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Case Report

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Case report on "conjoined embryos"

Abstract

Objective: To report an interesting incidental occurrence that may improve the understanding of the mysterious embryology of conjoined twins.

Methodology: The embryos were generated in-vitro through intracytoplasmic sperm injection ICSI. On day 3 of embryo development, the Zona of each 8-cell embryo was breached with LASER and the embryos were cultured in pairs per well in a culture dish in preparation for herniation for a day 5 trophectoderm biopsy for Pre-Implantation Genetic Diagnosis.

Results: On day 5, a particular pair of embryos was accidentally placed at very close proximity adjacent to each other. Interestingly, during herniation, the trophectoderm cells were observed to have joined and started developing together into "conjoined embryos".

Conclusion: The fact remains that there are more mysteries surrounding the origin or the embryology of conjoined twining. The current knowledge is that of two theories postulated of which the fission theory is the most widely accepted. This finding could support the fusion theory.

Keywords: trophectoderm, fission theory, conjoint embryo, mysteries

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Introduction

There are mysteries surrounding the spontaneous incidence of twinning, both dizygotic and monozygotic in different mammalian species. Conjoined twins are an extremely rare type of twins, specifically a form of monozygotic twins who are usually of same sex. Conjoined twinning is currently believed to arise when the twinning event occurs at about the primitive streak stage of development, at about 13-14days after fertilization in the human, and is exclusively associated with the mono-amniotic mono-chorionic type of placentation.[1] This type of placentation is linked with a twinning event that occurs during the early post-implantation period, and is the most common form of placentation observed in human monozygotic twinning, occurring in about 60–70% of cases.[2] It is believed that the highest incidence of conjoined twinning is encountered in the human with an increasing number of cases.

While the twinning rates vary considerably in different parts of the world, the overall incidence of twinning is said to be about 1:87 of all births with a worldwide incidence of monozygotic twinning rate at about 3.5 per 1,000 or about 1:286 pregnancies.[1] An increase in the number of monozygotic twins has also been reported following assisted reproduction.[3]

Monozygotic twinning and conjoined twins

In human, it is generally postulated that the embryo may divide to form monozygotic or identical twins at one of three stages of development. The earliest time is when the embryo is undergoing cleavage, at the morula or during the zona-intact blastocyst stage, following fission or cleavage, two genetically identical embryos would be expected to result, and following zona lysis each would implant and develop as two distinct genetically identical embryos. Accordingly, the type of placentation observed would be di-chorionic and diamniotic. This group accounts for about 30-40% of monozygotic twins in humans.[2] If the inner cell mass divides into two functional units within the blastocyst during the peri-implantation period, each of the resultant embryos would be expected to develop within its own amniotic cavity, although they would share a common placenta. Accordingly, the type of placentation seen would be mono-chorial and diamniotic. This group accounts for about 60–70% of monozygotic twins in humans.[2] In the most uncommonly encountered group, division occurs at the primitive streak stage, around day 13 to 14 of development when the embryonic axis is believed to be stimulated to divide into two parallel axes, giving rise to two embryos that develop within a single amniotic sac.[1] The type of placentation seen is of the mono-chorionic and mono-amniotic. Hence, the case of conjoined twining has been hypothesized to be a timing of the twining event rather than the twining event itself, although reasons and cause are unclear, it is reasoned that only partial separation of the embryonic axes occurs at a slightly later time during development than when complete splitting of the embryonic axis normally occurs.

There are basically two theories that have been postulated to explain the embryology of conjoined twining namely

a. **Fission theory of conjoined twinning:** Here, it is believed that if the split occurs more than twelve days' post conception, the embryos may not fully divide and then embryo may form with an anatomical connection resulting in conjoined twins. This is known as the "fission theory".[1,4] Explanations are not given as to why the zygote divides, or why the process is interrupted in such a way that the embryo remains connected and develops as conjoined twins.

b. **Fusion theory of conjoined twinning:** Another theory regarding conjoined twinning is known as the "fusion" or "collision" theory.[5–8] This implies that stem cells from one twin adhere to like stem cells from the other twin, fusing together and developing conjoined. Here also, there is no identified explanation for why this would happen. The degree of the connectivity between conjoined twins varies and determines the expected outcome for the individuals in terms of survival.

