A systematic review of noninvasive preimplantation genetic testing for aneuploidy

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Noninvasive and minimally invasive preimplantation genetic testing for aneuploidy (PGT-A) is a tool that may one day become the gold standard for embryonic chromosomal screening. Investigations on this topic have ranged from studying the culture media of embryos to the fluid inside the blastocoel, all in an attempt to find a reliable source of DNA without the need to biopsy the embryo. There is great interest across the board, both from those for and against biopsy, in a reliable test process that would give the patient and provider the same information possible from a biopsy without the risk. We aim to explore the current available research to better understand the utility and accuracy of PGT-A with these new sampling techniques. General concordance rates in comparison with biopsy-based PGT-A are promising, but it is clear that additional research and understanding are needed before adopting noninvasive and minimally invasive PGT-A as a widely used tool with strong clinical utility. (Fertil Steril® 2023;120:235–9. ©2023 by American Society for Reproductive Medicine.)

Key Words: Noninvasive embryo testing, preimplantation genetic testing for aneuploidy (PGT-A), spent blastocyst media (SBM), in vitro fertilization, aneuploidy

Noninvasive and minimally invasive preimplantation genetic testing for aneuploidy (miPGT-A) is a tool that may one day become the gold standard for embryonic chromosomal screening. Compared with traditional PGT-A, which removes cells from the trophectoderm (TE), noninvasive PGT-A (niPGT-A) focuses on the use of spent blastocyst media (SBM), whereas miPGT-A uses blastocoel fluid (BF). As can be seen in Figure 1 (A), (B), and (C), these different methods involve varying procedures for embryo sampling. Noninvasive embryo assessment efforts first became public knowledge in 2013, when a group published their findings from obtaining genetic information in embryonic spent culture media instead of an embryo biopsy (1). Because of this breakthrough approach, there has been a flurry of research on this topic. Investigations have ranged from studying the culture media of embryos to the fluid inside the blastocoel, all in an attempt to find a reliable source of DNA without the need to biopsy the embryo. Many recent studies have focused on this technology with conflicting conclusions, with some showing its promise, although others showcase its difficulties.

Since the inception of PGT-A, concerns regarding the ramifications of embryo biopsy have been raised. As with any technology, PGT-A techniques continue to evolve in an effort to optimize the procedure and mitigate risk. The current standard for sampling involves a blastocyst biopsy on days 5, 6, or 7 that removes an average of five cells, whereas prior techniques included a day 3 embryo biopsy with the removal of only one cell.

Practitioners, embryologists, scientists, and patients alike have voiced concern that this removal of actively growing cells from an embryo may create more risk than the ostensible reward of knowing the chromosomal makeup of that embryo (2, 3). Although some clinicians remain divided on this, there are many who accept the small risk of embryo biopsy and pursue PGT-A and other embryonic tests. There is great interest across the board, both from those for and against biopsy, in a reliable test process that would give the patient and provider the same information possible from a biopsy without the risk (2).

The challenges associated with establishing a noninvasive and miPGT-A technique, in addition to confirming concordance between samples compared with the TE biopsy, lie in the DNA source and protocol changes necessary to support this technique. The DNA collected using SBM is fragmented and typically in low

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concentration (approximately 8% of the culture medium contains embryonic DNA) (4). To accommodate changes in PGT-A, standard embryology protocols may or may not require alteration as well. This includes, but is not limited to, consideration of physical steps like embryo hatching and potential complications such as the admixture of maternal DNA in the sample (4).

Because the culture medium is the liquid in which the embryo will grow during its important first days of development, this complex solution has an important role to play. This role becomes increasingly important when the DNA shed within it is used for chromosome analysis. Ideally, this culture medium enhances the environment for the developing embryo, producing an optimal outcome. Studies have shown that the choice of media can have a long-range effect, impacting parameters such as fetal growth and birth weight. This may be because of the effect the media has on gene expression for factors such as apoptosis, protein degradation, metabolism, and cell cycle regulation (5). It is therefore conceivable that the media may have also an impact on cell-free DNA availability. In addition to the composition of the media, the amount of time the embryo is exposed to the media has been the subject of many studies (6). Currently, both two-step (sequential) media systems and one-step media systems exist. In a sequential process, the media are changed before transfer and cryopreservation, whereas in a one-step media system, the embryo remains in the same fluid for the duration of the culture. Currently, the impact each system could have on noninvasive and miPGT-A is unclear.

