

Commentary

Seeing is believing: Visualising the Formation of Monozygotic Twins in IVF

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Monozygotic (MZ) twins, identical clones developed from a single embryo, have long been a subject of curiosity, with their formation remaining largely unexplored [1]. In *In Vitro* Fertilisation (IVF) clinics, MZ twinning occurs at a relatively high rate up to 5%, compared to about 0.4% in natural pregnancies [2]. MZ twins' pregnancy pose high risks for both the mother and the fetus, including twin-to-twin transfusion syndrome and twin reversed arterial perfusion. Therefore, understanding the exact mechanisms of MZ twinning during IVF procedure is crucial for predicting and potentially preventing it before embryo transfer.

Studying MZ twins has been challenging due to the low rate of MZ twinning in natural pregnancies and the inaccessibility to observe their *in vivo* development. Furthermore, there has been a lack of suitable animal models to study their formation in research. However, recent rapid advancements in Assisted Reproductive Technologies (ARTs) have reduced these barriers, offering valuable insights into the formation of MZ twins. ARTs allow for the development of human embryos outside the body before implantation. Along with cutting-edge live embryo imaging techniques, it is now possible to record and monitor real-time human embryo development while maintaining optimal development conditions within the incubator [3-5]. Consequently, an increase in MZ twin cases, observed through embryo imaging, has been reported following assisted reproduction [3-12], providing a clearer view on when and how a single embryo splits into two.

Drawing on the insights from IVF clinics, Jin et al. recently published a comprehensive review in *Human Reproduction Update*, detailing the current understanding of the cellular mechanisms behind MZ twin formation [13]. In this commentary, we discuss how live embryo imaging implicated the observation of cases of MZ twinning

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and highlight key considerations for IVF clinics on how to potentially improve the prediction and reduction of MZ twinning events throughout IVF treatment processes. We also suggest potential directions for future MZ twin studies, where researchers can gather more evidence and insights into this phenomenon from human twin live imaging data. Ultimately, we wish to emphasize the increasing need for collaborations between researchers and IVF clinicians to enhance our understanding of MZ twinning.

Breach in the Zona Pellucida Separates Embryos at 2-Cell

Various factors can lead to MZ twinning at different stages of embryonic development, with some instances occurring as early as the 2-cell stage. During the early stages of development, the Zona Pellucida (ZP) serves as a crucial barrier to prevent multiple sperm entry and the fission of embryos. A breach in the ZP at the 1-cell stage led to the development of twin blastocysts during *in vitro* culture [3]. After successful fertilisation and the first cleavage into 2-cell stage, time-lapse imaging enabled the visualisation of how one of the cells was expelled from the slit in the ZP while the other remained inside. The ZP then acted as a barrier, allowing the two cells to develop into two individual blastocysts, one inside and one outside the ZP [3]. Although the twin blastocysts were not transferred, it is likely that this could have led to dichorionic MZ twins. This case highlights the importance of avoiding slits or openings in the ZP during early embryo manipulation in IVF clinics to prevent unintended consequences. If accessible, this phenomenon would be of great interest for researchers to explore the dynamics of cell junctions before compaction, and the forces that lead to cell separation when a breach occurs in the ZP.

Inner Cell Mass Division through Blastocoel Expansion

Advancements in *in vitro* culture technologies have made it feasible to culture human embryos until the blastocyst stage, a cavitated, ball-like structure. It contains the Inner Cell Mass (ICM), a group of pluripotent cells that can develop into any cell type in the human body. Typically, cells of the ICM are clustered as a single group. However, cases of two or three ICM groups within one blastocyst have been reported [5-7,14], which is a significant indicator of monochorionic MZ twins or triplets. Time-lapse imaging of cavitating blastocysts has revealed that the repeated cycles of collapse and re-expansion typically occurs in blastocysts during cavitation, demonstrating its highly dynamic nature over a period of time [15]. However, if the ICM cells are loosely connected during blastocoel expansion, it is highly likely that the ICM can separate into multiple groups [5,16]. This finding suggests that blastocysts should be carefully examined from all 3D perspectives to determine whether they contain two or more distinct groups of ICMs before transferring embryos for IVF treatments, as this could indicate a potential risk for MZ twinning. Aiding in the consistent detection of such features indicative of potential MZ twinning, artificial intelligence tools could also serve as powerful resources for analysing live-imaged embryo phenotypes.

Using human embryos in research raises significant ethical and legal concerns. Yet, in a very exciting era of revolutionary advancements in stem cell technology which have led to the development of blastoids, *in vitro* structures that mimic human blastocysts, innovative alternative models may be enabling some extent of human embryo-like studies [17]. Recently, the creation of twin blastoids has provided a valuable model for studying ICM separation *in vitro* [18]. These twin blastoids exhibited a high rate of double ICM formation, which separated during cavity expansion, closely mimicking the monozygotic twinning observed in human cases, addressing the lack of a suitable animal model. This model enables live imaging of the entire ICM splitting process during cavitation, while also serving as a valuable tool for studying the previously elusive process of MZ twin embryo implantation *in vitro*.

