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Multi-nucleation in human embryos should not be used as a marker for their elimination from candidates for transfer

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Study question: Is the appearance of multinucleated blastomeres (MNBs) a marker for developmental failure of human embryos?

Summary answer: Appearance of MNBs is not always a predictor of chromosomal aneuploidy. The blastulation rate of embryos with MNBs is similar to that of embryos without MNBs. Blastocysts developed from embryos with MNBs have a full term developmental competence to healthy babies.

What is known already: MNBs are frequently observed in human embryos with poor morphology. Its appearance would be associated with low pregnancy rates following transfer probably due to chromosomal aberration or chromosome mosaicism. Although such morphological abnormalities implicate developmental failure of embryos, it remains unknown whether these properties lead to aneuploidy and implantation failure.

Study design, size, duration: This study was approved by the ethical committee of the Japan Society of Ob/Gy. We assessed relationships between the appearance of MN in human embryos and their development in vitro using 139 donated embryos and in vivo by retrospective analysis of time-lapse images of 46 embryos which were singly transferred.

Participants/materials, setting, methods: Donated pronuclear ova were injected with a mixture of mRNAs encoding EGFP- α -tubulin and mRFP1-histone-H2B. Dynamic changes of their chromosomes were monitored continuously using a confocal microscope inside an incubator for 120 h. Chromosome analysis was performed using microarray-CGH. Time-lapse images of embryos in clinical study were captured using PrimoVision (Vitrolife).

Main results and the role of chance: Fluorescent imaging study revealed that 74% of embryos (80/108) showed MNBs at 2-cell stage after RNA injection. Nevertheless, embryos with MNBs developed to the blastocyst stage (45%, 36/80) and

morphologically good blastocysts (15%, 12/80). These values were similar to those obtained from embryos without MNBs (blastulation rate 50%, good blastocyst rate 18%). The duration between first and second cleavage was 8.5-12.0 h for embryos with MNBs that developed to the blastocyst stage. Seventy percent of blastocysts developed from embryos with MNB were found to be euploid (14/20). This value was also similar to that of embryos without MNBs (75%). In clinical study, sixteen embryos (35%) with MNBs transplanted following transfer and four healthy babies were born at the present time (January 5, 2015).

Limitations, reasons for caution: Multi-nucleation of embryos at 2-cell stage which had normal developmental competence appeared late at night. Thus, it is difficult to observe multi-nucleation of normal embryos based on conventional observation without time-lapse system. Further studies are required to make critical evaluation of normal and abnormal embryos showing multi-nucleation.

Wider implications of the findings: This study suggests that the appearance of MNB at first mitosis does not directly correlate with chromosomal aneuploidy and subsequent embryo development. It would be of negligible significance to assess the presence of MNBs compared with the observation of timing of mitosis.

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