Research Article

Expansion Grade and Morphokinetic Parameters Associated with Aneuploidy Status of Embryo Fifth Day

Tingkat Ekspansi dan Parameter Morfokinetik yang Diasosiasikan dengan Status Aneuploid pada Hari ke lima Sebuah Embrio

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Abstract

Objective: To determine the potential of examining embryo morphology, and morphokinetic parameters in predicting the chromosomal status of embryos.

Methods: This study is a cross-sectional that requires patients undergoing IVF followed by chromosome examination with NGS that was conducted at the IVF Center at Pondok Indah Hospital and Morula IVF Center at Bunda Hospital from December 2021 to December 2022. Each embryo that reaches the blastocyst stage on day 5 or 6 will be washed and put into a PCR tube for a week; then, embryologists annotate them to determine morphological assessment and morphokinetic parameters using Time-Lapse Microscopy. The chi-square test was used to analyse bivariate variables.

Results: One hundred twenty-four samples were collected on day 5 of patients undergoing the IVF procedure. 50.8% of the samples were aneuploid chromosomes, and 49.2% were euploid. The morphokinetic characteristics median was 3.86 fold. It was found that expansion grade, time to pro-nuclear fading, and time to the synchrony of the third cell cycle were significantly associated with euploid status (p = 0.000; 0.041 and 0.036).

Conclusion: The expansion grade has been proven as the most influential component for accurately predicting the ploidy status of embryos.

Keyword: blastocyst, embryo, euploid status, expansion grade, morphokinetics.

Abstrak

Tujuan: *Untuk mengetahui potensi pemeriksaan morfologi embrio, dan parameter morfokinetik dalam memprediksi status kromosom embrio.*

Metode: *Penelitian ini merupakan penelitian potong lintang yang memerlukan pasien yang sedang menjalani program bayi tabung yang dilanjutkan dengan pemeriksaan kromosom dengan NGS yang dilakukan di Pusat IVF RS Pondok Indah dan Pusat IVF Morula RS Bunda pada bulan Desember 2021 hingga Desember 2022. Setiap embrio yang mencapai tahap blastokista pada hari ke 5 atau 6 akan dicuci dan dimasukkan ke dalam tabung PCR selama seminggu; kemudian, ahli embriologi membuat anotasi untuk menentukan penilaian morfologi dan parameter morfokinetik menggunakan Mikroskop Time-Lapse. Uji chi-square digunakan untuk menganalisis variabel bivariat.*

Hasil: *Seratus dua puluh empat sampel dikumpulkan pada hari ke 5 dari pasien yang menjalani prosedur IVF. 50,8% sampel adalah kromosom aneuploid, dan 49,2% adalah euploid. Median karakteristik morfokinetik sebesar 3,86 kali lipat. Ditemukan bahwa tingkat ekspansi, waktu menuju pemudaran pro-nuklir, dan waktu hingga sinkronisasi siklus sel ketiga berhubungan secara signifikan dengan status euploid (p = 0,000; 0,041 dan 0,036).*

Kesimpulan: *Tingkat ekspansi telah terbukti sebagai komponen yang paling berpengaruh dalam memprediksi status ploidi embrio secara akurat.*

Kata kunci: *blastosist, embrio, status euploid, tingkat ekspansi, morfokinetik.*

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INTRODUCTION

In vitro fertilization (IVF) is an assisted reproductive technology used to achieve pregnancy on infertile couples. The pregnancy rate of IVF is between 30-40%, with endometrial recetivity and embryo being the most influential factors for successful implantation.[1, 2] Therefore, additional methods needs to be performed on embryos to increase implantation success and result in pregnancy and birth.[3] Study compared the pregnancy rate between day-2 and day-3 embryos found no significant difference. However, embryo quality was identified as a potential factor affecting pregnancy rates.[4] One way to ensure that the embryo quality is through the use of Preimplantation Genetic Testing for Aneuploidy (PGT-A) or Preimplantation Genetic Screening (PGS). These procedures are carried out to ensure the embryo does not have any aneuploid chromosomes. This procedure requires a biopsy of the blastomeres in the 8-cell phase or the trophectoderm in the blastocyst phase. It also requires high expertise as well as expensive equipment and reagents. In addition, there is no clear evidence yet that this invasive procedure does not cause injury to the embryo, and there is the possibility of undetected mosaic or segmental aneuploidy.[3] This has led IVF practitioners to consider non-invasive embryo selection methods.

