Preimplantation genetic testing: polar bodies, blastomeres, trophectoderm cells, or blastocoelic fluid?

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Objective: To investigate the blastocoelic fluid (BF) for the presence of DNA that could be amplified and analyzed; the extent to which its chromosomal status corresponds to that found in trophectoderm (TE) cells, polar bodies (PBs), or blastomeres; and the identification of segmental abnormalities.
Design: Longitudinal cohort study.
Setting: In vitro fertilization unit.
Patient(s): Fifty-one couples undergoing preimplantation genetic screening or preimplantation genetic diagnosis for translocations by array-comparative genomic hybridization on PBs (n = 21) or blastomeres (n = 30).
Intervention(s): BFs and TE cells were retrieved from 116 blastocysts, whose chromosome status had already been established by PB or blastomere assessment. Separate chromosome analysis was performed in 70 BFs.
Main Outcome Measure(s): Presence of DNA in BFs, evaluation of the chromosome condition, and comparison with the diagnosis made in TE cells and at earlier stage biopsies.
Result(s): DNA detection was 82%, with a net improvement after refinement of the procedure. In 97.1% of BFs, the ploidy condition corresponded to that found in TE cells, with one false positive and one false negative. The rate of concordance per single chromosome was 98.4%. Ploidy and chromosome concordance with PBs were 94% and 97.9%, respectively; with blastomeres, the concordances were 95% and 97.7%, respectively. Segmental abnormalities, which were detected in PBs or blastomeres of 16 blastocysts, were also identified in the corresponding BFs.
Conclusion(s): BF represents to a good extent the blastocyst ploidy condition and chromosome status when compared with TE cells. If the proportion of clinically useful BFs is improved, blastocentesis could become the preferred source of DNA for chromosomal testing. (Fertil Steril® 2016;105:676–83. ©2016 by American Society for Reproductive Medicine.)
Key Words: Blastocyst, blastocoel, blastomere, polar bodies, preimplantation genetic screening

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After compaction, the embryo starts to form a cavity in a process whereby cells differentiate into inner-cell mass (ICM) and trophectoderm (TE) and separate and migrate to distinct locations. In a normally developing embryo, TE cells give rise to the extraembryonic tissues that make up the placenta and the amnion, while ICM, made of undifferentiated embryonic stem cells, becomes the embryo proper (1). After polarization of TE cells and the formation of a belt-line seal made by tight junctions, the accumulation of blastocoelic fluid (BF) transported by TE cells begins (2, 3). As far as cavitation progresses, the accumulation of the fluid and the continuous cell mitoses produce enlargement of the blastocyst and thinning of the zona pellucida (ZP). At this point, hatching of the blastocyst occurs through a natural breach in the ZP, making the embryo ready to implant.

The BF represents the natural medium supporting the development of

Received July 15, 2015; revised October 28, 2015; accepted November 9, 2015; published online December 1, 2015.
M.C.M. has nothing to disclose. A.P. has nothing to disclose. G.C. has nothing to disclose. M.V. has nothing to disclose. A.C. has nothing to disclose. A.P.F. has nothing to disclose. L.G. has nothing to disclose.
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Fertility and Sterility® Vol. 105, No. 3, March 2016 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2015.11.018

the ICM and gives rise to the yolk sac (1, 4). Since both the differentiation process and the process of self-renewal are guided by proteins (5), the identification of metabolites and proteins in BFs is not surprising (6, 7). A recent study identified 286 proteins in the BF including heat shock proteins, ZP proteins, vitamin D–binding protein, retinol-binding protein, and proteins regulating ciliary assembly and function (7). In addition, eight previously uncharacterized proteins were found, which have not yet been assigned a function. In all, the distribution of these proteins according to their biological function was quite similar to that found in the cells forming the corresponding blastocyst. These findings suggest the derivation of BF proteins from the blastocyst cells through transcellular transport and/or through the release of components from ICM and TE cells (8). It is now believed that the BF plays a critical role in supporting cellular processes during embryo development other than merely providing a compartment for cell migration (9).

