Lights and shadows of preimplantation genetic testing for aneuploidy: better focusing on the accurate report of nonmosaic aneuploidies

Meiotic aneuploidies are undoubtedly the most prevalent genetic abnormalities in human embryos and the single most significant factor associated with in vitro fertilization failure. Recently, a study by Eva Hoffman's group provided compelling evidence that fertility in humans is shaped by full chromosome gains and losses in the oocytes (1). Accordingly, preimplantation genetic testing for aneuploidy (PGT-A) should aim at the accurate detection and report of these karyotype abnormalities.

This issue of *Fertility and Sterility* includes a study by Kim et al. (2) that provides additional data to assess the reliability and reproducibility of blastocyst stage PGT-A. These investigators conducted 4 additional trophectoderm (TE) biopsies of the same size of a clinical biopsy trophectoderm (cTE) from 300 blastocysts, producing the largest rebiopsy dataset published so far. They adopted a targeted next-generation sequencing (NGS) protocol for the analysis and subclustered the rebiopsy results according to the initial cTE diagnosis.

In case of euploid cTE, the rebiopsies yielded 99.5% per biopsy and 98.5% per embryo concordances. In case of full chromosome aneuploid cTE, the rebiopsies yielded 98% per chromosome and 97% per embryo concordances. Both these subanalyzes confirm that targeted NGS can be considered highly reliable when reporting uniform aneuploidies (or their absence). An evidence in line with the previous nonselection study conducted from the same group and with the same technique was found, which showed a 65% prediction on sustained implantation when a cTE was blindly diagnosed euploid and a 100% (95% confidence interval, 97.6%–100%) prediction on implantation failure when a cTE was blindly diagnosed aneuploid with full chromosome nonmosaic imbalances (3). With a different NGS protocol and including pure inner cell mass (ICM) samples, another recent study reported a 98% confirmation rate for euploid or full chromosome aneuploid cTE diagnoses (4).

A different scenario was instead outlined in Kim et al.'s (2) investigation when whole chromosome mosaic (WCM) aneuploidies were reported in the cTE on the basis of intermediate copy numbers (ICNs). Astonishingly, only <20% WCM aneuploidies were confirmed, resulting into approximately 2% of the embryos where the same chromosomal abnormality was confirmed in all rebiopsies and approximately 40% of them where ≥1 rebiopsy was concordant. Almost identical results were achieved in case of segmental mosaic aneuploidies (segM) diagnosed in the cTE on the basis of the ICN. Of note, no reciprocal whole chromosome aneuploidy was shown among multiple biopsies, thereby excluding a corroboration of ICN with causative mechanisms. Whether ICN originate primarily from technical errors or from the presence of highly localized mosaicism is still an open question deserving further research. All these evidences further confirm how, even when reported with targeted NGS (previous reports are based on array comparative genomic hybridization or whole genome amplification NGS), ICNs are mostly associated with false-positive calls of chromosomal mosaicism and are not enough validated to be applied clinically. Kim et al.'s (2) data are also echoed in our recent report where the ICN of 20%–50% in the cTE (medium-low mosaic) corresponded to fully euploid blastocysts in >96% of blastocysts donated to research, disgregated, and analyzed (5).

The most interesting evidence arising from Kim et al.'s (2) study, in our view, derives from cTE diagnosed with segmental aneuploidies (segA) not in the "mosaic range." These embryos yielded approximately 60% per chromosome concordance and 76% and 42% of cases when the segA was confirmed in ≥1 and all rebiopsies, respectively. In other terms, segA are the consequence of a mitotic issue more probably than the ICN, therefore more indicative of genuinely mosaic blastocysts. Still, approximately 40% of segA may be of a meiotic origin, therefore being constitutively present in the whole embryo. This is mirrored also by Girardi et al.'s (4) previous investigation, where approximately 70% of the blastocysts diagnosed with a segA on a cTE, disaggregated, and analyzed in all the other sections (including a pure ICM) showed a constitution compatible with mosaicism (4).

