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Pregnancy and perinatal outcomes after transfer of binucleated or multinucleated frozen–thawed embryos: a case–control study

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KEY MESSAGE

Acceptable pregnancy results and healthy newborns are obtained after transfer of embryos with binucleation or multinucleation. Binucleation or multinucleation in frozen–thawed embryos *per se* would not be considered a contraindication for cryopreservation or embryo transfer, especially if embryos with normal nuclei are not available.

A B S T R A C T

Blastomere multinucleation in human embryos is a common phenomenon, but data on its effect on pregnancy outcome and the health of newborns are scarce. In this case–control study, we assessed pregnancy and perinatal outcomes from 136 binucleated and multinucleated frozen–thawed embryo transfer cycles against a control group of 136 non-binucleated and multinucleated frozen embryo transfer cycles. Clinical pregnancy and live birth rates were lower among the case group (29.4% versus 44.1%, $P = 0.012$; 22.1% versus 36.0%, $P = 0.011$, respectively), but perinatal outcomes (gestational week at delivery, birth weight, placental weight and occurrence of congenital anomalies) were similar. Live birth rates among patients receiving embryos with multinucleation compared with binucleation was not significantly different (24.7% versus 13.2%). Consequently, frozen–thawed cleavage-stage embryos with bi- or multi-nucleation have lower than normal but still acceptable implantation potential and ability to produce healthy pregnancies and newborns. The study is limited by its retrospective nature. Time-lapse monitoring would be a more sensitive method of detecting multinucleation. Controls and cases were matched only by age at the time of oocyte retrieval, and other characteristics were only interpreted statistically. Although larger than previously reported, the number of cases is limited.

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Introduction

A significant proportion of cleaving embryos exhibit blastomere multinucleation, often in a transient manner. Multinucleation is related to factors contributing to poor embryo development, for instance, delayed or uneven cleavage and high degree of fragmentation, as well as impaired blastocyst formation (Egashira et al., 2015; Hardarson et al., 2001; Van Royen et al., 2003; Yakin et al., 2005). Multinucleation is found to decrease implantation potential, but the degree of the negative effect is under debate. Some suggest that multinucleated embryos should be automatically discarded, whereas others assert that, in the absence of good-quality embryos, multinucleated embryos could be transferred (Jackson et al., 1998; Van Royen et al., 2003; De Cássia Savio Figueira et al., 2010; Fauque et al., 2013; Yilmaz et al., 2014; Aguilar et al., 2016; De Desch et al., 2016).

Morphological features of the oocyte and the developing embryo and their correlation with live birth rate (LBR) and the health of the newborns is of growing interest (Desai et al., 2016; Shaw-Jackson et al., 2014). Aneuploidy is suggested to be more common in multinucleated embryos than in mononucleated and binucleated embryos (Kligman et al., 1996; Meriano et al., 2004; Yilmaz et al., 2014). Although chromosomal abnormalities usually result in developmental arrest in cleaving embryos, concerns regarding potentially increased risk for congenital anomalies and disorders in pregnancies arising from multinucleated embryos have been raised. Only a few reports of patient series after a transfer of either fresh or frozen–thawed multinucleated embryos have been published, all with deliveries of healthy newborns (Balakier et al., 2016; Egashira et al., 2015; Hashimoto et al., 2015; Yilmaz et al., 2014), although two cases of conjoined twins have also been reported (Mankonen et al., 2015; Serapinas et al., 2016). Therefore, the systematic evaluation of the effect of multinucleation on the health of newborns is scarce.

In this case–control study, we aimed to evaluate pregnancy and perinatal outcomes after transfer of binucleated and multinucleated frozen–thawed embryos. We hypothesized that the pregnancy and perinatal outcomes would justify the transfer of binucleated and multinucleated embryos. A subanalysis of the pregnancy results comparing transfers of binucleated with multinucleated embryos was also conducted.

