The karyotype of the blastocoel fluid demonstrates low concordance with both trophectoderm and inner cell mass

Running title: Genomic profiling of BF, TE and ICM

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Capsule

Blastocoel fluid displays high levels of mosaicism and has low karyotype concordance between the embryo inner cell mass and trophectoderm, making it unsuitable for diagnostic purposes.

Abstract

Objective: To compare the genomic profiles of blastocoel fluid (BF), inner cell mass (ICM) and trophectoderm (TE) cells derived from the same blastocyst.

Design: Prospective study.

Setting: Academic and *in vitro* fertilization units.

Patient(s): Sixteen donated cryopreserved embryos at blastocyst stage.

Intervention(s): BF, TE and ICM cells were retrieved from each blastocyst for chromosome analysis using next-generation sequencing (NGS).

Main Outcome Measure(s): Aneuploidy screening and assessment of mosaicism in BF, TE and ICM samples with subsequent comparison of genomic profiles between the three blastocyst compartments.

Result(s): Out of 16 blastocysts 10 BF samples and 14 TE and ICM samples provided reliable NGS data for comprehensive chromosome analysis. Only 40.0% of BF-DNA karyotypes were fully concordant to TE or ICM, compared to 85.7% between TE and ICM. In addition, BF-DNA was burdened with mosaic aneuploidies and the total number of affected chromosomes in BF was significantly higher compared to the TE and ICM ($P < 0.0001$).

Conclusion(s): BF-DNA can be successfully amplified and subjected to NGS, but due to increased discordance rate between ICM and TE, BF does not adequately represent the status of the rest of the embryo. To overcome biological and technical challenges, associated with BF sampling and processing, blastocentesis would require improvement in both laboratory protocols and aneuploidy calling algorithms. Therefore, TE biopsy remains the most effective way to predict embryonic karyotype, while the use of BF as a single source of DNA for preimplantation genetic screening is not yet advised.

Key Words: blastocentesis, preimplantation genetic screening, mosaicism, blastocoel fluid, next-generation sequencing

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Introduction

Chromosomal aneuploidy in human preimplantation embryos is considered to be a major cause of implantation failure and substandard IVF success rate (1-3). As such, preimplantation genetic screening (PGS) has been implemented into the clinics to identify euploid and aneuploid embryos prior to their transfer to the uterus. Thus, PGS has the capacity to prevent adverse IVF and pregnancy outcome, especially in women with advanced age (4-7). In assisted reproductive technology (ART) different biopsy methods are used to obtain the material for genetic analysis, including polar body biopsy of the oocyte, single blastomere biopsy of cleavage-stage embryos and trophectoderm (TE) biopsy of blastocysts. Polar body biopsy was shown to be the least efficient way of predicting embryo status, as it allows screening for maternal meiotic errors only, without taking into account paternally-derived and/or mitotic aneuploidies (8, 9). In contrast, blastomere biopsy directly evaluates embryonic genome, but it may not adequately represent the genomic status of the rest of the embryo due to the high-degree of post-zygotic chromosomal mosaicism at cleavage-stages of development that can be observed even in young fertile couples (10). Moreover, cleavage-stage embryos with abnormal cells may also develop into normal blastocysts (11). Hence, the genomic analysis has steadily shifted towards TE biopsy that is now widely adopted for PGS. In addition, TE biopsy is thought to be less harmful to the overall developmental capacity of the embryos, and currently chromosome analysis from the blastocyst stage may provide the most reliable representation of the embryonic genome due to the lower impact of mosaicism (12-15).

Recently, the discovery of the amplifiable cell-free DNA in blastocoel fluid (BF) made it the object of attention by representing new source of DNA for genetic analysis (16). BF can be removed from the blastocyst prior to vitrification to protect the embryo from membrane damage arising from ice-crystal formation and improve embryo survival following cryopreservation (17, 18). Although the volume of retrieved BF is usually relatively small, the study by Palini et al (16) successfully used the DNA from BF (BF-DNA) for whole-genome amplification (WGA), PCR and array comparative genomic hybridization (aCGH) for comprehensive chromosome analysis. Similarly, BF-DNA was also successfully subjected to next-generation sequencing (NGS) (19), supporting the idea that the aspiration of BF, a procedure termed blastocentesis (20), can become an alternative less invasive approach for blastocyst biopsy. However, given the remarkable genomic plasticity of early embryogenesis, the origin of genetic material in

