Assessment of Responsiveness and Sensitivity of the Spindle Assembly Checkpoint during the First Cell Division of the Human Embryo

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Human pre-implantation embryos frequently exhibit chromosomal abnormalities with the majority arising during the first mitotic divisions. In somatic cells, the spindle assembly checkpoint (SAC) ensures proper chromosome segregation during mitosis. The chromosomal passenger complex (CPC) ensures correction of non-bipolar kinetochore-microtubule attachments and signals the SAC to initiate anaphase onset, thereby preventing chromosome missegregation together. However, whether the SAC is fully operational in the human zygote is uncertain. Human zygotes have a pronounced epigenetic asymmetry for trimethylation of histone H3 at Lysine 9 (H3K9me3) on paternal (sperm) and maternal (oocyte) chromatin. Maternal chromatin shows global H3K9me3-enrichment whereas on paternal chromatin it is only retained at the pericentromeric regions. CPC localization to the inner centromeres is important for its function. A previous study from our group observed H3K9me3-rich maternal chromatin to have an advantage in CPC recruitment. This raises the question if paternal chromosomes may be more prone to misalignment. We aim to assess SAC responsiveness and sensitivity during the first cell division of the human embryo and whether differences exist in susceptibility to misalignment between maternal and paternal chromosomes.
This study utilized human surplus mono- and tripronuclear zygotes with approval from the Dutch Central Committee on Research Involving Human Subjects (CCMO, NL38053.000.11/ NL82597.000.22). Zygotes were cultured in a time-lapse incubator (EmbryoScope, Vitrolife) in culture medium with or without low-dose (5nM) or high-dose (25 or 50nM) GSK923295, or 62.5nM Cpd-5. Zygotes were imaged throughout the first cell cycle or fixed in 4% formaldehyde (MeOH-free) one hour post nuclear envelope breakdown for immunofluorescent staining for the centromeres, centrosomes, H3K9me3 and microtubuli. Spindles were imaged using the Leica SP8 STED Stellaris confocal microscope (Leica Microsystems) and 3D-analysed using Huygens software (Scientific Volume Imaging, The Netherlands). First, the kinetochore-binding microtubule motor protein CENP-E was inhibited using GSK923295 to disrupt chromosome congression. Analysis of fixed spindles showed that the average percentage of misaligned chromosomes increased from 6.2% in the controls (n=6) to 8.5% in low (n=8) and 36.6% in high-dose (n=7), respectively. No difference in misalignment frequency was found when differentiating between maternal and paternal chromosomes. Time-lapse imaging of the first cell cycle revealed no difference in cell cycle progression between the control (n=21) and low-dose (n=19) condition, but a severe delay or complete arrest at M-phase was observed at the high-dose conditions (n=21). Second, a low concentration Cpd-5, an inhibitor of mitotic kinase Mps1 responsible for recruiting components for SAC assembly, compromises SAC establishment and attachment error correction in somatic cells. In zygotes, low dose Cpd-5 resulted in early anaphase onset in 30% of the analysed fixed zygotes and induced misaligned chromosomes in 50% (n=12). Preliminary findings reveal a higher proportion of misaligned

paternal chromosomes. However, more spindles should be analysed to confirm the susceptibility of paternal chromosomes to misalign. To conclude, this study demonstrates that the SAC is responsive in the first cell division of the human embryo. However, the sensitivity of the SAC seems low as only high proportions of misaligned chromosomes cause a delay or arrest at M-phase. Besides, our preliminary data suggest Mps1 inhibition increases the susceptibility of paternal chromosomes to misalign, but not CENP-E inhibition. This is in line with our previous findings that paternal chromosomes have a disadvantage in recruiting CPC proteins and are thereby more prone to misalignment which seems independent of congression by CENP-E.