Embryo visual assessment is a well-established and commonly used method for selecting embryos by examining their morphology under a microscope at specific time points. Various parameters are evaluated at different stages of development to gather information about the embryo's quality. Although the scoring criteria may vary among fertility clinics, most assess cleavage-stage embryos based on: fragmentation, presence, number, and nuclei size. Similarly, blastocyst-stage embryos are assessed based on the number and symmetry of blastomeres. It is crucial to standardize the timing of these assessments. Embryonic development is highly dynamic; even assessments conducted on the same day can yield different results between day and night. The highlights are the importance of conducting frequent embryo examinations to gather more comprehensive information about their developmental progress and viability over time.[5-7] Nevertheless, it is important to acknowledge that this assessment necessitates the removal of embryos from the culture medium within the incubator and relies on the expertise of an embryologist. Therefore, the involvement of human factors such as embryologist's expertise cannot be eliminated.[8, 9]

Considering the natural variations in embryo development speed, the time element becomes crucial when assessing embryo morphology; consequently, evaluations at specific time points need to be performed daily. Time-lapse microscopy techniques have been devised to continuously analyses embryo morphology at regular intervals, spanning just a few minutes. Time-lapse microscopy holds significance as it allows for the observation of embryos without necessitating their removal from the incubator, there by maintaining the optimal conditions for embryo development.[10] The study demonstrated that using time-lapse technology resulted in a significantly higher pregnancy rate than conventional methods (67.32% vs. 57.22%; $p = 0.0410$) and live birth rate (65.37% vs. 55%; $p = 0.0380$).[11]

The time-lapse imaging displays the changes occurring in the nuclear and cytoplasmic components within the culture medium. The recorded videos are annotated at specific time points and developmental stages. The morphokinetic assessment of the embryo begins during the early stages, starting from the moment of syngamy and progressing through the stages of 2-cell, 3-cell, 4-cell, 5-cell, 6-cell, and 9-cell embryos (t1 – t9), and later at the time of partial compaction. In the final stages, the morphokinetic assessment encompasses genomic activation, the morula stage, blastulation, blastocyst formation, and blastocyst expansion. An embryo is referred to as a morula when over 90% of it has undergone compaction; while a blastocyst is characterized by a crescent-shaped embryo with fluid filling the central cavity.[12]

Many studies have been conducted to build non-invasive models by associating embryo morphology, morphokinetics, and euploidy status. Some studies have reported that euploid status is significantly related to the morphological or morphokinetic characteristics of the embryo. The euploid rate was significantly higher for embryos with good morphology (73.2%) than for those with average (50%) or poor morphology (40%; $p=0.001$). It was also found that the inner cell mass (ICM) and trophectoderm (TE) are strongly associated with the euploidy rate.[13] Moreover, study reported that blastocyst expansion was the most significant factor associated with euploid

status (p = 0.045).[14]

The study showed that the mean of time to pro nuclear fading (tPNf), Time to 2 cells (t2), Time to 5 cells (t5), Duration of second cells cycle (cc2), Duration of second cells cycle (cc3), Difference between t5 and t2 (t5-t2) were significantly delayed in aneuploidy embryos.[15] Additionally, it was observed that the early breakdown of pronuclei is a potential marker of euploid embryos. A study conducted suggests that t3 and t5-t2 associate significantly with chromosome abnormality. Aneuploid embryos appeared to have delayed tSC, tSB, and tB compared to euploid embryos.[10]

The invasiveness of the procedure, cost, and fear of embryo damage caused by PGT-A examination to determine the ploidy status of an embryo's chromosomes have created a need for another method that is simpler, cheaper, and non-invasive, and that has at least the same ability to predict aneuploidy in embryos. Morphological assessment alone is ineffective since embryos that have good morphology do not necessarily have normal chromosomes. Therefore, this study aims to determine the potential of examining embryo morphology, and morphokinetic parameters in predicting the chromosomal status of embryos.

METHODS

This research is a cross-sectional study to determine the relationship between embryo morphology and chromosomal status, and blastocyst morphology. Research has been done on embryo culture from patients undergoing in vitro fertilization (IVF). Samples were taken at the IVF Centre at Pondok Indah Hospital and Morula IVF Centre at Bunda Hospital from December 2021 – December 2022. Examinations were conducted at the PGS/PGD Cluster Human Reproduction, Infertility and Family Planning (HRIFP) IMERI FMUI laboratory and the Diagnostic and Research Centre biomolecular laboratory FMUI to analyse the Next Generation Sequencing (NGS). Non-probability consecutive sampling was used, in total 124 samples were analyzed. Samples included in this study are the embryo on day 5 of patients undergoing IVF, followed by chromosome examination with NGS. Samples were excluded if the embryo did not turn into a blastocyst on day 5 of culture, failed to take part in the chromosome examination due to the absence of embryos that could be biopsied, biopsy showed a mosaic embryo, and if the embryo were not cultured.