blastocoel cavity awaits elucidation. In addition, the potential use of BF-DNA for PGS remains questionable, as few of the preliminary studies showed contradictive results regarding aneuploidy detection rates and karyotypic concordance between BF and different biopsied samples. So far, only one group was able to achieve high concordance rate when comparing genomic profiles of BF with TE cells, polar bodies and blastomeres (20, 21), while in other studies the discordance in karyotypes reached up to 50% between BF and TE biopsy or the rest of whole embryo (22-24). However, by using aCGH to compare the genomic consistency between BF-DNA and TE biopsies or the rest of the whole embryo, previously published studies were not able to investigate the occurrence of embryonic mosaicism, which is currently a prominent topic in PGS. Therefore, because of the inconsistent results and lack of data on blastocyst stage mosaicism, additional studies are warranted to investigate the potential use of BF-DNA for diagnostic purposes.

Recently, NGS techniques were implemented in PGS, proving to be a more sensitive method for aneuploidy screening in embryos, because of the ability to reliably detect chromosomal mosaicism (25, 26). In the present study we utilized the most widely used VeriSeq™ PGS platforms for NGS-based comparative chromosome analysis of BF-DNA and TE and ICM cell populations. To our knowledge, this is the first pilot study to simultaneously evaluate molecular karyotypes of three different populations of cells derived from single blastocysts using high-resolution next generation sequencing. By analysing full and mosaic aberrations in different embryonic compartments, we aimed at unravelling to which extent the genomic profiles of BF, TE and ICM reflect each other at the blastocyst stage and to identify the source of DNA in blastocoel cavity. The data presented here provides novel insight into the feasibility of using BF-DNA in routine clinical practice.

Materials and Methods

Validation of mosaicism with mixing experiments

First, we performed a proof-of-principle mixing experiment to evaluate the sensitivity of the Illumina VeriSeq™ NGS platform (Illumina, USA) in detecting mosaicism, as described recently (27, 28). Briefly, we obtained fibroblast cell lines with previously characterized karyotypes from the NIGMS Human Genetic Cell Repository at the Coriell Institute of Medical Research (USA). Aneuploid cell lines included trisomy 13 (47,XY,+13; GM02948), trisomy 18 (47,XY,+18;

GM01359) and trisomy 21 (47,XX,+21; GM04616). The cells were then cultured and passaged once as recommended by the supplier. Subsequently, individual cells from various cell cultures were isolated under dissecting microscope by EZ-Grip micropipette using 125 µm capillary (Research Instruments LTD, UK) and combined in different ratios, creating a mixture of six cells with different proportions of abnormal alleles of interest (0%, 17%, 33%, 50%, 66%, 83% and 100%). Proof-of-principle experiments were performed in at least three replicates, each time by creating new cell mixtures.

Embryo biopsy and sampling

Embryo biopsy and sample collection was performed at the Tomsk regional perinatal center (Tomsk, Russia) and the Krasnoyarsk Center for Reproductive Medicine (Krasnoyarsk, Russia). The study was approved by the Bioethics Committee of the Biological Institute of the National Research Tomsk State University and all the patients have signed an informed consent. All micromanipulations were performed under a hood in a high-quality standard IVF laboratory

This study used 16 cryopreserved blastocysts, donated for research by patients who have undergone IVF treatment. Cryopreservation and thawing of blastocysts were done according to the manufacturer's VT601-TOP/VT602-KIT protocol (Kitazato Corporation, Japan). Blastocyst morphology was evaluated using to the criteria set by Gardner and Schoolcraft, which is based on the assessment of blastocoele expansion and hatching status, size and compaction of the ICM, and number of TE cells and the presence of a cohesive layer (29). According to the study design, BF was first aspirated, and subsequently ICM and TE cells were isolated and collected for separate chromosome analysis. Blastocyst micropuncture and aspiration of BF was performed by previously described methods (20, 30). Briefly, the blastocysts were immobilized by a holding pipette, mounted on a micromanipulator, and BF from the cavity of expanded blastocysts was aspirated by an intracytoplasmic sperm injection (ICSI) micropipette (Origio, Denmark), which was inserted between the TE cells to minimize the possible cell damage. The use of ICSI micropipette also minimizes the risk of cross-contamination by intact TE or ICM cells. A single aspiration was performed, avoiding aspiration of any cellular material. A volume of ~1µl aspirated fluid was retrieved from each blastocyst. The retrieved fluid was then expelled into the 2.5 µl of 1x PBS, after which the end of the ICSI micropipette was broken into the tube to avoid any loss of the material. Subsequently, OCTAX Laser Shot™ microsurgical laser system (MTG,

Germany) or ZILOS-tk® (Hamilton Thorne, USA) were used to separate TE and ICM cells that were placed in separate tubes, containing 2.5 µl of 1x PBS. All biopsied materials were immediately whole-genome amplified and were stored in -20°C until further processing.

