O-154 First mitotic spindle formation led by sperm centrosome-dependent microtubule organising centres may cause high incidence of zygotic division errors in humans

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Study question: Why do multinucleated blastomeres appear at high frequency in two-cell-stage embryos in humans?

Summary answer: Failure in microtubule assembly during the first mitotic spindle body formation by sperm centrosome-dependent microtubule organising centres (MTOCs) may lead to chromosomal instability.

What is known already: Unlike that in mice, multinucleated blastomeres appear at high frequency in two-cell-stage embryos in humans. However, the underlying mechanism remains elusive. In mice, multiple acentriolar MTOCs appear around the male and female pronuclei after pronuclear disappearance and contribute to dual-spindle formation, engulfing each parental chromosome. This spindle formation may ensure an error-free division, keeping the chromosomes stable during the first cleavage, as observed in mice, but it is unclear whether a similar mechanism exists in humans.

Study design, size, duration: To examine how sperm centrosomes contribute to MTOC formation in humans, two types of 3PN zygotes derived fromeither conventional *in vitro* fertilization (c-IVF, n = 30) or intracytoplasmic sperm injection (ICSI, n = 10) were used. The zygotes were collected from October 2018 to January 2020. MTOC and mitotic spindle formation at consecutive stages of development during the first cleavage were analysed under static and dynamic conditions using immunofluorescence assay and fluorescent live-cell imaging.

Participants/materials, setting, methods: Under ethics approval, 3PN zygotes were donated by infertile couples undergoing c-IVF or ICSI cycles at the Yamashita Shonan Yume Clinic in Japan. All participants provided informed consent. Immunofluorescence assay was performed using antibodies against αtubulin, pericentrin, and H3K9me3 after fixation with MTSB-XF solution. Fluorescent live-cell imaging was performed using TagGFP2-H2B mRNA (chromosome marker) and FusionRed-MAP4 mRNA (microtubule marker).

Main results and the role of chance: Immunofluorescence revealed that while 3PN zygotes derived from c-IVF showed four pericentrin dots, those derived from ICSI exhibited two pericentrin dots. In pro-metaphase, an independent group of chromosomes derived from each pronucleus and MTOCs were formed by the sperm centrosome at the core. Microtubules from each MTOC extended toward the chromosomes in the early metaphase; a quadrupolar spindle was formed in the c-IVF-derived zygotes, and a bipolar spindle was formed in the ICSI-derived zygotes by the MTOCs at the zygote apex after chromosome alignment. In pro-metaphase, the microtubules extended from the MTOCs to the nearest chromosome. Since microtubule assembly was found on oocyte-derived chromosomes, we hypothesised that whether a chromosome is surrounded by microtubules depends on the location of the MTOCs, irrespective of its origin. Live-cell imaging of histone H2B and MAP4 revealed that four MTOCs appeared around the three pronuclei just before the disappearance of the pronuclear membrane; microtubules then extended from the MTOCs toward the chromosomes, beginning to form a mitotic spindle as the chromosomes moved to the centre of the oocyte. Interestingly, one of the three assembled chromosome groups showed no microtubule assembly in the pro-metaphase. Similar results were obtained in all six 3PN zygotes subjected.

Limitations, reasons for caution: We demonstrated the high risk of developing bare chromosomes not surrounded by microtubules during the formation of the first mitotic spindle, using human tripronuclear zygotes. However, owing to unavailability of normal fertilized oocytes for this study because of the clinical use, we were unable to confirm this in normal zygotes.

Wider implications of the findings: Although two sperm centrosome-dependent MTOCs are expected to be formed in normal fertilized oocytes, these MTOCs are not sufficient to completely enclose physically separated female and male chromosomes with the microtubules. This explains the high frequency of zygotic division errors that lead to unstable human chromosomes.

Trial registration number: not applicable