Journal of Assisted Reproduction and Genetics (2019) 36:819–826
https://doi.org/10.1007/s10815-019-01437-6

COMMENTARY

Current status of spent embryo media research for preimplantation genetic testing

Denice Belandres[1] · Mousa Shamonki[2,3] · Nabil Arrach[1]

Received: 30 November 2018 / Accepted: 7 March 2019 / Published online: 21 March 2019
© Springer Science+Business Media, LLC, part of Springer Nature 2019

Abstract

In recent years, a growing body of literature has emerged investigating the clinical utility of spent embryo media (SEM) for preimplantation genetic testing for aneuploidy (PGT-A) (Hammond et al. in Fertil Steril. 107(1):220–8, 2017; Xu et al. in Proc Natl Acad Sci USA. 113(42):11907–12, 2016; Shamonki et al. in Fertil Steril. 106(6):1312–8, 2016; Feichtinger et al. in Reprod BioMed Online. 34(6):583–9, 2017; Vera-Rodriguez et al. in Hum Reprod. 33(4):745–56, 2018; Kuznyetsov et al. in PLoS One. 13(5):e0197262, 2018; Ho et al. in Fertil Steril. 110(3):467–75, 2018; Capalbo et al. in Fertil Steril. 110(5):870–9, 2018). Most of these studies have reported moderate success rates, suggesting the need for improvements in sensitivity and specificity. The concordance between spent media and embryo biopsy or whole embryo was reported to be between 30.4 and 90%, with 50–70% correlation being the most representative (Xu et al. in Proc Natl Acad Sci USA. 113(42):11907–12, 2016; Shamonki et al. in Fertil Steril. 106(6):1312–8, 2016; Feichtinger et al. in Reprod BioMed Online. 34(6):583–9, 2017; Vera-Rodriguez et al. in Hum Reprod. 33(4):745–56, 2018; Kuznyetsov et al. in PLoS One. 13(5):e0197262, 2018; Ho et al. in Fertil Steril. 110(3):467–75, 2018). Here, we will analyze all spent media testing strategies including SEM collection methods, whole genome amplification (WGA) strategies, chromosome copy number detection, and bioinformatics analysis tools. We will propose improvements to further increase the accuracy and sensitivity of the assay before bringing PGT-A with SEM into the clinical sphere.

Keywords Preimplantation genetic screening · Spent embryo media · Non-invasive · PGT-A · Cell-free DNA · cfDNA

Introduction

In the context of in vitro fertilization (IVF), SEM refers to the medium surrounding a growing embryo during culture. The length of time an embryo maintains contact with its surrounding medium depends on the standard operating procedures in a given IVF laboratory. While some laboratories culture embryos in a single-step monophasic medium, others opt for sequential media changes once or twice between fertilization and blastulation. These differences, in turn, may affect the quantity of cell-free DNA (cfDNA) as well as the quality or ability to analyze these samples for comprehensive chromosomal screening (CCS). Currently, it is unclear which system provides the ideal SEM sample for non-invasive preimplantation genetic testing for aneuploidy (PGT-A).

Multiple studies have demonstrated the ability to detect, extract, and amplify cfDNA from SEM at the cleavage and blastocyst stages, albeit with varying rates of amplification success [1–8]. The biological mechanism for this release of embryonic cfDNA into the medium is not clearly defined. Some have proposed the idea of embryonic "self-correction" for aneuploidies [9, 10]. Those who subscribe to this hypothesis suggest it as a possible explanation for artificially inflated or otherwise discordant PGT-A results in SEM samples compared to their trophectoderm (TE) biopsy or whole blastocyst (WB) counterparts. Still, the notion that a growing embryo will programmatically and preferentially discard aneuploid cells rather than other cell types has not yet been unequivocally proven [5, 9, 10].

Despite these uncertainties, SEM represents a preferable analyte for PGT-A due to its non-invasive nature. TE biopsies have become a standard protocol in IVF labs that routinely perform PGT-A. The procedure requires technically proficient

✉ Nabil Arrach
narrach@progenesis.com

[1] Progenesis Inc., 4150 Regents Park Row, Suite 245, La Jolla, CA 92037, USA

[2] Fertility and Surgical Associates of California, 325 Rolling Oaks Drive, Suite 110, Thousand Oaks, CA 91361, USA

[3] University of California, Los Angeles, CA 90095, USA

laboratory staff and additional instrumentation such as laser equipment. In comparison, SEM collection requires no specialized training and imposes negligible disruption to the embryo. Other non-invasive techniques to predict or assess IVF outcomes include time-lapse morphokinetics, proteomics, and metabolomics [11, 12]. However, the indirect nature of these techniques and the minimal evidence for their ability to significantly improve IVF outcomes renders them relatively unpopular in comparison to a more robust alternative.

