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Perivitelline threads associated with fragments in human cleavage stage embryos observed through time-lapse microscopy

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KEY MESSAGE

Time-lapse microscopy revealed perivitelline threads (PVT) that developed at the two-cell stage and were associated with fragmentation. No correlation was found between PVT and implantation potential or ploidy. Further studies are required to clarify the relationship between PVT and fragmentation, and determine the origin, composition and function of these structures.

A B S T R A C T

Perivitelline threads (PVT) are defined as thin filaments that extend across the perivitelline space connecting the zona pellucida with the oolemma or, in some cases, blastomere membrane. This is the first report of PVT in human embryos. Time-lapse imagery from 525 blastocysts with either tested ploidy, known implantation status, or both, were reviewed for the presence of PVT, the cell stage when PVT were first observed, association with fragmentation, ploidy or implantation potential; PVT were observed in most embryos (404/525 [77%]). The euploidy rate was similar in embryos with PVT (61/152 [40%]) and without PVT (17/35 [49%]). Implantation rates were also similar in embryos with PVT (64/259 [25%]) and without PVT (25/90 [28%]). In the embryos in which PVT were observed, 98% (396/404) developed at the two-cell stage. In most embryos (384/404 [95%]), PVT were observed to directly pull fragments from the embryo. Fragmentation occurred significantly less frequently in embryos without PVT compared with PVT (81/121 [67%] versus 388/404 [96%]; $P < 0.001$). These data suggest an association between PVT and fragmentation. This study is limited in that PVT were not characterized so their nature and origin remain unknown and to be determined in future studies.

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Introduction

Time-lapse technology captures images every 5–15 min throughout embryo development in culture, allowing for the identification of morphological features that may not be possible to detect through the traditional daily observations of embryos in a microscope outside the incubator. Some of these features have been shown to be associated with embryo viability, such as multinucleation (Desai et al., 2014), reverse cleavage (Desai et al., 2014) and direct cleavage from one blastomere to three or more blastomeres (Zhan et al., 2016).

The EmbryoScope™ culture system to culture human IVF-derived embryos in a clinical practice was used, and perivitelline thread-like structures were observed. Previously unpublished, perivitelline threads (PVT) are projections that extend across the perivitelline space connecting the zona pellucida with the oolemma or blastomere membrane. Anecdotal observations have suggested a possible relationship between PVT and fragmentation.

Fragmentation has been associated with irregular cell division (Prados et al., 2012), initiation of apoptosis in neighbouring cells leading to disruption of blastulation (Sathananthan et al., 1999; Alikani, 2005; Stone et al., 2005; Keltz et al., 2006) and loss of cytoplasmic volume (Alikani et al., 1999; Hardy et al., 2002; Keltz et al., 2006) associated with a reduction in implantation potential (Kovacic et al., 2004). Therefore, fragmentation is an established marker of viability used in most IVF laboratories as part of their embryo selection procedures.

It is proposed that selecting euploid embryos for transfer increases pregnancy rate and decreases abortion rate (Sermon et al., 2016), suggesting that identification of chromosomal euploidy, by using preimplantation genetic screening, can be a useful marker of viability, in addition to implantation potential. Recent studies have demonstrated that morphokinetic features observed during routine clinical culture, are correlated with ploidy (Campbell et al., 2013; Fragouli et al., 2014; Mumusoglu et al., 2017). Time-lapse technology is facilitating the identification and monitoring of morphokinetics, allowing for the assessment of the impact of these features on implantation potential and ploidy.

As PVT have not been reported, their prevalence and clinical significance is unknown.

This preliminary, retrospective observational study aims to determine the following: the incidence of PVT in embryos capable of blastulating; the cell stage at which PVT are first observed; and whether a relationship exists between PVT, implantation, ploidy, fragmentation, or both.

Materials and methods

Ethics

This study was conducted in a clinic with full compliance with the Human Fertilisation and Embryology Authority. This observational study assessed images from embryos from patients who consented to the use of time-lapse technology and for images and data to be included as part of the quality control process to continually improve embryo selection procedure. According to our clinic's research policy ethical approval was not required for this study.

Table 1 – Inclusion and exclusion criteria used to select embryos for analysis.

Inclusion criteria	Exclusion criteria
Blastocysts	Has not had transfer, biopsy, or both
Known implantation data, known ploidy status, or both	Unknown implantation
	If transferred earlier than day 5
	If video is inadequate, e.g. poor lighting, whole embryo not visible
	If unable to make accurate assessment of embryo or perivitelline threads.

Embryo cohorts

This retrospective cohort study took place at a private fertility clinic (Boston Place Clinic, The Fertility Partnership, London). Embryos were selected from a database of 6577 embryos cultured between 2013 and 2015 in the EmbryoScope™ incubator (described in Desai et al., 2014). Only embryos capable of blastulating (n = 525), either with known implantation data (KID) only (n = 338), with KID and known ploidy status (n = 11) or of known ploidy status only (n = 176), were selected for this study (**Table 1**). All embryos meeting the inclusion criteria between 13 September 2013 and 14 November 2013 and between 28 November 2014 and 7 March 2016 were assessed. Videos were analysed from insemination to day 6 and included embryos from IVF and intracytoplasmic sperm injection (ICSI) cycles from patients aged 23–46 years. Type of insemination was assessed as a possible confounding factor using the chi-square test.

A KID-positive embryo is defined as equal or more fetal hearts being observed at 6 weeks via ultrasound scan as the number of embryos transferred, whereas KID negative embryos are defined as transferred embryos not leading to a fetal heart. Ploidy was assessed via Next Generation Sequencing at Reprogenetics UK. Euploid rate is defined as proportion of euploid embryos over total number of embryos with detectable amplification.

To avoid bias, the practitioner assessing embryos for PVT was blinded to ploidy and implantation status. A second operator verified 50 videos for both the presence of PVT and their association with fragmentation. High inter-operator agreement was reached on identification of PVT, association with fragmentation and cell stage (**Table 2**).

Table 2 – Kappa agreement analysis between two observers. Agreement defined as; 'good agreement' = Kappa coefficient 0.6–0.8, 'very good agreement' = Kappa coefficient 0.8–1.0, 'perfect agreement' = Kappa coefficient 1.0.

Parameter	n	Relative observed agreement	Kappa coefficient	Agreement
Presence of perivitelline threads	56	0.96	0.84	Very Good agreement
Association with fragmentation	48	0.98	0.66	Good agreement
Cell stage	46	1	1	Perfect agreement

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