addition, we only examined embryos at two developmental stages (cleavage embryos and blastocysts), not the stages from oocytes to blastocysts [8]. However, the cases in our study were clinical PGD/PGS cases, and they are our center's initial clinical application of CGH and SNP microarrays. Of the six couples, five had normal/balanced blastocysts to transfer, and they all delivered healthy, chromosomally-balanced babies. After these six cycles, only trophectoderm cells biopsy is performed in the PGD/PGS cycles in our center.

In this study, we applied CGH and SNP microarray analysis to a small number of cells to characterize chromosomes at different embryonic developmental stages. Our results show that most blastocysts maintain chromosomal stability during embryonic development, and blastocyst stage may provide more reliable aneuploidy results.

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