GENETICS

Pushing the limits of detection: investigation of cell-free DNA for aneuploidy screening in embryos

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Objective: To determine the accuracy of cell-free DNA (cfDNA) in spent embryo medium (SEM) for ploidy and sex detection at the cleavage and blastocyst stages. To determine if assisted hatching (AH) and morphologic grade influence cfDNA concentration and accuracy.
Design: Prospective cohort.
Setting: Academic fertility center.
Patient(s): Nine patients undergoing IVF; 41 donated two-pronuclei embryos and 20 embryos from patients undergoing preimplantation genetic testing for aneuploidy (PGT-A).
Interventions(s): In a donated embryo arm, SEM was collected on days 3 and 5, with one-half of the embryos undergoing AH before and one-half after. In a clinical arm, SEM was collected on day 5 before trophectoderm (TE) biopsy. Samples underwent PGT-A with the use of next-generation sequencing. cfDNA results were compared with corresponding whole embryos and TE biopsies.
Main Outcome Measure(s): Concordance rates, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for ploidy and sex detection with the use of cfDNA.
Result(s): Of 141 samples, cfDNA was amplified in 39% and 80.4% of days 3 and 5 SEM, respectively. Concordances for ploidy and sex, respectively, were 56.3% and 81.3% between day 3 cfDNA and whole embryos, and 65% and 70% between day 5 cfDNA and TE biopsies. Day 5 cfDNA sensitivity and specificity for aneuploidy were 0.8 and 0.61, respectively. PPV and NPV were 0.47 and 0.88, respectively. Timing of AH and morphology did not influence cfDNA concentration or accuracy.
Conclusion(s): cfDNA is detectable on days 3 and 5, but more accurate on day 5. Although our data suggest moderate concordance rates, PGT-A with the use of cfDNA must be further optimized before clinical implementation. (Fertil Steril® 2018;■:■–■. ©2018 by American Society for Reproductive Medicine.)
Key Words: Cell-free DNA, preimplantation genetic testing for aneuploidy, next-generation sequencing

Selecting the best embryos is crucial for improving patients' chance of achieving a live birth with the use of in vitro fertilization (IVF). Blastocyst transfer and preimplantation genetic testing for aneuploidy (PGT-A) have led to improved outcomes (1–3). Although transferring euploid embryos improves implantation rates, this may be a less viable option for older women or those with severely diminished ovarian reserve (4, 5). This patient population may produce fewer or no blastocysts during an IVF cycle, with reported no-blastulation cycle rates ranging from 7.6% to 32.0% (6, 7). For these reasons, there is increasing interest in noninvasive methods for embryo assessment. Well studied tools include time-lapse morphokinetics and metabolomics of spent embryo medium (SEM). However, there is limited evidence that these approaches improve clinical outcomes (8–12).

Studies have investigated nucleic acids in SEM and demonstrated different microRNA and mitochondrial DNA profiles in embryos based on quality and implantation potential (13–15). The

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next logical application for SEM is the use of cell-free DNA (cfDNA) for aneuploidy screening. Recent studies have documented the ability to detect and sequence cfDNA in SEM with the use of cytogenic techniques such as array comparative genomic hybridization (aCGH) and modern sequencing platforms such as single-nucleotide polymorphism (SNP) sequencing and next-generation sequencing (NGS) (16–18). Although results have been encouraging, further studies are needed to assess the validity of this tool on different sequencing and bioinformatics platforms before clinical implementation. In the present study, our primary aim was to investigate the accuracy of cfDNA in SEM with the use of an improved method for DNA capture followed by NGS. We also sought to determine other factors that may influence accuracy of cfDNA, such as the timing of SEM collection, timing of assisted hatching (AH), and morphologic grade of the embryos. We also sought to establish a threshold concentration for which cfDNA could be detected and used to accurately predict the chromosomal status of an embryo. We hypothesized that cfDNA would have >90% concordance with whole embryos and trophectoderm (TE) biopsies. We also hypothesized that AH would increase cfDNA concentration and the accuracy of sequenced cfDNA for aneuploidy screening. We predicted that poor morphologic grade would be associated with higher DNA shedding and higher accuracy of cfDNA for aneuploidy screening.