With so many variables at play, assessing the question, "Where are we today with IVF spent media research?" is a unique challenge. In the last 3 years alone, nearly a dozen studies have been published on the efficacy of non-invasive strategies for PGT-A and/or PGT-M. Analytes studied have included SEM, blastocoel fluid (BF), and polar body biopsy [2–8, 13–16]. Focusing on PGT-A studies, research groups are asking different study questions, employing different embryo culturing protocols, and measuring outcomes in different ways. Furthermore, differences in analytes, duration of an embryo's contact with the collected medium, performance of assisted hatching (AH), amplification methods, CCS methods, sequencing platforms, bioinformatics analyses, and strategies for identifying maternal contamination all contribute to answering the question—can we reliably use SEM for PGT-A?

Culturing methods

Differences in culturing systems result in variability in the length of time that an embryo is in contact with the medium to be tested for PGT-A. These differences impact both the quantity of cfDNA and performance of the CCS assay. Monophasic or single-step medium techniques allow for the longest exposure to the growing embryo, theoretically increasing the quantity of cfDNA. Two SEM studies using single-step culturing systems showed successful amplification in 81.8% and 100% of tested samples [4, 7]. A significant drawback of this method, however, is increased cfDNA degradation over time decreasing the quality of the cfDNA. We have suggested collection of media at standard time points throughout culture may mitigate this issue; however, daily SEM collection has yet to be evaluated [3]. The use of sequential media may represent an alternative for minimizing DNA degradation in SEM. One study found that 24–48 h of contact with the embryo was sufficient to obtain amplification of cfDNA from SEM [6]. Our group and others using sequential media for SEM collection have reported amplification success rates between 91.1 and 100% [2, 3, 5, 6]. Currently, sequential media appears to be a more common choice for the SEM–PGT-A workflow, with slightly increased WGA success rates over single-step methods.

Previously frozen donated whole embryos have been utilized in many studies. However, the effects of freeze-thawing embryos have not been thoroughly evaluated in the context of SEM. It is plausible that the process of vitrification can lead to a higher rate of apoptosis and therefore an increased amount of cfDNA in the media. In two studies, the amount of DNA after amplification was lower in freshly cultured embryos compared to previously frozen embryos; however, these differences were not statistically significant [6, 7]. Freezing and thawing SEM may cause increased DNA degradation, though this has yet to be systematically evaluated.

The composition of the medium used is an important consideration when collecting SEM for PGT-A. The ideal analyte for non-invasive PGT-A is medium that contains a sufficient amount of cfDNA that is of sufficient quality for downstream amplification and CCS. Perhaps the balance between media that is just aggressive enough to encourage minimal cell lysis while gentle enough not to harm the embryo will play an important role in identifying the optimal medium for SEM analysis.

The optimal day for collecting SEM has not yet been established. While most research groups are collecting at the blastocyst stage, observed amplification rates from D5 SEM vary from 81.8 to 100% [2–7]. Ho et al. were the first group to investigate PGT-A on SEM collected both prior to and after blastulation [7]. In this study, SEM was collected on D3 and D5. DNA was detectable in 97% of samples collected at D3, though only 39% of amplified samples produced enough sequencing reads to yield a PGT-A result. In this same study, SEM collected on D5 had an amplification rate of 97% with 80% of amplified samples producing sufficient yields for a reliable PGT-A result [7]. This suggests that there is an optimal collection date for SEM in order to maximize quantity of cfDNA without sacrificing quality. Perhaps more important than SEM collection day is the duration of contact with the embryo, which is determined by culturing methods as previously discussed.

Another source of variability in PGT-A with SEM is assisted hatching (AH)—the technique of thinning or rupturing the zona pellucida (ZP). AH is widely practiced in IVF labs today to improve implantation and pregnancy rates and as a standard protocol for PGT-A [17, 18]. We have hypothesized that breaching the ZP would increase amounts of cfDNA in the surrounding media, thereby providing better sampling of the embryo and increased accuracy of the test [3]. However, our subsequent study comparing hatched versus unhatched embryos did not reveal a significant difference in cfDNA concentrations [7]. More studies are required to assess the impact of AH on the accuracy of non-invasive PGT-A.

The gold standard

Perhaps the biggest question we face is whether cfDNA in SEM uniformly and completely represents the embryonic

genome. The "gold standard" is defined differently in most SEM–PGT-A studies. When considering accuracy and concordance rates between SEM and the "gold standard" of choice, comparisons between cfDNA and D5 whole embryos (blastocysts) perhaps make the strongest cases for concordance. While WBs may arguably best represent the whole embryonic genome, these donated WBs are not always readily available.

Today in IVF, TE biopsy is a standard clinical practice for PGT-A. Thus, in clinical SEM studies, TE biopsies are generally used as the gold standard. TE biopsy has been criticized as mathematically incapable of accurately determining ploidy status due to the relatively few cells sampled, which therefore may not accurately represent the ploidy of the untested inner cell mass [19]. Although the invasive nature of the procedure elicits significant opposition, TE biopsy for PGT-A remains a widely employed practice in IVF, preferable to the riskier and less-accurate blastomere biopsy [20, 21]. In the current literature of SEM, TE biopsy represents the most frequently utilized gold standard for comparison of SEM PGT-A results.