Materials and methods

This study included 1335 frozen–thawed embryo transfers (FET) that were carried out at The University Hospital of Turku, Finland, between January 2009 and December 2015. Laboratory documents were searched for single- and double-embryo transfers, with exclusively binucleated and multinucleated embryos to form the case group. Altogether, 136 (10.2%) binucleated and multinucleated FET derived from 129 IVF and intracytoplasmic sperm injection (ICSI) stimulations in 124 women were identified. For each binucleated and multinucleated FET, a control with a transfer of mononucleated embryos was randomly selected among women matching for age at the time of oocyte retrieval. A total of 136 mononucleated FET in 136 women formed the control group. Factors known to contribute to the success of assisted reproductive technologies (ART) were recorded. Characteristics of the study populations and FET cycles are presented in **Table 1**. Ethical approval was obtained for retrospective review of data collected during clinical IVF–ICSI treatments from the Ethical Committee of the Hospital District of Southwest Finland on 15 April 2014 (reference no. 34/1801/2014).

Table 1 – Demographics of women and cycle characteristics.[a]

Variable	Cases (n = 136)	Controls (n = 136)
Female age at oocyte retrieval, years	32.3 (4.2)	32.5 (4.3)
Body mass index (kg/m^2)	24.1 (3.9)	24.0 (3.7)
Duration of infertility at oocyte retrieval (months)	47.7 (23.9)	47.4 (30.9)
Primary infertility, n (%)	71 (52.2)	89 (65.4)
Previous pregnancies, n	0.8 (1.2)	0.6 (1.0)
Previous deliveries, n	0.3 (0.6)	0.3 (0.6)
Antral follicle count	21 (10.4)	19 (9.2)
Cause of infertility, n (%)		
Female	58 (42.6)	64 (47.1)
Male	42 (30.9)	32 (23.5)
Combined	23 (16.9)	15 (11.0)
Unknown	12 (8.8)	24 (17.6)
Not available	1 (0.7)	1 (0.7)
Long stimulation protocol, n (%)	76 (55.9)	75 (55.1)
Live birth from fresh embryo transfer, n (%)[b]	30/125 (24.0)	24/124 (19.4)
All embryos frozen (n)	11	12
FET during natural menstrual cycle, n (%)	79 (58.1)	95 (69.9)
FET during artificial cycle, n (%)	55 (40.4)	41 (30.2)
FET during ovulation induction, n (%)	2 (1.5)	0
Average number of transferred embryos	1.1 (0.3)	1.1 (0.3)
Single embryo transfer	121/136 (89.0)	119/136 (87.5)
FET at cleavage stage, n (%)	130 (95.6)	126 (92.6)
Completely survived embryos, n (%)	116/151 (76.8)	118/153 (77.1)
Normally cleaved embryos, n (%)	77/122[c] (63.1)	74/109[c] (67.9)
Detection of binucleated and multinucleated enbryos		
Two- cell stage	1/151 (0.7)	
Four-cell stage	63/151 (41.7)	
Eight-cell stage	87/151 (57.6)	

[a] Values are mean (SD) unless otherwise indicated.
[b] From mononucleated embryos.
[c] Number of embryos cultured overnight after thawing.
FET, frozen embryo transfer, multinucleated, multinucleation.

Embryo freezing and thawing

At the cleavage stage, embryos with less than 25% fragmentation or difference in blastomere size were considered suitable for freezing. Binucleated and multinucleated embryos were frozen if these criteria for cryopreservation were met. Freezing was carried out predominantly after the second cleavage at the four-cell stage (74.2% of cases and 78.4% of controls). In a minority of cases, freezing was carried out after the third (19.2% and 16.3%, respectively) or first cleavage (3.3% and 2.6%, respectively) or at the zygote stage (3.3% and 2.6 %, respectively). Embryos were frozen using a slow freezing cryopreservation kit according to the manufacturer's protocol (Sydney IVF Cryopreservation kit, K-SICS-5000, Cook Australia years 2009–2013 and Freeze Kit Cleave, 10166, Vitrolife Sweden years 2014–2015). The cooling rate was controlled with a freezer (Planer Kryo 10-MRV, Planer PLC, Sunbury_on_Thames, UK). Thawing was carried out by rapid warming in a 30°C water bath and rehydration in a series of media with decreasing cryoprotectant concentrations according to

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