

FIRST CLEAVAGE POLYMORPHISM GRADING AND TIMING OF CELL DIVISION PREDICTS BLASTULATION RATE: A RETROSPECTIVE TIME-LAPSE STUDY

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Background: Abnormal division patterns during early cleavage stages are reported to be associated with decreased developmental potential and aneuploidy.

Objective: The aim of this study was to understand the implications of various morphokinetic markers in preimplantation stage embryos during the first cleavage to predict embryo development potential and ploidy status.

Materials and Methods: This retrospective cohort study included 112 patients undergoing in vitro fertilization (IVF) cycles with prenatal genetic testing (PGT) between January 2018 and January 2023. Using a time-lapse culture system, individual embryos were assessed by conventional morphologic evaluation, and the time to pronuclear stage (PN), 2 cells (2C), and 4 cells (4C) progression were recorded. Between 2C and 4C, we further documented the timing of early 2C, the start and end of irregular 2C, and proper 2C. Embryos that underwent direct cleavage to 3 or more cells without the 2C stage were excluded from the analysis. The first cleavage polymorphism (FCP) grading system was developed based on irregular cleavage axis, delayed cytokinesis, irregularly shaped blastomeres, and cytoplasmic fragments, assigning each embryo to FCP grades I-IV. Correlations between FCP grading, blastocyst development, and chromosome ploidy status were analyzed.

Results: Out of 1140 embryos from 132 IVF cycles, 1129 were analyzed after excluding 11 transferred without PGT. Of these, 744 embryos (65.9%) developed into blastocysts and were biopsied. Higher FCP grade with severe polymorphism was associated with a higher chance of embryonic arrest and, thus, a lower blastulation rate (90.2%, 87.4%, 77.2%, and 36.5% for grades I, II, III, and IV, respectively, $p < 0.01$). Discarded embryos showed slower division rates between PN-early 2C, early 2C-4C, and proper 2C-4C stages than cryopreserved embryos ($p < 0.05$). While euploid embryo incidence did not significantly differ between FCP grades, a slight increase in non-euploid embryos was noted in grades III-IV (51.8%, 53.6%, 62.9%, and 60.1% for grades I, II, III, IV respectively, $p = 0.103$). Non-euploid embryos exhibited slower division rates between PN and early 2C stages compared to euploid embryos (15.7 ± 3.9 vs 14.9 ± 3.3 hours, $p = 0.006$).

Conclusions: The FCP grading system and cell division timing, monitored via a time-lapse culture system, are promising early indicators of embryo development and potential predictors of ploidy status, aiding in the selection of transferrable embryos.

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References:

1. Eze K et al. Prolonged blastomere movement induced by the delay of pronuclear fading and first cell division adversely affects pregnancy outcomes after fresh embryo transfer on Day 2: a time-lapse study. *Reprod Biomed Online*. 2019 May;38(5):659-668.
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Table 1. Time-lapse data and embryo outcomes based on the first cleavage polymorphism grade.

FCP grade	I (n=92)	II (n=254)	III (n=377)	IV (n=406)	Total (n=1129)	P-value
Blastulation rate, n (%)	83 (90.2)	222 (87.4)	291 (77.2)	148 (36.5)	744 (65.9)	<0.001*
Non-transferrable embryos, n (%)	43/83 (51.8)	119/222 (53.6)	183/291 (62.9)	89/148 (60.1)	434/744 (58.3)	0.1033
Time to PN, hrs	10.6 (2.4)	10.4 (2.5)	10.7 (2.6)	10.8 (2.7)		0.364
Time from PN to early 2C, hrs	15.1 (3.6)	15.2 (3.8)	15.8 (3.9)	18.8 (9.4)		<0.001*
Time from early 2C to proper 2C, hrs	4.0 (2.7)	4.6 (2.6)	5.3 (3.3)	5.6 (3.9)		<0.001*
Time from proper 2C to 4C, hrs	8.6 (4.1)	8.1 (3.5)	7.5 (5.8)	8.1 (7.3)		0.333
Irregular 2C duration, hrs	3.3 (2.6)	3.8 (2.5)	4.6 (3.2)	5.1 (3.8)		<0.001*

Abbreviations; FCP: first cleavage polymorphism, hrs: hours, PN: pronuclear stage, 2C: 2-cell, 4C: 4-cell, continuous variables expressed in mean (SD), Non-transferrable embryos include aneuploid, mosaic, and no result embryos