Discordance among blastomeres renders preimplantation genetic diagnosis for aneuploidy ineffective

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Abstract *Purpose:* To investigate the contribution of discordance among blastomeres from the same embryo in the interpretation of blastomeres biopsied from day 3 embryos.

Methods: 228 IVF embryos had two blastomeres removed and fluorescent in situ hybridization (FISH) was used to detect aneuploidy of chromosomes 13, 15, 16, 18, 21, 22, X and Y. Of the 228 embryos, 102 had complete FISH results for both blastomeres.

Results: When the 2 blastomeres of 102 embryos with successful FISH results were compared, 26 (25.5%) were concordant for all 8 chromosomes and 76 (74.5%) were discordant for one or more chromosomes. Among the 102 embryos, 12 (12%) were disomy in both blastomeres and 37 (36%) were disomic in all 8 chromosomes in one of the two blastomeres.

Conclusion: Discordance among blastomeres from the same embryo appears to present a significant problem in interpreting results of embryos biopsied on day 3 and analyzed by FISH especially when most PGD's are done on single blastomeres.

Keywords Preimplantation genetic diagnosis · Aneuploidy screening · Discordance among blastomeres · Chromosomal discordance

Introduction

Preimplantation genetic diagnosis (PGD) is a technique used to identify genetic defects in embryos created through in vitro fertilization (IVF) before transferring them into the uterus. In 1989 in London, Handyside and colleagues [1] reported the first unaffected child born following PGD performed for an X-linked disorder. Since then, the indications for performing PGD have been expanded to include screening for aneuploidy, especially in older women undergoing assisted reproductive techniques [2, 3]. The increase in aneuploidy rates with maternal age has been found in cleavage-stage embryos [4–6] as well as products of conception from spontaneous abortions and live offspring [7]. Aneuploidy is a major factor contributing to low implantation and high abortion rates in young women experiencing recurrent implantation failure and recurrent spontaneous abortion even when “good quality” embryos are transferred [8]. Thus, with the introduction of PGD for aneuploidy screening to assisted reproduction programs, it was expected that an increase in implantation rates and a decrease in abortion rates would be observed. While some centers reported improvement in pregnancy outcome after embryos were screened with PGD for aneuploidy [3, 9–12], others could not demonstrate an increase in implantation, pregnancy or live birth rates [13–16]. This observation raised the questions of not only the reliability of the procedure to distinguish chromosomally balanced from chromosomally unbalanced embryos but also the effectiveness of aneuploidy screening with PGD.

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A major pitfall of PGD for aneuploidy screening with a single blastomere biopsy is the existence of mosaicism [17–21]. Chromosomally normal and abnormal blastomeres can coexist within the same early-cleavage-stage embryo [19]. Mosaicism is common in human embryos generated in vitro and can lead to erroneous results [17, 18]. A recent report described the rate of mosaicism among day 3 embryos to be 50% [21]. In addition, fluorescence in situ hybridization (FISH) results from embryos biopsied day 3 and day 6 showed confirmation in 54% to 60% [20, 21]. Because of the question of a high rate of mosaicism in day 3 embryos, we examined the discordance rate when two blastomeres were biopsied from the same embryo on day 3.

Materials and methods

Two hundred twenty eight fresh embryos obtained from 23 couples were biopsied on day 3 post-fertilization following intracytoplasmic sperm injection. Indications for the PGD were advanced maternal age in 12 (>37 years old), recurrent implantation failure in 5 (more than 8 cleaved embryos or 4 blastocysts transferred without successful pregnancy) and recurrent pregnancy loss in 6 couples (>2 consecutive spontaneous abortions). On the morning of day 3 of development, embryos were biopsied in calcium and magnesium free Quinn's Advantage Medium with HEPES (Sage Biopharma, San Clemente, California) supplemented with 0.05 mol/L sucrose, non-essential amino acid and 3% SSS. The zona pellucida was entered mechanically and an attempt was made to remove two blastomeres. The Tween-20/HCl fixation method was used [22]. Fluorescent in situ hybridization with directly labeled probes (Abbott/Vysis; Des Plaines, IL) for chromosomes 13, 15, 16, 18, 21, 22, X and Y was performed in two cycles by a national east-coast genetics center. The genetics laboratory performing the FISH was blinded to which blastomeres were paired. The criteria for signal scoring were as previously described by Munne et al. [23].

