Accurate single cell 24 chromosome aneuploidy screening using whole genome amplification and single nucleotide polymorphism microarrays

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Objective: To develop and validate a whole genome amplification and single nucleotide polymorphism (SNP) microarray protocol for accurate single cell 24 chromosome aneuploidy screening.
Design: Prospective, randomized, and blinded study.
Setting: Academic reproductive medicine center.
Patient(s): Multiple euploid and aneuploid cell lines were obtained from a public repository and blastomeres were obtained after biopsy of cleavage stage embryos from 78 patients undergoing IVF.
Main Outcome Measure(s): Accuracy of copy number assignment and consistency of individual SNPs, whole chromosomes, and single cell aneuploidy status were determined.
Intervention(s): None.
Result(s): Single cells extracted from karyotypically defined cell lines provided 99.2% accuracy for individual SNPs, 99.8% accuracy for whole chromosomes, and 98.6% accuracy when applying a quality control threshold for the overall assignment of aneuploidy status. The concurrence for more than 80 million SNPs in 335 single blastomeres was 96.5%.
Conclusion(s): We have established and validated a SNP microarray-based single cell aneuploidy screening technology. Clinical validation studies are underway to determine the predictive value of this methodology. (Fertil Steril® 2010;94:2017–21. ©2010 by American Society for Reproductive Medicine.)

Key Words: Aneuploidy screening, microarray, preimplantation genetic diagnosis, single cell, single nucleotide polymorphism

Despite the promise that aneuploidy screening could improve outcomes for patients undergoing IVF, all randomized clinical trials evaluating fluorescence in situ hybridization (FISH)-based methodologies of aneuploidy screening have failed to demonstrate the expected benefit (reviewed in Ref. 1). It is possible that the negative impact of embryo biopsy or sampling error due to mosaicism represent the root cause of this failure. Although new technologies may not overcome these important factors, they may improve on other limitations including the lack of comprehensive analysis of all 24 chromosomes. Indeed, current FISH methods of aneuploidy screening evaluate less than half of the human chromosomal complement (2–5) and may result in the transfer of reproductively incompetent embryos with aneuploidy for chromosomes not analyzed. In addition, new technologies may provide more accurate and easily interpretable results than current methods.

Some of the most promising progress toward developing a comprehensive 24 chromosome analysis method has been made possible through the combination of whole genome amplification (WGA) and either conventional comparative genome hybridization (CGH) (6–8) or array-based CGH (9–12). Although these methods represent exciting and potentially important advances toward improved preimplantation genetic diagnosis for aneuploidy screening, limited information is available regarding the overall accuracy based on blinded and randomized evaluation of samples with known abnormalities. In addition they have not demonstrated consistency in a large sample size of human blastomeres. To establish these parameters and to begin producing a highly validated methodology, we have combined WGA and high density (>262,000) single nucleotide polymorphism (SNP) microarrays for single cell aneuploidy screening. We have performed a series of prospective, randomized, and blinded studies that should allow full characterization of the precision of a new single cell 24 chromosome aneuploidy screening method.

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MATERIALS AND METHODS

Experimental Design

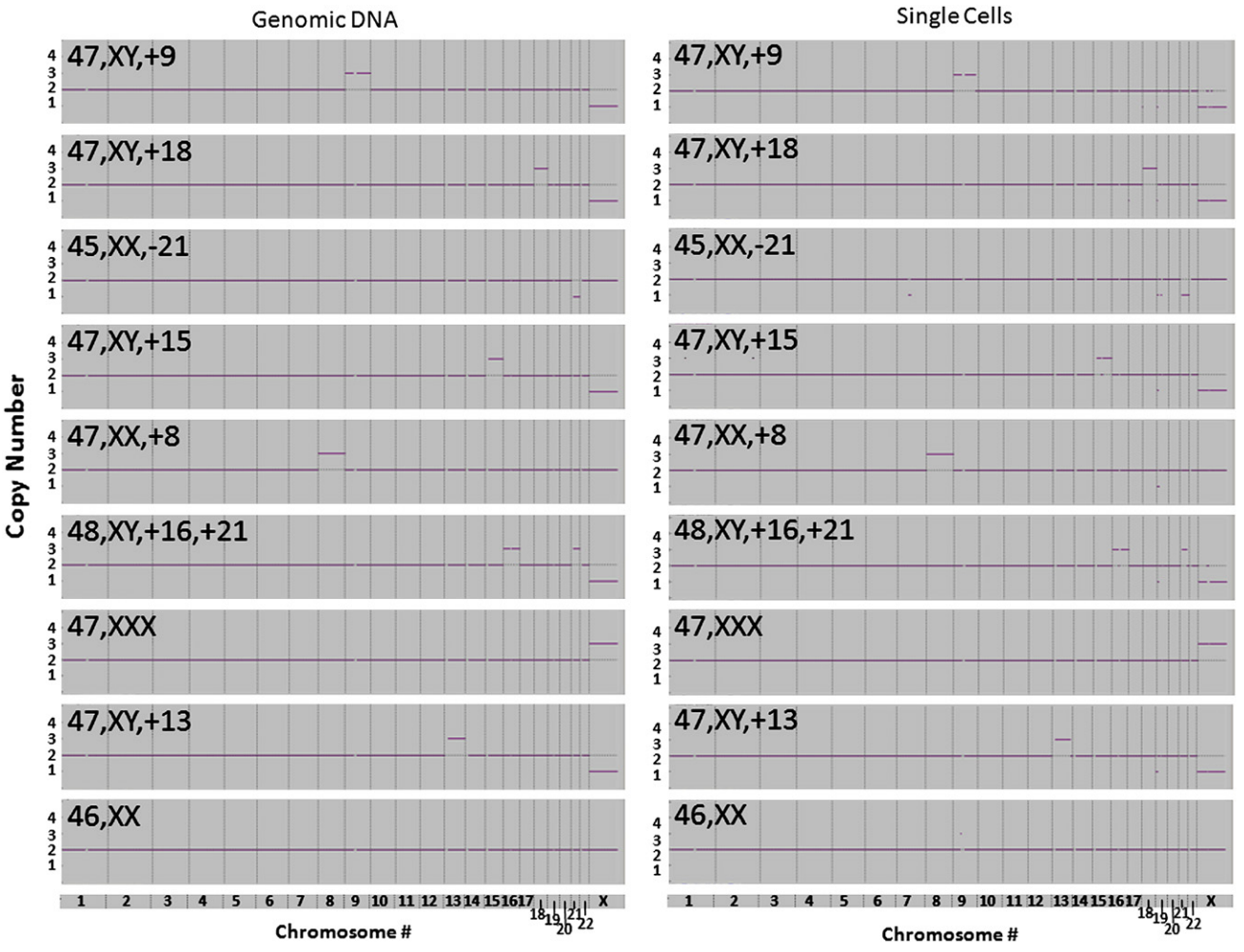
This study was organized into three phases of analysis. Phase I was designed to develop and define a method of analysis that could accurately determine the copy number state of chromosomes in single cells from karyotypically defined cell lines. This phase is effectively a calibration of the methodology. Phase II evaluated the accuracy of the method by testing single cells from karyotypically defined cell lines in a prospective, randomized, and blinded manner. Finally, phase III involved analysis of single human blastomeres to establish similar levels of chromosome-specific SNP copy number assignment concurrence as those observed in cell line single cells.

