Blastocoel fluid from differentiated blastocysts harbors embryonic genomic material capable of a whole-genome deoxyribonucleic acid amplification and comprehensive chromosome microarray analysis

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Objective: To obtain embryonic molecular karyotypes from genomic DNA (deoxyribonucleic acid) isolated from blastocoel fluid (BF) and to compare these karyotypes with the karyotypes from the remaining inner cell mass (ICM) and trophectoderm (TE) of the blastocyst.
Design: Prospective cohort study.
Setting: Academic center and preimplantation genetics laboratory.
Patient(s): Ninety-six donated cryopreserved embryos.
Intervention(s): Embryo biopsy, BF aspiration, DNA analysis using a comparative genomic hybridization microarray (aCGH).
Main Outcome Measure(s): The aCGH of a single blastomere, BF-DNA, and ICM-TE.
Result(s): The BF-DNA samples resulted in a successful aCGH in 63% of cases. Discordance in karyotypes was found between the BF-DNA and the ICM-TE in 52% of cases. A total of 70% of aneusomic (mosaicism), cleavage-stage embryos differentiated into euploid blastocysts. Probabilities for diagnostic accuracy were calculated and demonstrated the following: sensitivity of 0.88 (95% confidence interval [CI]: 0.62–0.98); specificity of 0.55 (95% CI: 0.39–0.70); positive predictive value of 0.41 (95% CI: 0.25–0.60); negative predictive value of 0.92 (95% CI: 0.75–0.99).
Conclusion(s): Genomic DNA from the BF can be amplified and characterized by comprehensive chromosome microarrays. The results demonstrated that aneusomic cleavage-stage embryos differentiated into euploid blastocysts, possibly using a mechanism that marginalizes aneuploid nuclei into the blastocoel cavity. In addition, owing to the high discordance between the karyotypes obtained from the BF-DNA and the ICM-TE, using BF-DNA for preimplantation genetic testing is not yet advised. (Fertil Steril® 2015;104:418–25. ©2015 by American Society for Reproductive Medicine.)
Key Words: Blastocoel fluid, preimplantation genetic testing, microarray, comparative genomic hybridization, preimplantation genetic screening

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Human embryogenesis is a complex process that is not fully understood. Since the advent of in vitro fertilization (IVF), the fundamentals of embryo development continue to expand. After activation of the embryonic genome, the embryo

can undergo compaction and differentiation into a blastocyst (1). After differentiation, the blastocyst consists of 3 principal morphological components: the inner cell mass (ICM) or future fetus: the trophectoderm (TE); and the blastocoel fluid (BF). Although investigations of the ICM and TE have contributed to the understanding of embryogenesis and affected clinical IVF outcomes (2–6), the potential use of the BF-DNA (deoxyribonucleic acid) has been largely neglected, with relatively few studies completed.

Preliminary investigations of human BF have been limited in scope and number. In 1 BF study, mass spectrometry was performed on BF-metabolites that were directly aspirated from the blastocoel cavity, using techniques similar to those previously completed on residual embryo culture media. They identified multiple metabolic proteins that have been suggested to correlate with embryo metabolism (7). However, apart from identifying the metabolic products, no direct clinical or embryologic quality outcomes were assessed. An additional BF study used proteomic analysis to identify numerous proteins within the BF (8); however, these were not found to be correlated with embryologic quality or clinical outcomes. Although these studies demonstrated that BF can be analyzed in a manner similar to that for embryo culture media, using metabolomics or proteomics, current findings have not yielded any correlation with clinical utility.

In an attempt to use BF for direct clinical applications, 4 prior studies (9–12) obtained embryonic BF-DNA and analyzed it for single genes and/or molecular karyotypes. Palini et al. (9) used a method described by D'Alessandro et al. (7) to obtain BF by creating a micropuncture through the TE, using an intracytoplasmic sperm injection (ICSI) pipette, followed by aspiration of the fluid until the blastocoel cavity fully collapsed around the pipette. The study targeted the glyceraldehyde 3-phosphate dehydrogenase (*GADPH*) gene as well as *TSPY1* and *TBC1D3*, using real-time polymerase chain reaction analysis. They next attempted a whole-genome microarray on 5 amplified DNA products obtained from the BF, and found a karyotype in only 2 samples.

