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Article

Early Spontaneous Twinning Recorded By Time-Lapse

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Abstract

Monozygotic twins (MZT) are 2.5 times more frequent in ART than in natural conceptions. A number of ART-related mechanisms have been probably linked with MZT. Studies that retrospectively analyze the time-lapse (TL) records resulting in MZT suggest that some morphokinetic traits of the inner cell mass and the trophectoderm could be predictors of MZT, but results are controversial. We present the complete TL record of one case of MZT that split itself at the very moment of the division into two cells, with one of the cells coming out through a hole in the zona pellucida (ZP). Both resulting embryos developed normally, and were vitrified. It is suggested that the hole in the ZP may facilitate the extrusion of some cells of the <day 4 embryo and that this cell development is not constrained by being inside the ZP. Despite the lack of the inhibition of the ZP itself or the influence of the other embryo cells, the totipotent cell was then able to develop correctly from the start. Moreover, the embryo inside the ZP compensated for the loss of this cell apparently without problems. Our findings are discussed in the context of previous literature and ethical problems are addressed.

Keywords: Blastocyst; cleavage–stage embryo; embryo splitting; embryonic development; monozygotic twins; time-lapse recording

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Twin pregnancies occur in near 1/90 natural pregnancies (Matorras et al., 2005), but are much more common in assisted reproductive technique (ART) pregnancies. There are two different types of twin pregnancies: dizygotic twins (DZT) and monozygotic twins (MZT). DZTs are the consequence of the implantation of two embryos resulting from the fertilization of two different oocytes by two different spermatozoa. ART DZT may be prevented in IVF with single embryo transfer (SET), and in intrauterine insemination (IUI) with monofollicular cycles (Prieto et al., 2022). MZT occur when a single embryo splits itself into two embryos and each one of them implants. MZT, compared with singleton and dizygotic twin pregnancies, have an increased risk of fetal growth restriction, preterm delivery, birth weight discordance and perinatal mortality (Hviid et al., 2018; Vitthala et al., 2009).

It is generally accepted that the day of embryo division determines the chorionicity of MZT. If the division occurs 1–4 days after fertilization, the fetuses will be dichorionic diamniotic; if it occurs 4–8 days after fertilization, the gestation will become monochorionic diamniotic; and if it occurs 8–12 days after fertilization, it will result in monochorionic monoamniotic twins (Hviid et al., 2018; Skiadas et al., 2008). Thus, MZ dichorionic twins are formed prior to the differentiation into two distinct cell lines, inner cell mass (ICM) and trophectoderm (TE) (Cunningham et al., 2014; Gilbert, 2014).

The frequency of MZT is 2.5 times higher in ART than in natural conceptions (Busnelli et al., 2019; Gurunath et al., 2015). In one study, among MZT resulting from ART, 9% were dichorionic diamniotic and 91% monochorionic diamniotic (Knopman et al., 2014). However, as far as we know, there are no strategies to prevent MZT. Prolonged culture and blastocyst transfer is associated with an increased risk of MZT, of relatively low incidence (0.8–0.9% in ART vs. 0.4% in natural pregnancies; Vitthala et al., 2009; Wang et al., 2018). However, it is the preferred technique since blastocyst transfer has a notably higher implantation rate than the cleavage stage embryo, allowing single embryo transfer to be a widespread practice and avoiding double embryo transfers with their high rate of dizygotic gestational pregnancy. Additional theoretical advantages are better selection of higher quality embryos, better embryo-endometrium synchronization (Wang et al., 2018), simplification of preimplantation genetic testing protocols and increased efficiency of embryo freezing programs (Matorras et al., 2021).

In the last few years, time-lapse monitoring (TLM) systems have been increasingly used in standard ART practice to continuously monitor early embryo evolution (Meseguer et al., 2011). There are some retrospective TLM studies suggesting that some morphokinetic traits could be predictors of twin pregnancies (Franasiak et al., 2015, Otsuki et al., 2016). In other reports, a retrospective TLM analysis was abnormal in one case of triplet pregnancy (Sutherland et al., 2019) as well as in conjoined twins (Grøndahl et al., 2022). In a previous work with an early TLM prototype, the formation of two cases of twinning at the blastocyst stage was shown under experimental conditions (Mio & Maeda, 2008). As far as we know, this is the first report on record of a human embryo spontaneous twinning at the cleavage stage, during a standard IVF procedure.