Because the development of noninvasive PGT-A continues, a valuable experimental goal is to answer the question, "How do we know that the results are good enough?" Generally accepted answers to this question have to do with how well the new test and its results match a current well-accepted test, in this case, the invasive PGT-A. There are multiple ways to compare concordance, including the following: (1) which test has a higher accuracy; (2) is there a subset of the new test that performs as well as the previous test; and (3) what is the sensitivity and specificity (7). For niPGT-A, these parameters are still being discussed.

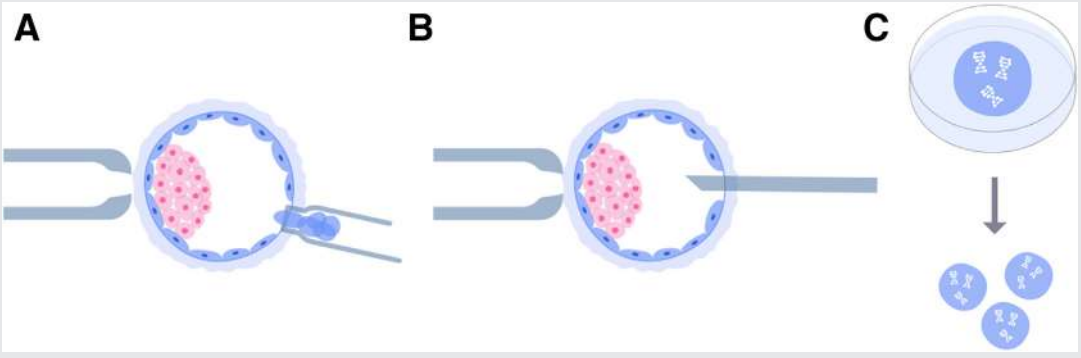
Another aspect of this consideration is the difference between general concordance and full concordance. In the studies cited, "general concordance" refers to the overall embryo result (euploid vs. aneuploid), i.e., PGT-A shows aneuploid and niPGT-A shows aneuploid. Full concordance not only requires general concordance but also the matching of specific chromosomal results. For example, an aneuploid embryo showing trisomy 5 on both platforms.

Perhaps the largest hurdle for commercially successful noninvasive PGT-A is being able to get results reliably and repeatedly from the culture medium that are as representative of the embryo as a TE biopsy. The general theory about testing spent biopsy media is that the embryo, as it grows, sheds DNA into the media. Although we do not know the source of the DNA, apoptotic and necrotic mechanisms may play a role in the release of fragmented DNA into the SBM (8). As Hammond et al. (8) state, hypotheses have ranged from those that believe the DNA being shed matches that of the embryo to the belief that the shed DNA is "abnormal" and that the embryo is discarding it.

This discussion highlights early investigations demonstrating a limited correlation between the embryo biopsy results and those from the culture media. A summary of the recent research conducted on this topic can be seen in Table 1. It is important to note that the currently published concordance rates are not near the accuracy available currently from the gold standard of invasive PGT-A. Some easily comparable data, such as embryo sex, have been the focus of articles because it provides a logical place to compare the two different methods (9). In theory, the chromosomal sex of an embryo should be easy to decipher. Starting as early as fertilization, an embryo that is 46XX should not have a Y chromosome present at any point in the development process. Although these studies show overlap and some concordance between the sexes of embryos from both DNA sources, the lack of complete concordance also leads to more questions regarding the composition of the DNA that is shed into the culture medium (9, 10).

Despite the growing interest in the use of SBM for niPGT-A, there are studies that focus on its challenges rather than its successes. Hanson et al. (11) have shown no evidence of a

FIGURE 1



(A) Trophectoderm biopsy for traditional PGT-A. **(B)** Blastocoel fluid biopsy for minimally invasive PGT-A. **(C)** Spent culture media. PGT-A = preimplantation genetic testing for aneuploidy.

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TABLE 1

Studies providing result concordance rates between trophectoderm biopsy and noninvasive sampling.

