Detection of segmental aneuploidy and mosaicism in the human preimplantation embryo: technical considerations and limitations

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Whole-chromosome aneuploidy screening has become a common practice to improve outcomes and decrease embryonic transfer order in patients undergoing treatment for infertility through in vitro fertilization. Despite implementation of this powerful technology, a significant percentage of euploid embryos fail to result in successful deliveries. As technology has evolved, detection of subchromosomal imbalances and embryonic mosaicism has become possible, and these serve as potential explanations for euploid embryo transfer failures. Cases involving a parent with a balanced translocation provide a unique opportunity to characterize the capabilities and limitations of detecting segmental imbalances with a variety chromosome screening platforms. Adaptation of these methods to de novo imbalances now represent an ongoing challenge in the field of preimplantation genetic screening as additional factors including mosaicism, clinical predictive value, and distinguishing true imbalances from technical artifacts must be more carefully considered. (Fertil Steril® 2017;107:27–31. ©2016 by American Society for Reproductive Medicine.)

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Comprehensive chromosome screening (CCS) use has grown considerably over the last several years. A clear benefit to the success of in vitro fertilization (IVF) has been demonstrated in multiple randomized controlled trials (1–3) and subsequent meta-analysis (4). Although not all IVF patients may benefit to the same extent, the opportunity to improve the likelihood of implantation of a singleton on the first attempt and to reduce the risk of a failed implantation or a clinical miscarriage after embryo transfer is of great interest to many patients. However, despite these advances, a number of morphologically normal euploid embryos fail to produce a live birth. There are many possible explanations originating from embryonic, endometrial, or epigenetic factors, but many point to subchromosomal abnormalities—segmental aneuploidies—or embryonic mosaicism as possible explanations for the subset of embryo transfers designated as euploid that fail to produce live births. As clinicians and scientists consider the appropriate application of new CCS methods, it is important to understand the capabilities and limitations associated with the ever-growing number of platforms available.

The appearance of subchromosomal aneuploidy and embryonic mosaicism on laboratory reports has provided a possible explanation for prior embryo transfer failures as well as raised questions about how to discuss these findings with patients. A critical component to this counseling is a firm understanding of the CCS platform used to make the diagnosis and knowledge of the limitations that may exist. There are many platforms for CCS, many of which now claim the ability to diagnose segmental aneuploidy and embryonic mosaicism; each demonstrates capabilities and possible limitations. Comprehensive chromosome screening can include the use of any of multiple available types of amplification, such as GenomePlex whole-genome amplification (WGA), SurePlex WGA, RepliG multiple displacement amplification (MDA)–based WGA,

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multiple annealing and looping based amplification cycles (MALBAC)–based WGA, and custom and targeted multiplex polymerase chain reaction (PCR). There are also multiple downstream methods of quantitation, including single-nucleotide polymorphism (SNP) arrays, 24sure array comparative genomic hybridization (aCGH), quantitative real-time polymerase chain reaction (qRT-PCR), MiSeq-based next-generation sequencing (NGS), and personal genome machine (PGM)–based NGS.

The bioinformatics of these methods can vary dramatically. For example, SNP array data analysis can be performed with parental support, karyomapping, or simple copy number analysis tools. Data from NGS can be analyzed using default settings or custom analysis of BlueFuse software (Illumina), with Ion Reporter software (Thermo Fisher Scientific), or with fully customized bioinformatics. Final interpretation of data and abnormality thresholds can also be impacted by clinically driven data to maximize the predictive value of the diagnosis for the actual clinical outcome.

We will discuss the capabilities and limitations associated with the detection of segmental aneuploidy and embryonic mosaicism in the preimplantation human embryo. Whenever possible, publicly deposited data will be used to illustrate some major concepts surrounding the detection of segmental aneuploidy and embryonic mosaicism.

SEGMENTAL ANEUPLOIDY

The recent emphasis on the ability to detect segmental imbalances in the embryo has stemmed from the growing amount of data that can be obtained from new methods for CCS. Estimates of frequency range from 4% to 58% (Table 1). Biologic limitations, in terms of the clinical significance of the segmental imbalance, and technical limitations, primarily in terms of the level of resolution of detection of the segmental imbalance, exist. Initial evidence of the ability to detect segmental errors derives from the application of CCS to patients who carry a balanced translocation (13–26). Embryos derived from such patients can inherit unbalanced chromosomes, resulting in subchromosomal copy number changes. In this situation, the size of the imbalance depends on where the breakpoints are located within the chromosomes involved in the original translocation. By evaluating data over multiple cases, each platform can be assessed for its size-specific capability—that is, the minimum size of a subchromosomal imbalance that is necessary for the platform to successfully detect it.

