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Conforcal live-cell imaging of multinuclear blastomeres in human embryos and embryo development

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Introduction: Multinuclear blastomeres (MNB) are often observed in human IVF embryos at the cleavage stage. However, all the MNB are not always detected in temporary observations using an inverted light microscope. The development of a confocal imaging system that includes an embryo culture system has made it possible to obtain time-lapse images of chromosome dynamics in mice (Yamagata et al., 2010). Using this technology for human embryos, we reported several aspects of chromosome abnormalities including the formation of multinuclei, micronuclei, and abnormal nuclear division. In this study, we observed the chromosome dynamics in embryos from the 2-cell stage to the blastocyst stage using the conforcal live-cell imaging system and investigated the relationship between the presence of MNB in 2- and 4-cell stage embryos and their development.

Material & methods: Thirty-three frozen-thawed pronuclear embryos intended for disposal were used after obtaining the informed consent of the patients and the approval of the Japan Society of Obstetrics and Gynecology research ethics committees. A mixture of mRNAs encoding enhanced green fluorescent protein coupled with α-tubulin and monomeric red fluorescent protein I fused with histone H2B was injected into the cytoplasm of the pronuclear embryos using a Piezo drive manipulator. The pronuclear embryos were cultured in KSOM^{AA} medium under an atmosphere of 5% O_2, 5% CO_2 and 90% N_2. Time-lapse images were captured at 15-minute intervals until the day 5 stage using an all-in-one confocal imaging system.

Results: Out of the 33 pronuclear embryos with injected RNA, 29 embryos (88%) developed to at least the 4-cell stage and 11 (33%) developed to the blastocyst stage. Of the 29 cleavage stage embryos, 5 embryos did not have MNB (17%) at the 2-cell stage and 10 (34%) did not have MNB at the 4-cell stage. Three of the 5 embryos (60%) without MNB at the 2-cell stage developed into blastocysts. At the 4-cell stage, 9 of the 10 embryos without MNB (90%) developed into blastocysts, while only 2 of the 19 embryos with MNB (11%) developed into blastocysts. Of the 11 blastocysts, a higher incidence of MNB (73%, 8/11) was detected at the 2-cell stage, compared to that (18.2%, 2/11) at the 4-cell stage. Seven embryos with MNB at the 2-cell stage did not contain MNB at the 4-cell stage. All the embryos in which one blastomere divided into 3 or more daughter blastomeres with abnormal nuclear divisions had MNB at the cleavage stage and did not develop to the blastocyst stage.

Conclusions: We succeeded in observing the nuclear dynamics of human live embryos until the blastocyst stage using the confocal imaging system. A high incidence of MNB at the 2-cell stage was observed not only among the poor embryos, but also among the viable ones. However, at the 4-cell stage, the embryos without MNB showed a high rate of blastocyst formation. These data suggest that the presence of MNB at the 4-cell stage is closely associated with embryo development.