Manipulation before collection	Study	Culture time	Manipulation details	General concordance (%)
None				
	Galluzzi et al.[15] (2015)	D0–D3 (3 d)	-	1/2 (50)
		D3–D5/6 (2–3 d)	-	2/2 (100)
		D3–D5/6 (2–3 d)	-	5/5 (100)
	Liu et al.[16] 2017	D0–D5 (5 d)	-	26/31 (83.9)
	Capalbo et al.[17] (2018)	D1–D5 or D3–D5 (2–4 d)	-	27/72 (37.5)
	Rubio et al.[6] (2019)	D4–D5 (1 day)	-	17/27 (63)
		D4–D6/7 (2–3 d)		68/81 (84)
	Rubio et al.[6] (2019)	D4–D6/7 (2–3 d)	-	866/1108 (78.2)
	Chen et al.[18] (2021)	D3–5/6 (2–3 d)	-	190/256 (74.2)
	Xie et al.[19] (2022)	D4–D5/6	-	111/147 (75)
	Ho et al.[20] (2018)	D1–D5 (4 d)	-	10/12 (83.3)
Assisted hatching				
	Shamonki et al.[21] (2016)	D3–D5/6 (2–3 d)	Assisted Hatching	2/2 (100)
	Feichtinger et al.[22] (2017)	D0–D5 (5 d)	Assisted Hatching	13/18 (72.2)
	Vera-Rodriguez et al.[23] (2018)	D3–D5 (2 d)	Assisted Hatching	17/56 (30.4)
	Yeung et al.[24] (2019)	D3–D5 (2 d)	Assisted Hatching on day 3	38/50 (76)
		D3–D6 (3 d)	Assisted Hatching on day 3	47/66 (71.2)
	Lledo et al.[25](2020)	D3–D5/6 (2–3 d)	Assisted Hatching on day 3	62/83 (74.6)
				60/83 (72.3)
	Hanson et al.[11] (2021)	D5/6/7 (1–2 d)	Assisted Hatching on day 3	62/104 (59.6)
	Lei et al.[26] (2022)	D3–D5/6 (2–3 d)	Assisted Hatching on day 3	76/111 (68.5)
	Ho et al.[20] (2018)	D1–D5 (4 d)	Assisted Hatching on day 3	16/28 (57.1)
	Kuznyetsov et al.[4] (2020)	D4–D5/6 (1–2 d)	Assisted Hatching on day 4	88/90 (97.8)
Assisted hatching plus vitrification				
	Huang et al.[2] (2019)	D5–D6 (1 day) D6–D7 (1 day)	Assisted Hatching on day 3 plus day 5/6 Vitrification	41/46 (89.1)
Vitrification				
	Xu et al.[27] (2016)	D3–D5 (2 d)	Vitrification	36/42 (85.7)
Blastocoel collapse				
	Kuznyetsov et al.[28] (2018)	D5–D6 (1 day)	Double Blastocoel Collapse	27/28 (96.4)

Abbreviation: D = day.

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correlation between biopsy-based PGT-A and SBM-based niPGT-A. This, of course, confounds the potential efficacy of this testing technique and whether it may be a good option for patient care in the future.

A significant variable in the study of embryo spent culture media is that there is no standardized approach to DNA collection from the media. Although studies are trying to determine concordance, they are also trying to determine an efficient protocol. Length of time in culture, single vs. co-culture, amount of media, and even collection protocol for the media itself are all under review. Some investigational approaches ask a participating study laboratory to change their existing embryo culture protocol. This creates more variables on the laboratory side of this process compared with their standard, internally validated approach. These considerations lead to many proposed techniques, vastly increasing the difficulty of the original task, the evaluation of noninvasive PGT-A (12).

