Title: MULTINUCLEATION PER SE IS NOT ALWAYS SUFFICIENT AS A MARKER OF ABNORMALITY TO DECIDE AGAINST TRANSFERRING HUMAN EMBRYOS IN VITRO

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OBJECTIVES: The present work describes dynamic change of chromosomes in human embryos monitored by a confocal laser microscope (CLM) after an injection of mRNAs. We also retrospectively analyzed the clinical data to assess the relationship between the appearance of multi-nuclei in human embryos and their development.

METHODS: This study was approved by the ethical committee of Japan Society of Obstetrics and Gynecology. In confocal imaging study, pronuclear ova donated from couples who had given informed consent (n: 143) were injected with a mixture of mRNAs encoding EGFP-EB1 and mRFP1-histone-H2B. Dynamic changes of their chromosomes were monitored continuously using a CLM in an incubator. Chromosomal analysis was performed using microarray-CGH. In clinical study, time-lapse images of embryos were captured using a light microscope (n: 282). Single embryo transfer was conducted on day 3 based on their morphology.

RESULTS: Abnormally-cleaved embryos at 1st mitosis showed multi-nuclei at least in one blastomere. The developmental competence of abnormally-cleaved embryos was significantly low compared with that cleaved normally in both confocal imaging and clinical studies. Low development of embryos with multi-nuclei was caused by abnormal cleavage.

Confocal imaging study revealed that the presence of multi-nuclei did not affect the rates of blastulation, morphologically-good blastocyst and euploid, showing that chromosomes in multi-nuclei congressed after nuclear envelope breakdown to form a bipolar spindle and equally segregated into two daughter blastomeres. Clinical study revealed that the implantation potential of embryos with multi-nuclei at 2-cell stage was similar to that without multi-nuclei (40% vs. 39%, respectively). Nine healthy babies were born from embryos with multi-nuclei.

CONCLUSIONS: The present work clearly showed that the appearance of multi-nuclei would not always lead to chromosomal aberration and low implantation potential in human embryos.