Whole-genome amplification and next-generation sequencing

Whole-genome amplification of all cell mixtures and biopsied samples was performed by commercial PCR-based PicoPLEX kit according to manufacturer's protocol (Rubicon Genomics, USA). The quality of DNA amplification was controlled by 1% agarose gel electrophoresis and the amount of DNA was quantified by Qubit® dsDNA HS Assay kit (Thermo Fisher Scientific, USA). Subsequent processing of successfully amplified material and library preparations were done according to the manufacturer's VeriSeq™ PGS kit protocol, after which the samples were sequenced with Illumina MiSeq® system. Data analysis and genome-wide profile visualization was performed by applying standard settings on Illumina BlueFuse Multi v4.3 Software with embedded aneuploidy calling algorithm. The detection sensitivity and the degree of mosaicism in mixture experiments and later in embryos were determined by BlueFuse Multi v4.3 numerical values.

Statistical analysis

Statistical calculations were performed using GraphPad Prism 6 software (GraphPad Software Inc., USA). The prevalence of chromosomal aberrations, including mosaic aneuploidies, in BF, TE and ICM was assessed with Chi-square test and the difference in the number of affected chromosomes between the embryo biopsies was considered to be statistically significant, when *P*-value was <0.002. To determine the potential value of BF-DNA use for aneuploidy screening two-tailed Fisher's exact test was applied, when comparing the karyotype concordance of ICM between either BF or TE.

Results

We first performed mixing experiments to mimic possible mosaic aneuploidies observed in embryos. Internal validation of our mixing experiments revealed that NGS technique is able to distinguish mosaic losses and gains that are present in at least 20% of cells (Supplementary Fig.

S1), which is concordant to recent comprehensive validation studies on mosaicism detection using next-generation sequencing (27, 31).

Next, a total of 16 cryopreserved embryos were biopsied with subsequent amplification of ICM-, TE- and BF-DNA for NGS analysis. After WGA, sufficient amount of DNA was detected in all of ICM and TE samples, but only in 14 out of 16 BF biopsies (87.5%). Following sequencing and initial quality control, BF chromosome profile could not be determined in four embryos. Out of those four embryos, two embryos also had an inconclusive result for either TE or ICM, so they were discarded from further investigation. Therefore, in 10 out of initial 16 (62.5%) embryos chromosome copy number profiling was obtained for BF and compared to TE and ICM, while the comparison of TE and ICM was performed in 14 embryos out of 16 (87.5%).

Based on the data of our mixing experiments and by using obtained numerical values of each embryo biopsy, we were able to determine the percentage of aneuploid cells present in BF, ICM, TE cells. However, because the detection of low-grade mosaicism within an embryo may be influenced in some degree to sampling error and technical artifacts (26, 32), we have classified our embryos according to the current Preimplantation Genetic Screening International Society (PGDIS) guidelines. Namely, embryos showing mosaicism of <20% were considered to be euploid and >80% were considered as aneuploid embryos with full chromosome losses or gains, while all the aneuploidies in the range of 20%-80% were classified as mosaic (Table 1). Importantly, when evaluating the data, overall noise ratio was also taken into account and mosaic aneuploidy calling was done with caution, as amplification artifacts can cause fluctuation in the genomic profiles that may be difficult to distinguish from low-level mosaicism, especially in the BF samples. We then compared the karyotypes of various biopsy types taken from the same embryo and classified our results as was performed previously: (1) full concordance was reported, if all biopsied samples were euploid or if the same chromosomes were affected in biopsied samples (including mosaic and/or reciprocal losses and gains); (2) partial concordance was reported, when at least one chromosome corresponded in both biopsies under comparison, but the overall genomic profile did not completely match; and (3) discordance was reported, when none of the affected chromosomes in one biopsy corresponded to other biopsies (21). Based on the results of our study, a full chromosome concordance between the three cellular populations was observed only in four embryos, of which three were uniformly euploid and one had a reciprocal mosaic aneuploidy (e.g Embryo 1 in Table 1; Fig. 1). In general, reciprocal

