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ARTICLE

Causes and estimated incidences of sex-chromosome misdiagnosis in preimplantation genetic diagnosis of aneuploidy

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Abstract Preimplantation genetic diagnosis of aneuploidy (PGD-A) with comprehensive chromosome analysis has been known to improve pregnancy outcomes. Accuracy in detecting sex chromosomes becomes important when selecting against embryos at risk for sex-linked disorders. A total of 21,356 PGD-A cycles consisting of day-3 (cleavage) or day-5 (blastocyst) biopsies were received at the same laboratory for PGD-A via fluorescence in situ hybridization (FISH) or array comparative genome hybridization (aCGH) from multiple fertility centres. The misdiagnosis rates were 0.12% (Wilson 95% CI 0.05 to 0.25%) in day-3 FISH cycles, 0.48% (Wilson 95% CI 0.19 to 1.22%) in day-3 aCGH cycles and 0.0% (Wilson 95% CI 0 to 0.26) in day-5 aCGH cycles. Although rare, the likely causative biological event for true misdiagnosis is embryonic XX/XY mosaicism. Reanalysis of 1219 abnormal cleavage-stage research embryos revealed a 73% incidence of minor and major mosaicism. Only four (0.3%) embryos were found to be diploid and contained XX and XY cells that could potentially account for the misdiagnosis of sex. Our investigation identified errors leading to misdiagnosis and their attribution to specific events during PGD-A testing. The reported misdiagnosis rates suggest that PGD-A for sex determination is highly accurate, particularly when using aCGH applied to blastocyst biopsies.

KEYWORDS: discrepancy, misdiagnosis, mosaicism, PGD-A

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Introduction

Preimplantation genetic diagnosis for aneuploidy (PGD-A) is used to improve assisted reproduction technique outcomes (Colls et al., 2007; Hodes-Wertz et al., 2012; Munné et al., 1993, 1999; Platteau et al., 2005; Verlinsky et al., 2005) by selecting for chromosomally normal embryos. Fluorescence in-situ hybridization (FISH) using three to 12 chromosome probes combined with day-3 blastomere biopsies was the method of choice (Colls et al., 2007, 2009; Munné, 2006; Munné et al., 1993, 1998; Rubio et al., 2009, 2010; Verlinsky et al., 2005) until 2010, after which improved blastocyst culture conditions and vitrification methods allowed for blastocyst biopsies to be applied clinically without compromising implantation potential (Schoolcraft et al., 2010). Apart from variability in results depending on techniques used in cell fixation (Velilla et al., 2002), embryos could be more easily damaged at the cleavage stage than at the blastocyst stage (Cohen et al., 2007; Munné et al., 2007; Scott et al., 2013). Conflicting data have been reported, with some studies demonstrating improved results after PGD-A at the cleavage stage (Garrisi et al., 2009; Gianaroli et al., 1999; Munné et al., 1999, 2003; Rubio et al., 2009, 2013; Verlinsky et al., 2005) and others, not (Hardarson et al., 2008; Mastenbroek et al., 2007; Platteau et al., 2005; Schoolcraft et al., 2009; Staessen et al., 2004).

The advent of vitrification methods (Cobo et al., 2009; Van Landuyt et al., 2011), better blastocyst culture media and improved microarray technologies (array comparative genome hybridization [aCGH], single nucleotide polymorphism [SNP] arrays and quantitative polymerase chain reaction [PCR]) have transformed the field of PGD-A, with several studies, including randomized controlled trials reporting significant improvements in assisted reproduction technique results (Forman et al., 2013; Hodes-Wertz et al., 2012; Scott et al., 2013; Yang et al., 2012). Currently, most aneuploidy screening procedures are conducted using blastocyst biopsies and microarrays. In patients of advanced maternal age who have experienced recurrent pregnancy loss, and even in young patients with good prognosis, PGD-A with comprehensive chromosome analysis, either by CGH, aCGH, SNP arrays or quantitative PCR, has been associated with improved pregnancy outcomes (Fragouli et al., 2010; Hodes-Wertz et al., 2012; Scott et al., 2013; Yang et al., 2012). Although PGD-A for sex chromosomes is used in some countries for family balancing (also known as social sexing), accuracy in detecting sex becomes especially important when selecting against embryos at high risk of sex linked diseases.

Misdiagnosis rates have been reported for all the above mentioned techniques; FISH produces the greatest variation, with error rates ranging from 3.7% (Magli et al., 2007) to 50% (Baart et al., 2004). Unsurprisingly, groups with high error rates often report poor assisted reproduction technique outcomes, as there is a higher chance of the inadvertent discard of potentially viable euploid embryos and transfer of abnormal embryos. Error rates associated with aCGH, calculated by comparing biopsied cells to the rest of the embryo, have been lower, and the spread of results tighter, from 1.8% in single blastomeres (Gutierrez-Mateo et al., 2011) to 6.0% in polar bodies (Geraedts et al., 2011).

Generally, misdiagnosis is considered to be the classification of a normal euploid embryo as abnormal or vice-versa. Misdiagnoses can occur as a result of biological or technical issues. Technical issues can involve errors in sample processing, including transfer of an incorrect embryo and contamination from other sources. Biological events that could provide plausible explanations for the discrepancy are mosaicism, chimerism, and in-vivo conception during in-vitro treatment. In the event of an apparent diagnostic error, current protocol dictates that the original amplified DNA specimen be re-tested to confirm the diagnosis. Quality control parameters for the affected case are then reviewed to rule out any possible errors or deviations from standards. Moreover, a range of tests are conducted to identify the origin of the discrepancy. Samples are generally tested for zygosity, sex chromosome confirmation, DNA fingerprint comparison between fetus and parents, and finally, DNA comparison between fetus and embryos tested.

To our knowledge, and with the exception of early studies using PCR (Harper et al., 2008) or FISH (Kligman et al., 1996) to assess small numbers of embryos, no other PGD-A reports have described sex misdiagnosis, i.e. classifying a female embryo as male or *vice versa*. This study aims to establish the incidence of true sex misdiagnosis in PGD-A cycles using FISH and aCGH, and to investigate possible causes to identify any aspects of the procedures that are particularly susceptible to error.

Materials and methods

Patient population

Patients that underwent PGD-A between 2006 and 2013, and whose biopsied samples were processed by Reprogenetics (Livingston, NJ), were included in the study. No exclusion criteria based on clinical reason for PGD-A, age, or other clinical factors, were enforced as these were not expected to alter the frequency of sex chromosome misdiagnosis. Embryo biopsy was carried out either on the day-3 embryo or at the blastocyst stage. Following strict standard protocol procedures, samples were processed either by FISH for 9–12 chromosomes (Colls et al., 2009) or using aCGH. Of the total 21,356 cycles involved in the analysis, 18,565 cycles used day-3 (cleavage stage) biopsies, whereas 2791 cycles involved trophectoderm biopsies. The breakdown of patient population by sample type, historical pregnancy rates, and reported discrepancies using each technique are shown in **Figure 1**. Reports were then sent to the respective referring fertility centre for embryo transfer in a fresh embroy transfer or vitrified-warmed embroy transfer.

Fluorescence in-situ hybridization

Blastomeres were fixed on a glass slide as previously described, dehydrated in 70%, 85% and 95% ethanol (2 min each), and hybridized with chromosome-specific probes (Abbot Molecular, Illinois, USA) following the most current protocol at the time, either for nine chromosomes (Colls et al., 2007) or 12 chromosomes (Colls et al., 2009).

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