Many argue that if fusion, rather than fission, accounted for all cases of conjoined twins, the prevalence of "mirror-imaging" should be the same in all mono-amniotic twins, whether they are conjoined or not.[1] If the incidence of mirror-imaging is higher in conjoined twins than in separate twins as it is found to be, the fusion hypothesis cannot be acceptable. Therefore, it follows to hypothesize that in conjoined twinning the twinning event occurs at a later time during development than the twinning event that leads to two separate mono-amniotic twins. Most DNA typing studies of conjoined twinning demonstrated mono-zygosity in support of the fission theory, however, an exceptional case was reported by Logrono et al.[5] where DNA typing demonstrated di-zygosity rather thanmono-zygosity.

Case presentation

Here, we present an interesting case of two distinct embryos that later became conjoined at day 5 blastocyst stage. The embryos were generated in-vitro through intracytoplasmic sperm injection ICSI for a couple undergoing male sex selections using PGS method with biopsy taken at the blastocyst stage. The embryos were derived using donor oocyte and the husband's sperm, 19oocytes were retrieved from the 25-year-old donor out of which 16 of them were metaphase II oocytes, 1 metaphase I, 1 germinal vesicle and 1 atretic oocyte. The 46-year old husband's sperm parameter was 70million/ml in concentration and 60% in motility with rapid progressive movement. All the metaphase II oocytes were fertilized by ICSI, all of them fertilized normally as evident by the presence of 2 pronuclei after 19hours of fertilization. The zygotes were then cultured in one-step SAGE media.

On day 3 of embryo development, all 11 fertilized zygotes developed to become good quality 8-cell stage cleavage stage embryos with less than 10% fragmentation. The Zona of each 8-cell embryo was breached with LASER by creating a hole of about 5-10um in preparation for herniation in order to take a day 5 trophectoderm biopsy. The embryos were cultured in pairs per well in a culture dish except the 11th embryo which was cultured singly, after LASER hatching the dish was then placed in the incubator for two days until the blastocyst stage.

On day 5, a particular pair of embryos which must have been placed at very close proximity of less than 2um with the zona pellucida adjacent to each other, interestingly during herniation, the trophectoderm cells joined together and started developing together into a structure we have termed "conjoined embryos" as illustrated in (Figure 1) (Figure 2) below. Eight other good quality blastocysts were formed from the other embryos, five of which pre-implantation genetic screening PGS by next generation sequencing certified to be chromosomally normal embryos.

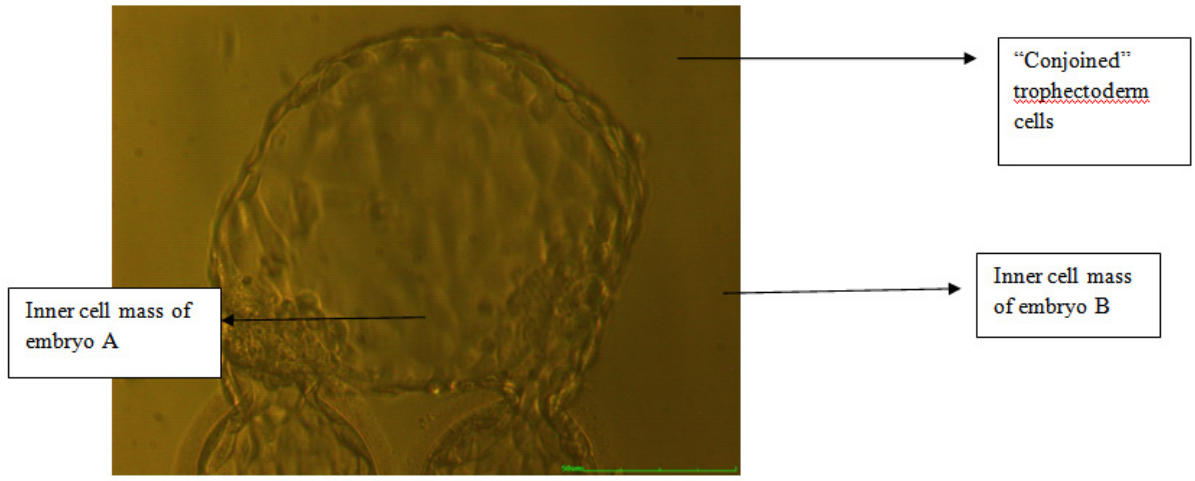

Figure 1 Showing fusion of trophectoderm cells from two distinct embryos (hatching blastocysts). (Nordica Fertility Center, Lagos, Nigeria).

