Asian Journal of Andrology (2021) 23, 227–228
www.asiaandro.com; www.ajandrology.com

Open Access

LETTER TO THE EDITOR

Male Infertility

Preimplantation genetic testing for a new abnormal cleavage behavior

Ming-Zhao Li*, Hai-Yan Bai*, Xia Xue, Juan-Zi Shi

Asian Journal of Andrology (2021) **23**, 227–228; doi: 10.4103/aja.aja_26_20; published online: 03 July 2020

Dear Editor,

Time-lapse systems (TLSs) have increasingly been introduced to fertility laboratories to improve the identification of embryo quality. TLSs detect some forms of abnormal division, such as direct and reverse cleavage divisions. Direct cleavage occurs when one blastomere divides directly into three or more daughter blastomeres. It strongly correlates with impaired preimplantation development, ploidy, and implantation capacity.[1–3] Reverse cleavage (RC) occurs when cytokinesis fails or when two blastomeres fuse into a hybrid cell containing two nuclei. The embryo is either entirely polyploid or mosaic according to the number of fused cells per embryo and the time-point when RC occurs. The effect of RC on embryo quality, ploidy, and implantation capacity may differ between embryos.[4] Other abnormal division characteristics, such as more than 50% fragmentation, big fragmentation, uneven blastomeres, delayed division, and distorted cytoplasm movement, also exert adverse effects.[5] We reveal a new abnormal blastomere division and report the effect of this abnormality on preimplantation development, ploidy, and implantation capacity. Few such cases have been reported in the literature.

A 29-year-old female was infertile for 2 years with no abnormal results for clinical examination. Semen samples from her husband were abnormal and he was diagnosed with severe oligozoospermia; further examination showed complete azoospermia factor c region (AZFc) deletions. Preimplantation genetic testing (PGT) was recommended and carried out since June 2019 at the Reproductive Center of the Northwest Women and Children's Hospital in Xi'an, China. This study was approved by the Ethics Review Board of the Northwest Women and Children's Hospital (2018002). The whole process of treatment was not intervened. The patients were informed of the abnormal cleavage behavior.

For the woman, a total of 9 metaphase II oocytes were retrieved then inseminated by intracytoplasmic sperm injection. Six two-pronuclear zygotes were obtained and three developed into blastocysts. We observed a new type of abnormal division by TLS (**Figure 1a**). In the two-cell stage, one blastomere failed to undergo complete cleavage. The other blastomere divided then formed into the blastocyst. The blastocysts underwent self-collapse in the process of blastocyst formation. The embryo's ability to achieve the blastocyst stage could be predicted with a high sensitivity and specificity, by having cc2 (the length of the 2nd cell cycle, cc2 = t3–t2) of 7.8–14.3 h and s2 (synchronicity of the 2nd round of divisions, s2 = t4–t3) of 0–5.8 h.[5] For the blastocysts in this case, the timings of cc2 were within the optimal range, but the s2 timings fell outside the optimal range. The embryos spent a long time between the second and third divisions. Parameters such as cc2 and s2 were also used for selection of embryos with the highest implantation and pregnancy potential.[6]

Embryos display different cleavage dynamics depending on their ploidy. Some research showed that ploidy could be predicted based on developmental time-points, such as the interval between the two- and five-cell stage, the duration of the 3rd cell cycle, start of blastulation, and formation of a full blastocyst.[7] However, the relationship between embryo ploidy and morphokinetic parameters remains controversial. In our study, PGT results showed that the three blastocysts with abnormal cleavage included one euploid and two aneuploid blastocysts (**Figure 1b**), suggesting that embryo ploidy could not be defined by this type of abnormal cleavage.

Two months later, natural frozen embryo transfer was conducted with the euploid blastocyst. Unfortunately, no pregnancy was obtained. Previous studies showed that blastocysts that exhibited collapse were less likely to implant and concluded that embryos not exhibiting strong blastocyst collapse should be preferred for transfer to increase implantation rates.[8] We predict that the abnormal cleavage identified in our study may be associated with the occurrence of blastocyst collapse. The molecular mechanisms of this process remain unknown. In the two-cell stage, one blastomere failed to cleave and ultimately led to mechanical stress that impeded blastocyst expansion. The excessive energy consumption may adversely affect their subsequent implantation.

