

SHORT REPORT

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Comprehensive chromosome analysis of blastocysts before implantation using array CGH

Mi Kyung Chung^{1†}, Hyeon Jeong Jeong¹, Jung Hyun Lee¹, Sang-Jin Park^{2†}, Hee-Doo Chung³ and Ho-Young Kang^{2*}

Abstract

Background: Chromosomal abnormalities are common in embryos produced *in vitro* and cause implantation failure, miscarriage, and serious medical problems in infants. Because preimplantation genetic screening (PGS) is increasingly being used to detect aneuploidy in embryos with the purpose of improving implantation rates after IVF (*in vitro* fertilization), we aimed to validate the usefulness of array CGH for the preimplantation genetic screening (PGS) of embryos at the blastocyst stage of development.

Results: A total of 150 blastocysts were biopsied from couples undergoing IVF and analyzed using array CGH. We found that 54.5% (73/134) of the blastocysts were euploid embryos, whereas 45.5% of the embryos (61/134) had chromosomal abnormalities. Multiple chromosome abnormality was most frequently observed (34.4%), and dual aneuploidy was observed in 26.2% of the embryos. Monosomy (21.3%) appeared more frequently than trisomy (18%).

Conclusion: Chromosomal microarray analysis provided clinically significant cytogenetic information regarding the frequency and variety of chromosomal abnormalities observed in embryos at the blastocyst stage, suggesting that this is a useful tool for comprehensive aneuploidy screening in IVF.

Background

During IVF procedures, a preimplantation genetic diagnosis (PGD) is used to eliminate embryos carrying genetic diseases prior to implantation. The first application of PGD was successfully performed for couples at risk for transmitting recessive X-linked diseases to male offspring [1], whereby polymerase chain reaction (PCR) was used to determine the sex of the embryos.

Unlike PCR methods, preimplantation genetic screening (PGS) aims to provide a means for identifying potentially viable euploid embryos *i.e.*, screening that may improve pregnancy rates. PGS was first described by Verlinsky *et al.* [2] and Munne *et al.* [3]. Although previous methods for embryo screening used fluorescence *in situ* hybridization (FISH) to analyze chromosomes [4,5], the FISH approach is limited because the technique is unable to screen all chromosomes simultaneously. Conventional comparative genomic hybridization (CGH) has

been used to comprehensively screen for aneuploidy in oocytes and embryos [6,7]. However, although useful for selecting euploid embryos, the CGH protocol is not generally used because it is time consuming and complicated. At present, both array CGH (aCGH) and single nucleotide polymorphism (SNP) arrays have been validated as accurate methods for producing comprehensive analyses of chromosome in embryos that are compatible with day-3 biopsies and day-5 replacements in a fresh cycle [8-12]. The difference in mosaicism between embryos at days 3 and 5 has led to a preference for biopsies at the blastocyst stage [13,14].

Here, we describe the results of an embryo analysis and the details of the chromosomal abnormalities found.

Results

In total, we analyzed 150 blastocysts from 49 couples undergoing IVF (Table 1). Amplification was not detected in 11 (7.3%) embryos, and noisy profile results were obtained for 3.6% (5/139) of the embryos. Euploidy was found in 54.5% of the embryos (73/134), whereas chromosomal abnormalities were found in 45.5% (61/134) of the embryos.

* Correspondence: ceo@mgmed.com

†Equal contributors

²MGMED, Inc., Elysia bldg, 173, digital-ro, Gasan-dong, Seoul, Korea
Full list of author information is available at the end of the article

Table 1 Array-CGH results

	Number
Embryos analyzed	150
Euploid embryos	73
Aneuploid embryos	61
Embryos without amplification	11
Embryos with noisy profile	5

The details of the array CGH results derived from aneuploid embryos (n=61) are summarized in Table 2. The type of chromosomal abnormality that was most frequently observed was multiple chromosomal abnormality (34.4%), and the second most frequent was dual chromosomal abnormality (26.2%). Monosomy (21.3%) appeared more frequently than trisomy (18%). Examples of array CGH profiles are shown in Figure 1.

