STRUCTURAL AND CHROMOSOMAL CONDITIONS RESOLVED WITH WHOLE GENOME SEQUENCING (WGS) OF EMBRYOS

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Background:
As preimplantation genetic testing (PGT) technologies advance, so too does their resolution and ability to identify conditions that were previously undetectable[1]. High-depth whole genome sequencing (WGS) now provides the capability to screen for chromosomal abnormalities missed by standard PGT for aneuploidy, including polyploidy, complete uniparental isodisomy (UPD, molar pregnancy), and microdeletions/microduplications[2]. Embryos carrying such chromosomal abnormalities that are incorrectly classified as euploid may have serious health implications for the resulting fetus but also the individual carrying the pregnancy[3].

Objective: To present case studies that demonstrate how PGT-WGS can identify chromosomal conditions often missed by traditional PGT-A. We showcase three cases where chromosomal abnormalities were identified that could be overlooked with conventional methods, as well as a fourth case that highlights the use of PGT-M for structural variants, which would typically be rejected by other labs.

Materials and Methods: Embryos from 4 separate couples were biopsied by trained embryologists at their respective In-vitro Fertilization (IVF) centers and sent to Orchid laboratory for testing. Each embryo biopsy underwent whole genome amplification using a lab-developed method followed by low-pass whole genome sequencing on the Illumina platform. Embryos marked as euploid were further analyzed by 30x whole genome sequencing.

Result(s): In the first case, nine embryos were biopsied, and five were classified as euploid and, therefore, further analyzed by 30x WGS. One of the five euploid embryos was identified as having genome-wide UPD, consistent with a molar embryo. This finding was made through the review of allele tracks without requiring parental samples.

In the second case, five embryo biopsies were received, with two identified as euploid and sent for further analysis. One of these embryos was identified as aneuploid: 69, XXX (triploidy).

The third couple had 14 embryos, of which 11 were identified as euploid. Further analysis revealed that 6 of these embryos had a ~1.5 Mb microduplication at Xp22.31. This duplication has been observed in the general population at a rate of approximately 0.32-0.41% and has been associated with various neurodevelopmental phenotypes, most commonly seizures and learning disabilities[4,5]. Maternal inheritance was suspected since all the affected embryos were male (46, XY). Further counseling uncovered the patient's personal history of seizures, suggesting that the mother also exhibited symptoms. Confirmatory testing for the mother was recommended, along with a referral to a medical geneticist for additional health recommendations.

Finally, a fourth couple aimed to screen for a ~412 kb duplication on chromosome 10, associated with split-hand/foot syndrome, carried by the affected male partner. Since the duplication was de novo, traditional PGT-M probe development was not feasible, and other labs rejected their case. Of the three embryos tested, the two euploid embryos were negative for chromosome 10 duplication.

Conclusion(s):
PGT-WGS enables the detection of chromosomal and structural variants that may be missed by traditional PGT-A, such as molar pregnancies and clinically significant microdeletions/duplications. Additionally, PGT-WGS offers the possibility of PGT-M for challenging structural variants without requiring additional informative familial samples and/or probe development. This provides an option for families who other labs might otherwise reject due to technical limitations.

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