Comparisons between cfDNA and polar body biopsies have also been studied, but with the important limitation of only detecting meiotic aneuploidies of maternal origin. Polar body analysis is incapable of detecting paternally inherited meiotic aneuploidies or mitotic aneuploidies [4]. Blastocoel fluid (BF) has also been explored as a non-invasive PGT-A analyte, both alone and in combination with SEM [6, 8, 13]. Research in BF analysis has shown inconsistent performance for DNA amplification rates and concordance, 34.8–76.5% and 37.5–97.4% respectively [8, 13].

The PGT field has been marked with significant improvements to create and optimize safer, more accurate testing options. It remains to be seen if improvements in non-invasive PGT-A can eliminate the need for biopsy in the future. An unbiased assessment of the efficacy of non-invasive PGT-A using SEM requires standardization across a number of variables, including the gold standard to which we compare cfDNA PGT-A results.

Whole genome amplification methods

Differences in amplification methods such as multiple displacement amplification (MDA), PCR-based techniques, and multiple annealing- and looping-based amplification cycles (MALBAC) appear to have an effect on amplification success rates of cfDNA derived from SEM. Furthermore, maternal DNA contamination in these samples complicates the interpretation of amplification success, where amplification of maternal DNA rather than embryonic DNA may provide an inaccurate measure of performance.

MDA is an isothermal amplification reaction that utilizes the high processivity and strand-displacement ability of the phi29 DNA polymerase [22]. Random hexamer primers anneal to the template DNA, resulting in amplicon products greater than 10 kb in length [23]. Whole genome sequencing coverage using MDA has been reported as high as 72% and as low as 10% in whole exome sequencing [24, 25]. Despite the high fidelity of the polymerase, amplification bias due to non-linear amplification remains a significant weakness of this technique.

PCR-based amplification using a single degenerate oligonucleotide primer (DOP-PCR) was among the first WGA methods employed to successfully detect aneuploidy using aCGH [26, 27]. Relatively low coverage of the genome and high incidence of allele dropout (ADO) renders DOP-PCR inferior to more modern WGA techniques [28]. Methods using a combination of displacement pre-amplification and PCR-amplification such as the PicoPlex/SurePlex WGA system are widely used in PGT-A today and have been reported to cover ~ 30% of the genome [25].

MALBAC is a technique first described in 2012 that uses a quasi-linear pre-amplification step followed by multiple annealing and looping based amplification cycles. The strategy was effectively used to identify copy number and single nucleotide variants in single cancer cells, with ~ 93% genomic coverage and ~1% ADO [29]. MALBAC on SEM has produced PGT-A sensitivity of 88.2% [2]. Despite this increased coverage, the lack proofreading capability from the pre-amplification polymerase may contribute to increased incidence of false positives [30]. Of additional concern, the high coverage reported has not been replicated and published by other labs.

Current literature on spent media has shown a broad representation of different WGA techniques. Our study employing MDA on SEM showed an amplification rate of 97%; however, only 2% of amplified samples yielded a reliable PGT-A result. Re-amplification of the samples increased the quantity of DNA but still failed to produce high-quality PGT-A results [3]. To the best of our knowledge, no other studies have published data on non-invasive PGT-A using MDA. Therefore, further investigation into the efficiency of MDA to amplify whole genomes from SEM is necessary.

The SurePlex/PicoPlex system is a popular choice in PGT-A. In our recent study, PicoPlex WGA was used on two sets of SEM collected on D3 and D5. WGA has shown amplification rates of 97% for both sets of samples. Only 39% of amplified samples from D3 produced sufficient sequencing reads to generate a PGT-A result; meanwhile, 80% of amplified samples from D5 yielded PGT-A results [7]. Another group using the SurePlex amplification method reported amplification rate of 81.8% in 22 SEM samples, with all amplified samples producing a PGT-A result by aCGH [4]. Double amplification on SEM using two consecutive rounds of this WGA method yielded 91% amplification rate in 56 PGT-A SEM samples, with all amplified samples producing reliable PGT-A results

[5]. One publication studying combined blastocoel fluid (BF) and SEM employed SurePlex WGA and found 100% amplification rate in 28 samples, with all producing PGT-A results [6].

While these PCR-based approaches are widely employed today, newer techniques such as MALBAC have also emerged. Amplification rate on SEM using MALBAC has been reported at 100% in 42 samples, with all samples producing a PGT-A result [2]. Importantly, the issue of significant maternal contamination demonstrated in SEM samples may inflate amplification rates. Further studies with MALBAC on SEM are necessary to assess the reproducibility of this result and determine if this technique is the ideal option for WGA before non-invasive PGT-A.

