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ARTICLE

The spatial arrangement of blastomeres at the 4-cell stage and IVF outcome

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Goedele Paternot obtained her Masters in biomedical sciences in 2007 at the Catholic University of Leuven, Belgium. In 2011, she finished her PhD in medical sciences entitled ''Selecting the embryo with the highest implantation potential regarding the morphology and developmental potential in a clinical setting'' at the Leuven University Fertility Centre (ISO 9001:2008 certified). The main research topics of the centre are embryo scoring and the optimization of in-vitro culture conditions, quality of patient care and endometriosis.

Abstract This study compared the developmental and implantation potential of tetrahedrally arranged versus non-tetrahedrally arranged 4-cell-stage embryos. If the cleavage planes of a 4-cell-stage embryo were perpendicularly orientated, blastomeres were defined as tetrahedrally arranged, while embryos with parallel-orientated cleavage axes were considered as non-tetrahedral embryos. The 4-cell-stage embryos ($n = 862$) examined in this study were obtained from 299 patients aged <36 years. A total of 299 embryos were transferred as a single-embryo transfer on day 3. This study showed that tetrahedral embryos developed into a 8-cell-stage embryo on day 3 more frequently (307, 45% versus 42, 24%; $P < 0.0001$) and also developed more frequently into good-quality embryos (461, 67% versus 67, 38%; $P < 0.0001$) and into excellent-quality embryos (290, 42% versus 34, 19%; $P < 0.0001$). Tetrahedral embryos had a significantly higher implantation potential (98, 38% versus 9, 21%; $P = 0.038$), ongoing pregnancy rate (84, 33% versus 7, 16%; $P = 0.032$) and live birth rate (84, 33% versus 7, 16%; $P = 0.032$). In conclusion, tetrahedral 4-cell-stage embryos on day 2 developed into embryos of better quality on day 3 with a higher implantation potential and live birth rate compared with non-tetrahedral 4-cell-stage embryos.

KEYWORDS: 4-cell-stage embryos, embryo quality, embryo selection, IVF, live birth rate, spatial arrangement

Introduction

The mechanisms behind the development of mammalian embryos from a single cell, the fertilized oocyte, into a complex structure of a blastocyst consisting of different cell lineages has been debated for the last few years (Zernicka-Goetz, 2006). The mouse model has been used to understand the mechanisms underlying the earliest stages of development by different research groups. As mentioned by Cooke et al. (2003), clear definitions are needed for terms like polarity and poles when assessing development poles and planes. While polarity defines an ongoing axis of symmetry

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throughout pre- and post-implantation embryo development, the term ''pole'' (animal or vegetal) defines two distinguishing parts of the oocyte from which the A–V plane is formed by a meridional cleavage (Cooke et al., 2003).

It has been stated that cell fate decisions in the embryo are crucial for the further development (Edwards and Beard, 1997; Zernicka-Goetz et al., 2009). The first cell fate decisions in the embryo can be explained by different points of views. A recent review focused on the different opinions (Johnson, 2009) and concluded that a combination of the early asymmetric hypothesis (Johnson and Ziomek, 1981), the inside–outside hypothesis (Tarkowski and Wroblewska, 2006) and the polarization hypothesis (Plusa et al., 2005) can explain the differentiation processes and the formation of the axis of symmetry in early development. In addition, studies on transcription factors confirmed the complexity of the process indicating the presence of different mechanisms (Plachta et al., 2011; Vermilyea et al., 2011; Zernicka-Goetz, 2011).