aneuploidies in BF were observed in three embryos, indicating a post-zygotic nature of chromosome abnormalities due to chromosome non-disjunction during mitosis. We have also detected a potentially polyploid partially concordant embryo with multiple reciprocal losses and gains in all three embryo compartments (Embryo 3 in Table 1; Fig. 2A). Such genomic profile could be a consequence of chromosome missegregations during the first post-zygotic cleavages that accumulated throughout the preimplantation development, resulting in an unviable embryo. At the same time, we have observed another chaotic profile in the BF-DNA only, while the corresponding TE and ICM had a euploid karyotype (Embryo 4 in Table 1; Fig. 2B). Similarly to this, also embryo 7 showed multiple aneuploidy profile in the BF, while both ICM and TE were normal. Owing to such differences in karyotypes, it comes as no surprise that in total the overall number of affected pairs of chromosomes (22 pairs of autosomes and one pair of sex chromosomes), including the potentially polyploid biopsies with whole affected genome, was higher in ten BF samples (79/230), compared to corresponding TE (34/230) or ICM biopsies (26/230) (both $P<0.0001$), while no such difference was observed between the available 14 ICM (27/322) and TE biopsies (35/322) (P=ns). As such, BF karyotype was discordant from ICM in 30.0% (3/10) of the cases and from TE in 20.0% (2/10) of the cases. Thus, BF-DNA karyotype reached full concordance between either ICM or TE in 40.0% (4/10) of the embryos (Table 2). In contrast, full concordance between ICM and TE was observed in 85.7% (12/14) of the embryos, making TE more representative of embryonic chromosomal status than BF ($P<0.03$). Therefore, our data suggests that using BF-DNA as a single source of DNA for PGS can potentially lead to an increased rate of false positive findings. This means that a viable embryo with euploid genome can be discarded based only on the aberrant BF-DNA karyotype, leading to suboptimal IVF success rate.

Discussion

Blastocyst culture has become a milestone in ART and is now widely used for selection of viable embryos for transfer. The design of our study enabled us to simultaneously compare for the first time the molecular karyotypes of cells from three major blastocyst components: inner cell mass, trophectoderm and blastocoel fluid, also taking into account the mosaic nature of embryos at this late stage of preimplantation development. In our cohort of embryos, the BF-DNA karyotype was fully concordant to ICM or TE cells in only 40.0% of cases, compared to 85.7% between

ICM and TE. This result is similar to previously published report, demonstrating 48% concordance rate between BF and the rest of the analyzed ICM and TE cells (24), although it drastically contrasts with the high concordance rates achieved by another group (20, 21). Such contradictory outcome may be explained by different types of material analyzed and in our case also by a different technological approach. The genomic profile of blastocysts may include mosaicism that can be missed by aCGH, which is able to detect only high-degree mosaicism, when >50% of cells are aneuploid (33). By using NGS method with improved resolution and sensitivity, we were also able to determine embryos carrying 20-40% of abnormal cells. Moreover, the reciprocal nature of some of the chromosome abnormalities indicate that what we observed were not technical artifacts, but rather true biological events that happened during post-zygotic cleavages. In addition, we have detected two embryos with the same full chromosome aberrations in all three embryonic biopsies, indicating the meiotic nature of these aneuploidies (e.g. Embryo 2 and Embryo 5).

The presence of embryonic DNA in BF suggest that potential mechanisms might exist by which the genetic material is released into the blastocoel cavity, like cell lysis, apoptosis and elimination of cellular debris (34). Interestingly, the intact ICM karyotype in the presence of aneuploidy in BF-DNA in some of the embryos also seems to support the idea that aneuploid cells are progressively depleted from the developing embryo through apoptosis, ensuring the genomic integrity of the future fetus (35). This phenomenon might also be one of the biological explanations of the high concordance rate previously observed between BF-DNA and blastomeres (21), as aberrant cells may be marginalized into the blastocoel cavity at later stages of development. Such mechanism may also likely explain why transfer of mosaic embryos can lead to live birth (36), although the impact of embryonic mosaicism on pregnancy outcome is currently under intense investigation (25, 27). Therefore, the biology behind data interpretation, especially the one derived from BF-DNA, must be adequately elucidated to provide proper patient counselling in everyday clinical practice.

