

**P-253 Description of a rare spontaneous monozygotic blastocyst splitting into two discrete euploid blastocysts in vitro detected with time-lapse imaging and preimplantation genetic testing (PGT)**

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**Study question:** Can spontaneous and complete blastocyst splitting into two, *in vitro*, be investigated using time-lapse imaging and biopsy of each trophoctoderm, for inference of ploidy?

**Summary answer:** Time-lapse imaging combined with PGT-A gives insights into the incidence, dynamics and timing of rare blastocyst splitting and the ploidy status of each resulting blastocyst.

**What is known already:** It is well known that multiple births occur more often with Assisted Reproductive Technologies (ART) than spontaneous conception, even after single embryo transfer. The mechanism of Monozygotic Twinning (MZT) during ART is still unclear but cryopreservation, extended culture, PGT, maternal age and assisted hatching are reported risk factors. MZT is a rare phenomenon, with an incidence of 0.4% in natural conception compared with up to 4.9% in ART. The timing of embryo splitting dictates the type of twinning, in terms of chorionicity and amnionicty, and this is officially determined using ultrasound scanning.

**Study design, size, duration:** This is a case study describing the detection of the complete splitting of an IVF blastocyst at 140 hours post insemination (hpi), using time-lapse imaging.

The 40-year-old patient previously experienced biochemical pregnancy and several miscarriages; an ectopic molar pregnancy and a probable cornual ectopic. The 39-year-old male partner was normozoospermic.

**Participants/materials, setting, methods:** Facilitative laser breaching was performed, according to standard operating procedure, of the morula at 96hpi of embryo development, prior to PGT. Images were collected every 10 minutes and developmental events and embryos morphology annotated using the EmbryoScope+™ time lapse incubator and software.

**Main results and the role of chance:** Over 50,000 hatching blastocysts have been time-lapse imaged, scrutinised and annotated within this group of fertility clinics. This is the first time that such a rare blastocyst splitting event has been recorded and studied.

Following observation of two pronuclei following IVF and typical cleavage development to blastocyst, with facilitative zona breaching on, at 106.7hpi, the full blastocyst's trophoctoderm (TE) began to herniate and hatch. By 114.3hpi a second internal blastocoel cavity formed appearing to divide the inner cell mass (ICM) within the *zona pellucida* (ZP). This resulting blastocyst proceeded to hatch as its discrete ICM migrated out of the ZP, along with its TE. TE cells from the original blastocyst then began to hatch at 117.5hpi at the same breached site in the ZP with its ICM visibly evacuating the ZP.

By 140hpi the blastocyst had split into two discrete blastocysts while hatching from the ZP. Both resulting blastocysts had clear and separate ICMs and TEs present. Biopsy of approximately 5 cells was performed for each TE, and the blastocysts were vitrified individually. Next Generation Sequencing (NGS) reported both blastocysts to be euploid.

**Limitations, reasons for caution:** This case may have been detectable without time-lapse imaging, as the splitting was completed prior to biopsy. More expert scrutiny of the images may result in earlier signs of twinning in progress being detected.

**Wider implications of the findings:** The nature of this detectable *in vitro* blastocyst splitting, indicates these embryos (if they implanted) to be monozygotic, dichorion-diamniotic 'identical' twins. However – as single embryo transfer is the preferred treatment plan; they may be born years apart. These observations could shed light on the debated models of monozygotic twinning.

**Trial registration number:** not applicable