Assisted Hatching - Slit and Split

After the formation of the blastocyst, the embryo undergoes hatching, emerging from the ZP as the final preparation for implantation into the uterus. In IVF clinics, assisted hatching is commonly employed by creating a hole or slit in the ZP to facilitate the blastocyst's exit. While assisted hatching can enhance the success of IVF treatments by promoting embryo hatching and implantation, it also carries the risk of MZ twin pregnancies. During the hatching of the embryo from the small slit, the cavitated blastocyst can be squeezed into an 8-shaped structure [19]. Time-lapse imaging of the hatching process revealed that the ICM splits into multiple groups if it is located near the hatching slit, with part of the ICM remaining inside the ZP [4]. Two scenarios could occur if the ICM separates during hatching. If only the ICM separates, it can result in monozygotic MZ twins [4]. On the other hand, if the TE splits together with the ICM, it can result in dichorionic MZ twins [9-11]. Therefore, it is crucial to ensure that the hatching point is not created near the ICM when performing assisted hatching in IVF clinics. If 8-shaped hatching is observed before embryo transfer, the embryo should be carefully examined to determine the location and number of ICMs per blastocyst. Such cases could allow researchers to explore further what type of slits or openings lead to the separation of the ICM alone, and what forces might cause the ICM and TE to separate together. Consequently, the gained knowledge could provide clinicians with key insights on how to create the optimal size or type of opening during assisted hatching to avoid unintended separation of the ICM and TE.

Fresh or Frozen? The Risk of Dichorionic MZ Twin Formation

With the development and widespread application of cryopreservation techniques in IVF laboratories, frozen blastocyst transfers have resulted in higher pregnancy rates compared to fresh blastocyst transfers [20]. Intriguingly, live imaging during a frozen-thaw cycle captured the formation of a dichorionic MZ twin embryo [8]. During the re-expansion of the thawed blastocyst, some TE cells remained connected rather than separating, resulting in two small cavities, each containing an ICM. After transfer, this led to a dichorionic MZ twin pregnancy [8]. Thus, embryos should be carefully screened for structural integrity to avoid twinning after thawing. Some scientists are even calling for a return to fresh embryo transfer, advocating for a reduction in the use of cryopreservation techniques in IVF clinics due to potential complications [21]. It may need to be noted that blastocyst cryopreservation may pose a higher risk of MZ twinning compared to cleavage-stage freezing, as it involves two stages of blastocoel

dynamics: expansion of the blastocoel during the fresh cycle, followed by collapse during cryopreservation, and then re-expansion after thawing. These changes may increase the likelihood of ICM and TE splitting, potentially raising the risk of MZ twinning. Further investigations on how the cryopreservation process affects cell junctions in the blastocyst might be needed. Furthermore, exploring how potential additives in thaw media could promote normal blastocoel re-expansion while preventing the separation of ICM and TE cells could help to bring the field of reproductive medicine forward.

The First Case of Time-Lapse Imaging of a Conjoined MZ Twin Embryo

Conjoined twins pose extremely high health risks to the offspring and mother, and impose a substantial life-long financial burden on their families. However, understanding the mechanisms behind the formation of conjoined twins is even more challenging than non-conjoined MZ twins due to their extremely low occurrence, representing only 1-2% of MZ twin births [22]. The prevailing theory is that conjoined twins result from the late separation of the ICM either after hatching or even after implantation [23]. However, this theory remained a hypothesis, as the actual development of embryos leading to conjoined MZ twins had not been visualised in real time. Until recently, the first time-lapse recording of a conjoined twin pregnancy revealed that one of the cells at the 2-cell stage fragmented during subsequent development, and the other non-fragmented cell developed into a small blastocyst, resulting in a conjoined twin pregnancy after transfer [12]. Astoundingly, the entire process of preimplantation development of the embryo was recorded, but no separation of the ICM was detected, suggesting it may have occurred after transplantation. The report highlighted the first observed case where poor-quality embryos, even those with only half of the original cell mass, can still develop into conjoined MZ twins. This case challenges our traditional understanding that conjoined twin embryos may appear normal until blastocyst stage. The conjoined twin embryos may exhibit early-stage defects such as cell fragmentation during cleavage, potentially indicating their developmental trajectory toward conjoined twinning. So, the embryos with severe fragmentation should not be the optimal choice for transfer. While only one such case has been observed through live imaging to date, it provides valuable insights for future studies on conjoined twins, urging researchers to investigate not only the later stages after blastocyst formation but also the very early stages within the first 48 hours post-fertilisation.

Conclusion

Live imaging is a powerful tool, for both researchers and clinicians, to record and monitor preimplantation embryogenesis in real-time. It offers valuable insights into the mechanisms of MZ twinning during the earliest stages of life, which otherwise would not be visible. It also highlights potential critical precautions for IVF treatments to help reduce the occurrence of MZ twins. By analysing these cases and accumulating data in the future, further insights can be gained that could aid in lowering MZ twinning rates in IVF clinics, ultimately promoting better health outcomes for newborns, mothers and families.