In addition to biological challenges, technical limitations can also restrict the use of BF as a source of DNA for PGS using NGS, as BF can contain a variable amount of cell-free DNA, which may vary in size (19). Moreover, BF-DNA can become fragmented or degraded, which can affect whole-genome amplification rates. In addition, 10the limited quantity of available starting material may be prone to uneven amplification and allele drop-out (37). In addition,

library preparation methods and technical artifacts can result in an altered representation of the genome that will reduce the reliability of chromosome analysis (31). Because VeriSeq™ protocol is not suitable for handling DNA fragments less than 300bp, smaller fragments present in the cell-free DNA may be lost upon library preparation for sequencing, leading to genomic underrepresentation and overall higher noise ratio of the sequenced data. In contrast, the amplification rate and quality of the data were much higher in TE and ICM cells, and our results showed that TE is quite representative of ICM. In addition, no evidence of preferential allocation of aneuploid cells to trophectoderm was observed.

Based on our data, the genomic profiles of TE and ICM showed either generally lower level of mosaicism or the absence of aneuploidy at all, if compared to BF-DNA profiles. Hence, from the clinical and diagnostic point of view the use of insensitive to mosaicism aCGH platform (that detects only >50% mosaicism) might seem like a more suitable approach for the analysis of BF-DNA that could potentially increase the karyotypic concordance rate between different embryo compartments, because the biologically irrelevant low-grade mosaicism in BF would not be detected. On the other hand, embryos with normal TE and ICM karyotype also showed high-grade mosaic aneuploidies in BF that would likely be interpreted as false positive finding using aCGH, thus leading to misdiagnosis. Because such discordance was also evident in previous studies using aCGH (22-24), together our results suggest that at this point chromosome analysis of TE biopsy remains a more optimal and effective way of predicting the karyotype of the blastocyst. However, a more sophisticated bioinformatical approaches are still warranted to overcome the challenges in mosaic aneuploidy calling and help refine the criteria for embryo selection for transfer without compromising the treatment success rate by excluding mosaic embryos capable of resulting in viable pregnancies.

The limiting aspect of our pilot study was the number of embryos analyzed. In addition, when looking at TE cells, we sequenced the whole trophectoderm cell population. This is opposite to TE biopsy, when only a small number of cells are analyzed that may not necessarily represent the karyotype of the rest of the embryo. In our case, euploid cells could have potentially normalized the genomic profile of TE samples, making low-level mosaicism undetectable. Similarly, a mixture of cells with monosomy and trisomy of the same chromosome (reciprocal aneuploidies) can also result in genomic normalization below the level of mosaicism detection, leading to a false diagnosis of disomy. Finally, we also acknowledge that the

aspiration of BF was performed after embryo thawing, which can potentially affect the quality of DNA and subsequent results. Another important consideration is whether any contaminating genetic material from culture medium or extracellular vesicles can arise during BF isolation. Perhaps, advanced genome-wide haplotyping technologies can shed some light on the true origin of BF-DNA in blastocoel cavity.

In conclusion, we have corroborated that BF-DNA can be amplified and applied for next-generation sequencing. However, based on the observations of this study, the results obtained from BF-DNA do not seem to be comparable to those obtained via standard TE biopsies, and BF-DNA does not adequately represent the rest of the embryo, making it diagnostically unacceptable, at least using current methods and protocols. In addition, although the impact of BF sampling seems less invasive, functional studies on the effect of BF biopsy on embryo viability may be warranted, as blastocoel may contain proteins crucial for embryonic development (38). Nonetheless, the potential use of blastocoel sampling cannot be ruled out in the future, although the improvement of current sample handling protocols and development of novel bioinformatical tools are required. Therefore, all the limitations must be carefully evaluated before BF-DNA can be used as a single alternative approach for embryonic aneuploidy screening.

