

# Time-lapse recordings reveal why IVF embryos are more likely to develop into twins

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Evidence gathered from time-lapse recordings of the formation of early embryos (blastocysts) in the laboratory has revealed why embryos created via IVF and undergoing extended culture are more likely to develop into twins than those created via natural conception.

Furthermore, the research has shown that the culture in which the IVF embryos are formed is possibly responsible for the embryos dividing into twins.

Dianna Payne, a visiting research fellow at the Mio Fertility Clinic, Yonago, Japan, told the 23rd annual meeting of the European Society of Human Reproduction and Embryology today (Monday 2 July) that about three pairs of twins per thousand deliveries occurred as a result of natural conception, but many more were born after IVF, even when only one embryo had been transferred to the mother (approximately 21 pairs per thousand deliveries). However, it was not known why this happened.

Using 33 surplus frozen-thawed embryos that had been donated for research, Ms Payne and her colleagues used computer software called MetaMorph [1], which creates a free-running film from single images taken every two minutes with a digital camera attached to a microscope. They then used the software to analyse data from the film.

After thawing, 26 of the 33 embryos (most of which were composed of between two and ten cells) developed to blastocyst stage in which the blastocoele is formed. This is a fluid-filled cavity in the blastocyst and is formed on about day four or five when the embryo forms tight junctions

between the cells around its periphery. These outer cells (the trophoctoderm) begin to pump fluid into the blastocoelic cavity where a micro-environment is formed in which the cells that will go on to develop into the body of the embryo (the inner cell mass or ICM) develop.

The time-lapse recording showed that at this stage the blastocoele collapsed at least once in 25 of the 26 embryos (96%). “The frequency and degree of collapse varied, but the embryos that died tended to be those that had bigger and more frequent collapses,” said Ms Payne.

She explained the mechanism that underlies blastocoelic collapse and re-expansion. “The fluid in the cavity must be under positive pressure as this pressure is the motive force for expansion of the blastocyst. The trophoctoderm maintains the pressure by pumping the fluid into the cavity. I believe that the collapses occur when some of the junctions between the cells fail – possibly due to localised cell death, or maybe due to a structural weakness in the junction itself – and the blastocoelic fluid leaks out. These collapses occur quite quickly – far more quickly than a pump could manage. The magnitude of the collapses is determined by the number of failed junctions. The greater the number of failed junctions, the more severe the collapse. In some cases the embryo cannot re-establish the junctions and the blastocyst is unable to re-expand and thus dies.”

Seventeen of the 33 embryos went on to become fully formed blastocysts and 11 either started to hatch or hatched completely from the zona pellucida (the gelatinous protective coating around the blastocyst).

Fifteen embryos degenerated during culture and 11 of them did not re-expand after a collapse and subsequently degenerated. There was no evidence of embryo splitting during the hatching – which was one of the theories as to how twins were formed from a single blastocyst. However,

two of the 26 embryos (8%) had two distinct ICMs and a third had a possible second ICM. The most common form of monozygotic twinning (identical twins resulting from the dividing of one embryo fertilised by a single sperm) is monochorionic/diamniotic, when two ICMs form before hatching.

Ms Payne said: “The second ICM was evident early in blastocyst formation in both embryos, and appeared to be the result of some ICM cells relocating and adhering to the opposite trophectoderm wall, seeded during an early collapse of the blastocyst. Both these embryos with two ICMs hatched completely.”

She continued: “Up until now, blastocyst collapse in the laboratory was thought to be a normal feature of blastocyst development. However, our findings that collapse was associated with degeneration of blastocysts as well as the formation of the second ICM suggest that these episodes in which blastocoele volume cannot be maintained may be an artefact of culture. Furthermore, our findings suggest that the formation of two ICMs during blastocyst development may be the cause of the high monozygotic rate after extended culture. This hypothesis fits well with the long established cause of the most common form of natural monozygotic twins.”

This research is, to Ms Payne’s knowledge, the first systematic study by time-lapse recording of blastocyst formation. It was time-consuming, with each recording taking up to five days, and required specialist equipment and knowledge. However, she said it should enable embryologists to work towards avoiding twin pregnancies during IVF. “This could be another tool in their armoury,” she said. “Those embryos that are at risk of twinning could be easily identified by counting the number of ICMs. Then, either a decision could be made about whether to transfer those embryos that are likely to give rise to twins, or, if the choice of embryos is limited, the mother could be prepared for the

likelihood of twins and given appropriate clinical advice.”

In addition, more information about the cause of monozygotic twins could be collected in the future by a straightforward check of the numbers of placentas and sacs when the babies are born. The chorion and amnion are foetal membranes that contribute to the placenta and the foetal sac respectively. When twinning has occurred because two ICMs have developed (monochorionic/diamniotic twins) there is one placenta and two sacs, and these can be counted at birth.

Source: European Society for Human Reproduction and Embryology

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