In the current case study, the infertile couple included a healthy woman and a man with complete AZFc deletions. Few studies in the literature have reported the influence of Y chromosome microdeletions upon embryo cleavage patterns. Previous research suggested that 80% MII oocytes retrieved from stimulated cycles had one or more abnormal morphological characteristics.[9] The use of TLS confirmed that abnormalities of embryo division were increased by abnormal oocyte morphology.[10] Therefore, we believed that the abnormal cleavage patterns of the embryos were mostly resulted from the poor quality of MII oocytes.

Although an euploid embryo with this type of abnormal cleavage could be obtained after intracytoplasmic sperm injection plus PGT, there was no success. The blastocysts may exhibit self-collapse from mechanical stress due to the failed cleavage of the

The ART Center, Northwest Women and Children's Hospital, Xi'an 710003, China.
*These authors contributed equally to this work.
Correspondence: Dr. X Xue (xuexia91011@163.com) or Dr. JZ Shi (szzxsjz@163.com)
Received: 14 November 2019; Accepted: 09 April 2020

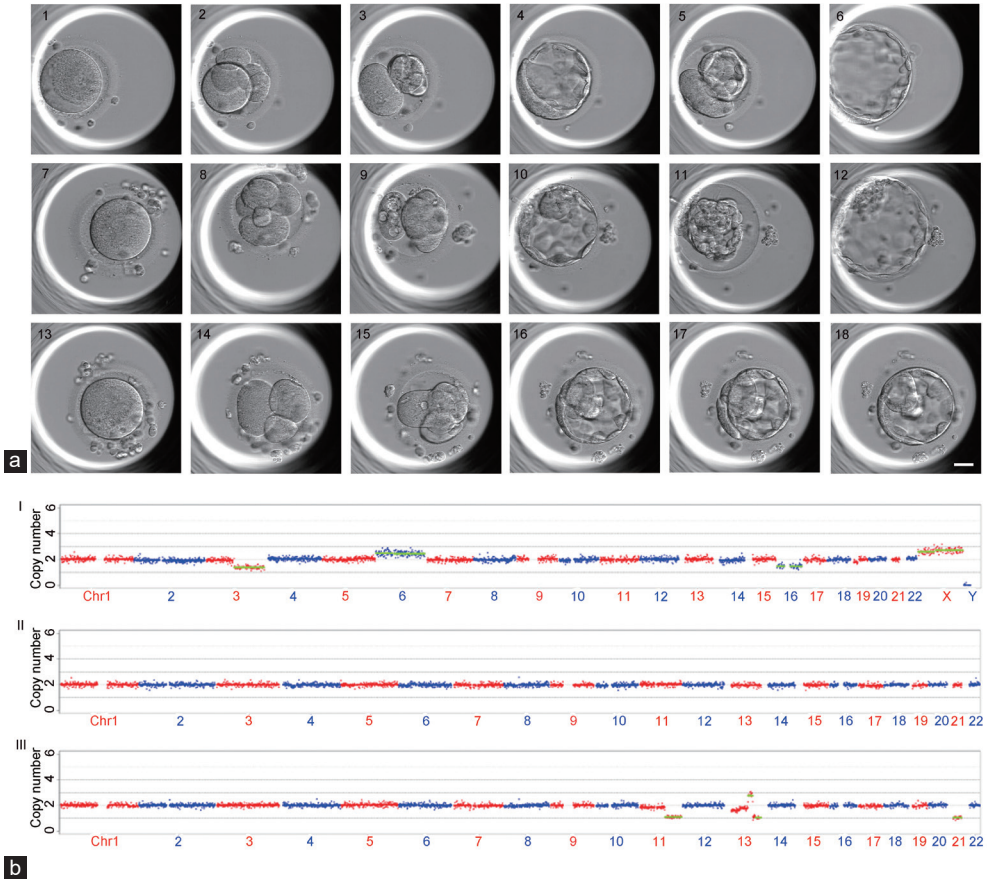

Figure 1: (**a**) Imaging of three examples of abnormal cleavage failure by using TLS. For three examples, one blastomere failed to undergo complete cleavage in the two-cell stage. The other blastomere divided then formed into the blastocyst. Three blastocysts underwent self-collapse in the process of blastocyst formation (scale bar = 30 μm). 1–6: imaging of example 1; 7–12: imaging of example 2; 13–16: imaging of example 2. (**b**) Whole genome of copy number variation (CNV) test results. I: 46,XX,+Xp(×3,mos,~60%),+Xq(×3),-3q(×1,mos,~60%),+6p(×3,mos,~50%),+6q(×3,mos,~50%),-16p(×1,mos,~50%),-16q(×1,mos,~50%); example 1: abnormal. II: example 2: normal. III: 45,XN,11q(q14.1→q25,~53Mb,×1),+13q(q21.33→q31.1,~16Mb,×3),-13q(q31.2→q31.3,~7Mb,×1),-13q(q32.1→q34,~19Mb,×1),-21(×1); example 3: abnormal.

blastomere, likely to reduce the implantation rate. More research is needed to confirm a relationship between this abnormal cleavage and blastocyst collapse. If future work confirms such a relationship, we should consider removing the blastomere lacking division in a proper time.

AUTHOR CONTRIBUTIONS