Oocyte in metaphase II stage, which have been taken by a specialist in obstetrics and gynecology, were then carried out to do intracytoplasmic sperm injection (ICSI) procedure. The oocytes that have been injected with sperm cells will have the pronucleus examined 18-20 hours post-ICSI to determine whether the oocytes are fertilized or not. Fertilized oocytes will form 2 pronuclei (2pn) and then be cultured until the third day. On the fifth day, the oocytes will be cultured separately in blast assist without phenol red media. Embryo developments will then be recorded and photographed every day to assess its morphological quality using the morphokinetic time-lapse. Embryos that reach the blastocyst stage will undergo a trophectoderm biopsy.

The morphology of the inner cell mass was categorized into three groups: A, B, and C. Group A represented a good morphology, characterized by a high number of compacted cells. Group B indicated a moderate morphology with fewer cells and less compaction. Group C represented a poor morphology with very few cells and an absence of compaction. Similarly, the trophectoderm morphology was categorized into groups A, B, and C. Group A denoted a good morphology with a cohesive layer formed by numerous cells. Group B indicated a moderate morphology with fewer cells and loosely arranged structure. Group C represented a poor morphology with a small number of larger cells. The expansion grade was classified on a scale from 1 to 6. Grade 1 indicated the initial stages of blastocyst development. Grade 2 represented a blastocoel occupying more than 50% of the embryo. Grade 3 denoted a blastocoel occupying the entire embryo, indicating a complete blastocyst. Grades 4, 5, and 6 indicated the expanding blastocyst with thinning trophectoderm, the embryo starting to hatch, and the embryo fully hatched, respectively.

We conclude the morphokinetic characteristic. To enhance the accuracy of a morphokinetic evaluation, it is essential to have a device capable of documenting the evolving embryo with precision. Time-lapse microscopy (TLM), which involves the utilization of a specialized microscope capable of capturing microscopic changes, allows for closer observation of embryos, enabling the tracking of their development and changes over time. TLM comprises various key components, including a microscope equipped with phase contrast or fluorescent capabilities, a real-time imaging digital camera, computer software controlling the

camera, and an incubator providing an optimal environment for the embryo.[16] In this study TLM assessed for the embryo begin from the early stages (pro-nuclear fading) to the final stages (blastocyst). The duration of cycle and time of synchrony were also evaluated.

Each embryo that reaches the blastocyst stage on day 5 or 6 will be informed to the patient. A biopsy will be performed if the patient wishes to undergo a chromosomal examination. Each embryo will be placed in a separate medium. Then, 4 to 10 cells from the trophectoderm will be taken from each embryo by perforating the pellucida zone with a laser under microscope, creating a hole that is by 6-9 µm in size. The collected trophectoderm cells will then be washed in a drop containing PBS and 10% PVP solution. The washed cells will be placed into a PCR tube containing 2.5 µl of the same medium. The cells will then be stored with the temperature of -20oC for a week, before the chromosomes were examined using the NGS method. The morphology of day five embryos obtained by time-lapse microscopy were annotated by embryologists to carry out morphological assessments. All variables that have been assessed to predict the chromosomal status of the embryo will then be included in creating a prediction model to determine which model has the best diagnostic ability. Finally, all data were analyzed using percentages, means, and medians for abnormal distributions. Bivariate analysis employed the chi-square test for categorical variables, unpaired t-tests and the Mann-Whitney test for numerical variables.

ETHICAL CONSIDERATION

This study has been approved by the ethics committee of the Faculty of Medicine Universitas Indonesia.

RESULTS

One hundred forty-eight samples were collected from embryo culture and embryo biopsies on day 5 of patients undergoing the IVF procedure. Samples were screened based on exclusion and inclusion criteria resulting in 24 samples being excluded from the study because the PGT-A results showed mosaic chromosomes. Final data processing and analysis were carried out on 124 samples. Morphological characteristics and PGT-A results were described in Table 1. Out of the total samples, 50.8% exhibited aneuploid chromosomes, while 49.2% exhibited euploid chromosomes. The morphology of the inner cell mass is predominantly Category A (54%); morphology of the trophectoderm showed that Categories A and B are slightly different (37.9% and 40.3%, respectively), and 36.5% of embryos reach expansion grade 3.

