Noninvasive Preimplantation Aneuploidy DNA Test Shows Promise But Concerns Remain

Jun 27, 2019 | Andrew P. Han

NEW YORK – A noninvasive genetic test for aneuploidy in embryos, to be used as part of the *in vitro* fertilization process, may be more reliable than the current standard, according to a study published this week. But critics pointed out several flaws, including related to the study design.

The study, led by Sunney Xie of Harvard University and Peking University and Catherine Racowsky, director of the IVF lab at Brigham and Women's Hospital, compared the next-generation sequencing-based test, which looks at cell-free DNA in spent embryo culture medium, with trophectoderm (TE) biopsy — a prevailing method for preimplantation genetic testing for aneuploidy (PGT-A) that is invasive to the embryo. Cell-free methods can analyze DNA from the inner cell mass (ICM), from which the fetus develops, while trophectoderm cells develop into the placenta.

The test was developed by Yikon Genomics of Shanghai, a startup cofounded by Xie. Earlier this year, Yikon signed an agreement with the Center for Reproductive Health & Gynecology in Beverly Hills, California, to launch the test commercially.

In a paper published this week in the *Proceedings of the National Academy of Sciences*, the authors reported a false negative rate of zero and a positive predictive value and specificity for determining aneuploidy that were "much higher" than with TE biopsy PGT-A.

"This technique holds the promise of a more precise test than the current method," Racowsky said. "The results show that the test is more sensitive and, because it is noninvasive, is potentially safer." The study is the first phase of a three-phase plan she and her co-authors have laid out to further validate this kind of test, she said.

According to Marcelle Cedars, director of the University of California, San Francisco Center for Reproductive Health, it looks like the authors have made advances in methods to remove potential sources of contaminating DNA and in the whole-genome amplification of cell-free DNA from the embryo. However, because these advances appear to be proprietary, they were not provided in detail, she said. Xie did not respond to requests for comment before deadline.

The paper adds to a "growing body of evidence" that noninvasive PGT (niPGT) might be possible by analyzing cell-free DNA, Joris Vermeesch, head of the Laboratory for Cytogenetics and Genome Research at Belgium's KU Leuven, said in an email. "Nevertheless, this is not the definitive paper showing that niPGT is ready for primetime."

Joyce Harper, head of the reproductive health department at University College London, said her field "disagrees in general. About everything." She noted that research on human embryos is difficult to conduct and the authors "have done what they could with the samples they had."

Cedars said that the study design was appropriate for the circumstances. "But the question is, if I were going to apply this in a clinical setting, how would I need to modify the protocol and would that itself have an impact that is negative on the embryo?" she said.

Yikon Genomics is leading just one of several efforts to develop niPGT-A and improve upon PGT-A by TE biopsy. Biopsy sacrifices cells, which could be harmful to fetal development, and it doesn't get at the cells that develop into the fetus. Moreover, PGT-A is complicated by mosaicism. "In theory, each cell should have identical DNA content," explained Alan Penzias, a reproductive endocrinologist at Boston IVF. "But some do not." This raises the risk of a false positive, where mosaicism is confined only to the TE but the embryo is declared abnormal and discarded, as well as a false negative, where the embryo is mosaic but only normal euploid TE cells are sampled and the embryo is saved or used.

A 2017 study showed that PGT-A reduces the number of transferable embryos or embryos selected for implantation, compared to testing for inheritable diseases alone. But researchers have cautioned for years that embryos testing positive for aneuploidy can still result in healthy babies.

Norbert Gleicher, medical director and chief scientist of the Center for Human Reproduction in New York, suggested in an email that the paper downplayed the fact that healthy babies have been conceived from embryos that were deemed abnormal due to mosaicism. He took issue with the paper's mention of "several reports" of such births; he said there have been at least 200 such babies and that he had a paper in press that would increase that number by 400.

Furthermore, the general clinical protocol of sampling embryos at day five or six does not take into account reports that in mammalian mosaic embryos, aneuploid cells can die off soon after, he said, resulting in a viable embryo for implantation. If Yikon's test was performed as described in the paper, it would be "clinically worthless," he said, "since why would one test embryos which later, in a high percentage of cases, will still self-correct?"

"This is a deeply flawed paper for technical as well as biological reasons," Gleicher said.

Racowsky responded that the ability of a high percentage of embryos to self-correct "begs the question why PGT-A with TE biopsy was being performed at all." She added that her team was also concerned about false positives and that their paper had shown an improvement by lowering the false positive rate compared to TE biopsy, to 20 percent from 50 percent, in their study samples.

"It represents a potentially new way to evaluate embryos with less risk of discarding a viable embryo," she said.

"We see this noninvasive testing as being more of a rule-in test than a rule-out test," she added.

Both Vermeesch and Gleicher suggested that because the TE biopsies were performed by different labs, many of which did not present an analysis of mosaicism, the paper was comparing apples to oranges. They also criticized the paper for asserting without references that the ICM and TE leak DNA into spent media.

Racowsky countered that the reference lab procedures have been validated and that the paper showed how her team had developed an evidenced-based threshold for calling aneuploidy in mosaic embryos, where TE biopsy analysis will make that call based on an arbitrary cutoff, if it even does so at all.

She also said that the study was proof-of-concept that cell-free DNA in the culture media came from the embryo, since the test's chromosome copy number results were concordant with results of the same

whole embryo in the 83 percent of cases, compared with 62 percent of the cases with TE biopsy.

In the paper, the authors reported four euploid embryos whose results were deemed too noisy and excluded them from analysis. Cedars said it's worth questioning whether euploid embryos would shed enough cell-free DNA into the spent culture media. Moreover, the noisy results made her "nervous" and she wondered what would happen in future studies where the prevalence of aneuploid embryos would be lower. "For any test, when you take it into a low-prevalence population, the positive predictive value goes down," she said.

Racowsky acknowledged the issue and stressed that her team was limited by the samples that patients had donated to them. She said this would be addressed in the next phase of their investigation, where they will be collecting the spent culture media between days three and five, prior to the embryos being biopsied.

"There's going to be a high proportion of euploid embryos in these samples, allowing us to address whether they're skewing the false positive or false negative rate," she said.

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