Beside proteins, DNA has also been found in BFs, whose origin is still under investigation (10–12). It could be free DNA or in particulate forms like microparticles, which are membrane-bound vesicles containing nuclear molecules released by membrane blebbing during cell death and replication (13). Whatever the form is, this DNA can be submitted to amplification and analyzed to identify mutations responsible for genetic diseases or chromosomal abnormalities in preimplantation genetic screening (PGS) or preimplantation genetic diagnosis (PGD) programs (11, 12).

We already presented our preliminary data in a report where DNA was found and analyzed for aneuploidy in 39 out of 51 tested BFs accounting for an efficiency of 76.5% (12).

In this study, we extended our experience with the aims of [1] verifying the presence of DNA in BFs, [2] estimating whether its chromosomal status corresponded to the ploidy condition predicted by polar bodies (PB) or blastomeres and TE cells, and [3] investigating whether segmental abnormalities could also be detected in BFs.

MATERIALS AND METHODS

Plan of the Study

This study included 51 couples (maternal age 38.1 ± 3.2 years) undergoing 24-chromosome array-comparative genomic hybridization (-CGH) in PBs (21 patients) or blastomeres (30 patients) in a PGS/PGD for a translocation program. Indications for PGS were advanced maternal age (n = 26) or repeated IVF failures (n = 13); PGD for translocation (n = 12) included both Robertsonian (n = 1) and reciprocal (n = 11) translocations.

To address the first aim of this study, 116 blastocysts from these couples were investigated for the presence of DNA in the BF. To address the second aim, 70 of the amplified BFs underwent 24-chromosome analysis, and the results obtained were compared with those from the corresponding PBs or blastomeres and TE cells. This analysis was done only on those samples that also had chromosomal results available from PBs or blastomeres and TE cells. Finally, to address the third aim of the study, 16 BFs were analyzed for their chromosomal condition including segmental anomalies, and the results obtained were correlated with those from the other stages.

The study included blastocysts with a chromosome status already defined by PB or blastomere analysis generated by patients, who signed a consent form allowing further chromosomal analysis on their supernumerary blastocysts. These blastocysts were of two types: [1] Euploid, eventually destined to cryopreservation. The BF was aspirated and the collapsed blastocyst was immediately vitrified; and [2] aneuploid and therefore nontransferrable. After BF aspiration, TE cells were biopsied from the reexpanded blastocysts for separate chromosomal analysis. Owing to a national regulation that does not allow embryo donation for research, whole embryos (WE) were only tested when they turned out to be nonviable after extended culture (14). The study was approved by our Institutional Review Board (IRB no. 20110503).

Biopsy Procedures

Biopsy of PBs, blastomeres, TE cells, and BF was performed as described elsewhere in Hepes-buffered medium supplemented with protein (5 mg protein/mL) under oil (LifeGlobal Media) (12).

Briefly, PB1 and PB2 were sequentially biopsied after mechanical opening of the ZP. PB1 was removed immediately before intracytoplasmic sperm injection (ICSI), and PB2 was removed 6–9 hours later. Blastomere biopsy was performed at approximately 62–64 hours after ICSI in regularly developing embryos at the 6- to 8-cell stage (14). The ZP was opened by making a small hole using a laser (Saturn; Research Instruments) through which a nucleated blastomere was aspirated.

TE biopsy was performed by excising via laser pulses 3–5 cells, which had herniated through the breach previously opened in the ZP at the time of PB or blastomere biopsy.

Each biopsy was transferred to 0.2 mL polymerase chain reaction (PCR) tubes with 1 μL phosphate buffered saline kept on ice, spun, and stored at −80°C until further processing for chromosomal analysis (15).

BFs were aspirated from expanded blastocysts using an ICSI pipette paying great attention to avoid the aspiration of any cell. The retrieved fluids were transferred into empty PCR tubes kept on ice, which were spun and stored at −80°C. A volume of approximately 0.01 μL BF was retrieved from each blastocyst.

In case of nonviable blastocysts, the WE was transferred to a PCR tube with 1 μL buffer kept on ice and processed as indicated above.

Whole Genomic Amplification (WGA) and Array-CGH

WGA was performed in a class II Laminar flow cabinet using a PCR library–based method (SurePlex, Illumina). DNA amplification was determined by loading 5 μL of the final reaction onto a 1.5% agarose gel and was defined as “strong,” “weak,” or “failed” according to the band shape and intensity. An aliquot of the amplified DNA was used for 24-chromosome