Although these data are important to understand the CCS platform's size-specific capability of detecting segmental aneuploidies, there are important limitations that need to be considered. For example, because the origin of the imbalances from these particular cases is meiosis during the formation of the gamete from the translocation carrier, the errors can be expected to be uniformly present in the resulting embryo. This may not always be the case when a de novo segmental imbalance develops in an embryo. If the error occurs during mitotic cell division, the resulting embryo will possess mosaicism of the segmental error. That is, some cells may be chromosomally normal, and others may have a segmental gain or segmental loss of chromosomal material. A recent study demonstrated that this does in fact occur by providing evidence of reciprocal segmental errors in multiple blastomeres from the same embryo (Fig. 1) (27). Similar observations have been made with other platforms including aCGH (29).

Given that much of the field has moved toward analysis of the blastocyst trophectoderm biopsy for preimplantation genetic screening (PGS), the ability to detect mosaic levels of segmental errors within the biopsy must also be considered. Mixture models of a mosaic trophectoderm biopsy have been useful to establish the detection limits of various CCS platforms for whole-chromosome mosaic aneuploidy (28), which we discuss further later. However, similar data for detecting de novo mosaic segmental aneuploidy have yet to be published for any platform currently in clinical use. One of the more commonly used methods of NGS-based testing called VeriSeq PGS (Illumina) has been used to predict segmental aneuploidy by a number of reputable PGS reference laboratories. In each case, these laboratories have established their own criteria for reporting segmental aneuploidy based on the size of the imbalance and the copy number assignment within the segment.

TABLE 1

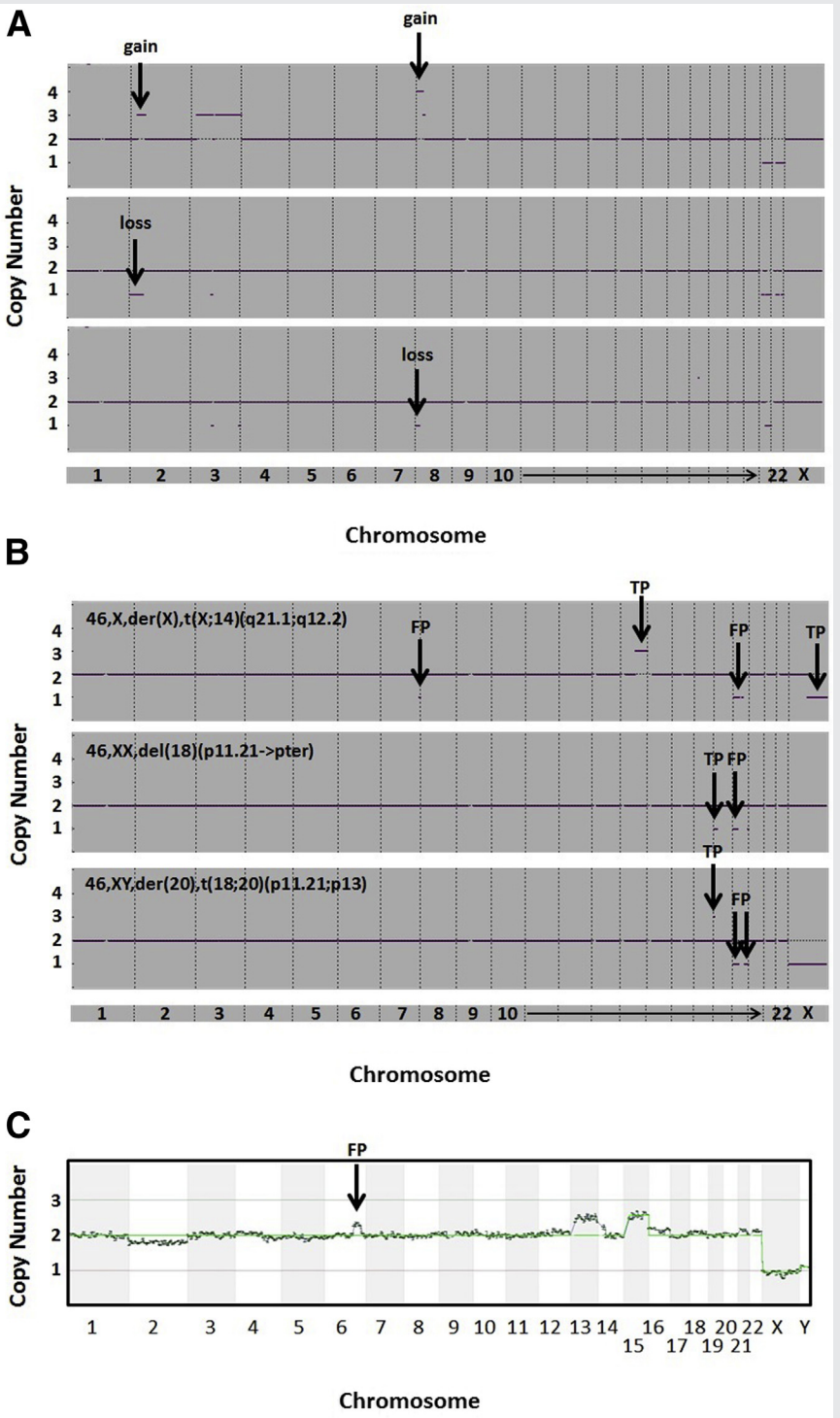
Selection of studies with data on the frequency of segmental aneuploidy at either the blastocyst stage or cleavage stage of embryo development.

