P-216 Successful pregnancies and deliveries in patients with a recurrent failure of ART treatments following artificial removal of the zona pellucida (ZP) at the pronuclear stage

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Study question: Can a novel embryo culture method that artificially removes the ZP at the pronuclear stage yield successful pregnancy in patients with poor-quality embryos and/or blastocysts?

Summary answer: A blastocyst transfer after ZP-free culture can result in pregnancy for patients who cannot obtain good quality blastocysts from conventional culture methods.

What is known already: Perivitelline threads are been associated with the formation of cytoplasmic fragments. We had previously observed perivitelline threads in the adhesive region between the ooplasm and the ZP at the first cleavage in human embryos. We removed the ZP at the pronuclear stage in 71 abnormally fertilized oocytes (zygotes with three pronuclei), donated after conventional IVF (c-IVF), and termed them ZP-free 3PN. We found ZP-free 3PN embryos could be cultured without losing blastomere adhesions. Furthermore, the rate of good quality embryos was significantly higher in ZP-free 3PN embryos compared with ZP-intact embryos (ZP-intact 2PN/2PB and 3PN embryos; $P < 0.05$).

Study design, size, duration: This study was conducted in two cases selected among patients who underwent ART treatment in our clinic between 2018 and 2019. Cases were selected if they lacked good quality blastocysts in previous c-IVF/Intracytoplasmic Sperm Injection (ICSI) cycles due to massive cytoplasmic fragmentation at the first and second cleavage. We performed a clinical trial of ZP-free culture from December 2019 to March 2020.

Participants/materials, setting, methods: Two cases were selected for this trial. Normally fertilized oocytes were grouped as ZP-free or ZP-intact. For the ZP-free group, 2PN embryos were placed in 0.125M sucrose-containing HEPES to reduce ooplasm size, then ooplasms were completely separated from ZPs by a laser and pipetting. ZP-free and ZP-intact embryos were cultured with time-lapse imaging for up to seven days. Resultant blastocysts were either transferred into uterus or cryopreserved on Day5/6/7 for future embryo transfer cycles.

Main results and the role of chance: The ZP-free culture method was applied to two patients (patient A and B) with recurrent failure of ART in our clinic due to poor-quality embryos and/or difficulties in obtaining good quality blastocysts. In both cases, blastocysts were successfully obtained and cryopreserved for all ZP-free culture cycles. In patient A, one good quality ZP-free blastocyst was freshly transferred five days after oocyte retrieval, and a live male baby (2925g) was delivered at 40 weeks of gestation by caesarean section). In patient B, a frozen/thawed ZP-free blastocyst transfer was conducted, and a live female baby (3225g) was delivered at 39 weeks of gestation by vaginal delivery. This shows ZP-free culturing may help obtain viable embryos in patients for which conventional in vitro culturing methods result in embryos characterized with severe cytoplasmic fragmentation and poor quality in the early cleavage stage.

Limitations, reasons for caution: Although successful pregnancies and deliveries were confirmed in two cases, postnatal evaluations will be absolutely necessary for infants derived from ZP-free culture. In addition, the number of trial cases needs to be expanded, however careful selection of suitable patients is necessary for this novel culture method.

Wider implications of the findings: We found removing the ZP at the pronuclear stage improved embryo development and led to successful pregnancies and deliveries after blastocyst transfer. This indicates ZP-free culturing may be an effective method for decreasing cytoplasmic fragmentation caused by perivitelline threads or adhesion between the ooplasm and the zona pellucida.

Trial registration number: not applicable