Aneuploidy detection

As IVF and PGT co-evolve, notable improvements to CCS methods have been accomplished. These include the movement from fluorescent in situ hybridization (FISH) to more sensitive techniques such as quantitative polymerase chain reaction (qPCR), array competitive genomic hybridization (aCGH), single nucleotide polymorphism (SNP) microarrays, and next-generation sequencing (NGS).

Array CGH has long been considered the gold standard for CCS in the last decade, making significant improvements in aneuploidy detection rates compared to previous in situ methods [31]. More recently, the development and application of NGS for PGT-A have brought a greater level of sensitivity, precision, and accuracy [32–34]. With increasing popularity of single thawed euploid embryo transfer cycles, NGS has proven to increase implantation rates and live birth rates compared to aCGH [35, 36]. As mosaicism gains more interest and attention in the IVF community, NGS represents a favorable platform for PGT-A to detect mosaicism due to its increased sensitivity over aCGH [32, 34].

Bioinformatics analyses

As the chemistry and sequencing platforms mature, so must their complementary analytical tools. Out-of-the-box software for analysis of chromosome copy number seems to be a standard practice among researchers and PGT-A providers [3–7]. Still, variability between different software and quality control (QC) thresholds set by individual testing providers may lend additional bias during analysis. Depending on the platform used for CCS, a variety of QC metrics contribute to the final call on a PGT-A report. Most importantly, these analytics were designed for whole-cell DNA sequencing and thus may not apply to cfDNA assessment.

For NGS platforms, the number of reads per sample is a pertinent metric in determining quality of the test. One study employing the Ion Torrent sequencing platform allowed for 150,000–200,000 reads per sample [7]. Another study sequenced approximately 2 million reads per sample on an Illumina platform [2]. Notably, differences in read lengths between the two sequencing platforms may justify the differences in reads per sample, and overall coverage per base is comparable. Furthermore, differences in uniformity, GC-bias, reproducibility, and CNV detection for single-cell sequencing between Ion Torrent and Illumina technologies have been observed [37].

In-house pipelines developed from synthesizing publicly available tools for trimming, alignment, duplicate read removal, base recalibration, and downstream variant calling and copy number analyses are an alternative to commercial software and introduce more variability in the data-processing workflow.

Custom pipelines and commercial software to tackle the challenge of maternal DNA contamination are a requisite for implementing non-invasive PGT-A into clinical practice. Currently, in non-invasive prenatal testing (NIPT), calculating fetal fraction (ff)—the ratio of fetal DNA to total cfDNA—is a crucial step in determining the efficacy of the test. In general, performance of the assay gets better with increased ff [38]. There are many ways to calculate ff or quantify fetal DNA; these include differential methylation analysis, short-tandem repeat (STR) analysis, single nucleotide polymorphism (SNP) panels, indel polymorphism panels, real-time PCR (qPCR), droplet digital PCR (ddPCR), and whole-genome sequencing [39–43]. In SEM research, the prevalence of maternal contamination was investigated using a small SNP panel; importantly, maternal contamination was found in the majority of the samples tested [5]. Ultimately, it may be necessary to perform oocyte striping and ICSI to minimize the risk of maternal and paternal contamination if cfDNA assessment of embryos is planned. NIPT has taught us that not all bioinformatics approaches are equally powered to accurately differentiate fetal from maternal DNA in a cost-effective way. There may be additional value for couples enrolled in IVF to seek comprehensive genetic screening in order to generate parental haplotypes to rule out ADO and contamination events during non-invasive testing. Further large-scale studies are required to find a practical and cost-effective strategy for identifying maternal contamination. As with commercially available NIPTs, the ability to accurately distinguish between embryonic and maternal DNA is absolutely necessary to achieve a reliable PGT-A result.

Defining concordance

Rates of concordance between SEM and the gold standard of choice have been reported in several categories including

general ploidy status (aneuploid versus euploid), concordance for sex, whole chromosome number (WCN) concordance, concordance for all aberrations (including mosaicism and segmental aneuploidies), and concordance per single chromosome. For an added level of precision, the degree of concordance between two samples from the same blastocyst has also been described in several sub-categories. In most studies, perfect matches were few and far between. Most common were instances of imperfect or partial concordance. Some examples include the following: complementary concordance—a trisomy in one sample and a monosomy of the same chromosome in the corresponding sample; aneuploid-mosaic—a full trisomy or monosomy in one sample and a mosaic call affecting the same chromosome in the corresponding sample; aneuploid complex—at least one shared aneuploidy between two samples, but with other unshared aneuploidies; and aneuploid–aneuploid—both samples are aneuploid but for completely different chromosomes.