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Conflict of interests

None

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Figures and Figure Legends

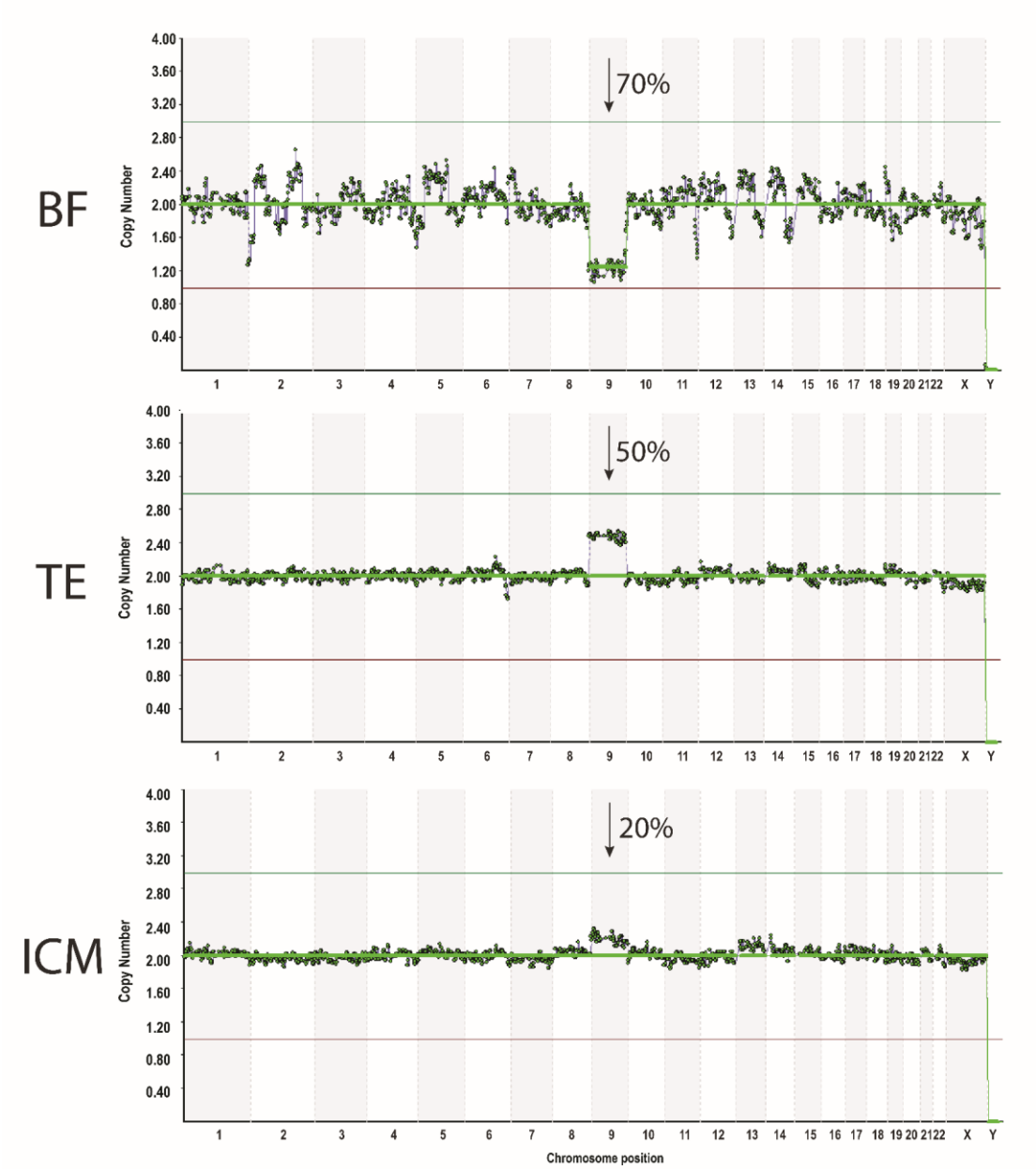


Figure 1. A fully concordant embryo with reciprocal mosaic aneuploidy

Example of an embryo (Embryo 1; Table 1) with a 70% mosaic loss of chromosome 9 in the blastocoel fluid (BF) and a reciprocal gain in the trophectoderm cells (TE; 50%) and inner cell mass (ICM; 20%). The decreased rate of mosaicism in the ICM suggests that aberrant cells may be marginalized from the ICM lineage.