The chromosomes that were most frequently detected to have aneuploidy were, in order, 15, 22, 21, 16, and 18. Chromosomes 4 and 12 were the least frequently found to have aneuploidy.

Discussion

Preimplantation genetic screening for aneuploidy is increasingly used to examine the chromosomes of embryos from couples undergoing IVF [8-10,15,16]. The purpose of PGS is to identify embryos that are free from chromosomal abnormalities. The main indications for PGS are maternal age, repeated implantation failure, and repeated miscarriage. We examined 150 embryos from 49 couples with these indications.

As shown in Table 1, we successfully analyzed 89.3% (134/150) of the embryos and found that 45.5% (61/134) of the embryos contained abnormal chromosomes. Although the array CGH method is robust and specific, we observed some failure in amplification and a noisy profile. Some cells containing degraded DNA or samples of low quality resulting from apoptosis can be obtained during the biopsy procedure, causing experimental error.

Table 2 Chromosome abnormality analyzed

Aneuploid types	Number (%)
Single chromosome loss	13 (21.3%)
Single chromosome gain	11 (18.0%)
Dual chromosomal abnormality	16 (26.2%)
Multiple chromosomal abnormality	21 (34.4%)

The results showed excessive single chromosome loss *versus* single chromosome gain (Table 2); frequent abnormalities in chromosomes 15, 22, 21, 16, and 18; and rare aneuploidy in chromosomes 4 and 12, which are similar to the results of previous reports [17-19]. Although the frequency of chromosomal abnormalities varied, aneuploidy occurred in all of the 24 chromosomes (data not shown), suggesting that PGS is necessary for selecting healthy embryos during IVF procedures. Previous studies have shown the importance of screening embryos with improved pregnancy success as a result [19].

In conclusion, array CGH is a useful technique for the detection of chromosomal abnormalities during IVF procedures, as previously described [18]. However, embryo cultures up to days 5 or 6 should be established before performing array CGH experiments on blastocysts, and further evidence is required to determine whether PGS results in enhanced delivery rates[14].

Methods

Patient materials

A total of 150 blastocysts were collected from 49 couples who visited the clinic center to undergo IVF between September 2011 and December 2012. All patient materials were obtained and evaluated with informed patient consent and under approval from the Ethics Committees of MGMed clinic center and Seoul Rachel Fertility Center. All patients were provided with counseling regarding PGS using array CGH and signed an informed consent prior to entering the study.

Experimental procedures

The biopsied cells were washed in PBS and collected into PCR tubes. Whole-genome amplification was performed using a kit and following the manufacturer's instructions (Sigma-Aldrich, Saint Louis, MO).

Approximately 3 µg of amplified DNA was used in the array CGH experiments, as described, with slight modifications [20]. Briefly, the amplified DNA was labeled with Cy-3 and Cy-5 dCTP for 3 h using a random priming method. The labeled DNA was purified, dissolved in hybridization buffer, and hybridized overnight. The slides were washed several times and dried as described [21]. Images of the slides were acquired with a GenePix4000B dual-laser scanner (Axon Instruments, Union City, CA) and analyzed with MacViewer software [21].

