



**P-193 First cleavage division perpendicular to the pronuclear axis adversely affects the clinical outcome in human embryos**

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**Study question:** Does the direction of formation for the first cleavage plane relative to the pronuclear axis affect clinical outcome?

**Summary answer:** A first cleavage division perpendicular to the pronuclear axis adversely affects the rate of embryo utilization for transfer or cryopreservation and the pregnancy outcome.

**What is known already:** It remains unclear how the first cleavage plane is determined in human embryos. By using time-lapse monitoring, our previous study (presented in ESHRE 2019) suggested that both the axis and locations of male and female pronuclei are involved in determining the first embryonic cleavage plane. Furthermore, by using immunofluorescence analysis, it was also revealed that most analyzed zygotes showed two pericentrin signals aligned around the interface between the male and female pronuclei. Our findings suggest that the pronuclear axis strongly influences the positions of the centrosomes, which become mitotic spindle poles and define the first cleavage plane. Study design, size, duration: From January 2015 to December 2017, time-lapse imaging (EmbryoScope®) of 3397 intracytoplasmic sperm injection (ICSI) oocytes was conducted. Of those, the relationship between the pronuclear axis and the first cleavage plane was analyzed in 607 normally fertilized embryos that cleaved to two cells and were obtained in 2015. Furthermore, of 3397 ICSI oocytes, 749 transferred embryos were classified based on the first cleavage patterns relative to the pronuclear axis, and the pregnancy rate was examined.

**Participants/materials, setting, methods:** A straight line connecting the centers of the pronuclei was defined as the 2PN axis. Based on the direction of the first cleavage relative to the 2PN axis, embryos were classified into three groups: parallel, perpendicular and intermediate. Fresh embryos were transferred on Day 2/3 (fresh-ET). Frozen and thawed embryos were transferred on Day 2/3 or Day 5 (F/T-ET). Clinical pregnancy was defined as confirmed gestational sac in the uterine cavity.

**Main results and the role of chance:** Of 607 analyzed embryos, 506 produced suitable images and were assigned to one of three groups: parallel (84.4%, n=427), perpendicular (9.7%, n=49) and intermediate (5.9%, n=30). Embryos that formed a cleavage furrow parallel to the 2PN axis were significantly more frequent than others (perpendicular, intermediate) ( $P < 0.001$ ). The embryo utilization rate for transfer or cryopreservation was significantly lower in the perpendicular group than in the parallel group (30.7% vs. 69.3%,  $P < 0.01$ ). Furthermore, of 749 transferred embryos, 504 assigned to the parallel and perpendicular groups were selected (n=470 and n=34, respectively), and the pregnancy outcome was analyzed. The mean maternal age was not significantly different between groups. The pregnancy rate of embryos was 24.2% (n=45/186) from fresh-ET and 39.4% (n=112/284) from F/T-ET in the parallel group, and 0% (n=0/14) from fresh-ET and 15.0% (n=3/20) from F/T-ET in the perpendicular group. Regardless of the types of embryo transfer (fresh or F/T), the pregnancy rate was significantly lower in the perpendicular group than in the parallel group ( $P < 0.01$ ). In addition, one of three patients who became pregnant from the transfer of an embryo in the perpendicular group had a miscarriage.

**Limitations, reasons for caution:** Since only ICSI embryos were analyzed in this study, the influence of fertilization methods on subsequent development could not be investigated. Further studies including preimplantation genetic testing for aneuploidy may help determine the reasons why pregnancy rates differ between groups.

**Wider implications of the findings:** We suggest that the 2PN axis is essential for determining the first cleavage plane because it seems to be involved in positioning the mitotic spindle poles. The direction of the first cleavage plane relative to the 2PN axis can be an important indicator for predicting embryo development and pregnancy outcome

**Trial registration number:** none