ORIGINAL PAPER

doi: 10.5455/medarh.2025.79.4-8
MED ARCH. 2025; 79(1): 4-8
RECEIVED: MAR 05, 2025
ACCEPTED: APR 02, 2025

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The Relationship Between Abnormal Morphokinetic Embryos, Genetic Testing Results, and Clinical Outcomes

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ABSTRACT

Background: Embryo quality is a crucial factor in the success of in vitro fertilization (IVF). Morphokinetics, which refers to the timing and sequence of embryonic cell division and development, has gained attention as a potential indicator of embryo viability and genetic competence.. **Objective** This study evaluates the relationship between abnormal embryonic morphokinetics and genetic analysis results, and their impact on clinical outcomes in assisted reproductive technology (ART). **Methods:** Conducted at Duc Phuc Hospital with Hanoi Medical University from January to December 2023, the prospective study included 152 patients undergoing in-vitro fertilization (IVF). A total of 968 blastocysts were analyzed using preimplantation genetic testing for aneuploidy (PGT-A). Time-lapse monitoring assessed cell division milestones and abnormal morphokinetic patterns, including direct cleavage, reverse cleavage, multinucleation, and vacuole. Patients received a single euploid embryo transfer. Clinical outcomes were tracked to the live birth stage, analyzed using SPSS 20.0, with p-values < 0.05 considered significant. **Results:** Of 583 blastocysts, 294 (50.4%) showed abnormal cleavage patterns. The aneuploidy rate was higher in embryos with reverse cleavage (56.1%) and multinucleation (50%), while direct cleavage and vacuolization showed no significant correlation. Early blastocyst formation (≥100 hours) was linked to a higher aneuploidy rate (60.8%). Nonetheless, clinical outcomes, such as β-hCG positivity and live birth rates, were similar between abnormal and normal cleavage groups when euploid embryos were transferred. **Conclusion:** Abnormal morphokinetic patterns are linked to higher aneuploidy rates, but do not significantly affect clinical outcomes when euploid embryos are selected. Integrating genetic testing with morphokinetic assessment can optimize ART success rates.

Keywords: abnormal cleavage, morphokinetics, aneuploidy, preimplantation genetic testing, in vitro fertilization, embryo selection.

1. BACKGROUND

Embryo quality is a crucial factor in the success of in vitro fertilization (IVF). Morphokinetics, which refers to the timing and sequence of embryonic cell division and development, has gained attention as a potential indicator of embryo viability and genetic competence. Abnormal morphokinetic patterns, such as delayed cleavage or irregular blastocyst formation, have been associated with decreased implantation rates and poor pregnancy outcomes (1). ecent studies have highlighted the importance of euploidy the presence of the correct number of chromosomes in determining the viability of embryos. Euploid embryos have been shown to have significantly higher implantation potential compared to their aneuploid counterparts (2). However, the relationship between morphokinetic characteristics and euploidy remains an evolving area of investigation. Some studies suggest that abnormal morphokinetic behavior is correlated with an increased likelihood of aneuploidy (3), while others argue that morphokinetics alone may not be definitive in predicting genetic normality (4). Understanding the euploidy rates among embryos exhibiting abnormal morphokinetic patterns is vital for optimizing embryo selection strategies. Such insights could lead to improved outcomes in assisted reproductive technology (ART) by refining the criteria for embryo transer.

2. OBJECTIVE

This study aims to evaluate the euploidy rates of embryos with abnormal morphokinetics and correlate these findings with established embryonic developmental patterns and genetic assessments and also, to evaluates the relationship between abnormal embryonic morphokinetics and genetic analysis results, and their impact on clinical outcomes in IVF.

3. MATERIAL AND METHODS

This study was approved by the Institutional Review Board (IRB) of Hanoi Medical University (number: 839/GCN-HĐĐĐNCYSH-ĐHYHN, date of approval 13 March 2023). Written informed consent was obtained from all participants prior to study enrollment. This research received no external funding. The authors declare no conflicts of interest.

Study Design

This prospective study was conducted at Duc Phuc Hospital in collaboration with Hanoi Medical University from January 2023 to December 2023.