Unfortunately, Kim et al.'s (2) study design did not entail a pure ICM sample. However, assuming that abnormal cells are randomly assorted in a truly mosaic embryo, this does not substantially affect the clinical translation of their data. Conversely, a clear limitation exists in the reproducibility and translation of this study across different molecular platforms because the analytic scheme adopted to outline WCM and segM is poorly detailed in the manuscript.

How can we translate all these information clinically? A reasonable way is via blinded nonselection studies. Up to date, these studies showed that blastocysts diagnosed with full chromosome aneuploidies on the basis of a cTE have a negligible chance of being reproductively competent (0–2%) (3) and the ICN (20%–50%, i.e., low-to-medium risk of apparent mosaicism) in the cTE, instead, do not associate with any lower chance of a live birth or higher risk for a miscarriage compared with euploid cTE. In other terms, blastocysts showing up to 50% ICNs on a cTE can be considered as competent as euploid ones in the clinical setting. As a matter of fact, then, the most important issue clearly deserving future investigations are cTE diagnosed with segA (not in the mosaic range). These chromosomal errors must be thoroughly characterized from a basic science perspective, so to gradually outline a clinical workflow aimed at the translation of this information when counseling a couple after PGT-A. In Girardi et al.'s (4) manuscript, we have attempted to outline a workflow on the basis of a second confirmatory cTE and the size of the segA (≤80 or >80 Mb). These features, along with a careful counseling by the geneticist on the risks of transferring in utero blastocysts showing a given segA, may support the management of the related couples (4), but more data are required.

In conclusion, on the basis of the current level of evidence, it is probably time in PGT-A to stop focusing on the ICN (a.k.a. "mosaic aneuploidies") and invest more efforts into novel challenges and further improvements. These may span from further optimization of genetic technologies to reveal true mosaicism on the basis of genotyping data to the development of novel molecular and clinical tools for also underpinning de novo segA in cTE.

Danilo Cimadomo, Ph.D.[a]
Laura Rienzi, M.Sc.[a]
Filippo Maria Ubaldi, M.D., Ph.D[a]
Antonio Capalbo, Ph.D.[b]
[a] Clinica Valle Giulia, GeneraLife IVF, Rome, Italy; and
[b] Igenomix, Marostica, Italy

https://doi.org/10.1016/j.fertnstert.2021.12.002

DIALOG: You can discuss this article with its authors and other readers at https://www.fertstertdialog.com/posts/34340

REFERENCES

1. Gruhn JR, Zielinska AP, Shukla V, Blanshard R, Capalbo A, Cimadomo D, et al. Chromosome errors in human eggs shape natural fertility over reproductive life span. Science 2019;365:1466–9.
2. Kim J, Tao X, Cheng M, Steward A, Guo V, Zhan Y, et al. The concordance rates of an initial trophectoderm biopsy with the rest of the embryo using PGTseq, a targeted next-generation sequencing PGT-A platform. Fertil Steril 2022;117:315–23.
3. Tiegs AW, Tao X, Zhan Y, Whitehead C, Kim J, Hanson B, et al. A multicenter, prospective, blinded, nonselection study evaluating the predictive value of an aneuploid diagnosis using a targeted next-generation sequencing-based preimplantation genetic testing for aneuploidy assay and impact of biopsy. Fertil Steril 2021;115:627–37.
4. Girardi L, Serdarogullari M, Patassini C, Poli M, Fabiani M, Caroselli S, et al. Incidence, origin, and predictive model for the detection and clinical management of segmental aneuploidies in human embryos. Am J Hum Genet 2020; 106:525–34.
5. Capalbo A, Poli M, Rienzi L, Girardi L, Patassini C, Fabiani M, et al. Mosaic human preimplantation embryos and their developmental potential in a prospective, non-selection clinical trial. Am J Hum Genet 2021;108: 2238–47.