Reproductive BioMedicine Online (2011) 22, 362–370

www.sciencedirect.com
www.rbmonline.com

ARTICLE

The nature and origin of binucleate cells in human preimplantation embryos: relevance to placental mesenchymal dysplasia

L Xanthopoulou [a,*], JDA Delhanty [a], A Mania [a,1], T Mamas [a], P Serhal [b], SB SenGupta [a], A Mantzouratou [a,b]

[a] *UCL Centre for PGD, Institute for Women's Health, University College London, 86–96 Chenies Mews, London WC1E 6HX, UK;* [b] *Centre for Reproductive and Genetic Health, University College London Hospital, The New Wing, Eastman Dental Hospital, London WC1X 8LD, UK*

[*] Corresponding author. *E-mail addresses:* l.xanthopoulou@ucl.ac.uk (L Xanthopoulou), j.delhanty@ucl.ac.uk (JDA Delhanty), anastasia.mania@imperial.nhs.uk (A Mania), t.mamas@ucl.ac.uk (T Mamas), paul.serhal@uclh.nhs.uk (P Serhal), s.sengupta@ucl.ac.uk (SB SenGupta), a.mantzouratou@ucl.ac.uk (A. Mantzouratou). [1] Present address: IVF Hammersmith, Hammersmith Hospital, Du Cane Road, London W12 0HS, UK.

L Xanthopoulou obtained her BSc in biology in 2003 and her MSc in prenatal diagnosis and fetal medicine in 2005 at University College London. Leoni joined UCL Centre for PGD in January 2006 as a part-time PhD student and part time clinical scientist. Her clinical work works up protocols for carriers of structural chromosomal abnormalities, whereas her research focuses on molecular cytogenetics of human preimplantation embryos. Her main research interests include the origin and formation of binucleate cells and the investigation of microsatellite instability in human preimplantation embryos, as well as role of mutations in PGS patients with unexplained infertility.

Abstract Cleavage-stage embryos often have nuclear abnormalities, one of the most common being binucleate blastomeres, which may contain two diploid or two haploid nuclei. Biopsied cells from preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS) cycles were studied to determine the relative frequency of binucleate cells with two haploid versus two diploid nuclei. The frequency of mononucleate haploid biopsied blastomeres was also recorded. In the chromosomal PGD cycles 45.2% of the biopsied binucleate cells were overall diploid and 38.7% were overall tetraploid, compared with 50.0% and 29.2% for the PGS group, respectively. Placental mesenchymal dysplasia is a rare condition associated with intrauterine growth restriction, prematurity and intrauterine death. Recent work suggests that androgenetic diploid/haploid mosaicism may be a causal mechanism. There are two possible origins of haploid nuclei, either the cell contained only one parental genome initially or they may be derived from the cytokinesis of binucleate cells with two haploid nuclei. Binucleate formation therefore may be a way of doubling up the haploid genome, to produce diploid cells of androgenetic origin as seen in placental mesenchymal dysplasia.

KEYWORDS: binucleate, diploid, fluorescent in-situ hybridization (FISH), placental mesenchymal dysplasia (PMD), preimplantation genetic diagnosis (PGD), tetraploid

1472-6483/$ - see front matter
doi:10.1016/j.rbmo.2011.01.001

Introduction

Nuclear abnormalities in human preimplantation embryos

Cleavage-stage embryos often appear to have nuclear abnormalities, i.e. contain blastomeres that are anucleate, multinucleate or binucleate (**Figure 1**), that contribute to arrested embryo development (Winston et al., 1991). Such nuclear abnormalities, one of the most common being binucleate blastomeres, i.e. one blastomere containing two nuclei (Hardy et al., 1993), have often been associated with poor embryo morphology and development and lower pregnancy rates (Chatzimeletiou et al., 2005). However, binucleate blastomeres have been observed in both arrested and normally developing embryos at all stages of preimplantation embryo development, including the blastocyst stage (Chatzimeletiou et al., 2005). Embryo culture conditions and changes in temperature have been implicated in the formation of multinucleated blastomeres (Pickering et al., 1995; Winston et al., 1991) but interestingly, the occurrence of blastomeres with nuclear abnormalities has also been observed in embryos that have been fertilized *in vivo* (Hertig et al., 1954 cited in Delhanty et al., 1997) suggesting therefore that it is not a phenomenon seen only after superovulation regimes and culture conditions used in an IVF setting.