Perloe et al. (10) reported preliminary data, at the American Society of Reproductive Medicine (ASRM) annual meeting in 2013, from an attempt to validate the use of BF-DNA for preimplantation genetic testing (PGT) using comparative genomic hybridization microarrays (aCGH). They analyzed DNA from the BF of 32 embryos; however, only 9 (28%) produced an interpretable molecular karyotype, and of those 9, only 3 karyotypes were concordant with the corresponding embryo's TE microarray analysis. In an additional study presented at the 2013 ASRM annual meeting, Poli et al. (11) reported results on the use of DNA fingerprinting for 8 chromosomes from 11 paired samples (BF-DNA separated from the whole-embryo DNA) to determine the concordance of polymorphic loci between paired samples. These results showed that 100% of the loci from the whole embryo successfully amplified, but that the DNA from the BF was of inferior quality and insufficient to complete DNA fingerprinting analysis.

Additionally, they performed aCGH on 4 paired samples, and obtained a karyotype on each sample analyzed. Their results showed discordance between the DNA from the BF and the whole embryo in 3 of 4 samples analyzed. They concluded that BF contains a variable amount of DNA and that the results were insufficient for reliable single-gene preimplantation genetic diagnosis.

In contrast to the studies just described, which were mostly unsuccessful attempts at BF-DNA analysis, Gianaroli et al. (12) recently published their successful attempt at investigating the potential of BF and PGT. This group analyzed BF that had been removed from differentiated blastocysts, and compared the ploidy status of the BF-DNA with either polar bodies or a single blastomere and TE cells. They used 51 blastocysts from 17 couples, which resulted in DNA detection from the BF in 76.5% of the samples. They identified a total ploidy concordance of 94.9% between the BF-DNA and either polar body or blastomere cells. Additionally, they identified a 97.4% ploidy concordance between the BF-DNA and the TE cells. They concluded that BF represents an accurate alternative source of embryonic DNA that could be used for chromosome testing.

As these preliminary studies demonstrated inconsistent and conflicting results, additional studies are required to determine the potential role of BF-DNA in PGT.

The aim of this study is to determine if embryonic DNA can be isolated from cells within the BF, and thereby a whole-genome microarray obtained. The results of this study provide insight into embryogenesis during differentiation and data on the feasibility of using BF-DNA for PGT.

MATERIALS AND METHODS

This study used 96 cryopreserved embryos that were donated for research, primarily owing to their unsuitability for clinical use, by patients who had undergone previous IVF cycles. Institutional review board (IRB) approval from Johns Hopkins Medical Institutions was obtained. As required by the IRB, all samples were deidentified at the time of informed consent and donation, and no patient demographics were available.

All embryos used in this study had been previously cryopreserved using vitrification at either the cleavage stage or the blastocyst stage of embryo development. Cryopreserved embryos first underwent a slow-thaw laboratory protocol. After thawing, those embryos that were initially cryopreserved at the cleavage stage were biopsied, using a laser, to remove a single blastomere. The embryos were continued in culture (continuous single culture, with Gentamicin, Irvine Scientific) until they differentiated to the blastocyst stage of embryo development. After differentiation to the expanded blastocyst stage, approximately 1 μL of BF was removed from each embryo, using an ICSI micropipette (5.5 μm, 30 degrees, Wallace, Smith Medical), via an established technique; the BF was placed in 2.5 μL of 1x phosphate buffered saline (7, 9).

Figure 1A–1C demonstrates this technique in 3 separate stages. Only those embryos that had expanded with an identifiable blastocoel cavity were aspirated. The remaining ICM-TE were removed from the media droplet and placed in a separate tube. All embryos that failed to differentiate to the blastocyst stage after thaw, or did not have a discernable blastocoel cavity, were excluded from the study.

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