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Abnormal spindles in human blastocyst increase with maternal age

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Study question: Does the incidence of morphologically abnormal spindle in human blastocysts increase with maternal age?

Summary answer: The incidence of morphologically abnormal spindle increased and the implantation competence decreased with an increase of maternal age. The appearance of abnormal spindle adversely affected the cell cycle. The increase in morphologically abnormal spindles would be one of the critical causes of age-related infertility.

What is known already: An increase of chromosomal aneuploidy in oocytes with maternal age is the dominant contributor of age-related infertility. However, the infertility could not be explained simply by the increased aneuploidy.

Study design, size, duration: We performed a retrospective cohort study including 727 single vitrified-warmed blastocyst transfers that developed to the blastocyst stage on day 5 after insemination between 2010 and 2012, and 2 experimental studies using 154 day-5 blastocysts and 36 pronuclear embryos donated for research. The local IRB approved this study.

Participants/materials, setting, methods: We analyzed the relationship of the pregnancy rate of vitrified-warmed day 5 blastocysts with maternal age. In experimental studies, vitrified blastocysts were immunostained with anti- α -tubulin antibody, anti- γ -tubulin antibody and DAPI. Pronuclear embryos were injected with cRNA encoding mRFP1 fused with histone H2B.

Main results and the role of chance: The clinical study revealed that the pregnancy rate of day 5 blastocysts on 9 weeks of gestation decreased with maternal age ($R^2 = 0.7982$, $P < 0.0001$). We analyzed spindles of totally 392 specimens. The incidence of morphologically abnormal spindles increased with maternal age ($R^2 = 0.59$, $P = 0.0038$). Dynamic changes of chromosomes of the cRNA-injected embryos were monitored using a confocal microscope inside an incubator. Seventeen embryos developed to blastocysts after imaging (47.2%). More than half embryos appeared to form abnormal spindles and multi-nuclei. The cell cycles of such blastomeres were

found to delay or arrest. Comparative genomic hybridization (CGH) analysis showed that 12 blastocysts were euploid (70.6%) though morphologically abnormal spindles were observed during their development.

Limitations, reasons for caution: Further studies are required to clarify the relationship between an increase in morphologically abnormal spindle and a decrease in embryonic implantation potential.

Wider implications of the findings: This study provides new insights into the implications of a decrease of fertility with maternal age.

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