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Validation of a targeted next generation sequencing-based comprehensive chromosome screening platform for detection of triploidy in human blastocysts

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KEY MESSAGE

In this study a tNGS-based CCS platform for detection of triploidy in human blastocysts from a TE biopsy was validated. It was established that ICSI-derived triploid blastocysts are rare, with a frequency of approximately 0.5%, and that, as expected due to virtual exclusion of polyspermy, are mostly of maternal origin.

A B S T R A C T

Triploidy accounts for ~2% of natural pregnancies and 15% of cytogenetically abnormal miscarriages. This study aimed to validate triploidy detection in human blastocysts, its frequency and parental origin using genotyping data generated in parallel with chromosome copy number analysis by a targeted next generation sequencing (tNGS)-based comprehensive chromosome screening platform. Phase 1: diploid and triploid control samples were blinded, sequenced by tNGS and karyotype predictions compared for accuracy. Phase 2: tNGS was used to calculate the frequency of triploidy in 18,791 human blastocysts from trophectoderm (TE) biopsies. Phase 3: parental origin of the inherited extra alleles was evaluated by sequencing parental gDNA to validate triploidy predictions from Phase 2. All karyotypes and ploidy in controls from Phase 1 were correctly predicted by two independent methods. A blastocyst triploidy frequency of 0.474% (89/18,791) was observed in Phase 2 of the study. Finally, five suspected triploid blastocysts with parental DNA available were confirmed to be triploid and of maternal origin. tNGS provides higher sequencing depth in contrast to other contemporary NGS platforms, allowing for accurate single nucleotide polymorphism calling and accurate detection of triploidy in TE biopsies. Triploidy in intracytoplasmic sperm injection-derived blastocysts is rare and mostly of maternal origin.

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Introduction

Chromosomal aneuploidy is the most common genetic abnormality in humans and the main genetic cause of infertility (Treff and Zimmerman, 2017). The prevalence of aneuploidy in pre-implantation embryos increases dramatically with maternal age (Franasiak et al., 2014; Hassold et al., 2007), which in turn explains why infertility rapidly declines after 35 years of age in female patients. Therefore, several technologies have been developed in order to perform comprehensive chromosome screening (CCS) from a single biopsy of the pre-implantation embryo allowing for selection of euploid embryos for transfer. All randomized controlled trials published to date highlight the benefit of performing this intervention to increase implantation rates and reduce miscarriage rates (Dahdouh et al., 2015; Forman et al., 2013; Rubio et al., 2017; Scott et al., 2013; Yang et al., 2012). In addition, single embryo transfers coupled with CCS have been reported to be more cost-effective than transfers of unscreened embryos (Neal et al., 2016a). Nevertheless, although improvements in clinical outcomes seem unquestionable, current CCS platforms still face the challenge of detecting polyploidy from a single trophectoderm (TE) biopsy (Marin et al., 2017), a phenomenon known to result in negative clinical outcomes (Hassold et al., 1980; Levy et al., 2014).

Triploidy refers to the presence of an extra copy of every chromosome, resulting in 69 instead of the normal 46 chromosomes in humans. The prevalence of triploidy in natural pregnancies ranges from 1 to 3% (McFadden and Robinson, 2006; Rosenbusch, 2008). Its frequency in spontaneous abortions rises to around 8% (Hassold et al., 1980; Jacobs et al., 1978; Wang et al., 2014) and accounts for approximately 15% of all cytogenetically abnormal miscarriages (Hassold et al., 1980; Levy et al., 2014; Wang et al., 2014). Furthermore, triploid pregnancies can present aberrant phenotypes depending on the origin of the extra set of chromosomes. If the origin happens to be paternal (diandric), pregnancies may develop into partial hydatidiform moles, whereas if maternal in origin (diginic), they most commonly result in asymmetric intrauterine growth restriction, marked adrenal hypoplasias, or non-molar placentas (McFadden and Robinson, 2006). While triploid gestations culminating in live births are extremely rare, they present numerous congenital abnormalities and do not survive after the neonatal period (Takabachi et al., 2008).