It has been proposed that there are different mechanisms resulting in multinucleation in human preimplantation embryos (Pickering et al., 1995). The most widely proposed mechanism involves the formation of binucleate cells during the cleavage divisions due to erroneous mitosis, whereby karyokinesis is not synchronized with cytokinesis (Coonen et al., 2004). Hardy et al. (1993) argue that binucleate cells arise from failure of cytokinesis in a diploid cell after correct DNA replication and karyokinesis therefore suggesting that the binucleate blastomeres would be overall tetraploid, consisting of two diploid nuclei. However, cell fusion could also lead to the same effect (Balakier et al., 2000; Benkhalifa et al., 1993). Other errors during the cleavage divisions could result in the failure of the chromosomes to segregate properly due to asymmetric cytokinesis or due to the presence of abnormal spindles, i.e. spindles of abnormal shapes that do not have well-defined poles (Chatzimeletiou et al., 2005). Indeed, DNA replication and failure of cytokinesis, chromosome malsegregation and erroneous chromosome packaging, as well as nuclear fragmentation, have all been implicated in multinucleation (Munné and Cohen, 1993; Pickering et al., 1995).

The lack of cell-cycle control mechanisms during those early stages of preimplantation embryo development could be an important factor involved in the formation and accumulation of binucleate blastomeres (Delhanty and Handyside, 1995; Harrison et al., 2000), until the activation of the embryonic genome, at which point such abnormalities may cause developmental arrest (Munné and Cohen, 1993). Due to the loose regulation of the cell cycle at the early cleavage stages, the earlier the event leading to binucleation takes place, the more likely it is that the maternal transcripts present in the oocyte are responsible for the binucleation. Apart from oocyte maturation, abnormalities in sperm function could also be involved (Balakier and Cadesky, 1997) since the spindle for the first embryonic division is organized by the centrosome which is paternally inherited (Sathananthan et al., 1991). It has also been reported that the earlier binucleation occurs, the worse the fate of the embryo, as a greater incidence of chromosomal abnormalities is observed (Kligman et al., 1996). The fact that nuclear abnormalities are often seen in conjunction with chromosomal abnormalities further supports the involvement of abnormal spindles. An abnormal number of centrosomes as well as centrosome dysfunction would lead to abnormal cell division (Sathamanthan et al., 2001, Palermo et al., 1994) through abnormal spindle formation (Chatzimeletiou et al., 2008).

According to all these proposed mechanisms, binucleate cells are overall tetraploid, since DNA replication has taken place correctly, followed by acytokinesis and either correct chromosome segregation and packaging or malsegregation and defective packaging. If such a binucleate cell was to divide, two mononucleate diploid cells would form or, in the case of chromosome malsegregation, the daughter cells would exhibit reciprocal chromosome gains or losses.

There have been reports, however, of binucleate cells that are overall diploid. Iwarsson et al. (2000) reported a binucleate cell containing two haploid nuclei, whereas Delhanty et al. (1997) reported on five binucleate blastomeres, four of which consisted of two haploid nuclei for the chromosomes tested, arguing that the origin of haploid cells in human preimplantation embryos could be associated with the formation of binucleate blastomeres. In this case, a diploid mononucleate cell would undergo a meiotic type of division, whereby the chromosomes segregate without previously being replicated and karyokinesis takes place without cytokinesis. In the case that such a binucelate cell undergoes cytokinesis, two haploid mononucleate cells would form.

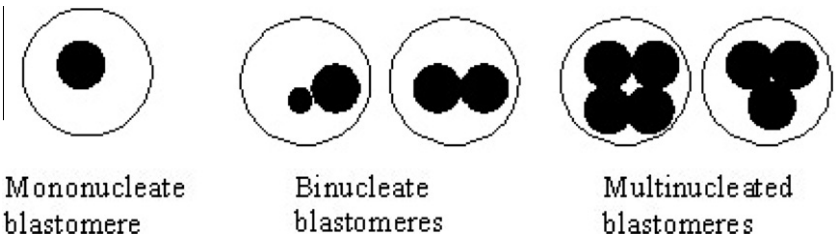

Figure 1 Nuclear abnormalities seen in human preimplantation embryos.