Mosaicism presents a unique challenge in the definition of concordance in the context of SEM. Discordant results for PGT-A with SEM are often attributed to mosaicism [2, 5, 6]. However, due to the nature of the DNA source and relatively low embryonic fraction, current methods for non-invasive PGT-A do not appear to be sufficiently sensitive to detect mosaicism [5, 7]. This further complicates the definition of true concordance between SEM and its corresponding biopsy or embryo.

General ploidy concordance rates between cfDNA from SEM and the corresponding WB or TE biopsy have been published as low as 30.4% and as high as 87.5% [2, 5]. Other studies have published moderate rates in the 55–75% range [4, 7]. One study measuring cfDNA from a mixture of BF and SEM found 100% ploidy concordance between cfDNA and TE biopsy in 19 freshly cultured embryos, with 98.2% concordance per chromosome [6]. The great variability between these rates and the relatively small sample sizes in each study point to large-scale studies as a crucial next step for non-invasive PGT-A.

Discussion

In the vast majority of publications on SEM and PGT-A, concordance between two samples is "achieved" when two samples have the same general ploidy status. For example, TE biopsy yielding a PGT-A result of 45, XY, − 16 is "concordant" with its corresponding cfDNA sample result of 44, XX, − 16, − 17, though the whole chromosome number is discordant between the two samples. Similarly, a WB with karyotype 46, XX, + 4 is "concordant" with its corresponding cfDNA sample with karyotype 50, XX, + 1, + 5, + 10, + 13, though there are no shared aneuploidies between the two samples. Although the different results may converge on the same clinical decision—not recommended for transfer—there is a clear issue of imprecision between the two samples. In these examples, unshared aneuploidies between the samples prompt the question—how can we be convinced that cfDNA is an accurate representation of the growing embryo? A more troubling question: what if cfDNA is actually more accurate and representative of the entire embryo? Preliminary data suggest otherwise, but large-scale studies are needed to confirm this.

It has been demonstrated that the prevalence of aneuploidy increases with maternal age [44, 45]. Recently, a randomized, controlled trial showed a clinical benefit for women 35 years and older to have PGT-A [46–48]. Notably, findings from this multicenter study did not support a benefit for PGS in all patient age groups. It is possible that this may be attributed to lack of standardization across laboratory protocols, which result in variable false-positive rates across centers. To demonstrate a clinical benefit of PGT-A with SEM, accuracy and reliability of the result must meet a minimum threshold of 95%. The creation and subsequent validation of a standardized protocols for PGT-A with SEM represent significant short-term goals. The initial stages of a validation with a clinical trial would primarily study concordance with TE biopsy results. Many clinical parameters discussed in this review including culture media, culturing system, assisted hatching, WGA chemistry, and sequencing platform must all be interrogated in the validation to establish standardized procedures for PGT-A. Once standard procedures have shown accuracy and reliability of SEM compared to conventional methods, follow-up studies measuring clinical outcomes such as ongoing pregnancy rate, miscarriage rate, and live birth rate will be necessary. This proposed validation of the protocols established in the initial study phase requires the participation of multiple IVF centers and multiple PGT-A laboratories. Analysis of the data by age group represents an important step in understanding the clinical utility of the test. Parallel testing of the corresponding TE biopsy during this phase will be performed as a control.

Maternal contamination is a common theme among discordant results. Full maternal contamination was observed in TE euploid males that had corresponding SEM result with euploid female karyotype, indicating that the Y chromosome was not sampled in the cfDNA [5]. The majority of false-negative results have been attributed to partial or full maternal contamination. Clinically validating non-invasive PGT-A requires the development of strategies—whether in silico or in practice—to parse maternal and embryonic DNA in order to report accurate aneuploidy calls. Additionally, interpreting success of amplification may be further complicated by the extent to which non-embryonic DNA is present and amplified.

Mosaicism has drawn significant attention in the IVF community in recent years. The debate over the impact of transferring mosaic embryos is ongoing. False-positive and otherwise discordant results in non-invasive PGT-A with SEM have

been hypothesized to have arisen from mosaicism [2, 5, 6]. One study found a majority of samples with imperfect or partial concordance between SEM and TE biopsy originated from mosaic blastocysts. FISH analysis of these WB showed concordance with the TE result in most cases, suggesting a potential preferential release of certain types of aneuploid cells [5]. The underlying biology remains unknown.

Other applications for spent media

Spent media has been studied in the context of preimplantation genetic testing for monogenic diseases (PGT-M) such as alpha- and beta-thalassemia [14, 15]. Though contamination of the medium and ADO due to low embryonic DNA input are significant concerns, concordance has been reported between SEM and biopsies [14, 15]. Studies that follow clinical outcome after testing are a necessary next step for non-invasive PGT-M.

Additionally, it has been shown that embryonic microRNAs (miRNAs) are differentially secreted into the culture medium with distinct expression profiles [49]. Evidence has also been published supporting the role of miRNA in embryo implantation, ploidy status, and pregnancy outcome [49–51]. A clinical application for this may involve selection of embryos with miRNA expression profiles consistent with euploidy and increased implantation potential. Further clinical investigation is needed to determine the efficacy of miRNAs as biomarkers for non-invasive embryo selection.