Participants

A total of 152 patients undergoing in vitro fertilization (IVF) cycles were recruited. Patients had 2169 oocytes picked up, 1809 oocytes were cultured and monitored in a time-lapse system, 968 blastocysts were analyzed by preimplantation genetic testing for aneuploidy (PGT-A). There were 583 blastocysts having enough information. 68 euploidy blastocysts were single embryo transferred.

Inclusion Criteria:

Patients must meet at least one of the following indications for preimplantation genetic screening:

- Advanced maternal age (>35 years),
- History of failed embryo transfers ≥ 2 consecutive times,
- History of miscarriage or stillbirth ≥ 2 consecutive times,
- Severe sperm abnormalities,
- History of live birth with congenital anomalies.

Patients must voluntarily consent to participate in the study.

Transfer of one euploid blastocyst during the endometrial preparation cycle using hormone replacement therapy.

Exclusion Criteria:

- Severe uterine abnormalities,
- Any medical condition or health status that could compromise patient safety or adherence during the study.

Embryo Culture and Time-lapse Monitoring

Embryos were cultured in a time-lapse incubator, which captured continuous images to track cleavage events and morphological changes. Abnormal morphokinetic events recorded included direct division, reverse division, multinucleation, and vacuole formation.

- Direct Division: Defined as the process in which, during each cell division, a single cell divides into more than two daughter cells.
- Reverse Division: Defined as the phenomenon where two blastomeres, after forming, merge back together to create a new blastomere.
- Multinucleation: Identified when more than one nucleus appears within a single cell at the two-cell stage.
- Vacuoles: Identified during the compacted embryo stage.

Genetic Testing (PGT-A)

Trophectoderm biopsy was performed, and chromosomal analysis was conducted using next-generation sequencing (NGS) to determine euploidy status. Embryos were classified as either euploid, aneuploid, or mosaic based on genetic results.

Data Collection and Statistical Analysis

Patient characteristics, embryonic development parameters, and genetic testing results were collected from medical records. The relationship between abnormal morphokinetics and euploidy status was analyzed using SPSS 20.0. Chi-square and Fisher's exact tests were applied to assess categorical variables, while continuous data were compared using the Student's t-test or Mann-Whitney U test, as appropriate. A p-value < 0.05 was considered statistically significant.

4. RESULTS

The study was conducted on 152 couples. The average maternal age and paternal age were 36 and 39 years old, respectively. On average, 14 oocytes were collected per cycle, with 12 subjected to Intracytoplasmic Sperm Injection (ICSI), yielding a high fertilization rate of 88.9%. Furthermore, the cleavage embryo rate was impressive at 98.3%, and the blastulation rate was 60.3%.

A total of 583 embryos were analyzed, of which 289 embryos had the normal division (49.6%) and 294 embryos had the abnormal division (50.4%). The rates of euploid, mosaic, and aneuploid in the abnormal division group were 45.2%, 13.6%, and 41.2%, respectively.

The prevalence of direct division is 18%, reverse division is 29.7%, multinucleation account for 23.3%, and vacuoles are observed in 2.9% of embryos.

The relationships between aneuploidy rates and 4 types of abnormal morphorkinetic (Direct division, Reverse division, Multinucleation, Vacuoles) were described in Table 1. In the group with abnormal division, the rate of chromosomal aneuploidy (56.1%) was higher compared to the rate of euploidy (32.4%) and mosaicism (11.6%). In the multinucleated group, the rate of chromosomal aneuploidy (50%) also exceeded the rate of euploidy (36%) and mosaicism (14%). These differences were sta-

	Euploiday	Mosaic	Aneuploidy	N	p
Direct division	39/1055 (37.1%)	13/105 (12.4%)	53/105 (50.5%)	105	P > 0.05
Reverse division*	56/173 (32.4%)	20/173 (11.6%)	97/173 (56.1%)	173	P < 0.05
Multinucleation*	49/136 (36%)	19/136 (14%)	68/136 (50%)	136	P < 0.05
Vacuoles	7/17 (41.2%)	1/17 (5.9%)	9/17 (52.9%)	17	P > 0.05

Table 1. The relationship between aneuploidy rates and 4 types of abnormal morphorkinetic

	Euploidy	Aneuploidy	N	
R1*	48/142 (33.8%)	94/142 (66.2%)	136	P < 0.05
R2	22/64 (34.4%)	42/64 (65.6%)	79	P > 0.05
R (1 and 2)*	9/33 (27.3%)	24/33 (72.7%)	142	P < 0.05

Table 2: The relationship between aneuploidy rates in reverse division group. R1: Reverse division at the first division. R2: Reverse division at the second division.

