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CONFOCAL LIVE CELL IMAGES WITH FLUORESCENT PROTEINS REVEAL MULTINUCLEATION OF MORPHOLOGICALLY GOOD DAY 3 EMBRYOS IMPAIRS SUBSEQUENT DEVELOPMENT

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OBJECTIVE:
Serial observations of morphological changes using time-lapse cinematography have improved the outcome of assisted reproductive technology. However, the relationship between morphological changes and nuclear dynamics is not fully understood.
In this study, we observed the nuclear status of morphologically good day 3 embryos by confocal microscope and investigated the relationship between their morphological changes and their nuclear dynamics from the pronuclear to the blastocyst stage. Chromosomal analysis was also conducted.

DESIGN: Laboratory assessment

MATERIALS AND METHODS: Seventy-two frozen-thawed pronuclear embryos intended for disposal were used, after obtaining the informed consent of the patients and the approval of 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 pronuclear embryos. These embryos were cultured in KSOMaa medium under 5% O_2, 5% CO_2, and 90% N_2 atmosphere. Time-lapse images were captured at 15-min intervals for 5 days using a confocal microscope. Morphology of day 3 embryos was assessed 48 h after initiation of culture. Chromosomal analysis of blastocysts was conducted by array comparative genomic hybridization.

RESULTS: Of the 41 morphologically good day 3 embryos, 28 (68.3%) had multinuclei. Abnormal mitosis in which one cell divided into three or more cells was observed in 7 embryos at the first cleavage (17.1%) and in 6 embryos at the second cleavage (14.6%). Only 13 embryos (31.7%) showed normal nuclear status, and 15 embryos (36.6%) showed multinucleation without abnormal mitosis. Blastocyst formation rate of embryos with

abnormal mitosis was 14.3% (1/7) at the first cleavage and 33.3% (2/7) at the second cleavage. Of the embryos without abnormal mitosis, blastocyst formation rate was significantly higher in embryos with normal nuclear status (84.6%, 11/13) than in those showing multinucleation (26.7%, 4/15). Chromosomal abnormalities were observed in 50.0% (2/4) of the multinucleated embryos and 14.3% (1/7) of the embryos without multinuclei.

CONCLUSIONS:
Serial confocal images with fluorescent proteins revealed that abnormal nucleation was high in morphologically good day 3 embryos. Thus, multinucleation is a crucial determinant for selection of viable embryos on day 3.