Conclusions

Improvements in SEM collection methods, cfDNA amplification, and chromosome copy number screening technologies must be developed and optimized before clinical application of non-invasive PGT-A. Currently, many IVF labs are growing human embryos in group culture. Testing spent media requires each embryo to be cultured separately (single embryo culture). Uprooting the established culturing systems in an IVF lab is likely to be met with opposition, as these changes would require significantly more space, consumables, and time from laboratory staff.

Still, once optimized, the test offers significant benefits. Patients with fewer or more fragile embryos who elect to forego an invasive biopsy procedure will benefit greatly from spent media collection as an alternative for PGT-A. Older patients generally have low-quality embryos that are susceptible to degradation, stress, and viability when biopsied. A non-invasive technic such as SEM will alleviate stress on the embryos and offer a better embryo survival. Due to high aneuploidy rate among patients over 40 years old (65%), false-positive rate of SEM test will have less impact on the overall clinical outcome. For example, if SEM test produces a 10% false-positive rate, patients under 35 years old and aneuploidy rate of 34% will have a false-positive rate of 22.7% (10 out of 44 aneuploidy diagnoses is false). In contrast, for patients over 35 years old and aneuploidy rate of 65%, false-positive rate would be only 13% (10 out of 75 aneuploidy diagnoses is false).

Overall, our assessment is that PGT-A with SEM is not yet ready to be offered as a commercial or clinical test. Surely improvements to existing WGA chemistry will bring us closer to uniform, complete coverage of the genome with minimal amplification bias. Likewise, CCS technologies and their bioinformatics analysis pipelines must continue to evolve, especially to tackle the significant challenge of maternal contamination. Perhaps the future of embryo quality assessment and selection for transfer may employ SEM in a multifaceted approach by combining PGT-A, gene expression analysis, miRNA expression profiling, time-lapse parameters, and other non-invasive techniques.

References

1. Hammond ER, McGillivray BC, Wicker SM, Peek JC, Shelling AN, Stone P, et al. Characterizing nuclear and mitochondrial DNA in spent embryo culture media: genetic contamination identified. Fertil Steril. 2017;107(1):220–8.
2. Xu J, Fang R, Chen L, Chen D, Xiao JP, Yang W, et al. Noninvasive chromosome screening of human embryos by genome sequencing of embryo culture medium for in vitro fertilization. Proc Natl Acad Sci USA. 2016;113(42):11907–12.
3. Shamonki MI, Jin H, Haimowitz Z, Liu L. Proof of concept: preimplantation genetic screening without embryo biopsy through analysis of cell-free DNA in spent embryo culture media. Fertil Steril. 2016;106(6):1312–8.
4. Feichtinger M, Vaccari E, Carli L, Wallner E, Mädel U, Figl K, et al. Non-invasive preimplantation genetic screening using array comparative genomic hybridization on spent culture media: a proof-of-concept pilot study. Reprod BioMed Online. 2017;34(6):583–9.
5. Vera-Rodriguez M, Diez-Juan A, Jimenez-Almazan J, Martinez S, Navarro R, Peinado V, et al. Origin and composition of cell-free DNA in spent medium from human embryo culture during preimplantation development. Hum Reprod. 2018;33(4):745–56.
6. Kuznyetsov V, Madjunkova S, Antes R, Abramov R, Motamedi G, Ibarrientos Z, et al. Evaluation of a novel non-invasive preimplantation genetic screening approach. PLoS One. 2018;13(5):e0197262.
7. Ho JR, Arrach N, Rhodes-Long K, Ahmady A, Ingles S, Chung K, et al. Pushing the limits of detection: investigation of cell-free DNA for aneuploidy screening in embryos. Fertil Steril. 2018;110(3):467–75.
8. Capalbo A, Romanelli V, Patassini C, Poli M, Girardi L, Giancani A, et al. Diagnostic efficacy of blastocoel fluid and spent media as sources of DNA for preimplantation genetic testing in standard clinical conditions. Fertil Steril. 2018;110(5):870–9.
9. Barbash-Hazan S, Frumkin T, Malcov M, Yaron Y, Cohen T, Azem F, et al. Preimplantation aneuploid embryos undergo self-correction