Case Report

We report a case corresponding to an oocyte donation cycle performed with oocytes from an uneventful donor (aged 27) using

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Figiure 1. Separation of the two blastomeres after first cleavage through the ruptured zona pellucida. Yellow arrow indicates the break in the zona pellucida. Development time 25.7 h.

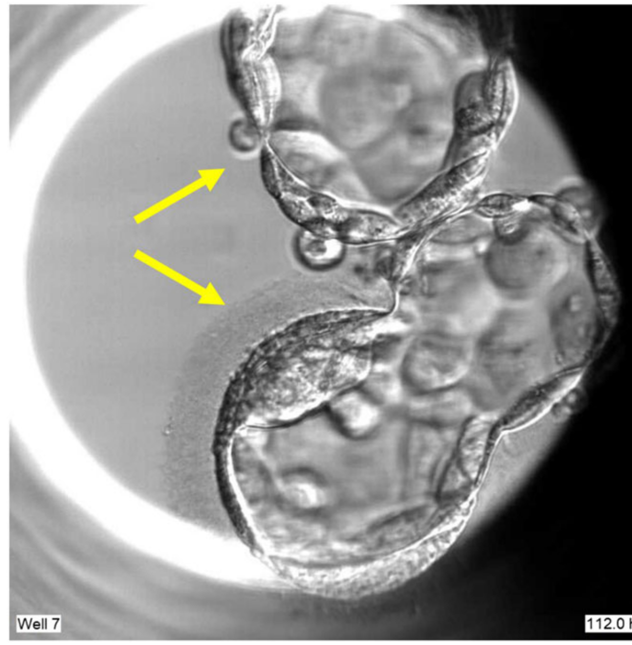

Figure 2. Development at 112.0 hours of the twin embryos. The embryo developed within the zona pellucida is hatching through the break in the ruptured zona pellucida. Yellow arrows indicate the two separate blastocysts.

normal sperm. The donor had no family history of twins. She had performed six previous oocyte donations, in which no incidences were recorded. Her fertilization rate was 75% (53/71). The resulting pregnancies were singletons. She had no natural pregnancies. Of the nine available oocytes (seven fresh and two devitrified) all nine were fertilized. All embryos were cultured for up to 5 or 6 days in an Embryoscope® time-lapse incubator (Vitrolife®, Canada) and evaluated based on the KIDscore^{TM} D5 algorithm (KS5), an embryo selection technology using an artificial intelligence algorithm with automatic embryo scoring. The score is obtained by means of annotations of the timing of pronuclear fading, the timing of 2-cell division, the timing of 3-cell division, the timing of 4-cell division, the timing of 5-cell division, and the timing of blastocyst formation; and morphological grade of ICM and TE are required. The model calculates a continuous score from 1.0–9.9.

The case we report corresponds to a fresh oocyte that was microinjected with the ZP already unintentionally broken. The cycle had no indication for preimplantation genetic test, so no assisted hatching was performed on any of the embryos. The whole TL record sequence can be seen at Supplementary Video 1. The second polar body extruded at 4.3 hours. The second pronucleus appeared at 8.1 hours and the two pronuclei disappeared at 21.9 hours. The division into two cells occurred at 24.9 hours. One cell remained inside the ZP while the other came out through the hole in the ZP (Figure 1). Both cells split simultaneously into two cells each. At the beginning, the cells that remained inside the ZP underwent division faster and had more fragmentation than the cells outside the ZP. The time of cleavage to a 5-cell embryo (T5) was 48.6 for the cells inside the ZP. The cells outside the ZP divided into 5, 6, 7 and 8 cells somewhat later, but compacted and reached the morula stage earlier (at 79.1 hours). At day 5 of embryo development, there were two good quality embryos, one inside the ZP and one outside, perfectly visible and distinct (Figure 2). The exterior cells had a higher quality score when reaching blastocyst. Both embryos presented a dense trophectoderm with many cells (type B). With respect to the ICM, there was a difference; while the blastocyst that was outside the ZP had small ICM, the ICM of the embryo that was inside the ZP was compact, large, and of good morphological appearance (grade B). The KIDScoreD5 v3.1 offered a score of 6.8 for the two embryos together. Of the other 8 oocytes from the same cohort (all of them with an unbroken ZP), there were three others reaching the blastocyst stage (with KIDscores of 6.1, 2.7 and 2.5). The nontwinned embryo with the highest score was selected for SET. The twinned embryos and the two nontwinned embryos were vitrified.

