Not even noninvasive cell-free DNA can rescue preimplantation genetic testing

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Huang et al. (1) suggest that noninvasive preimplantation genetic testing for aneuploidy (niPGT-A) of embryos (assessments of cell-free aneuploid DNA in spent media) may be more reliable than trophectoderm (TE) biopsy. We welcome their efforts but question their assumption that blastocyst-stage embryos by any means can be accurately diagnosed, since testing at the blastocyst stage does not account for self-correcting embryos downstream. The authors quote Bolton et al. (2) as demonstrating increased apoptosis in inner cell mass (ICM) in comparison to TE. They, however, fail to note that the primary observation in their paper was successful self-correction of embryonic cells within the ICM. Observations on apoptosis were only the biological explanations for how these self-corrections occur.

The possibility of self-correction renders almost every diagnostic procedure at the blastocyst stage, whether invasive or noninvasive, largely irrelevant. Such testing, moreover, creates false-positive diagnoses (3), leading to the mistaken disposal of large numbers of embryos with excellent live-birth potential. A recent survey of fertility centers, indeed, revealed over 400 normal pregnancies established from transfer of embryos by invasive PGT-A at the blastocyst stage diagnosed as aneuploid or mosaic (4).

Although the technical analysis of embryos is presented elegantly, it ultimately is at best only hypothesis-generating and does not offer validated evidence regarding niPGT-A's abilities to reliably define the ultimate karyotype of embryos, which may still be in flux. In addition, the authors' statistical analysis is based on unproven assumptions which do not take into account important biological realities, including the following. First, TE and ICM are assumed to leak into spent media, but TE is in direct contact with medium and ICM is not. Cell-free DNA detected in spent media may, therefore, primarily or exclusively only be TE-derived. As TE and ICM can be discrepant (5), where aneuploid DNA comes from is important, especially since TE produces the placenta, which even in euploid pregnancies at term can still contain aneuploid cell islands. Second, TE at the blastocyst stage is made up by *ca.* 200 to 250 cells but the ICM by only *ca.* 10 to 15% of that cell mass. Even assuming leakage from ICM cells, there was no mathematical adjustment made for this difference in cell numbers. Third, the authors contradict themselves, on one hand basing their diagnostic formula on the belief that increased apoptosis, whether from ICM or TE, must be associated with increased leakage, yet on the other hand arguing "at least for the euploid embryos, that leakage of DNA from the euploid cells outweighs that of the apoptotic aneuploid cells."

Two other weaknesses of their study are that next-generation sequencing is able to detect DNA from secondary cell lines only above 20% of total DNA, thereby missing lower levels of mosaicism. Also, spent media came from thawed blastocyst-stage embryos after 24 h of culture. These embryos, therefore, were 1 full day older than embryos undergoing PGT-A in clinical practice. They, therefore, may already be self-correcting.

In conclusion, while niPGT-A, theoretically, would be clearly preferable over TE biopsy, this study does not offer information warranting routine clinical use of either procedure.

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