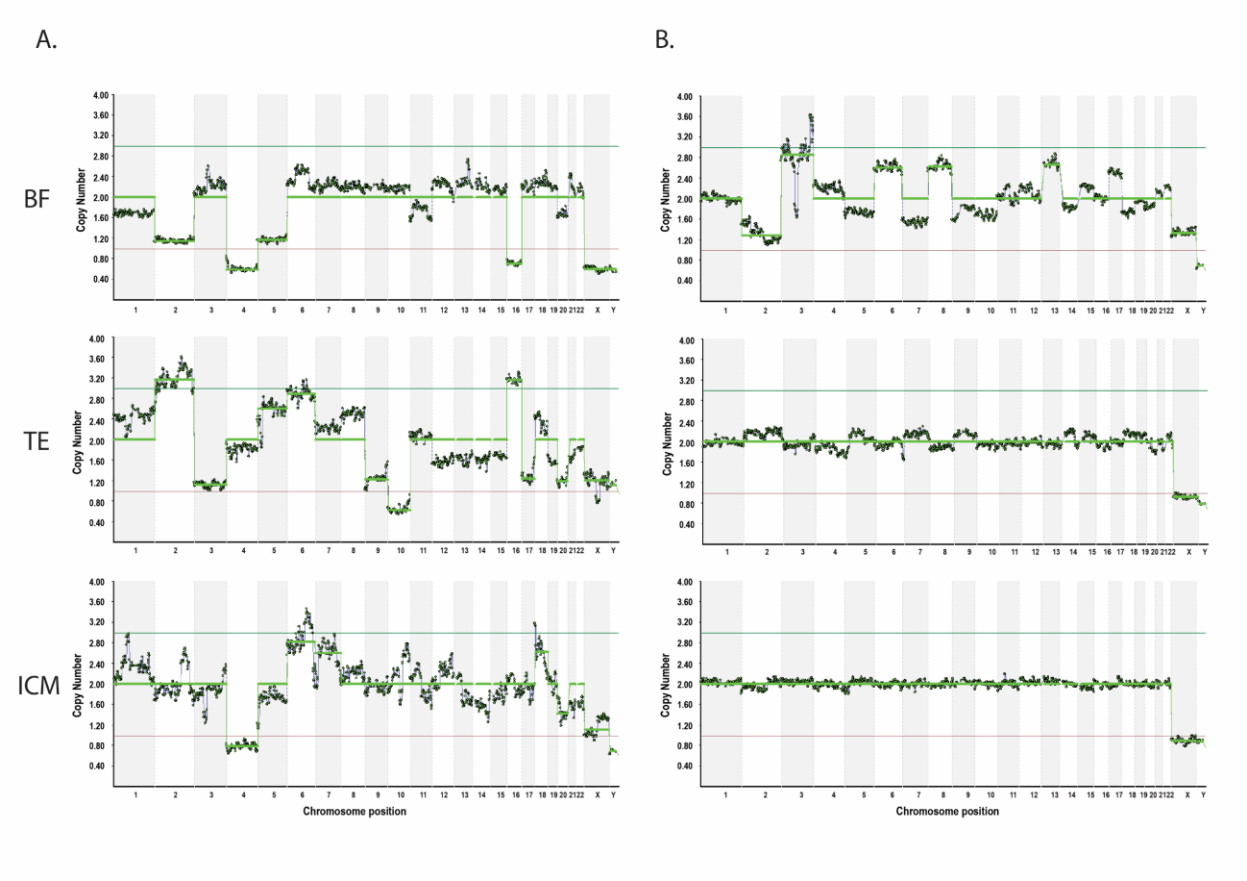
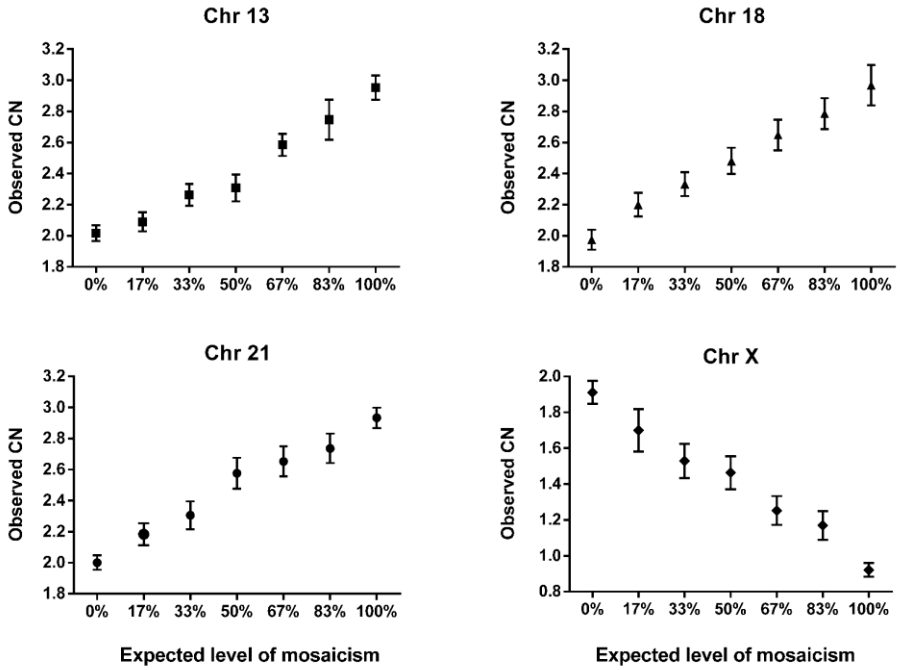


