Reproductive BioMedicine Online (2010) **20**, 92–97

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ARTICLE

Improved detection of aneuploid blastocysts using a new 12-chromosome FISH test

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Santiago Munné gained his PhD in genetics from Pittsburgh University and joined Dr Jacques Cohen at Cornell University Medical College, NY in 1991. He became the director of PGD at the Saint Barnabas Medical Centre in 1995. In 2001 founded Reprogenetics, a PGD laboratory that he directs and that has performed 20,000 PGD cycles.

Abstract Fluorescence in-situ hybridization (FISH) has been the principal method used for the identification and preferential transfer of chromosomally normal embryos, in the context of both preimplantation genetic diagnosis (PGD) and screening (PGS). Generally, the probe combinations used during PGS have focused on chromosomes frequently identified as abnormal in prenatal samples or material derived from first-trimester spontaneous abortions. Recent data, however, obtained with the use of comparative genomic hybridization (CGH), have suggested that commonly used PGS strategies may fail to detect a large number of aneuploidies affecting preimplantation embryos. Some chromosomes, which have been relatively neglected in PGS protocols thus far, display a disproportionate contribution to embryo aneuploidy and should be prioritized for screening. Using CGH data, it is possible to design new probe combinations that examine between 10 and 12 chromosomes and are capable of accurately diagnosing 89–91% of anomalies seen in embryos. At present, 24-chromosome tests, such as CGH, array CGH or single nucleotide polymorphism arrays, remain relatively costly and, in some cases, are yet to be fully validated. For these reasons, a cost-effective method, capable of accurately detecting almost all aneuploid embryos, represents an attractive alternative to comprehensive chromosome screening approaches.

KEYWORDS: aneuploidy, CGH, FISH, miscarriage, Preimplantation genetic diagnosis, RPL

Introduction

New methods for the cytogenetic examination of oocytes or preimplantation embryos, such as comparative genomic hybridization (CGH) and microarrays (Fragouli et al., 2006, 2008; Hellani et al., 2008; Le Caignec et al., 2006; Sher et al., 2009; Wells et al., 2002, 2008a; Wilton et al., 2003), allow imbalances affecting any chromosome to be identified. Preliminary data (Munné et al., unpublished data; Wells et al., 2008b) suggests that the improved

1472-6483/$ - see front matter
doi:10.1016/j.rbmo.2009.10.015

detection of aneuploidy achieved by these methods may increase the efficacy of preimplantation genetic screening (PGS), apparently improving IVF outcomes.

It is possible that comprehensive chromosome screening techniques will ultimately replace fluorescence in-situ hybridization (FISH) as the method of choice for the enumeration of chromosomes in human oocytes and embryos. However, at present FISH continues to offer several benefits. First, the FISH methodology is well understood and well described. A recent review of PGD methods describes the optimal methods that need to be used in order to obtain improved outcomes after PGD (Munné et al., 2009). Unfortunately, many well-intended clinically randomized trials that have appeared recently have not followed the original protocols that were shown to work (biopsy of two cells, using insufficient or inadequate probes, lack of biopsy skills, applying PGD to low responder cycles, unsuitable fixation and other deviations from optimal methods that have precluded improving pregnancy outcomes) (Hardarson et al., 2008; Mastenbroek et al., 2007; Staessen et al., 2004). The optimized techniques recently reviewed by Munné et al. (2009) have been fully validated, thousands of clinical cycles have been conducted and accuracy rates are well established (Colls et al., 2007; Kuliev et al., 2002; Magli et al., 2007; Munné et al., 1998, 2002). Second, the method remains unmatched in terms of value, costing substantially less than CGH or microarray approaches.