MATERIALS AND METHODS

We conducted a prospective study comparing the accuracy of aneuploidy screening with the use of cfDNA in SEM compared with TE biopsies and whole embryos by means of NGS. The study was composed of a pilot portion with two separate arms using donated research embryos, as well as a clinical portion using patient samples. Institutional Review Board approval was obtained from the University of Southern California (HS-15-00858).

Pilot Study

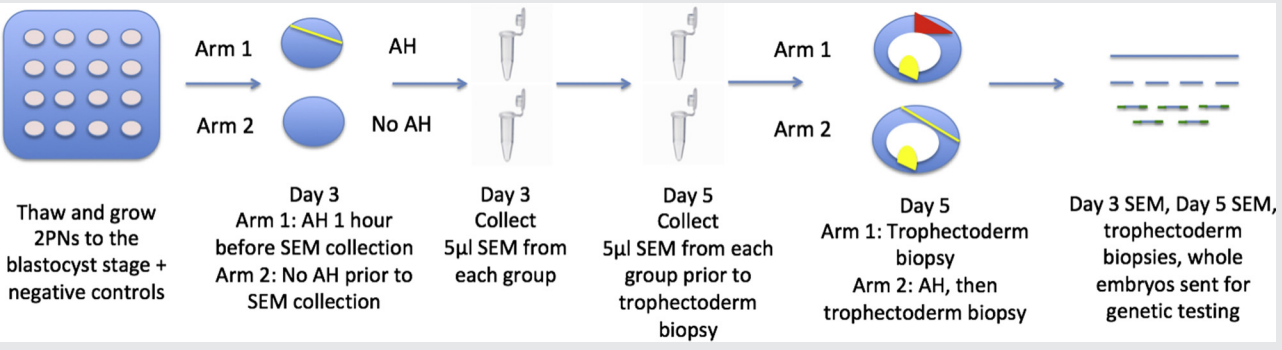
For the pilot study, previously cryopreserved embryos donated to research were used. All embryos were from previous oocyte donation cycles, previously fertilized by means of intracytoplasmic sperm injection (ICSI) and subsequently cryopreserved at the zygote stage by means of the slow-freeze technique with 1.5 mol/L propylene glycol and 0.1 mol/L sucrose (Irvine Scientific). Embryos were thawed with the use of Irvine Scientific embryo thaw kit with serial dilutions of cryoprotectant. After the thaw, embryos were placed in labeled wells corresponding to their study ID. All embryos were cultured in 25 μL continuous single-culture medium (CSC; Irvine Scientific) with the use of a Vitrolife Micro-Droplet dish overlayed with Liteoil (Life Global) at 37°C with 5% O_2 and 8% CO_2. They were removed from the incubator on days 3 and 5 for morphologic grading and for collection of SEM and TE biopsy.

We investigated factors that may potentially influence the concentration and accuracy of cfDNA, including timing of SEM collection and AH. In the first arm, AH was performed on day 3, after which 5 μL SEM was collected on both days 3 and 5. Blastocysts then underwent TE biopsy on day 5. In the second arm, we collected 5 μL SEM on days 3 and 5, before any AH. Embryos then underwent AH on day 5 after SEM collection, followed by TE biopsy. All corresponding whole embryos were saved for sequencing. All samples (days 3 and 5 SEM, TE biopsies, and corresponding whole embryos) were placed in RNase- and DNase-free polymerase chain reaction (PCR) tubes and stored at −30°C until ready for analysis. Negative control samples were culture medium placed in empty wells with the same incubation parameters. Pipette tips were changed between sample collections to avoid contamination. For all TE biopsies, the Lykos laser system (Hamilton Thorne) was used to remove approximately five cells (Fig. 1).

Clinical Study Design

For the clinical arm, we prospectively recruited patients planning to undergo PGT-A as a part of their IVF cycle from March through August 2017. All oocytes underwent stripping of all visible cumulus cells with the use of hyaluronidase before ICSI. Stripped mature oocytes were then fertilized by means of ICSI and cultured per clinical protocol in continuous single-culture medium (as described above). All blastocysts

FIGURE 1



Experimental diagram for pilot study. 2PN = two pronuclei; AH = assisted hatching; SEM = spent embryo medium.

Ho. Cell-free DNA for aneuploidy screening.. Fertil Steril 2018.