Study	Platform(s)	Detection limits	Frequency, % (blastocysts positive/total tested embryos)
Fiorentino et al. (5)	SurePlex WGA, aCGH, NGS	5 Mb	17 (33/192)
Fiorentino et al. (5)	SurePlex WGA, aCGH, NGS	Not defined	5 (18/208)
Fragouli et al. (6)	SurePlex WGA, aCGH	Not defined	7 (73/608)
Greco et al. (7)	SurePlex WGA, aCGH	Not defined	11 (2/18)
Tan et al. (8)	GenomePlex WGA, NGS, SNP array	Not defined	18 (21/119)
Fragouli et al. (6)	SurePlex WGA, aCGH	Not defined	15 (115/754)
Rabinowitz et al. (9)	SurePlex or MDA WGA, SNP array	15% of chromosome length	15 (41/274)
Rodrigo et al. (10)	SurePlex WGA, aCGH	10 Mb	4 (unclear)
Mertzanidou et al. (11)	MDA WGA, custom aCGH	18 consecutive probes	57 (8/14)
Vanneste et al. (12)	MDA WGA, aCGH, SNP array	Not defined	58 (7/12)

Note: aCGH = array comparative genomic hybridization; MDA = multiple displacement amplification; SNP = single-nucleotide polymorphism; WGA = whole-genome amplification.

Treff. Segmental aneuploidy and mosaics. Fertil Steril 2016.

FIGURE 1



(**A**) An example of single-nucleotide polymorphism (SNP) array results from three blastomeres from the same embryo with multiple reciprocal segmental aneuploidies (chromosomes 2 and 8) indicative of an error originating during mitosis and illustrating the potential importance of developing methods that can detect mosaic segmental aneuploidy within a trophectoderm biopsy. Data obtained from Kort et al. (27), NCBI GEO accession number GSE72150. (**B**) SNP array results from Vanneste et al. (12) that indicate numerous false-positive predictions from cell lines with known karyotypes (indicated within each plot), illustrating the possibility for technical artifacts introduced by whole-genome amplification (WGA). Data from Vanneste et al. (12), NCBI GEO accession number GSE11663. (**C**) VeriSeq data that indicate a false-positive segmental aneuploidy within a cell line mixture model, illustrating WGA-based artifacts with next-generation sequencing (NGS) technology. Data from Goodrich et al. (28).

Treff. Segmental aneuploidy and mosaics. Fertil Steril 2016.

Still, there has yet to be a study demonstrating that an observation within one biopsy of an embryo is predictive of the remaining embryo. This illustrates the importance of evaluating the specificity of segmental aneuploidy prediction from a trophectoderm biopsy, as WGA is expected to introduce artifacts that may be misinterpreted as true segmental imbalance. Unfortunately, the errors introduced by WGA have been largely overlooked in studies to characterize the performance of NGS-based segmental aneuploidy detection. For example, both Fiorentino et al. (30) and Vera-Rodriguez et al. (31) compared profiles generated by VeriSeq PGS to profiles from aCGH using the same WGA DNA. Both studies reported highly concordant data (~100%) between the two downstream methods, but it remains unclear whether the segmental aneuploidies observed were real or simply a result of the same WGA artifact (see Fig. 1).