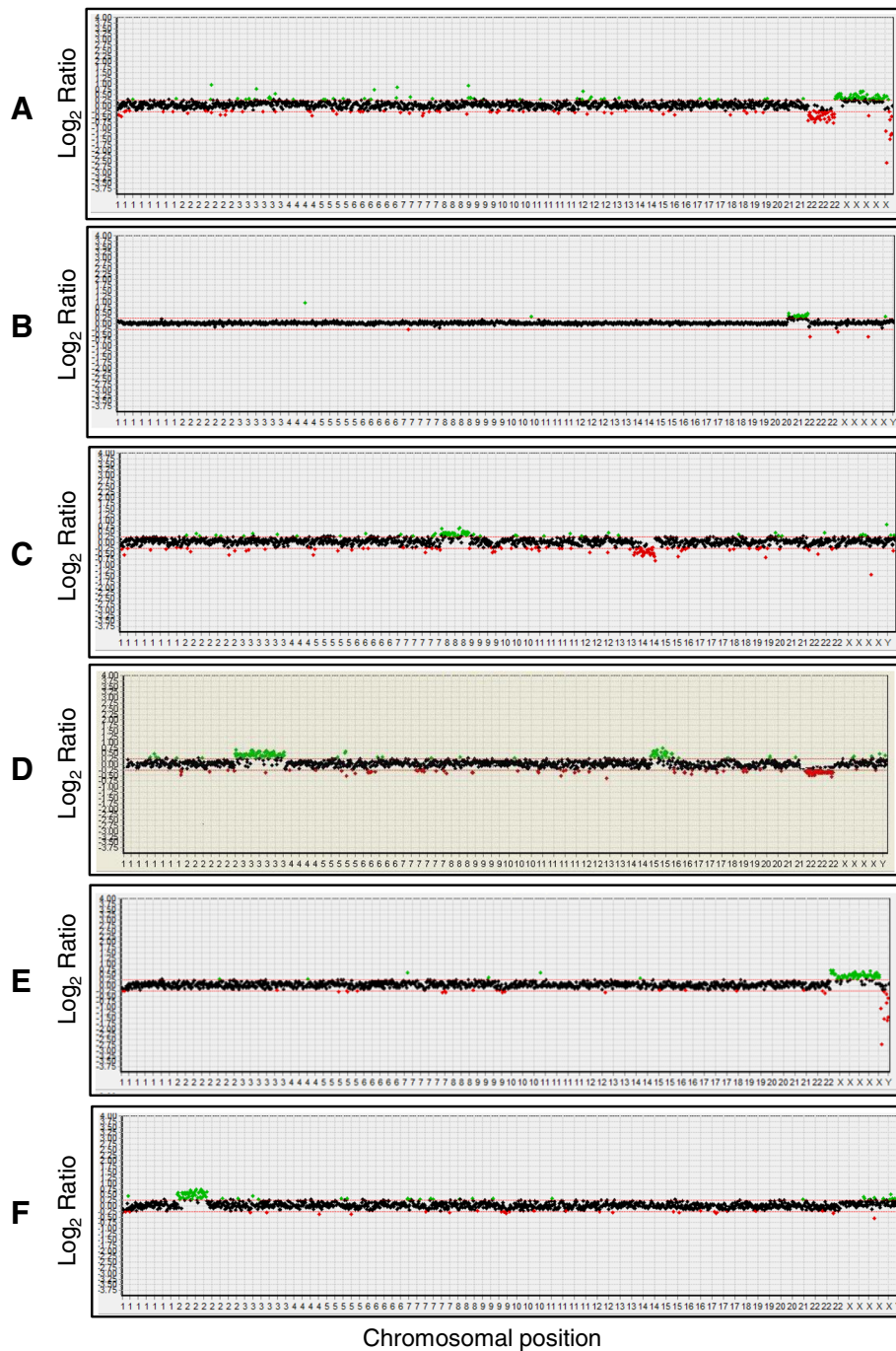


Figure 1 Examples of array CGH results are shown. (A) Array result displaying a female cell with the loss of chromosome 22. (B) A male cell with trisomy 21. (C) An XY cell showing two chromosomal abnormalities i.e., gain of chromosome 8 and loss of chromosome 14. (D) A male cell with multiple chromosomal abnormalities - gain of chromosomes 3 and 15 and loss of chromosome 22. (E) A normal female cell. (F) A cultured cell with a 2p duplication was used as a positive control.

Competing interest

The authors declare that they have no competing interests.

Authors' contributions

MKC and SJP analyzed the data for the paper. HDJ and HJJ helped with the discussion and data summary. JHL performed various experiments. HYK drafted the manuscript, conceived of the study, and also approved the manuscript. All authors read and approved the final manuscript.

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Author details

¹Seoul Rachel Fertility Center, 109 Mapo-daero, Seoul, Korea. ²MGMED, Inc., Elysia bldg, 173, digital-ro, Gasan-dong, Seoul, Korea. ³MGMED Clinic, 60-24 Gasan-dong, Seoul, Korea.

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