ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Proof of concept: preimplantation genetic screening without embryo biopsy through analysis of cell-free DNA in spent embryo culture media

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Objective: To assess whether preimplantation genetic screening (PGS) is possible by testing for free embryonic DNA in spent IVF media from embryos undergoing trophectoderm biopsy.
Design: Prospective cohort analysis.
Setting: Academic fertility center.
Patient(s): Seven patients undergoing IVF and 57 embryos undergoing trophectoderm biopsy for PGS.
Intervention(s): On day 3 of development, each embryo was placed in a separate media droplet. All biopsied embryos received a PGS result by array comparative genomic hybridization. Preimplantation genetic screening was performed on amplified DNA extracted from media and results were compared with PGS results for the corresponding biopsy.
Main Outcome Measure(s): [1] Presence of DNA in spent IVF culture media. [2] Correlation between genetic screening result from spent media and corresponding biopsy.
Result(s): Fifty-five samples had detectable DNA ranging from 2–642 ng/μL after a 2-hour amplification. Six samples with the highest DNA levels underwent PGS, rendering one result with a derivative log ratio SD (DLRSD) of <0.85 (a quality control metric of oligonucleotide array comparative genomic hybridization). The fluid sample and trophectoderm results were identical demonstrating (45XY, -13). Three samples were reamplified 1 hour later and tested showing improving DLRSD. One of the three samples with a DLRSD of 0.85 demonstrated (46XY), consistent with the biopsy. Overnight DNA amplification showed DNA in all samples.
Conclusion(s): We demonstrate two novel findings: the presence of free embryonic DNA in spent media and a result that is consistent with trophectoderm biopsy. Improvements in DNA collection, amplification, and testing may allow for PGS without biopsy in the future. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: In vitro fertilization (IVF), trophectoderm biopsy (PGS), comprehensive chromosomal screening (CCS), free embryonic DNA

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Although in recent years dramatic improvements in IVF success rates have been achieved, reproductive medicine remains fraught with inefficiency pertaining to risk of multiple pregnancy and miscarriage (1–3). Ideally, transferring one euploid embryo at a time would decrease the risk of both of these serious complications, yet simultaneously increasing the precision and efficiency of IVF (4, 5). One would therefore presume that preimplantation genetic screening (PGS) of the embryo would address our goals for increased precision and risk reduction (6).

Biopsy, an invasive technique, currently remains the only reliable approach to obtain sufficient embryonic DNA for genetic screening. A screening technique for embryonic competency without the need for biopsy would avoid any potential risk due to an invasive procedure and would thus be an important advancement in assisted reproduction. At present, there have been several approaches to evaluate embryos in the laboratory and determine embryonic viability without biopsy. These include morphologic assessment based on various characteristics noted at differential time points of embryonic development (7, 8), time-lapse imaging (9–11), assessment of DNA from the blastocele fluid (12), and assessment of the "secretome"

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within the spent IVF media, specifically metabolomics (13–16), proteomics (17–19), and analysis of micro-RNAs (20–22). None of these approaches have been reliable in accurately determining whether an embryo, once transferred, will undergo sustained implantation and lead to a healthy live birth.

Within the past 5 years, noninvasive prenatal screening has revolutionized the way obstetricians evaluate pregnant patients during the late first and early second trimester of pregnancy, demonstrating high sensitivities, specificities, and negative predictive values for detection of the most common aneuploidies (23–32). This method uses techniques to distinguish and assess free fetal DNA (from that of maternal origin) from a single maternal blood draw obtained at $\geq$10 weeks gestation. Initially, this approach was recommended for patients at high risk for fetal aneuploidy (33–35); however, a recent prospective randomized trial (32) demonstrated benefit for the general population. Free fetal DNA exists in maternal blood due to cell turnover as the placenta and fetus develop. It is unknown when free DNA is released, whether it starts as soon as an embryo begins to divide or at a later time point in embryonic development.

We hypothesize that free embryonic DNA is released early in embryonic development, soon after an embryo begins to divide. As such, we aimed to demonstrate that free embryonic DNA is present in spent IVF culture media. At present, there have been no peer-reviewed studies demonstrating presence of free embryonic DNA in spent media in which an embryo has developed. Furthermore, we hypothesize that a genetic screening result can be rendered similar to that of the corresponding trophectoderm biopsy.