Another source of error, as described by Van Der Aa et al. (32), involves S-phase artifacts, where single-cell DNA replication domains can result in copy number changes that may appear like segmental aneuploidy. Although it is less likely that an entire trophectoderm biopsy would have all cells in the same phase of DNA replication, even a few cells in the replication phase may appear as a mosaic segmental aneuploidy.

EMBRYONIC MOSAICISM

Embryonic mosaicism, the presence of more than one chromosome complement within a single embryo or embryo biopsy, is another prominent explanation put forth for failed euploid embryo transfer. This is quite plausible as the prevalence has been noted to be high throughout embryonic development, with an incidence as high as 70% noted (11). Many reproductive genetics laboratories are now routinely including embryonic mosaicism on their diagnostic reports. However, like segmental aneuploidies, both biologic and technologic limitations exist and must be understood to adequately guide patients during clinical care.

The first limitation is that of sampling. When assessing the biologic reality of embryonic mosaicism with a trophectoderm biopsy that samples only a few of several hundred cells, the question of threshold of detection becomes immediately apparent. This can only be accurately determined by using mixture models of aneuploid cell lines. It is important to note that this analysis paradigm will depend on the molecular platform used and the bioinformatics paradigm used to analyze the data. It is not transferrable between these platforms and paradigms.

Another technologic issue related to sampling of the embryo involves mitotic errors that result in mosaicism. These result in reciprocal whole-chromosome errors. Thus, if a particular trophectoderm biopsy contains a mixture of monosomy and trisomy of the same whole chromosome at a ratio that causes an "averaging" of the signal that is below the level of detection of mosaicism for that platform, a false diagnosis of disomy will occur.

However, the largest component when it comes to understanding the technical considerations of embryonic mosaicism is that of bioinformatics. For diagnosing embryonic mosaicism, it is important to point out that no additional molecular genetics techniques are applied—the laboratory process is unchanged; rather, it is the bioinformatics paradigm applied to the data afterward that yields the diagnosis. Before the diagnosis of mosaicism, the results yielded are simply monosomy, disomy, or trisomy. These diagnoses are based upon threshold values set to discriminate between statistically smoothed data points based upon standard deviations or multiples of the medium from calibration standards composed of known disomic samples. The diagnosis of embryonic mosaicism is a bioinformatics reassessment of these data points with a new category between disomy and trisomy or disomy and monosomy.

When setting these thresholds, aneuploid cell mixing studies are performed that allow for established cutoffs (7). When analyzing how these thresholds are set, it is important to note how these studies translate to clinical practice. For example, establishing the mosaicism cutoffs for aCGH mixing studies with 100 cells yielded tight confidence intervals and good discrimination between repeat samples. However, this is not a biologically realistic cell number when considering a trophectoderm biopsy. When these studies were performed with eight cells—a more realistic number to consider for a trophectoderm biopsy—the confidence intervals were much wider, and the sample-to-sample discrimination was nonexistent (33, 34).

The understanding of this technical limitation explains some of the findings published recently by Greco et al. (7), where the transfer of 18 embryos designated as mosaic resulted in six apparently normal live births. Given the previous discussion, it can be surmised that, although a subset of the embryos whose analysis results in a mosaic diagnosis truly possess a mixture of normal and abnormal cells, some of the embryos that fall into this category will truly be disomic and some will truly be trisomic. Thus, it may not be surprising that, although the reproductive competence of embryos in this category will clearly be diminished (35), there will be circumstances in which normal live births may result.

FUTURE DIRECTIONS

Segmental aneuploidy and embryonic mosaicism represent important new areas of research when it comes to determining the cause of failed implantation and delivery when an embryo that has been diagnosed as euploid is transferred. However, both of these diagnoses come with technical limitations, which must be understood when counseling patients and applying results in clinical situations. The combination of mitotic origins, WGA, and S-phase artifacts makes the clinical validation of the predictive value of CCS-based prediction of segmental aneuploidy extremely important. A better understanding of the sampling limitations and a refining of the bioinformatics algorithms when diagnosing embryonic mosaicism are clearly needed. The use of "nonselection" data, where embryos are biopsied and the samples are analyzed afterward and correlated with the clinical outcome, may help to better define the criteria used to make a clinical diagnosis.

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