MZL and JZS designed the study and revised the manuscript. HYB collected the clinical information. XX drafted the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

ACKNOWLEDGMENTS

This project was supported by Young Physicians Program of Chinese Medical Association (No. 17020470716).

REFERENCES

1 Rubio I, Kuhlmann R, Agerholm I, Kirk J, Herrero J, *et al*. Limited implantation success of direct-cleaved human zygotes: a time-lapse study. *Fertil Steril* 2012; 98: 1458–63.
2 Athayde Wirka K, Chen AA, Conaghan J, Ivani K, Gvakharia M, *et al*. Atypical embryo phenotypes identified by time-lapse microscopy: high prevalence and association with embryo development. *Fertil Steril* 2014; 101: 1637–48.
3 Vera-Rodriguez M, Chavez SL, Rubio C, Reijo Pera RA, Simon C. Prediction model for aneuploidy in early human embryo development revealed by single-cell analysis. *Nat Commun* 2015; 6: 7601.
4 Liu Y, Chapple V, Roberts P, Matson P. Prevalence, consequence, and significance of reverse cleavage by human embryos viewed with the use of the Embryoscope time-lapse video system. *Fertil Steril* 2014; 102: 1295–300.
5 Yang SH, Wu CH, Chen YC, Yang CK, Wu TH, *et al*. Effect of morphokinetics and morphological dynamics of cleavage stage on embryo developmental potential: a time-lapse study. *Taiwan J Obstet Gynecol* 2018; 57: 76–82.
6 VerMilyea MD, Tan L, Anthony JT, Conaghan J, Ivani K, *et al*. Computer-automated time-lapse analysis results correlate with embryo implantation and clinical pregnancy: a blinded, multi-centre study. *Reprod Biomed Online* 2014; 29: 729–36.
7 Milewski R, Ajduk A. Time-lapse imaging of cleavage divisions in embryo quality assessment. *Reproduction* 2017; 154: 37–53.
8 Marcos J, Pérez-Albalá S, Mifsud A, Molla M, Landeras J, *et al*. Collapse of blastocysts is strongly related to lower implantation success: a time-lapse study. *Hum Reprod* 2015; 30: 2501–8.
9 Ashrafi M, Karimian L, Eftekhari-Yazdi P, Hasani F, Arabipoor A, *et al*. Effect of oocyte dysmorphisms on intracytoplasmic sperm injection cycle outcomes in normal ovarian responders. *J Obstet Gynaecol Res* 2015; 41: 1912–20.
10 Faramarzi A, Khalili MA, Ashourzadeh S. Oocyte morphology and embryo morphokinetics in an intra-cytoplasmic sperm injection programme. Is there a relationship? *Zygote* 2017; 25: 190–6.