Results

A total of 228 embryos generated from 23 women with a mean age of 37.8 years undergoing 28 cycle of IVF were included in the study in which 2 blastomeres were taken from a single embryo on day 3 of development. Of the 228

Fig. 1 Number of discordant chromosomes within each embryo undergoing biopsy of two blastomeres on day 3 of development

embryos with 2 blastomere biopsies, 49 blastomeres had a fragmented nucleus, 9 blastomeres had no nucleus, 59 had only one blastomere FISH result and 9 had incomplete FISH results leaving 102 embryos that had successful FISH analysis for 8 chromosomes. Rates of concordance/discordance were determined for both the blastomeres from each of 102 embryos and the 816 chromosomes involved in the two blastomeres derived from the 102 embryos.

Concordance/discordance per embryo

Concordance of the two blastomeres obtained from each of 102 embryos was only 25.5% for the 8 chromosomes studied. The numbers of discordant chromosomes per embryo are shown in Fig. 1. Discordance for one chromosome pair occurred in 26% of blastomeres; for two of the chromosomes in 23% of blastomeres; for three chromosomes, 11%; for four chromosomes, 10% and for greater than 4 chromosomes, 9% of the blastomere pairs.

The types of discordance are classified into two major categories in Table 1: 1) embryos that had one blastomere indicating disomy and 2) neither blastomeres removed from the same embryo was disomy. Among the 102 embryos, 12 (12%) were disomy for both blastomeres and at least one of the two blastomeres was disomy in 37 (36%).

Concordance/discordance per chromosome

When comparing chromosomes, the discordance appeared to be randomly distributed, that is, the rate of discordance from chromosome to chromosome did not differ significantly, ranging from a low of 13% for the Y chromosome to

Table 1 Type of concordance or discordance observed when two blastomeres were biopsied from the same embryo on day 3 of embryonic development

	Number of embryos	Percent of embryos
Both blastomeres disomy	12	11.8
One blastomere disomy	25	24.5
Neither blastomere disomy	65	63.7

Table 2 Concordant and discordant rates for each of the chromosomes analyzed by FISH on 2 blastomeres from 102 embryos biopsied on day 3 of development

Chromosome	X	Y	13	15	16	18	21	22	Total
Concordant	80	89	76	72	72	74	73	81	617
Discordant	22	13	26	30	30	28	29	21	199
% Discordant	21.6	12.8	25.5	29.4	29.4	27.5	28.4	20.6	24.4

29% for chromosomes 15 and 16 (Table 2). Overall the percent of discordant chromosomes was 24% (199/816) and the ratio of concordant to discordant embryos was 3:1 (617/199).

Discussion

The present data demonstrate a 74.5% rate of discordance when 2 blastomeres biopsied from the same embryo on day 3 of development are analyzed for 8 chromosomes by FISH. Of special clinical interest is the observation that among the 102 embryos studied, only 12 (12%) were disomy for both blastomeres and at least one of the two blastomeres was disomy in 37 (36%). A rate of 12% euploidy in both embryos biopsied is less than that reported by Staessen [13] when at least one blastomere was biopsied from each embryo. However, these findings are in agreement with a previous report showing a rate of chromosome mosaicism of 50% after analysis of two blastomeres biopsied on day 3 [21]. When embryos, biopsied on day 3 and found to be aneuploid were rebiopsied on day 5 [21] or 6 [20], the overall cytogenetic confirmation rate was 54% and 60%, respectively. These observations were consistent with the discordant rates found in the present study since a majority (74%) of the aneuploid embryos did not survive to the blastocyst stage and therefore were not rebiopsied [20]. Also of importance in these two studies was the fact that embryos judged to be euploid on day 3 were not rebiopsied on day 5–6. With a 36% discordance rate between disomy and non-disomy embryos, it is difficult to say which blastomere would have been biopsied had a single blastomere biopsy been performed, raising the question of the reliability of a single blastomere biopsy on day 3 of development to represent the true chromosome complement of the developing embryo.

Explanations for the observed discordance include both technical and biological contributions. Discrepancies due to technical factors involve preparation of the blastomeres and the consequences of the FISH analyses including signal failure, overlap of FISH signals, split signals, cytoplasmic artifact that cover over FISH signals or, conversely, give a false positive signal, and loss of nuclear material during fixation or during cell preparation for FISH [23, 24]. Fixation techniques play a major role in discrepancies in FISH results [25]. The Tween HCl method used in the current study gave less “no results” in our hands compared with the acetic

acid/methanol method. Velilla [25], however, reported better nuclear quality, fewer overlaps and FISH errors with the acetic acid/methanol technique compared with the Tween 20 technique. If half of the discrepant results obtained in the current study were the consequence of technical problems, then one could make the case that the data represent the existence of many chaotic embryos.