Cell Lines and Patient Samples

A total of nine well-characterized cell lines were obtained from the Coriell Cell Repository (CCR, Camden, NJ) and cultured as recommended by the

FIGURE 1

Graphic representation of copy number changes observed in various aneuploid cell lines using Copy Number Analysis Tool 4.0.1. *Left*. Results from microarray analysis performed on "genomic DNA" extracted from cultured cell lines. *Right*. Results from whole genome amplified DNA from single cells derived from the same cell line as shown in the left panel. Each graph indicates the copy number assignments (0, 1, 2, 3, or 4) on the y-axis and the chromosome number on the x-axis. Gains (copy number state >2) and losses (copy number state <2) are seen as bars above and below the copy number state of 2, respectively. The likely karyotype is indicated for each sample and is consistent with the designated karyotype assigned by the Coriell Cell Repository using standard cytogenetic analysis.



Treff. Single cell 24 chromosome aneuploidy screening. Fertil Steril 2010.

supplier (CCR). Briefly, lymphocytes were cultured in RPMI 1640 medium (Invitrogen Inc., Carlsbad, CA) with 15% fetal bovine serum (FBS; Invitrogen) and fibroblasts were cultured in Minimum Essential Medium (Invitrogen) with 10%–20% FBS (Invitrogen) at 37°C and 5% carbon dioxide as recommended (CCR). Cell lines included a trisomy 8 female (GM04610), a trisomy 9 male (GM09286), a trisomy 13 male (GM02948), a trisomy 15 male (GM03184), a double trisomy 16 and 21 male (GM04435), a trisomy 18 male (GM01359), a monosomy 21 female (GM01201), a normal female (GM00321), and a trisomy X female (GM04626). Single cells from each cell line were processed by whole genome amplification as described later. Genomic DNA was isolated from approximately 1×10^6 cells from each cell line using the QIAgen DNeasy Tissue kit as recommended by the supplier (QIAgen Inc., Valencia, CA), and evaluated (without WGA) by microarray analysis in parallel with single cells as described later.

Three hundred and thirty five blastomeres were obtained, as previously described (13), from 235 cleavage stage embryos from 78 IVF patients. Multiple blastomeres were evaluated from the same embryo for 16 that arrested in culture. Arrested embryos were donated for research while the remaining embryos were biopsied for embryo DNA fingerprinting (13). All material was obtained with informed and written consent, and under Institutional Review Board approval.

Whole Genome Amplification

Fibroblast cell lines were treated with trypsin EDTA solution (Invitrogen) for 3 minutes at 37°C, followed by the addition of media containing FBS to inactivate trypsin as recommended (CCR). Lymphocyte cell lines (which are nonadherent) did not require trypsin treatment to resuspend cells. Cell lines were placed under a dissecting microscope to load single cells in a 1-μL volume into a 0.2-mL nuclease-free polymerase chain reaction (PCR) tube (Ambion Inc., Austin, TX) using a 100-μm stripper tip and pipette (Midatlantic Diagnostics, Mount Laurel, NJ). Blastomeres were also transferred in a 1-μL volume under a dissecting microscope. Samples were brought to an 8-μL volume with nuclease-free water (Sigma-Aldrich,