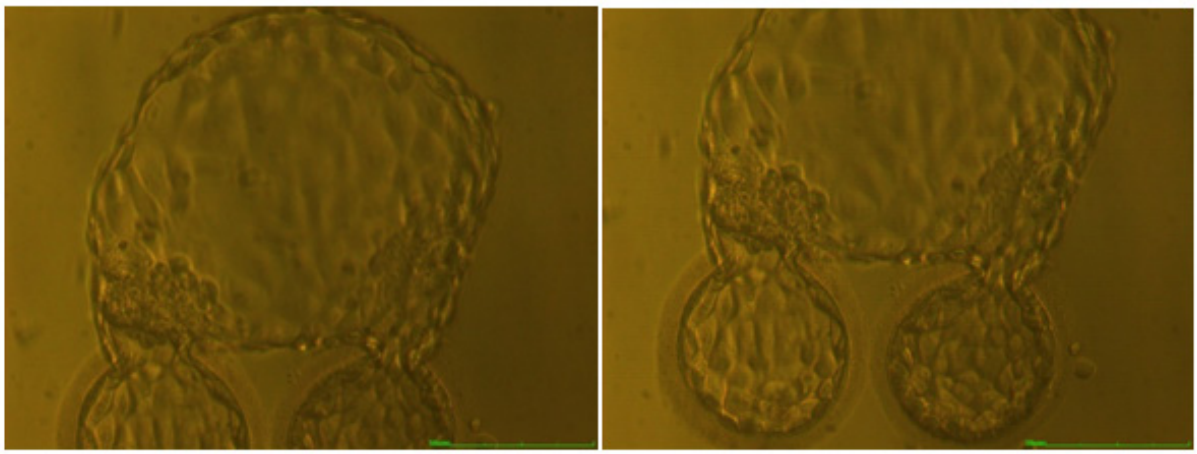

Figure 2 Two distinct embryos (hatching blastocysts) in close proximity. (Nordica Fertility Center, Lagos, Nigeria).

Discussion

The novel structure brings back to fore front the debate of the fission versus fusion theory of conjoined twining, it could mean that perhaps the fusion theory is more suitable to explain conjoin twinning, it might be possible to suggest that extremely close proximity of two distinct embryos can in some cases result in the fusion of cells which would then give rise to two embryos sharing structures. The fact that conjoined twins are mostly monozygotic could be explained by the fact that monozygotic twin embryos are more likely to be in closer proximity than dizygotic twin embryos and as such incidences of conjoin twining is more likely in the monozygotic, however, it is not entirely impossible to occur in dizygotic twin embryos as reported in a study mentioned earlier by Logrono et al.[5]

It could also be postulated that mirror-image twinning is more prevalent in conjoined twinning because they are likely to be positioned closely and as such the incidence of mirror image twins would be higher in conjoined twinning.

Conclusion

The fact remains that there are more mysteries surrounding the origin or the embryology of conjoin twining, the current knowledge is that of two theories postulated of which the fission theory is the most widely accepted, however, there is no embryological or genetic explanation as to why it happens.

Therefore, all of these postulations and theories need to be researched and scientifically proven or disproven, there is need to be able to scientifically investigate the possibility of inducing conjoin embryos *in vitro* by LASER hatching and keeping at close proximity as seen in this case. A number of studies have attempted to induce conjoined twins by exposing the embryos to some environmental agents that has been found to induce monozygotic twining; this however, has been most successful in non-mammalian species.

We then monitor real time fusion of these cells with the use of time-lapse incubator, animal models can be used, and then transfer can be done to evaluate if conjoin embryos give rise to conjoined twins. This can help us better understand the origin and embryology of conjoined twining.

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Conflict of interest

Author declares that there is no conflict of interest.

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