	Euploidy	Aneuploidy	N
tSB < 100h	188/384 (49%)	196/384 (51%)	384
tSB ≥ 100h	78/199 (39.2%)	121/121 (60.8%)	199
P < 0.05			

Table 3: The relationship between the abnormality rate and the timing of blastocyst formation initiation (tSB)

tistically significant. Conversely, in the direct division and vacuoles formation, no significant differences were observed among the rates of euploid, mosaic, and aneuploid embryos.

Table 2 describes the relationship between the timing of reverse division and the rates of chromosomal aneuploidy. In the group exhibiting reverse division during the first cleavage, the aneuploidy rate (66.2%) was significantly higher compared to the euploidy rate (33.8%), (P<0.05). A similar result was observed in the group where reserve division occurred during both of the first two cell divisions, with aneuploidy and euploid rates reported at 72.7% and 27.3%, respectively, (P<0.05). However, when reserve division occurred only during theThe relationship between chromosomal abnormalities and the timing of blastocoel formation is illustrated in Table 3. The aneuploidy rate in the tSB ≥ 100h group was 60.8%, which is higher compared to the tSB < 100h group (51%.) This difference was statistically significant.

The clinical outcomes revealed the βhCG positivity rate, the clinical pregnancy rate, and the live birth rate in two distinct groups of embryos classified by their morphokinetic patterns: normal and abnormal. In the group with abnormal morphokinetics, the βhCG positivity rate, the clinical pregnancy rate, and the live birth rate were 79%, 66%, and 52%, respectively. This difference was not statistically significant.

5. DISCUSSION

This study investigates the euploidy rates of embryos with abnormal morphokinetic patterns in the context of assisted reproductive technologies and their correlation with clinical outcomes in IVF procedures. The findings underscore the intricate relationship between morphokinetic and genetic integrity, highlighting critical considerations for embryo selection in ART.

The findings of this study, which involved a comprehensive analysis of 152 couples undergoing assisted reproductive technology, provide significant insights into the relationships between embryonic morphokinetics, chromosomal abnormalities, and clinical outcomes. The average ages of the participants, with mothers at 36 years and fathers at 39 years, highlight the demographic factors often pertinent to fertility treatments, this results is similar to other publics (5, 6). Notably, the study observed a high fertilization rate of 88.9% following Intracytoplasmic Sperm Injection (ICSI), with an impressive cleavage embryo rate of 98.3% and a blastulation rate of 60.3%, align with previous reports (7). These outcomes lay a solid foundation for evaluating the subsequent analysis of 583 embryos, which were categorized into those with normal division (49.6%) and abnormal division (50.4%). This result was different to the previous study of Amy Barrie with 11.4%. This different result may be attributed to differences in study design. Our study specifically examines four types of abnormal division behaviors: direct cleavage, reverse cleavage, multinucleation, and vacuole formation. In contrast, Amy's study encompasses direct cleavage, reverse cleavage, absent cleavage in the presence of karyokinesis, chaotic cleavage, and cell lysis (8).

The data outlined in Table 1 reveals a strong correlation between morphokinetic patterns and aneuploidy rates. Specifically, embryos exhibiting reverse division demonstrated a significantly higher chromosomal aneuploidy rate of 56.1% compared to the euploid rate of 32.4% and a mosaicism rate of 11.6%, with similar significant findings in the multinucleation group. In contrast, direct cleavage and vacuolization were not significantly associated with embryonic aneuploidy. This finding suggests that not all abnormal cleavage events have the same impact on the developmental fate of the embryo. Our findings align with those of previous studies (9). This variability warrants further exploration of the biological mechanisms underpinning these morphokinetic behaviors.