Figure 2. Examples of embryos with chaotic chromosome profiles

(A) Embryo 3 (Table 1) with numerous losses and gains observed in all three blastocysts compartments. Deviations in the numerical values of autosomes and sex chromosomes suggest a potential polyploid karyotype. This embryo was classified as partially concordant. (B) Chromosome plots of Embryo 4 (Table 1) with a discordant karyotype between the blastocoel fluid (BF) and trophectoderm (TE) or inner cell mass (ICM).



Supplementary Figure S1. NGS proof-of-principle mixing experiments

Cells with known aneuploid karyotype were mixed at different proportions with normal diploid cells to mimic mosaicism in embryos. Mixing experiments were done in at least three replicates and copy number (CN) value was evaluated. Each shape and error bar indicate mean and standard deviation of all independent measurements.

470 **Table 1. Molecular karyotypes of the blastocoel fluid, trophectoderm and inner cell mass**

Embryo #	Patient Age	Embryo Morphology	Blastocoel fluid (% of mosaicism)	Trophectoderm (% of mosaicism)	Inner Cell Mass (% of mosaicism)
1	39	3BB	46,XX Mosaic -9 (70%)	46,XX Mosaic +9 (50%)	46,XX Mosaic +9 (20%)
2	39	3-4BB	45,XY, -13 Mosaic +1 (60%) Mosaic -16 (30%) Mosaic -21 (40%)	45,XY, -13	45,XY, -13
3	39	3-4BB	Chaotic, likely polyploid	Chaotic, likely polyploid	Chaotic, likely polyploid
4	33	3-4AB	Chaotic, likely polyploid	46,XY	46,XY
5	33	4BB	45,XY, -7 Mosaic -1 (50%) Mosaic +8 (60%) Mosaic +11 (50%) Mosaic +18 (40%) Mosaic +20 (40%) Mosaic +21 (50%)	45,XY, -7	45,XY, -7
6	37	4-5BB	44, XX,-9,-9 Mosaic -3 (60%) Mosaic -10 (80%) Mosaic -12 (70%) Mosaic -13 (80%) Mosaic +14 (50%) Mosaic -15 (70%) Mosaic +16 (50%) Mosaic +17 (50%) Mosaic +19 (50%) Mosaic +20 (50%) Mosaic -22 (70%)	46,XX Mosaic +3 (20%) Mosaic +9 (50%) Mosaic +10 (30%) Mosaic +12 (20%) Mosaic +13 (30%) Mosaic +15 (20%) Mosaic -20 (30%) Mosaic +22 (30%)	46,XX
7	37	4-5AA	47,XY,+11 Mosaic +2 (80%) Mosaic -9 (30%)	46,XY	46,XY

			Mosaic -10 (30%) Mosaic -12 (30%) Mosaic -13 (40%) Mosaic +19 (50%) Mosaic -21 (50%) Mosaic –X (60%)		
8	32	5BB	ND	46,XY	46,XY
9	23	4BB	46,XX	46,XX	46,XX
10	23	5BB	46,XX	46,XX	46,XX
11	32	4BB	46,XX	46,XX	46,XX
12	32	4BB	ND	46,XX	46,XX
13	32	4BB	ND	46,XX	46,XX
14	42	3BC	ND	46,XY Mosaic -17 (80%)	46,XY Mosaic -17 (70%)

Note: The chaotic likely polyploid profiles of embryos 3 and 4 are depicted in Fig. 2.; ND, chromosome profile not determined

Table 2. Concordance levels between the blastocoel fluid (BF), trophectoderm (TE) and inner cell mass (ICM)

	Concordant	Partially concordant	Discordant
BF vs ICM	40.0% (4/10)	30.0% (3/10)	30.0% (3/10)
BF vs TE	40.0% (4/10)	40.0% (4/10)	20.0% (2/10)
TE vs ICM	85.7% (12/14)	7.1% (1/14)	7.1% (1/14)