American Society for Reproductive Medicine （70th Annual Meeting）
Honolulu（USA）、2014.10.18-22

ABNORMAL MITOSIS IN EARLY EMBRYOS AS A CAUSE OF MOSAICISM

Yoshiharu Nakaoka, Shu Hashimoto, Ami Amo, *Kazuo Yamagata, Takao Himeno, Tomoko Inoue, Keijiro Ito, Yoshiharu Morimoto

*IVF Namba Clinic　*Osaka University*

【Objective】

Embryo mosaicism is indicated by the presence of two or more genotypes within cells of a single embryo. The incidence of embryo mosaicism is high, even in morphologically sound embryos, with one report showing that 71% of day 3 embryos were mosaic. This phenomenon results from chromosomal segregation errors and cytokinetic errors during mitosis of an embryo after fertilization. One major cause of embryo mosaicism is abnormal mitosis in which one cell divides into three or more daughter cells. However, the detailed mechanisms of this phenomenon remain largely unknown. Obtaining time-lapse images of live embryos stained with fluorescent proteins enables analysis of the dynamics of nuclei and spindles. In this presentation, we aim to demonstrate the dynamics of the nuclei and spindles using a confocal imaging system.

【Methodology】

Frozen-thawed pronuclear embryos intended for disposal were used after obtaining informed consent from the patients and approval from 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 by using a Piezo-drive manipulator. We used an all-in-one confocal imaging system that contains an incubator with a confocal microscope. Four-dimensional images were generated from three-dimensional confocal images, which were acquired at 6-μm intervals in the z-axis. 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 for 5 days, using the all-in-one confocal imaging system.

【Conclusions】

Abnormal mitoses were observed not only at the first cleavage stage but also at the second or later cleavage stages. Before division into three cells, Y-shaped metaphase plates and tripolar spindles could be observed. However, the Y-shaped metaphase plate did not always lead to abnormal mitosis. The results of this study suggest that abnormal mitosis plays a key role in the occurrence of mosaicism.