In addition, the prevalence of zygotes with three pronuclei after IVF is not negligible and may result in a triploid pregnancy or failure to implant if transferred. The prevalence of three-pronucleated (TPN) zygotes ranges from 6.6 to 8.1% in conventional IVF cycles (Balakier, 1993; Porter et al., 2003) and from 2.5 to 6% in intracytoplasmic sperm injection (ICSI) cycles (Porter et al., 2003; Sachs et al., 2000). As routine practice in IVF laboratories, TPN zygotes are discarded. Nevertheless, there is strong evidence that around half of the TPN zygotes fertilized by ICSI can result in true diploids (Grau et al., 2011, 2015). Consequently, evaluating ploidy status in embryos developed from TPN zygotes might result in increasing the number of transferable embryos per patient. Therefore, a technology capable of discriminating between polyploid and normal embryos may be important to improve clinical outcomes in patients with infertility.

Given the considerable prevalence of this phenomenon in pre-implantation embryos, pregnancies and miscarriages, and its detriment to obstetric outcomes, it is clinically relevant to develop technologies that can detect this abnormality and potentially avoid pregnancies with a triploid karyotype. Although CCS platforms based on single nucleotide polymorphism (SNP) microarrays may be capable of identifying triploidy based on their genotyping data (Doody et al., 2016), most contemporary CCS platforms rely purely on copy number to evaluate chromosomal content. Furthermore, commercially available next generation sequencing (NGS) methods that involve whole genome amplification (WGA) only provide shallow sequencing in the standard protocols, preventing accurate genotyping and genotyping-based polyploidy assessment (Treff and Zimmerman, 2017), and only allow for the diagnosis of triploidy if the X and Y copy numbers fall into a step-wise pattern, which could be interpreted as two copies of the X chromosome, one of the Y and three for the rest of the autosomes.

Here we present a validation study of an NGS platform for CCS that instead of WGA follows an initial targeted amplification approach (targeted next generation sequencing, or tNGS), allowing for enough sequencing depth per locus to generate accurate genotyping data. We demonstrate that SNP genotyping data generated in parallel to chromosome copy number analysis by tNGS accurately detects triploidy in human blastocysts from a single TE biopsy. In addition, we calculate the frequency of triploid embryos derived by ICSI and develop an independent test to confirm the presence and origin of triploidy using sequencing data from parental DNA.

Materials and methods

Study design

Phase 1 – validation of detection of triploidy by tNGS

In Phase 1, a validation step was performed in order to evaluate whether our proprietary tNGS platform could discriminate between diploid and triploid samples based on genotyping data obtained in parallel with chromosome copy number analysis. In this phase, genomic DNA isolated from triploid products of conception (POC) and isolated genomic DNA from normal individuals were processed with tNGS. In addition, six-cell aliquots of well-characterized diploid and triploid cell lines were also processed with the same platform, in order to model the approximate number of cells in a TE biopsy (Neal et al., 2016b). All samples were processed blindly. Genotyping data from 2690 SNP, from which 2571 are in autosomes and 119 in sex chromosomes, were retrieved in variant call format files (.vcf) in order to analyse the alternative allele frequencies provided for each SNP and to predict whether the sample was diploid or triploid.

Predictions of polyploidy were performed following two independent methods. *Method 1-allele ratio (AR)*: First, from all 2571 autosomal SNP genotyped by tNGS, only those with a heterozygous call were selected for each sample. For each heterozygote SNP and for each sample, the allele with the highest read count was considered the major allele, and the one with the lowest the minor allele. A value referred to as the 'average allele ratio' was calculated for each sample. Because diploid specimens have two copies of each autosome, a 1:1 ratio is expected between the major and minor allele genotypes for a given heterozygous SNP. In contrast, if three copies of all chromosomes are present, then a 2:1 ratio is expected. Therefore, the average AR may be sufficient to discriminate between a diploid and a triploid sample irrespective of any $\log_2$ ratio values obtained after copy number analysis for aneuploidy. *Method 2-high throughput*: A second high-throughput set of criteria was also used whereby alternative allele frequencies in the range from 0.30 and 0.36 were predicted as triploid. All blinded samples were analysed using these

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