MATERIALS AND METHODS

Study Subjects

Institutional Review Board approval was obtained, and participation for enrollment was offered to patients undergoing IVF with trophectoderm biopsy for PGS/comprehensive chromosomal screening, at the University of California, Los Angeles. There were no exclusion criteria. Study subjects agreed to additional testing of the spent media with the understanding that this additional testing would not have any impact on fertility treatment timeline or outcome. There was no added patient time or expense for study participation and no known effect on pregnancy outcomes.

Preparation and Collection of Spent Media

On day 3 of embryo development, each embryo was separated from the G1 media in which it was developing with up to five embryos, and was placed in a separate single 15-μL droplet of G2 media and assisted hatching was performed. The purpose of assisted hatching was twofold: to allow for extrusion of trophectoderm by day 5/6 to facilitate trophectoderm biopsy, and to allow expulsion of free embryonic DNA, if present, through the opening created by hatching into the G2 media. Assisted hatching on day 3 of embryonic development is standard operating procedure in our laboratory for embryos intended for PGS. On day 5 or 6 of development, for each blastocyst that qualified for trophectoderm biopsy based on laboratory criteria (expanding blastocyst with clear distinction between the inner cell mass [ICM] and trophectoderm), the spent media in which it developed was collected in a sterile vial and frozen at −80°F (refer to Fig. 1 for a visual representation of these steps). All qualifying embryos then underwent trophectoderm biopsy, frozen with vitrification, and received a PGS result using array comparative genomic hybridization (aCGH) and ET was performed as planned. Spent media was frozen when collected, then evaluated by a separate genetic screening laboratory blinded to trophectoderm biopsy comprehensive chromosomal screening results.

FIGURE 1

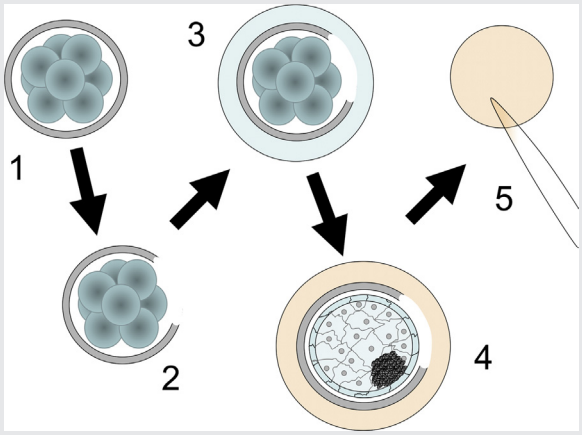

Illustration of preparation and collection of spent IVF media for noninvasive genetic screening. 1. On day 3 of embryonic development, each embryo was removed from G1 media; 2. the embryo underwent assisted hatching where an opening in the zona pellucida (ZP) was created using a laser; 3. the embryo was placed in isolation in a 15-μL droplet of G2 media; 4. the embryo was incubated from day 3 to the blastocyst stage by day 5 or 6. Once the embryo was removed to undergo trophectoderm biopsy for preimplantation genetic screening/comprehensive chromosomal screening; 5. the spent media droplet was collected, frozen, then eventually tested for presence of DNA and genetic screening performed to compare with the corresponding trophectoderm biopsy result.

Shamonki. Free embryonic DNA in spent IVF media. Fertil Steril 2016.

Evaluation of Media for DNA

A separate College of American Pathologists-accredited PGS laboratory blinded to any patient characteristics and trophectoderm biopsy results assessed all media used in the laboratory for incubation of egg, sperm, or embryo for DNA contamination. These media were all commercially manufactured, and included [1] oocyte retrieval flush media: modified human tubal fluid (HTF) medium (HTF *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES]-buffered with gentamicin) (Irvine Scientific), [2] oocyte retrieval media for washing oocytes during retrieval: G-MOPS (Vitrolife) supplemented with serum substitute supplement (SSS) (Irvine Scientific), [3] medias used during sperm processing: gradient: isolate (sperm separation medium) (Irvine Scientific) and wash: sperm rinse