THE CHALLENGE OF POLYPLOIDY AND COMPREHENSIVE CHROMOSOME SCREENING: VALIDATION OF A TARGETED NEXT GENERATION SEQUENCING (TNGS) PLATFORM FOR DETECTION OF TRIPLOIDY IN HUMAN BLASTOCYSTS

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OBJECTIVE: Current NGS-based CCS platforms perform an initial whole genome amplification step, which prevents simultaneous genotyping due to shallow sequencing depth. Therefore, triploidy detection by NGS remains challenging and has only been reported based on sex chromosome copy number ratios, which fail to account for XXX triploids, and may be prone to misdiagnosis due to sample contamination or technological artefacts. Using genotyping data generated in parallel with copy number analysis from a tNGS-based CCS platform, this study aims to validate triploidy detection in human blastocysts as well as its frequency and parental origin.

DESIGN: Retrospective validation study.

MATERIALS AND METHODS: Phase 1: Known diploid and triploid DNA samples were blinded and sequenced by tNGS. The average ratio of both allele frequencies of all heterozygous SNPs was calculated for each sample, where a 1:1 ratio is expected for diploid samples and 2:1 for triploids. A threshold of allele ratios (AR) was established between known triploid and diploid controls. Phase 2: The established threshold was applied to calculate the frequency of triploidy in 15,933 human blastocysts. Phase 3: The parental origin of the inherited extra alleles was evaluated by sequencing parental gDNA when available in order to validate triploidy predictions from phase 2.

RESULTS: Phase 1: Calculated average AR were sufficient to accurately discriminate between diploid and triploid control samples (diploid ARs 1.47 ± 0.09 and triploid 2.12 ± 0.09, $p<0.0001$). An intermediate AR value of 1.85 was established as a threshold to distinguish between diploid and triploid samples. Phase 2: A blastocyst triploidy frequency of 0.48% was observed, with 76 out of 15 933 embryos falling within the triploid range. Phase 3: All alleged triploid blastocysts with parental DNA available were confirmed to be triploid and of maternal origin by having inherited the extra allele exclusively from the mother in all heterozygous SNPs assessed. A similar frequency of triploid blastocyst (0.31%) was observed among 1598 embryos with available parental gDNA sequenced.

CONCLUSIONS: tNGS provides higher sequencing depth in contrast to other contemporary NGS platforms, allowing for accurate SNP calling and accurate detection of triploidy. Around 15% of genetically abnormal miscarriages present a triploid karyotype, stressing the importance of validating a CCS platform capable of detecting it. In addition, this technology could be used to evaluate restoration of diploidy and heteroparentality in embryos with 1 or 3 pronuclei, potentially increasing the number of normal available blastocysts for transfer.