Conflict of Interest

The authors declare no conflict of interest.

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References

- Herranz G (2015) The timing of monozygotic twinning: A criticism of the common model. *Zygote* 23: 27-40.
- Behr B, Fisch JD, Racowsky C, Miller K, Pool TB, et al. (2000) Blastocyst-ET and monozygotic twinning. *J Assist Reprod Genet* 17: 349-351.
- Matorras R, Vendrell A, Ferrando M, Larreategui Z (2023) Early Spontaneous Twinning Recorded By Time-Lapse. *Twin Res Hum Genet* 26: 215-218.
- Sutherland K, Leitch J, Lyall H, Woodward BJ (2019) Time-lapse imaging of inner cell mass splitting with monochorionic triamniotic triplets after elective single embryo transfer: A case report. *Reprod Biomed Online* 38: 491-496.
- Mio Y, Maeda K (2008) Time-lapse cinematography of dynamic changes occurring during *in vitro* development of human embryos. *Am J Obstet Gynecol* 199: 660.
- Noli L, Capalbo A, Ogilvie C, Khalaf Y, Ilic D (2015) Discordant Growth of Monozygotic Twins Starts at the Blastocyst Stage: A Case Study. *Stem Cell Reports* 5: 946-953.
- Meintjes M, Guerami AR, Rodriguez JA, Crider-Pirkle SS, Madden JD (2001) Prospective identification of an *in vitro*-assisted monozygotic pregnancy based on a double-inner-cell-mass blastocyst. *Fertility and Sterility* 76: 172-173.
- Shibuya Y, Kyono K (2012) A successful birth of healthy monozygotic dichorionic diamniotic (DD) twins of the same gender following a single vitrified-warmed blastocyst transfer. *J Assist Reprod Genet* 29: 255-257.
- Jundi SI, Pereira NCA, Merighi TM, Santos JFD, Yadid IM, et al. (2021) Monozygotic dichorionic-diamniotic twin pregnancy after single embryo transfer at blastocyst stage: A case report. *JBRA Assist Reprod* 25: 168-170.
- Sundaram V, Ribeiro S, Noel M (2018) Multi-chorionic pregnancies following single embryo transfer at the blastocyst stage: A case series and review of the literature. *J Assist Reprod Genet* 35: 2109-2117.
- Behr B, Milki AA (2003) Visualization of atypical hatching of a human blastocyst *in vitro* forming two identical embryos. *Fertil Steril* 80: 1502-1503.
- Grøndahl ML, Tharin JE, Maroun LL, Stener Jørgensen F (2022) Conjoined twins after single blastocyst transfer: A case report including detailed time-lapse recording of the earliest embryogenesis, from zygote to expanded blastocyst. *Hum Reprod* 37: 718-724.
- Jin H, Han Y, Zenker J (2024) Cellular mechanisms of monozygotic twinning: clues from assisted reproduction. *Hum Reprod Update*: dmae022.
- Lee SF, Chapman M, Bowyer L (2008) Monozygotic triplets after single blastocyst transfer: Case report and literature review. *Aust NZJ Obstet Gynaecol* 48: 583-586.
- Sciorio R, Meseguer M (2021) Focus on time-lapse analysis: Blastocyst collapse and morphometric assessment as new features of embryo viability. *Reprod Biomed Online* 43: 821-832.
- Payne D, Okuda A, Wakatsuki Y, Takeshita C, Iwata K, et al. (2007) Time-lapse recording identifies human blastocysts at risk of producing monozygotic twins. *Human Reproduction* 22: 9-11.
- Liu X, Tan JP, Schröder J, Aberkane A, Ouyang JF, et al. (2021) Modelling human blastocysts by reprogramming fibroblasts into iBlastoids. *Nature* 591: 627-632.
- Luijckx DG, Ak A, Guo G, van Blitterswijk CA, Giselbrecht S, et al. (2024) Monochorionic Twinning in Bioengineered Human Embryo Models. *Adv Mater* 36: 2313306.
- Gu YF, Zhou QW, Zhang SP, Lu CF, Gong F, et al. (2018) Inner cell mass incarceration in 8-shaped blastocysts does not increase monozygotic twinning in preimplantation genetic diagnosis and screening patients. *PLoS One* 13: 0190776.
- Wei D, Liu JY, Sun Y, Shi Y, Zhang B, et al. (2019) Frozen versus fresh single blastocyst transfer in ovulatory women: A multicentre, randomised controlled trial. *Lancet* 393: 1310-1318.
- Pier BD, Havemann LM, Quaas AM, Heitmann RJ (2021) Frozen-thawed embryo transfers: Time to adopt a more "natural" approach? *J Assist Reprod Genet* 38: 1909-1911.
- Hall JG (2003) Twinning. *Lancet* 362: 735-743.
- Johnston I (2001) Conjoined twins. *Lancet* 357: 149.



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