Blastocoel fluid, which is being also investigated as a potential material source for PGT-A, is a fluid that accumulates in the blastocoel cavity. The possibility of using BF as a replacement for TE biopsy has been shown to be worth exploring (1, 6). The gathering of BF involves a needle aspiration of the fluid within the blastocoel cavity rather than an embryo biopsy. The leading hypothesis is that there is DNA shed into this cavity, and it may be more representative of the embryo than that shed into the culture medium (1). Because of the use of the needle, which is meant to be inserted between cell junctions into the blastocoel cavity, this method may still be considered invasive, although it is seemingly well tolerated by the embryo (2). Fragments of DNA shed from the embryo and accumulating in this fluid result in a possible sample for PGT-A testing. The concordance rate between TE biopsy and BF has varied in recent articles, the most promising of which was reported by Gianaroli et al. (12). This study found a 97.4% concordance rate, the highest figure reported on the topic. These results differ from those of the spent culture medium, which is collected after the embryo has been removed from the medium and is truly noninvasive. However, studies on the BF have shown interesting results, ranging from an almost total lack of concordance with TE PGT-A to a significant correlation (13, 14). Various explanations for the wide range of concordance values for this methodology exist, including DNA source ambiguity.

A third approach to noninvasive testing is a combination of spent culture media and BF. There is increased power for

analysis when combining SBM and BF. Kuznyetsov et al. (4) report a 97.8% concordance between this miPGT-A technique and TE biopsy. This is an encouraging result for both patients and providers. It remains to be seen whether the effort required for a two-step embryonic DNA evaluation would be logistically feasible, at scale, for a busy embryology laboratory.

As can be seen in Table 1, study data has varied since 2015. In the ongoing studies on this topic, general concordance across all methodologies has not yet been shown to be at a level necessary to warrant a change in the gold standard of testing. General concordance rates in comparison with biopsy-based PGT-A are promising, but it is clear that additional research and understanding are needed before adopting noninvasive and miPGT-A as a mainstream, trusted tool with strong clinical utility.

Considerations for bringing a product to market are wide-ranging. There are patient, client, and financial decisions at stake. At this time, there are commercially available, noninvasive PGT-A options for providers to use for their patients. Although some clinicians have adopted these into their practice, it is likely that many providers believe that this technology is not yet ready for "prime time." Those who offer this test to patients do so either for all of their patients as a standard practice or for those whose embryos may be at greater risk of degradation because of embryo biopsy. In either case, fully educating patients regarding the choices before them ensures that patient autonomy is prioritized.

With PGT-A testing in general, genetic counseling plays a pivotal role in patient care. Without proper discussion and informed decision making, the utility of chromosomal screening for embryos falters. Because conversations such as mosaic embryo transfers and aneuploidy risks become more complex, so does the potential for use of these results. The application of these newer PGT-A sampling options in combination with genetic counseling will allow for broader information for patients and the fertility field, ideally without increased risks.

Ongoing research is promising on the topic of alternative methods to invasive biopsy for PGT-A, but as these investigations continue, it is pivotal to clarify its utility compared with the widely accepted traditional embryo biopsy. In the future, should noninvasive and minimally invasive methods prove to be good or better than traditional embryo biopsy, thoughtful and intentional changes will be required throughout the in vitro fertilization care process. This systematic review has shed light on the potential paths forward with these technologies and indicated what research may be needed to further the advancement toward noninvasive options for PGT-A.