Studies on basic embryology of embryos revealed that the oocyte consists of an animal and vegetal pole (Antczak and Van Blerkom, 1997, 1999; Edwards and Beard, 1997; Edwards, 2002). Although not morphologically visible, this polarization was found based on the differential expression of molecular factors (Antczak and Van Blerkom, 1997, 1999; Edwards and Beard, 1997; Van Blerkom, 2007). Maternal proteins like leptin and STAT3 are distributed differentially and are mainly expressed in the cortex of the animal pole (Antczak and Van Blerkom, 1997, 1999). Differences in distribution in human embryos was confirmed by the detection of high concentrations of animal-distributed leptin/STAT3 in one blastomere of a 4-cell embryo, in two blastomeres of the 8-cell embryo and finally to trophectoderm only (Antczak and Van Blerkom, 1999). Controlled cleavage planes leading to correct distribution of proteins might have an influence on important developmental aspects of the embryo (Hansis and Edwards, 2002). Indeed, the pattern of inheritance of polarized domains in daughter cells seems to be determined by how successive equatorial or meridional planes of cell division are oriented with respect to the domains (Antczak and Van Blerkom, 1999). The first meridionally orientated cleavage results in two daughter cells with the same polarity, meaning that both cells contain animal and vegetal cytoplasm. One cell divides meridionally orientated while the other cell divides equatorially (or after the second cleavage plane rotated through 90 degrees) which result in four cells with different polarity (Edwards and Hansis, 2005; Gulyas, 1975). The two daughter cells resulting from the meridionally orientated cleavage have again the full polarity (animal and vegetal cytoplasm). The daughter cells resulting from equatorially cleavage differ in polarity since one cell will contain mostly animal cytoplasm while the other contains mostly vegetal cytoplasm. These specific cleavage planes result in a tetrahedral arrangement of the blastomeres (Edwards, 2002). On the other hand, other researchers have claimed that the pattern of the second cleavage is random (Louvet-Vallée et al., 2005) and, as a consequence, other topologies were found in 4-cell-stage embryos. However, as mentioned by Gardner (2006) the validity of the latter study is questionable due to the method used. The only clear experimental evidence for the second cleavage was published by Gardner, who described tetrahedral and non-regular tetrahedral 4-cell-stage embryos in mice (Gardner, 2002). The last group was subdivided into two groups based on the positioning of the polar body (either in contact with all the blastomeres or lay between only two) (Gardner, 2002) which might result in blastulation failure (Gardner, 2002; Ebner et al., 2012).

The current manuscript focuses on the arrangement of blastomeres at the four cell stage (tetrahedral versus non-tetrahedral arrangement). In turn, this characteristic can be useful in embryo selection in a non-invasive way. Tetrahedral 4-cell-stage embryos have been described with a meridional axis and an equatorial axis during the second cleavage (Cauffman et al., 2010). Where the two cleavage planes are oriented otherwise, a non-tetrahedral arrangement of blastomeres may occur (Cauffman et al., 2010; Ebner et al., 2012). This can result in a different distribution of cytoplasm in the daughter cells (for example different distribution of membrane associated or cortical factors (Edwards, 2005) or mitochondria (Van Blerkom, 2007). The aim of this study was to compare in a large set of embryos the developmental and implantation potential of tetrahedral versus non-tetrahedral 4-cell-stage embryos.

Materials and methods

Patients

The 4-cell-stage embryos examined in this study resulted from 299 patients aged <36 years, who received a single-embryo transfer on day 3 at the Leuven University Fertility Centre. Patients entered their first ($n = 254$) or second ($n = 45$) IVF/intracytoplasmic sperm injection (ICSI) cycle and were only included once. The stimulation protocol used in this study has been published (Debrock et al., 2010). Patients were excluded when the cycle included biopsy for preimplantation genetic diagnosis or if donor spermatozoa/donor oocytes were used. In total, the dataset contained 862 4-cell-stage embryos of which 299 embryos were transferred on day 3. The study was approved by Institutional Review Board of the University Hospitals Leuven (ML4564, 16 November 2007).

Assisted reproduction treatment

After oocyte retrieval, the oocytes were washed through four wells and placed in a 4-well dish containing 500 μl fertilization medium (Cook medium; Sydney IVF, Queensland, Australia; or GM501 medium; Gynemed Lensahn Germany) at 37°C, pH 7.25–7.35 per well, under mineral oil. Spermatozoa used for the IVF procedure were prepared using standard density gradient procedures (Isolate; Irvine Scientific, USA). Sperm samples used for ICSI were diluted and were centrifuged twice for 10 min at 300 ***g***. Standard IVF/ICSI procedures were performed 2–6 h after oocyte retrieval. During the IVF procedure, oocytes were inseminated with 300,000 progressively motile spermatozoa per well (5 oocytes in 0.5 ml). In the case of ICSI cycles, injected oocytes were incubated together in a 20 μl culture medium droplet under oil. On day 1 (16–20 h after insemination/injection), fertilization was evaluated. Only normal