## P-248 Statistical estimation for incidence of blastocyst trophectoderm vesicles (TVs) and efficacy of assisted hatching (AH)

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**Study question:** The aim of this study is to analyse the association between blastocyst diameter and TVs development, and to examine the efficacy of AH. **Summary answer:** Blastocysts with a diameter of more than 170 μm leads to high incidence of TVs and AH applied from the incidence should be effective. **What is known already:** TVs are protrusion of trophectoderm cells often observed in expanding blastocyst stages. TVs can be observed in expanding blastocysts regardless of Intracytoplasmic sperm injection (ICSI) and Conventional-IVF (C-IVF), when the internal pressure of blastocysts increase. The rate of TVs incidence in blastocysts inseminated by ICSI is higher than that by C-IVF, due to penetration of the needle into the zona pellucida. Moreover, it has been reported that TVs may inhibit blastocyst hatching. However, the developmental timing of TVs is still unclear, and there is no study that has analysed the association between blastocyst diameter and the incidence of TVs.

**Study design, size, duration:** I) Diameters and TVs incidence of blastocysts by ICSI and C-IVF were measured, and the cut-off value and the area under the curve (AUC) of the receiver operating characteristic (ROC) curve were calculated to estimate the timing of TV incidence. 2) We analysed the clinical pregnancy rates of blastocysts with TVs treated by AH compared to those of blastocysts by C-IVF not subjected to AH.

**Participants/materials, setting, methods:** This study included 821 transferred frozen blastocysts ranging from March 2018 to November 2019. The embryos were cultured in a dry incubator after insemination by ICSI or C-IVF. Blastocyst freezing conditions were set at day5 to day7 with a diameter of more than 150  $\mu$ m in inner diameter of zona pellucida, and this was measured before freezing. The ROC curve was performed using EZR statistical analysis software.

Main results and the role of chance: I) The incidence of TVs in blastocysts by ICSI and C-IVF was 27.5% (117/424) and 14.6% (58/397) respectively. The rate of the incidence of TVs in blastocysts inseminated by ICSI and C-IVF; 8.6% (12/140) and 0.95% (1/105) in 150-159  $\mu m$ , 12.7% (14/110) and 8.2% (6/73) in 160-169  $\mu m$ , 40.6% (28/69) and 10.5% (6/57) in 170-179  $\mu m$ , 55.6% (30/54) and 25.5% (13/51) in 180-189  $\mu m$ , 66.7% (20/30) and 35.7% (10/28) in 190-199  $\mu m$ , and 68.4% (13/19) and 26.8% (22/82) in the diameter of more than 200  $\mu m$ . The cut-off value of the ROC curve was respectively 170  $\mu m$  (sensitivity 78.6% and specificity 73.0%) and 176  $\mu m$  (sensitivity 84.5% and specificity 59.6%) in the diameter; the AUC was 0.8 [95%CI:0.752-0.848] and 0.74 [95%CI:0.687-0.793] respectively. 2) The clinical pregnancy rate of TVs blastocyst vs C-IVF blastocyst was 52.7% (88/167) vs 57.8% (37/64) respectively. There is no significant difference between the two clinical pregnancy rates (P=0.556).

**Limitations, reasons for caution:** The findings of this study have to be seen in light of some limitations. Since this study aimed to analyse the incidence of TVs based on blastocyst size, we did not take into account the grade according to the Gardner classification and the number of trophectoderm cells.

Wider implications of the findings: Blastocysts inseminated by ICSI and C-IVF were highly likely to have TVs above 170  $\mu$ m and 176  $\mu$ m respectively. The clinical pregnancy rates of the blastocyst with TV treated by AH was similar to those of the C-IVF blastocyst.

Trial registration number: not applicable