Table 1. Morphological Embryo Characteristics

Characteristic		Description (n= 124)
Inner cell mass morphology	Category A	67 (54)
	Category B	42 (33.9)
	Category C	15 (12.1)
Trophectoderm morphology	Category A	47 (37.9)
	Category B	50 (40.3)
	Category C	27 (21.8)
Expansion grade	Grade 1	26 (21)
	Grade 2	41 (33.1)
	Grade 3	45 (36.3)
	Grade 4	9 (7.3)
	Grade 5	3 (2.4)
	Grade 6	0 (0)
PGT-A chromosomes status	Euploid	61 (49.2)
	Aneuploid	63 (50.8)

*Categorical variables are presented by percentage n (%)

Morphokinetics data were extracted from time lapse-monitoring to determine the length of time it takes for the embryo to divide and reach the blastocyst stage (tB). The morphokinetics characteristics were described in table 2. It can be seen that the embryos need about 100.49 ± 10.19 (107.94 – 111.57) hours to reach the blastocyst stage and becomes a complete blastocyst after 107.19 hours (91.2 – 140.20).

Table 2. Morphokinetics Characteristics

Characteristic	Description (n= 124)
Time to pro nuclear fading (hour); tNPf	20.91 (5.23 – 53.02)
Time to 2 cells (hour); t2	25.64 (6.56 – 64.02)
Time to 3 cells (hour); t3	36.00 (8.15 – 67.93)
Time to 4 cells (hour); t4	38.10 (10.73 – 68.43)
Time to 5 cells (hour); t5	48.52 ± 9.26 (46.88 – 50.17)
Time to 6 cells (hour); t6	52.22 (23.48 – 86.27)
Time to 7 cells (hour); t7	53.77 (28.15 – 88.02)
Time to 8 cells (hour); t8	60.91 (30.90 – 100.81)
Time to compact (hour); tSC	80.44 ± 11.77 (78.35 – 82.55)
Time to morula (hour); tM	88.10 ± 10.86 (86.10 – 90.03)
Time to start blastocyst (hour); tSB	100.49 ± 10.19 (107.94 – 111.57)
Time to blastocyst (hour); tB	107.19 (91.2 – 140.20)
Duration of second cells cycle (hour), cc2 (t3 – t2)	10.65 (1.34 – 17.67)
Time of the synchrony of the second cell cycle (hour); s2 (t4 – t3)	1.35 (0.00 – 16.5)
Duration of third cells cycle (hour); cc3 (t5 – t3)	12.69 (1.36 – 33.57)
Time of the synchrony of the third cell cycle (hour); s3 (t8 – t5)	15.34 (0.83 – 102.25)

*Numerical variables with normal distribution are presented in mean ± standard deviation and variables with a non-normal distribution are presented as the median.

Bivariate analysis was performed to assess variables related to euploid and aneuploid chromosomal status and were described in Table 3. It was found that expansion grade has significant value on predicting PGT-A chromosome status (p=0.000). The expansion grade was categorized in ordinal (Grade 1-2; Grade 3-6) and nominal (Grade 1 – 5); both categorizations showed that the expansion grade has significant value, consequently (p=0.000; p=0.001). However, several other variables, such as the morphology of the inner cell mass and the trophectoderm, did not show any significant differences in chromosome status.

Table 3. Results of Morphological Bivariate Analysis with PGT-A Chromosome Status

Variable	Euploidy PGT – A (n=61)	Aneuploidy PGT – A (n=63)	*P-value*	OR	(95%CI)
Inner cell mass morphology					
Category A	36 (53.7)	31 (46.3)	0.531[a]		
Category B	18 (42.9)	24 (57.1)			
Category C	7 (46.7)	8 (53.3)			
Inner cell mass morphology					
Category A	36 (53.7)	31 (46.3)	0.18[a]	1.48	0.73 – 3.02
Category B dan C	25 (43.9)	32 (56.1)		Comparison	
Trophectoderm morphology					
Category A	24 (51.1)	23 (48.9)	0.948[a]		
Category B	24 (48)	26 (52)			
Category C	13 (48.1)	14 (51.9)			
Trophectoderm morphology					
Category A	24 (51.1)	23 (48.9)	0.444[a]	1.12	0.54 – 2.33
Category B dan C	37 (48.1)	40 (51.9)		Comparison	
Expansion Grade					
Grade 1 – 2	23 (34.3)	44 (65.7)	0.000[a]	3.82	1.81 – 8.07
Grade 3 – 6	38 (66.7)	19 (33.3)		Comparison	
Expansion Grade					
Grade 1	10 (38.5)	16 (61.5)	0.001[a]		
Grade 2	13 (31.7)	28 (68.3)			
Grade 3	26 (57.8)	19 (42.2)			
Grade 4	9 (100)	0 (0)			
Grade 5	3 (100)	0 (0)			