REFERENCES

1. Palini S, Galluzzi L, De Stefani S, Bianchi M, Wells D, Magnani M, et al. Genomic DNA in human blastocoele fluid. Reprod Biomed Online 2013;26:603–10.
2. Huang L, Bogale B, Tang Y, Lu S, Xie XS, Racowsky C. Noninvasive preimplantation genetic testing for aneuploidy in spent medium may be more reliable than trophectoderm biopsy. Proc Natl Acad Sci U S A 2019;116:14105–12.
3. Alteri A, Cermisoni GC, Pozzoni M, Gaeta G, Cavoretto PI, Viganò P. Obstetric, neonatal, and child health outcomes following embryo biopsy for preimplantation genetic testing. Hum Reprod Update 2023;29:291–306.
4. Kuznyetsov V, Madjunkova S, Abramov R, Antes R, Ibarrientos Z, Motamedi G, et al. Minimally invasive cell-free human embryo aneuploidy testing (miPGT-A) utilizing combined spent embryo culture medium and blastocoel fluid-towards development of a clinical assay. Sci Rep 2020;10:7244.
5. Tarahomi M, Vaz FM, Van Straalen JP, Schrauwen FAP, Van Wely M, Hamer G, et al. The composition of human preimplantation embryo culture media and their stability during storage and culture. Hum Reprod 2019;34:1450–61.
6. Rubio C, Rienzi L, Navarro-Sánchez L, Cimadomo D, García-Pascual CM, Albricci L, et al. Embryonic cell-free DNA versus trophectoderm biopsy for aneuploidy testing: concordance rate and clinical implications. Fertil Steril 2019;112:510–9.
7. Kwiecien R, Kopp–Schneider A, Blettner M. Concordance analysis: part 16 of a series on evaluation of scientific publications. Dtsch Ärztebl Int 2011;108:515–21.
8. Hammond ER, Shelling AN, Cree LM. Nuclear and mitochondrial DNA in blastocoele fluid and embryo culture medium: evidence and potential clinical use. Hum Reprod 2016;31:1653–61.
9. Leaver M, Wells D. Non-invasive preimplantation genetic testing (niPGT): the next revolution in reproductive genetics? Hum Reprod Update 2020;26:16–42.
10. Rubio C, Navarro–Sánchez L, García-Pascual CM, Ocali O, Cimadomo D, Venier W, et al. Multicenter prospective study of concordance between embryonic cell-free DNA and trophectoderm biopsies from 1301 human blastocysts. Am J Obstet Gynecol 2020;223:751.e1–13.
11. Hanson BM, Tao X, Hong KH, Comito CE, Pangasnan R, Seli E, et al. Noninvasive preimplantation genetic testing for aneuploidy exhibits high rates of deoxyribonucleic acid amplification failure and poor correlation with results obtained using trophectoderm biopsy. Fertil Steril 2021;115:1461–70.
12. 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;102:1692–9.e6.
13. Shangguan T, He W, Li H, Shang X, Liu Y, Bai X, et al. Detection and analysis of DNA material in human blastocoel fluid. Biomed Genet Genomics 2017;2:1–5.
14. Tobler KJ, Zhao Y, Ross R, Benner AT, Xu X, Du L, et al. Blastocoel fluid from differentiated blastocysts harbors embryonic genomic material capable of a whole-genome deoxyribonucleic acid amplification and comprehensive chromosome microarray analysis. Fertil Steril 2015;104:418–25.
15. Galluzzi L, Palini S, Stefani S, Andreoni F, Primiterra M, Diotallevi A, et al. Extracellular embryo genomic DNA and its potential for genotyping applications. Future Sci OA 2015;1:FS062.
16. Liu W, Liu J, Du H, Ling J, Sun X, Chen D. Non-invasive pre-implantation aneuploidy screening and diagnosis of beta thalassemia IVSII654 mutation using spent embryo culture medium. Ann Med 2017;49(4):319–28.
17. 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;110:870–9.e5.
18. Chen Y, Gao Y, Jia J, Chang L, Liu P, Qiao J, et al. DNA methylome reveals cellular origin of cell-free DNA in spent medium of human preimplantation embryos. J Clin Invest 2021:131.
19. Xie P, Zhang S, Gu Y, Jiang B, Hu L, Tan YQ, et al. Non-invasive preimplantation genetic testing for conventional IVF blastocysts. J Transl Med 2022;20:396.
20. 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;110:467–75.e2.
21. 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;106:1312–8.
22. 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;34:583–9.
23. 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;33:745–56.
24. Yeung QSY, Zhang YX, Chung JPW, Lui WT, Kwok YKY, Gui B, et al. A prospective study of non-invasive preimplantation genetic testing for aneuploidies (NiPGT-A) using next-generation sequencing (NGS) on spent culture media (SCM). J Assist Reprod Genet 2019;36:1609–21.
25. Lledo B, Morales R, Ortiz JA, Rodriguez-Arnedo A, Ten J, Castillo JC, et al. Consistent results of non-invasive PGT-A of human embryos using two different techniques for chromosomal analysis. Reprod Biomed Online 2020;42:555–63.
26. Lei C, Fu J, Li X, Zhou J, Xiao M, Zhang S, et al. Re-denudation of residual cumulus cells on day 3 increases the accuracy of cell-free DNA detection in spent embryo culture medium. J Assist Reprod Genet 2022;39:1653–60.
27. 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 U S A 2016;113:11907–12.
28. 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:e0197262.