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

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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 by means of 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 with TE or ICM, compared with 85.7% concordance 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 with the TE and ICM.
Conclusion(s): BF-DNA can be successfully amplified and subjected to NGS, but owing to increased discordance with ICM and TE, BF does not adequately represent the status of the rest of the embryo. To overcome biologic 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, and the use of BF as a single source of DNA for preimplantation genetic screening is not yet advised. (Fertil Steril® 2018;109:1127–34. ©2018 by American Society for Reproductive Medicine.)
Key Words: Blastocentesis, preimplantation genetic screening, mosaicism, blastocoel fluid, next-generation sequencing

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Received November 13, 2017; revised January 22, 2018; accepted February 6, 2018.
O.T. has nothing to disclose. D.I.Z. has nothing to disclose. T.J. has nothing to disclose. N.A.S. has nothing to disclose. O.R.K. has nothing to disclose. V.G.A. has nothing to disclose. A.V.S. has nothing to disclose. K.T. has nothing to disclose. A.T. has nothing to disclose. A.S. has nothing to disclose. A.K. has nothing to disclose. I.N.L. has nothing to disclose.
Supported by: grant IUT34-16 from the Estonian Ministry of Education and Research; Enterprise Estonia grant no. EU48695; European Commission Horizon 2020 research and innovation program under grant agreements 692065 (project WIDENLIFE) and 691058 (MSCA-RISE-2015 project MOMENDO); grant from Russian Foundation for Basic Research (project no. 15-04-08265); and Program of the Federal Agency of Scientific Organizations of Russian Federation for supporting of bioresource collection in 2017 (project no. 0550-2017-0019).
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Fertility and Sterility® Vol. 109, No. 6, June 2018 0015-0282/$36.00

https://doi.org/10.1016/j.fertnstert.2018.02.008

Chromosomal aneuploidy in human preimplantation embryos is considered to be a major cause of implantation failure and poor in vitro fertilization (IVF) success rate (1–3). As such, preimplantation genetic screening (PGS) has been implemented into the clinics to identify euploid and aneuploid embryos before 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, because 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 owing to the high degree of postzygotic 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). Therefore, genomic analysis has steadily shifted toward TE biopsy, which 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 chromosome analysis from the blastocyst stage may provide the most reliable representation of the embryonic genome, owing 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 as a new source of DNA for genetic analysis (16). BF can be removed from the blastocyst before vitrification to protect the embryo from membrane damage arising from ice-crystal formation and to improve embryo survival after cryopreservation (17, 18). Although the volume of retrieved BF is relatively small, Palini et al. (16) successfully used the DNA from BF (BF-DNA) for whole-genome amplification (WGA), polymerase chain reaction (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 alternate, less invasive, approach for blastocyst biopsy. However, given the remarkable genomic plasticity of early embryogenesis, the origin of genetic material in the blastocoel cavity awaits elucidation. Another important consideration is whether any contaminating genetic material from culture medium or extracellular vesicles may be present in the blastocoel (21). In addition, the potential use of BF-DNA for PGS remains questionable, because the few preliminary studies showed contradictory results regarding aneuploidy detection rates and karyotypic concordance between BF and different biopsied samples. So far, only one group was able to achieve a high concordance rate between genomic profiles of BF and TE cells, polar bodies, and blastomeres (20, 22), whereas in other studies the discordance in karyotypes reached up to 50% between BF and TE biopsy or the rest of whole embryo (23–25). However, with the use of 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. 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, owing to the ability to reliably detect chromosomal mosaicism (26, 27). In the present study we used the most widely used Veriseq PGS platforms for NGS-based comparative chromosome analysis of BF-DNA and TE and inner cell mass (ICM) cell populations. To our knowledge, this is the first pilot study to simultaneously evaluate molecular karyotypes with the use out of high-resolution next generation sequencing of three different embryonic compartments (BF, TE, and ICM) derived from a single blastocyst. By analyzing full and mosaic aberrations in different embryonic compartments, we aimed at unraveling to what extent the genomic profiles of BF, TE, and ICM reflect each other at the blastocyst stage. The data presented here provide novel insight into the feasibility of using BF as a source of DNA for PGS in routine clinical practice.

MATERIALS AND METHODS

Validation of Mosaicism with Mixing Experiments

First, we performed proof-of-principle mixing experiments to evaluate the sensitivity of the Illumina Veriseq NGS platform in detecting mosaicism, as described recently (28, 29). Briefly, we obtained fibroblast cell lines with previously characterized karyotypes from the National Institute of General Medical Sciences Human Genetic Cell Repository at the Coriell Institute of Medical Research. 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 means of EZ-Grip micropipettes using 125 μm capillary (Research Instruments) 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 at least three times, each time with the creation of new cell mixtures.

Embryo Biopsy and Sampling

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