in correlation with their developmental potential. Fertil Steril. 2009;92(3):890–6.
10. Munné S, Velilla E, Colls P, Bermudez MG, Vemuri MC, Steuerwald N, et al. Self-correction of chromosomally abnormal embryos in culture and implications for stem cell production. Fertil Steril. 2005;84(5):1328–34.
11. Katz-Jaffe MG, McReynolds S, Gardner DK, Schoolcraft WB. The role of proteomics in defining the human embryonic secretome. Mol Hum Reprod. 2009;15(5):271–7.
12. Pribenszky C, Nilselid AM, Montag M. Time-lapse culture with morphokinetic embryo selection improves pregnancy and live birth chances and reduces early pregnancy loss: a meta-analysis. Reproductive biomedicine online. 2017;35(5):511–20.
13. Gianaroli L, Magli MC, Pomante A, Crivello AM, Cafueri G, Valerio M, et al. Blastocentesis: a source of DNA for preimplantation genetic testing. Results from a pilot study. Fertil Steril. 2014;102(6):1692–9.
14. Wu H, Ding C, Shen X, Wang J, Li R, Cai B, Xu Y, Zhong Y, Zhou C. Medium-based noninvasive preimplantation genetic diagnosis for human α-thalassemias-SEA. Medicine. 2015;94(12):e669.
15. Liu W, Liu J, Du H, Ling J, Sun X, Chen D. Non-invasive preimplantation aneuploidy screening and diagnosis of beta thalassemia IVSII654 mutation using spent embryo culture medium. Ann Med. 2017;49(4):319–28.
16. Magli MC, Pomante A, Cafueri G, Valerio M, Crippa A, Ferraretti AP, et al. Preimplantation genetic testing: polar bodies, blastomeres, trophectoderm cells, or blastocoelic fluid? Fertil Steril. 2016;105(3):676–83.
17. Practice Committee of the American Society for Reproductive Medicine, Practice Committee of the Society for Assisted Reproductive Technology. Role of assisted hatching in in vitro fertilization: a guideline. Fertil Steril. 2014;102(2):348–51.
18. Sathananthan H, Gunasheela S, Menezes J. Mechanics of human blastocyst hatching in vitro. Reprod BioMed Online. 2003;7(2):228–34.
19. Gleicher N, Metzger J, Croft G, Kushnir VA, Albertini DF, Barad DH. A single trophectoderm biopsy at blastocyst stage is mathematically unable to determine embryo ploidy accurately enough for clinical use. Reprod Biol Endocrinol. 2017;15(1):33.
20. Cimadomo D, Capalbo A, Ubaldi FM, Scarica C, Palagiano A, Canipari R, et al. The impact of biopsy on human embryo developmental potential during preimplantation genetic diagnosis. Biomed Res Int. 2016;2016:1–10.
21. Chuang TH, Hsieh JY, Lee MJ, Lai HH, Hsieh CL, Wang HL, Chang YJ, Chen SU. Concordance between different trophectoderm biopsy sites and the inner cell mass of chromosomal composition measured with a next-generation sequencing platform. MHR: Basic science of reproductive medicine. 2018;24(12):593–601.
22. Dean FB, Hosono S, Fang L, Wu X, Faruqi AF, Bray-Ward P, et al. Comprehensive human genome amplification using multiple displacement amplification. Proc Natl Acad Sci USA. 2002;99(8):5261–6.
23. Mai M, Hoyer JD, McClure RF. Use of multiple displacement amplification to amplify genomic DNA before sequencing of the α and β haemoglobin genes. J Clin Pathol. 2004;57(6):637–40.
24. Voet T, Kumar P, Van Loo P, Cooke SL, Marshall J, Lin ML, et al. Single-cell paired-end genome sequencing reveals structural variation per cell cycle. Nucleic Acids Res. 2013;41(12):6119–38.
25. Borgström E, Paterlini M, Mold JE, Frisen J, Lundeberg J. Comparison of whole genome amplification techniques for human single cell exome sequencing. PLoS One. 2017;12(2):e0171566.
26. Telenius H, Carter NP, Bebb CE, Nordenskjo M, Ponder BA, Tunnacliffe A. Degenerate oligonucleotide-primed PCR: general amplification of target DNA by a single degenerate primer. Genomics. 1992;13(3):718–25.
27. Voullaire L, Wilton L, Slater H, Williamson R. Detection of aneuploidy in single cells using comparative genomic hybridization. Prenat Diagn. 1999;19(9):846–51.
28. Blagodatskikh KA, Kramarov VM, Barsova EV, Garkovenko AV, Shcherbo DS, Shelenkov AA, et al. Improved DOP-PCR (iDOP-PCR): a robust and simple WGA method for efficient amplification of low copy number genomic DNA. PLoS One. 2017;12(9):e0184507.
29. Zong C, Lu S, Chapman AR, Xie XS. Genome-wide detection of single-nucleotide and copy-number variations of a single human cell. Science. 2012;338(6114):1622–6.
30. Navin NE. The first five years of single-cell cancer genomics and beyond. Genome Res. 2015;25(10):1499–507.
31. Wells D, Alfarawati S, Fragouli E. Use of comprehensive chromosomal screening for embryo assessment: microarrays and CGH. Mol Hum Reprod. 2008;14(12):703–10.
32. Fiorentino F, Bono S, Biricik A, Nuccitelli A, Cotroneo E, Cottone G, et al. Application of next-generation sequencing technology for comprehensive aneuploidy screening of blastocysts in clinical preimplantation genetic screening cycles. Hum Reprod. 2014;29(12):2802–13.
33. Zheng H, Jin H, Liu L, Liu J, Wang WH. Application of next-generation sequencing for 24-chromosome aneuploidy screening of human preimplantation embryos. Mol Cytogenet. 2015;8(1):38.
34. Tortoriello DV, Dayal M, Beyhan Z, Yakut T, Keskintepe L. Reanalysis of human blastocysts with different molecular genetic screening platforms reveals significant discordance in ploidy status. J Assist Reprod Genet. 2016;33(11):1467–71.
35. Friedenthal J, Maxwell SM, Munné S, Kramer Y, McCulloh DH, McCaffrey C, et al. Next generation sequencing for preimplantation genetic screening improves pregnancy outcomes compared with array comparative genomic hybridization in single thawed euploid embryo transfer cycles. Fertil Steril. 2018;109(4):627–32.
36. Lai HH, Chuang TH, Wong LK, Lee MJ, Hsieh CL, Wang HL, et al. Identification of mosaic and segmental aneuploidies by next-generation sequencing in preimplantation genetic screening can improve clinical outcomes compared to array-comparative genomic hybridization. Mol Cytogenet. 2017;10(1):14.
37. Chen D, Zhen H, Qiu Y, Liu P, Zeng P, Xia J, et al. Comparison of single cell sequencing data between two whole genome amplification methods on two sequencing platforms. Sci Rep. 2018;8(1):4963.
38. Canick JA, Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE. The impact of maternal plasma DNA fetal fraction on next generation sequencing tests for common fetal aneuploidies. Prenat Diagn. 2013;33(7):667–74.
39. Nygren AO, Dean J, Jensen TJ, Kruse S, Kwong W, van den Boom D, et al. Quantification of fetal DNA by use of methylation-based DNA discrimination. Clin Chem. 2010;56(10):1627–35.
40. Barrett AN, Xiong L, Tan TZ, Advani HV, Hua R, Laureano-Asibal C, et al. Measurement of fetal fraction in cell-free DNA from maternal plasma using a panel of insertion/deletion polymorphisms. PLoS One. 2017;12(10):e0186771.
41. Artieri CG, Haverty C, Evans EA, Goldberg JD, Haque IS, Yaron Y, et al. Noninvasive prenatal screening at low fetal fraction: comparing whole-genome sequencing and single-nucleotide polymorphism methods. Prenat Diagn. 2017;37(5):482–90.
42. Sillence KA, Roberts LA, Hollands HJ, Thompson HP, Kiernan M, Madgett TE, Welch CR, Avent ND. Fetal sex and RHD genotyping with digital PCR demonstrates greater sensitivity than real-time PCR. Clinical chemistry. 2015 Nov 1;61(11):1399-407.
43. Svobodová I, Pazourková E, Hořínek A, Novotná M, Calda P, Korabečná M. Performance of droplet digital PCR in non-invasive fetal RHD genotyping-comparison with a routine real-time PCR based approach. PLoS One. 2015;10(11):e0142572.