The SET performed with the nontwinned embryo led to a single pregnancy that resulted in a healthy term newborn infant. The infertile couple does not want more pregnancies, since they have a previous IVF newborn and the woman had a history of breast cancer.

Discussion

The frequency of MZT is 2.5 times higher in ART pregnancies than in natural pregnancies (Busnelli et al., 2019). It is unclear what might cause embryo splitting (Blickstein & Keith, 2007). A number of ART-related mechanisms have been proposed that could be linked with MZT: laboratory-related risks (creation of breaks in the ZP, culture media, overripe oocytes, fertilization delay, blastocyst stage embryo transfer, artificially assisted hatching), medical treatment risks (ovulatory drug-related hardening of the ZP), and patient-related risks (young woman's age) (Blickstein & Keith, 2007).

There are at least four theories concerning the mechanism of MZT. The 'cell repulsion hypothesis' says that cells in the developing zygote express subtle specific genetic differences that cause a repulsive force, leading to the zygote splitting (Hall, 1996). A second hypothesis postulates that whereas there is an axis that dominates normal embryo development, in MZT there is a codominant axis that leads to embryo splitting (Boklage, 2005). A third theory suggests that the triggering of embryo-splitting is due to depressed calcium levels in the early embryo (Steinman & Valderrama, 2001). The fourth theory establishes the existence of a blastomere herniation (Blickstein & Keith, 2007; Hall, 2003).

In recent years, a number of reports on TLM and twinning have been published. Some of them suggest that some morphokinetic traits of ICM and trophectoderm could be predictors of twin pregnancies (Franasiak et al., 2015; Otsuki et al., 2016). In one previous report, ICM grade A was associated with twins (Eliasen et al., 2021) but in another, the opposite was found (Otsuki et al., 2016). Other previously reported predictors of twinning are ICM looseness (Otsuki et al., 2016) and short s3 (time between 5-cell stage and 8-cell stage).

In the case we report, it is shown how at the 2-cell stage, reached at the normal time, 1 cell came out through a hole in the ZP and replicated independently, producing a blastocyst of normal appearance and with a normal morphokinetic pattern. We can speculate that a hole in the ZP may facilitate the extrusion of a cell from the <day 4 embryo and that this cell development is not constrained by being inside the ZP. Despite the lack of inhibition of the ZP itself or the influence of the other embryo cells, the totipotent cell was able to develop correctly from the start. Moreover, the embryo inside the ZP compensates for the loss of this cell apparently without problems. Previous experiments in animal cloning always gave importance to the ZP, either transferring the blastomere/s obtained by biopsy into a previously emptied ZP or bisecting the embryo and the ZP into two parts (Rahbaran et al., 2021).

In our case, the ZP breakage was not intentional and could be due to the manipulation during oocyte denudation. ZP breakage is not a common event but could well occur in isolated cases due to higher than normal aspiration pressures during oocyte pick-up, or poor oocyte quality, or due to the procedure of oocyte denudation or thawing.

Even though the twinned embryo had a higher score than the other blastocysts, the embryo selected for SET was a nontwinned one, to rule out any possible unknown risks. The pregnancy evolved uneventfully.

Embryo splitting during embryo culture is a very rare procedure. In our IVF laboratory, where we have evaluated more than 7000 embryos by TLM, this was the first case of embryo splitting that we observed among cases where assisted hatching was not performed. It does not appear that TLR can significantly reduce MZT at this time.

The existence of two genetically (almost) identical twins could give rise to some ethical and/or psychological problems. If two SETs were performed with them and both resulted in a newborn, we would have two (almost) identical persons with a few year's lag. This could be potentially distressing for the younger one and could attract media attention. Another option if a newborn is obtained with the first twin, would be not to transfer the second and keep it vitrified, in case in the future it could be of any help for stem cell transplantation. Neither of these options was considered in our case, since the social and medical situation of the woman precluded a new pregnancy. Although this is a very infrequent case, we highlight the importance of developing solid guidelines and regulations to control the clinical practice in these situations.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/thg.2023.24.

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Competing interests. None.

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