Discrepancies due to biologic factors would include spontaneous loss of chromosomes leading to monosomy or nullisomy and chromosome mosaicism due to failure of cell checkpoints to function early in embryogenesis [26]. Previous reports have implicated mosaicism as a source of discordance among blastomeres and have suggested that mosaicism is the major drawback in the efficiency and reliability of preimplantation genetic diagnosis [17, 18]. Chromosomally abnormal cells can coexist with normal cells and mosaic embryos can develop to morphologically [27] and chromosomally [20] normal blastocysts in vitro. It has been suggested that mosaicism in early embryos represents a capability of early embryonic development to “self correct” [20, 28]. Indeed, Munne et al. [28] has demonstrated by FISH analysis of day 12 cultures that chromosome self-normalization occurs in a significant proportion of chromosomally abnormal embryos.

Three mechanisms for the correction of trisomic embryos have been proposed. The first is anaphase–lag correction that results in one disomic and one trisomic daughter cell [29]. The second is nondisjunction correction which results in one viable disomic and one lethal tetrasomic cell leading to a reduction of the number of cells and delay in normal development [30]. The third mechanism proposed for embryonic self-correction of trisomy is chromosome demolition that consists of deliberate fragmentation of one of the three chromosomes during metaphase or anaphase resulting in two disomic daughter cells. Loss of trisomic chromosomes in embryonic tissue resulting in a diploid fetus and a completely trisomic placenta has been reported (fetal rescue) [31–33].

For aneuploidy screening using preimplantation genetic diagnosis to be clinically useful it must be both reliable and effective, that is, aneuploid screening should significantly improve the likelihood of a healthy newborn. While a few studies report a decrease in abortion rate after preimplantation genetic diagnosis with aneuploidy screening, most centers employing the technique have not been able to show an appreciable increase in live birth rates [8–16]. In the current

study, if embryo selection for transfer were based on a single blastomere biopsy and the error rate was defined only as the risk of transferring a diploid embryo with the potential of producing a viable aneuploid pregnancy, specifically, trisomies 13, 18 or 21 or monosomy X, an estimate of the clinical effectiveness can be calculated. Of 102, 22 embryos were identified with discordances for these four chromosomes with the potential for clinically adverse but viable pregnancy outcome. In this case, the maximum error rate in this cohort of embryos would be 21.6%. However, if embryo selection were to be random, then at least half of the time an embryo would be identified as aneuploid and not transferred, thereby making the mean error rate approximately 10.8%. This error rate is in agreement with the estimate of Wilton et al. [34] and Platteau [15] of 8%. An error rate of around 10% raises the question of the effectiveness of performing aneuploidy screening in preimplantation embryos.

A compilation of pregnancy outcomes from three programs performing aneuploidy screening reported that only three infants in 722 births or 0.4% were chromosomally misdiagnosed as preimplantation embryos [14]. In order to account for this discrepancy, an overall negative selection pressure of 80% for trisomies 13, 18, and 21 as well as monosomy X would be necessary. Although a negative selection pressure of 80% may be in agreement with the collective natural histories of these aneuploid states, it does appear counterintuitive to the very purpose of aneuploid screening of preimplantation embryos. To rely on natural selection to counterbalance the errors associated with aneuploid screening would seriously question the efficacy and validity of such testing.

It seems that preimplantation genetic diagnosis for aneuploidy screening utilizing a single blastomere biopsy on day 3 of embryo development is neither reliable in distinguishing chromosomally balanced and unbalanced embryos nor effective in improving the likelihood of a healthy newborn. Single and even double blastomere biopsies have yielded inconclusive results. Options for aneuploidy screening might include polar body biopsies (realizing only the oocyte contribution is being evaluated) or blastocyst biopsies (if the issue of acceptable rates of mosaicism can be determined as was done for chorionic villous sampling) as well as microarrays. This study, in conjunction with previous reports [15, 20, 35] demonstrates the need for prospective, multicenter, randomized trial for preimplantation genetic diagnosis of genetic disorders similar to previous trials related to chorionic villous sampling, amniocentesis and first trimester screening.

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