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ARTICLE

Chromosomal complement and clinical relevance of multinucleated embryos in PGD and PGS cycles

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Abstract The objective of this retrospective study was to investigate the incidence and clinical implications of multinucleation in blastomeres biopsied from cleavage-stage embryos obtained from patients undergoing preimplantation genetic screening (PGS) for aneuploidies or preimplantation genetic diagnosis (PGD) for translocations or single-gene defects (SGD). A total of 3515 embryos were obtained from 306 couples in 380 PGD or PGS cycles. Incidence of multinucleation, chromosomal complement in multinucleated (MN) and sibling embryos and the characteristics of MN embryos resulting in healthy births were investigated. Of all cycles, 41.3% involved at least one MN embryo. There were more uniformly diploid than uniformly haploid nuclei (22.0% versus 7.9%, $P < 0.01$). The most common form of abnormality was chaotic chromosomal complement (39.9%, 147/368). Transfer of embryos that had MN blastomeres free of the genetic abnormalities tested resulted in three healthy deliveries. It is concluded that, although the majority of MN blastomeres are chromosomally abnormal, healthy births are possible after transfer of embryos containing these blastomeres subjected to genetic analysis. As far as is known, this is the first report of healthy births after transfer of embryos with MN blastomeres tested for translocations or SGD in PGD cycles.

KEYWORDS: FISH, human embryos, multinucleation, preimplantation genetic diagnosis, preimplantation genetic screening, translocations

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Introduction

Genetic analysis for selection of genetically healthy embryos for transfer is an established treatment option offered to patients referred due to advanced maternal age, translocations or single-gene defects (SGD) (Harper and Sengupta, 2012; Munne, 2003). Preimplantation genetic screening (PGS) for aneuploidies or preimplantation genetic diagnosis (PGD) of SGD or translocations performed at the cleavage stages of embryonic development involves removal of blastomere(s) from the embryo. However, some of these removed blastomeres may contain more than one nuclei (i.e. bi- or multinucleated (MN)), complicating the genetic diagnosis because each nucleus may be haploid, diploid, aneuploid or chaotic (Xanthopoulou et al., 2011).

Multinucleation is one of the most common nuclear abnormalities seen in human embryos (Hardy et al., 1993). Much research effort has been spent to try to explain how and why multinucleation occurs. Karyokinesis without cytokinesis (Hardy et al., 1993) that may result from defects in structure and/or function of the extra- or intracellular elements, culture reagents or conditions (De La Fuente and King, 1998; Chatzimeletiou et al., 2005; Wang et al., 2000) or suboptimal ovarian stimulation regimes (Van Royen et al., 2003) have been proposed as plausible explanations. However, the fate of MN human embryos is still subject to controversy in the literature. Some authors have suggested that multinucleation may represent a major pathway leading to chromosomal chaos and subsequent developmental arrest (Chatzimeletiou et al., 2005) whereas others have reported the development of embryos fully binucleated on day 2 post insemination into chromosomally normal diploid blastocysts (Staessen and Van Steirteghem, 1998).

The current literature on chromosomal abnormality rates in MN embryos in PGD and PGS cycles is limited. Although chromosomal complement in MN embryos after fixation and staining of the nuclei (Xanthopoulou et al., 2011) as well as pregnancy after transfer of MN embryos not subjected to genetic analysis have been reported previously (Balakier and Cadesky, 1997), there are no reports of healthy births after the transfer of embryos with MN blastomeres tested for translocations or SGD in PGD cycles. Consequently, it is currently unknown what type, if any, of chromosomal complement in MN blastomeres may be associated with healthy births. This may become an important issue in PGD cycles where only MN embryos are available for transfer.

A thorough investigation of the frequency and clinical outcome of multinucleation in blastomeres sampled from preimplantation embryos may help not only embryologists and clinicians in identifying the type(s) of MN embryos that may result in healthy births but also basic researchers investigating cellular mechanisms leading to multinucleation and chromosome segregation in early human embryos. The aim of this study was to help to achieve both of these objectives by investigating frequency, type and clinical relevance of MN embryos in PGD and PGS cycles.

Materials and methods

Patient details

This study retrospectively analyzed data to obtain the multinucleation rate in 3515 cleavage-stage embryos obtained from 306 couples (219 PGS, 41 translocation and 46 SGD) who underwent 380 PGD or PGS cycles (267 PGS, 58 translocation and 55 SGD) at the McGill University Health Centre — Reproductive Centre (MUHC-RC) in Montreal, Quebec, Canada. The data were collected from March 1998 to November 2011. These projects were approved by the Royal Victoria Hospital — MUHC Office of Research Ethics (mosaicism: SUR-99-825, continuing review approved 20 December 2012; SGD: SUR-99-781, continuing review approved 30 March 2010).

Definitions

Blastomeres containing two or more nuclei or an embryo with at least one MN blastomere were considered MN. A cycle was defined as MN when at least one MN embryo was obtained. Diagnosis of multinucleation was based on microscopy as well as spreading and staining of nuclei in 3120 embryos tested for translocation or screened for aneuploidy. Diagnosis of multinucleation was based on microscopic evidence obtained using a high-power inverted microscope in 395 embryos tested for SGD. Each blastomere was carefully examined and only those with clearly more than one nucleus were included in the analysis. Based on fluorescence in situ hybridization (FISH) signals, embryos were classified as 'chaotic' whenever more than two chromosomes were aneuploid or complex segregation patterns were observed. Blastomeres were classified as 'uniformly haploid' or 'uniformly diploid' when each nucleus consisted of one or two sets, respectively, of all chromosomes tested. Embryo quality was visually assessed and recorded on day 3 post insemination and again on the day of transfer. Clinical pregnancy rate was defined as the number of cycles with at least one gestational sac divided by the total number of cycles. Implantation rate was obtained by dividing the total number of gestational sacs obtained in all cycles by the total number of embryos transferred.

Embryo biopsy, FISH and multiplex PCR

Embryos were biopsied on day 3 post insemination in a drop of Ca^{2+} and Mg^{2+}-free biopsy medium (Cook Canada) using an infrared diode laser in computer-controlled non-contact mode (Hamilton Thorn, MA, USA). A single blastomere was removed and spread on a glass slide using spreading buffer (0.1% Tween 20, 0.01 mol HCl; Zhang et al., 2010), except that two cells were removed from 42 embryos.

Genetic analysis was performed using multicolour FISH for PGS and testing for translocations. FISH was performed in two or three rounds using probes specific for chromosomes 13, 15, 16, 18, 21, 22, X and Y in aneuploidy screening cases. Only signals from CEP probes were included in the analysis of reciprocal translocations to remove effects of translocations on aneuploidy rates.