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Live cell imaging reveals chromosome dynamics during human embryo development

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Introduction: It has been shown that high potential embryos that develop to high-scoring blastocysts can be selected at Day 2/3 by assessment of the synchronization of early cleavage timing of each blastomere using a time-lapse imaging system composed of an individual culture method and a microscope inside the incubator (Hashimoto et al., Fertil Steril 2012). However, it is difficult to observe dynamic change of chromosomes in each blastomere based on only morphological change of embryos using a light microscope. Recently, chromosome dynamics of mouse embryos was observed using a confocal laser microscope (CLM) inside an incubator after an injection of a mixture of mRNAs (Yamagata et al., Hum Reprod 2009). In this study, dynamic change of chromosomes of human embryos was monitored by a CLM inside an incubator after an injection of mRNAs.

Materials and methods: This study was approved by an ethical committee of the Japan society of Obstetrics and Gynecology. All patients gave informed consent. A total of 33 frozen-thawed pronuclear embryos were injected a mixture of mRNAs encoding enhanced green fluorescent protein (EGFP) coupled with α-tubulin (EGFP–α-tubulin) and mRFP1 fused with histone H2B (H2B–mRFP1). Dynamic change of chromosomes of these embryos were monitored using a CLM inside an incubator (Yokogawa Electronic Co., Tokyo, Japan) for 120 h. Time-lapse images of chromosomal changes were captured 4 times per hour over 5 days. Developed blastocysts were categorized morphologically into high- and low-scoring blastocysts as previously reported (Hashimoto et al., Fertil Steril 2011). Two of high scoring blastocysts were tested using microarray-based comparative genomic hybridization (CGH) to assess the euploidy.

Results: Eleven of 33 embryos developed to the blastocysts stage after RNA

injection and capturing images. Of these, five embryos were categorized in high-scoring blastocysts (33.3%). Cleavage timing of each blastomere was synchronized in each high-scoring blastocysts. In embryos developed to low-scoring blastocysts, timing of cell cleavage was not always identical to that of chromosome division. However, in embryos developed to high-scoring blastocysts, timing of cell cleavage was identical to that of chromosome division. Eight of 11 embryos which developed to blastocysts had multinuclear blastomere (MNB) at 2-cell stage. However, five of them developed to high-scoring blastocysts (63%). In this case, a metaphase spindle was formed and was divided equally during mitotic division even in multinuclear blastomeres. CGH showed uniformly euploid in both of high-scoring blastocysts although MNBs were observed at 2-cell stage.

Conclusions: Data of the present study revealed that RNA injection into human embryo and confocal laser scanning for 5 days did not severely impair the developmental competence of the embryos and that chromosome dynamics of during early embryo development were obtained. Even when MNB was observed at 2-cell stage, the embryos developed to high-scoring blastocysts if the embryo cleaved normally.

This work opens new prospective on the analysis of dynamic change of chromosomes of human embryo.

Key words: dynamic change of chromosome, time-lapse image, euploidy, comparative genomic hybridization