[a]Categorical variables were analyzed using the chi square test with continuity correction, presented in the form of n (%), p value, OR and 95%CI

The results of morphokinetic bivariate analysis with PGT-A chromosome status are described in Table 4. Only tPNf and ss3 showed significant results, with p-value of 0.041 and 0.036, respectively.

Table 4. Results of Morphokinetic Bivariate Analysis with PGT-A Chromosome Status

Variable	Euploidy PGT – A (n=61)	Aneuploidy PGT – A (n=63)	*P-value*	Average Ranking	(95%CI)
Time to pro nuclear fading (hours); tNPf	19.88 (11.05 – 53.02)	21.91 (5.23 – 30.65)	0.041[a]		
Time to 2 cells (hours); t2	25.01 (19.67 – 64.02)	25.96 (6.56 – 33.43)	0.301[a]		
Time to 3 cells (hours); t3	35.13 (23.44 – 67.93)	36.36 (8.15 – 47.62)	0.397[a]		
Time to 4 cells (hours); t4	37.76 (26.13 – 68.43)	38.43 (10.73– 53.79)	0.430[a]		
Time to 5 cells (hours); t5	48.23 ± 10.54	48.81 ± 7.97	0.727[b]	-0.58	-3.88 – 2.72
Time to 6 cells (hours); t6	50.52 (32.99 – 86.27)	52.51 (23.48 – 79.85)	0.342[a]		
Time to 7 cells (hours); t7	54.28 (41.22 – 88.02)	53.67 (28.15 – 83.25)	0.986[a]		
Time to 8 cells (hours); t8	60.92 (44.34 – 100.81)	60.09 (30.9 – 100.35)	0.479[a]		
Time to compact (hours); tSC	81.17 ± 11.80	79.73 ± 11.79	0.498[b]	1.43	-2.75 – 5.63
Time to morula (hours); tM	88.17 ± 11.84	88.02 ± 9.91	0.938[b]	0.15	-3.72 – 4.03
Time to start blastocyst (hours); tSB	100.10 ± 10.04	100.88 ± 10.63	0.676[b]	-0.77	-4.45 – 2.90
Time to blastocyst (hours); tB	105.22 (91.2 – 138.25)	108.85 (96.36 – 140.20)	0.227[a]		
Duration of second cells cycle (hours); cc2 (t3 – t2)	10.63 (1.56 – 15.50)	10.67 (1.34 – 17.67)	0.446[a]		
Time of the synchrony of the second cell cycle (hours); s2 (t4 – t3)	1.42 (0.00 – 16.30)	1.33 (0.00 – 16.75)	0.492[a]		
Duration of third cells cycle (hours); cc3 (t5 – t3)	12.58 (1.36 – 33.57)	13.58 (3.73 – 27.21)	0.073[a]		
Time of the synchrony of the third cell cycle (hours); s3 (t8 – t5)	17.46 (1.42 – 47.67)	13.43 (0.83 – 102.25)	0.036[a]		

[a]Numerical variables with abnormal data distribution were analyzed using the Mann Whitney test, presented in the form of median (minimum – maximum), p-value and average ranking

[b]Numerical variables with normal data distribution were analyzed using unpaired t-test, presented in the form of mean ± standard deviation, p-value, mean difference and 95%CI

DISCUSSION

Morphological characteristics have been studies for determine embryo selection. A study conducted examined the relationship between morphology and the euploid status of the cleavage and embryo blastocyst. It was found that 40.6% of good embryo morphology are euploid, 29.3% were in moderate and 25.8% were in poor; no significant difference was found between the morphology of the cleavage phase and the euploid number (p=0.254). Assessment of embryos in the blastocyst phase found a significant difference in the euploid rate which were 73.2% with good morphology, 50% were moderate and 40.5% were poor (p=0.001). It was also found that the grade of inner cell mass and trophectoderm were related to the euploid number of embryos in blastocyst phase.[13] Carried out a research study that employed multiple logistic regression analysis to determine the most influential morphological grading factor affecting the euploidy status of embryos. Among the three grading factors evaluated (TE, ICM, expansion), the blastocoel expansion grade emerged as the most influential factor (odds ratio: 1.261; P = 0.045).[14] However, this study found that inner cell mass and trophectoderm morphology did not significantly correlate with euploid status (p = 0.531; 0.948). Interestingly, only the expansion grade showed a significant value with a p-value of 0.000 (OR 3.82; 95% CI 1.81 – 8.07).