44. Ata B, Kaplan B, Danzer H, Glassner M, Opsahl M, Tan SL, et al. Array CGH analysis shows that aneuploidy is not related to the number of embryos generated. Reprod BioMed Online. 2012;24(6):614–20.
45. Franasiak JM, Forman EJ, Hong KH, Werner MD, Upham KM, Treff NR, et al. The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening. Fertil Steril. 2014;101(3):656–63.
46. Munné S, Cohen J. Advanced maternal age patients benefit from preimplantation genetic diagnosis of aneuploidy. Fertil Steril. 2017;107(5):1145–6.
47. Munne S, Kaplan B, Frattarelli JL, Gysler M, Child TJ, Nakhuda G, et al. Global multicenter randomized controlled trial comparing single embryo transfer with embryo selected by preimplantation genetic screening using next-generation sequencing versus morphologic assessment. Fertil Steril. 2017;108(3):e19.
48. Rubio C, Bellver J, Rodrigo L, Castillón G, Guillén A, Vidal C, et al. In vitro fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized, controlled study. Fertil Steril. 2017;107(5):1122–9.
49. Rosenbluth EM, Shelton DN, Wells LM, Sparks AE, Van Voorhis BJ. Human embryos secrete microRNAs into culture media—a potential biomarker for implantation. Fertil Steril. 2014;101(5):1493–500.
50. Kropp J, Salih SM, Khatib H. Expression of microRNAs in bovine and human pre-implantation embryo culture media. Front Genet. 2014;5:91.
51. Cuman C, Van Sinderen M, Gantier MP, Rainczuk K, Sorby K, Rombauts L, et al. Human blastocyst secreted microRNA regulate endometrial epithelial cell adhesion. EBioMedicine. 2015;2(10):1528–35.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.