In our study, the prevalence of direct division is 18%. This result is consistent with previously published medical literature. Rubio et al. (2012) reported a reverse division rate of 13.7% in their study, while Hickman et al. (2012) also documented a reverse division rate of 18% (8, 10). Regarding the reverse cleavage, the result of reverse division was 29.7%. Yanhe Liu also reported 27.4% of embryos having reverse division during the first three cleavage cycles (11).

The insights from Table 2 further elucidate the timing of abnormal division, revealing that reverse division occurring during the first cleavage resulted in a notable aneuploidy rate of 66.2%, significantly higher than the euploidy rate (p <0.05). The findings reinforce the critical role of early embryonic development in determining chromosomal outcomes, with the aneuploidy rate increasing when reverse division occurs during both of the initial cleavages, while no significant difference was observed when it happened exclusively during the second cleavage. These results are consistent with previous publications (12, 13).

Additionally, Table 3 presents compelling evidence linking the timing of blastocyst cavity formation to chromosomal abnormality, indicating that embryos forming the cavity at or beyond 100 hours exhibit a higher aneuploidy rate (60.8%) compared to those forming it earlier (51%). Chien-Hong Chen in his study also found out the correlation between tSB and mosaicsm (14). This relationship emphasizes the importance of developmental timing in assessing embryonic viability. While the study reports promising βhCG positivity and clinical preg-

nancy rates in both normal (80%) and abnormal morphokinetics (79%), there was no statistically significant difference in live birth rates between the two groups, suggesting that the presence of abnormal morphokinetics may not preclude successful clinical outcomes in all cases. This finding invites further research to better understand the implications of morphokinetic assessments on live birth outcomes and the potential for improving embryo selection strategies in ART.

Overall, this study underscores the importance of morphokinetic analysis in the context of assisted reproduction, suggesting that early embryonic development characteristics can be predictive of chromosomal integrity and subsequent reproductive success. Further exploration of these relationships may enhance clinical practices and optimize outcomes in fertility treatments.

6. CONCLUSION

This study highlights that while certain morphokinetic abnormalities are associated with higher aneuploidy rates, their impact on clinical outcomes is mitigated when euploid embryos are selected for transfer. These findings support the continued use of PGT-A as a key tool in embryo selection while recognizing the complementary role of time-lapse monitoring. A comprehensive approach that combines genetic assessment with morphokinetic evaluation may optimize ART success rates and improve patient outcomes.

Study limitations and future directions

Despite its strengths, this study has some limitations. The sample size, while substantial, may still limit the generalizability of the findings, particularly in cases with rarer morphokinetic abnormalities. Future research should explore larger, multi-center studies to validate these findings and assess whether specific morphokinetic parameters could be integrated into embryo selection algorithms alongside PGT-A results. Additionally, further investigation into the mechanisms linking abnormal cleavage patterns to aneuploidy could provide deeper insights into early embryonic development and improve ART outcomes.

- **Acknowledgment**: We sincerely thank the medical and research teams at Duc Phuc Hospital and Hanoi Medical University for their support. We also appreciate the patients who participated in this study and the laboratory staff for their meticulous work.
- **Ethical Statement**: This is a retrospective study, collecting data from medical records. Information is kept confidential during the research process, with the review and approval of Hanoi Medical University.
- **Author's contribution**: Research conception and design: Diem Thi Yen. Project administration: Nguyen Thi Hue Giang. Data acquisition: Diem Thi Yen, Le Thi Quyen, Tran Thi Dieu Thuy, Doan Nhu Tho. Statistical analysis: Diem Thi Yen. Data analysis and interpretation: Diem Thi Yen, Nguyen Khang Son. Drafting of the manuscript: Diem Thi Yen and Nguyen Khang Son. Edit: Nguyen Xuan Hoi.
- **Conflict of interest**: The authors declare that there is no conflict of interest
- **Financial support and sponsorship**: The authors received no specific funding for this work.

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