There are two main reasons for moving away from FISH. First, there are concerns over accuracy, because FISH tests only one locus on each chromosome and thus any signal overlap or DNA loss may potentially result in a misdiagnosis. As such, the accuracy of FISH in single cells is very well known and is determined by reanalysing all the cells of embryos not replaced and previously diagnosed by PGD. The resulting error rate, not surprisingly, is centre- and technique-related and dependent on the study and laboratory: PGS error rates using FISH range from 5–7% in large studies (Colls et al., 2007; Magli et al., 2007) to 50% in some smaller investigations (Baart et al., 2004). Contrary to some previous and limited studies, later and more extensive ones have shown that the error rate is not related to the number of probes. For example, a new study by Colls et al. (2009) shows similar error rates in a 9-probe panel as in a 12-probe panel. Thus, the modification of the 12-probe panel should not result in higher error rates, just because there are more probes than in the standard 9-probe test. Second, data from spontaneous abortions suggests that 9- to 12-probe FISH assays detect only 57–67% of aneuploid embryos (Jobanputra et al., 2002; Lathi et al., 2007; Munné et al., 2004). Although no study to date has directly compared PGS outcomes for different probe selections, the study by Munné et al. (2009) indirectly strongly suggests that centres not using probes for chromosomes 15, 16 and 22 have poorer results than those using those probes.

This study asks whether PGS strategies using FISH could be improved by re-evaluating which chromosomes are screened. Traditionally, the subset of chromosomes chosen for analysis are those previously known to display the highest frequency of aneuploidy in material from first-trimester spontaneous abortions and prenatal samples. However, it has been observed that the aneuploidy frequencies for specific chromosomes change between the cleavage stage and the first trimester (Munné et al., 2004). Some abnormalities are common at the cleavage stage but may cause embryo demise at an early stage before or shortly after implantation and, as such, are not seen during prenatal testing. The detection of these abnormalities at the preimplantation stage could improve the ability of FISH-based PGS to identify the most viable embryos for transfer.

Until recently, the frequency of aneuploidy had only been determined for a small number of chromosomes and, as a result, FISH tests targeting the chromosomes most often affected by aneuploidy were a matter of guesswork. However, in the past year large numbers of human blastocysts have been analysed using CGH (Fragouli et al., 2008; Wells et al., 2008b), revealing the true incidence of aneuploidy affecting each chromosome on days 5/6 post fertilization.

Materials and methods

CGH patients

The present study includes PGD-CGH results from 79 infertile couples with an average maternal age of 38.2 years. These patients underwent IVF, blastocyst biopsy, blastocyst vitrification and PGD with CGH at the Colorado Centre for Reproductive Medicine. The patients consented to preimplantation screening for aneuploidy and the analysis was performed under the institutional review board approved protocols.

Blastocyst biopsy, cell processing and vitrification

Intracytoplasmic sperm injection (ICSI) was performed in all cases. Embryos were cultured in cleavage-stage medium in 30 μl drops in a 30 mm falcon culture dish, overlaid with 3 ml of Ovoil (Vitrolife, Sweden) incubated at 6% CO_2/5% O_2. The zona pellucida was breached 3 days after fertilization using a laser (Hamilton Thorne, USA) and biopsy of herniating trophectoderm cells was performed on day 5 or 6.

Biopsied cells were processed as outlined in Fragouli et al. (2006). The biopsy material of each blastocyst, consisting of three–10 cells (mean five), was placed in a microcentrifuge tube containing 0.5 μl of 0.1% polyvinyl alcohol, frozen at −20°C and sent to the Reprogenetics laboratory in Livingston, NJ, USA. Cell lysis took place by incubating the samples in 2 μl proteinase K (125 μg/ml; Roche, USA) and 1 μl sodium dodecyl sulphate (17 mmol/l; Sigma, USA), at 37°C for 1 h, followed by incubation at 95°C for 15 min to inactivate the proteinase K.

Comparative genomic hybridization

The CGH methodology was described previously (Fragouli et al., 2006). The reference DNA against which the blastocyst biopsies were hybridized and compared with was extracted from the blood of a normal male individual (46,XY). Whole genome amplification of the blastocyst biopsies and reference DNA was performed using the degenerate oligonucleotide primed polymerase chain reaction with the modification of Wells et al. (1999). Nick translation followed in order to fluorescently label the test blastocyst biopsies