A study conducted by Desai et al. reported that expansion grade had a stronger correlation with clinical pregnancy (OR 7.10; 95% CI, 2.73 – 19.5 ; p<0.001) and live birth rate (OR 7.19; 95% CI,

2.68 – 20.27 ; p<0.001) than other morphologic characteristics in frozen embryo transfer. A different significant implanted rate was reported within the same expansion grade on day 6 and 5 (36% vs 58%; p<0.001). In bivariate analysis, TE and ICM were also significant in affecting clinical pregnancy and live-birth rate. But both ICM and TE score were influenced by blastocyst expansion. Furthermore, the expansion grade was categorized as either an expanded blastocyst (grade 3 to 4) and a non-expanded blastocyst (grade 1 to 2). The result showed that the likelihood of clinical pregnancy and live birth were four times higher in expanded blastocyst. In conclusion, their study provides evidence that the expansion grade is the most crucial factor influencing blastocyst implantation in FET cycles.[12]

Another study showed that expansion score strongly predicted successful clinical pregnancy and live birth independently. Study demonstrated that expanded blastocyst with normal ICM and suboptimal TE had high implantation rate. Assume that TE did not independently impact implantation rate.[17]

Instead of predicting pregnancy and implantation rate, we correlated the expansion grade with euploidy status. In this context, aneuploid embryo has a lower chance of establishing a pregnancy and live birth compare with euploid embryo (5.8% vs 59.6%; 5.0% vs 46.7%).[18] A study reported that the kinetics of blastocyst expansion are associated with euploid status. The expansion of embryo affected by aquaporin water channels which allow extracellular liquid to flow into trophectoderm. The inflow results from the increase of ion concentration regulated by the sodium/potassium pump. Aneuploidy is a genomic condition characterized by an imbalance in chromosome number, resulting in both generalized and chromosome-specific cellular deficits. Moreover, imbalance chromosome causes slower cell division, which reduces cellularity and leads to less robust expansion of the blastocoel.[19-21]

Our study revealed only time to pro-nuclear fading (tPNf) and time of the synchrony of the third cell cycle (s3; t8 – t5) showed significant results (p = 0.041 and 0.036) operating Time-lapse Miscroscopy. In a retrospective study carried out embryos that underwent biopsy on the third day were subjected to morphokinetic assessment using time-lapse technology. The study observed that the most significant variables for predicting chromosomal abnormalities were the duration of division from 3 blastomeres to 5 blastomeres (cc3, OR=2.095; 95% CI=1.356 – 3.238) and the time interval between 2-cell and 5-cell stages (t5−t2, OR=2.095; 95% CI=1.763−4.616). A meta-analysis that incorporated various morphokinetic parameters to assess embryo development. According to their findings, embryos that exhibited rapid division and shorter durations of 2-cell and 3-cell stages had a higher rate of successful implantation. In a separate study it was demonstrated that time-lapse microscopy (TLM) combined with a specific algorithm that could identify the risk of aneuploidy without the need for Preimplantation Genetic Screening (PGS). TLM serves as a valuable selection tool in cases where PGS is recommended but not feasible due to factors such as economic, legal, social constraints, or even when PGS is not indicated for certain patients.[15, 22]

One of the studies assessing morphokinetic parameters associated with euploid or aneuploid status in embryos was conducted on 1730 blastocysts that were biopsied. There was a significant difference in the average time required to reach blastocyst (tB) between the euploid embryo group of 110.2 hours compared to the aneuploid group of 112.8 hours (p<0.001). Also obtained the difference between the time needed to reach expanded blastocyst (tEB) and the time to reach blastocyst hatching (tHB).[23]

Study reported that tPNa, tPNf, t2, t5, tSB, s3, s3 are significant associate with ploidy status of embryo (p = 0.010; 0.001; 0.010; 0.010; 0.040;0.006;0.040). The mechanism of aneuploidy embryo impact on morphokinetic remains elusive. Between the appearance of pronuclei and their fading, chromosome arrangement, histone modifications, and transcriptional activity occur. The variety length of the S and G2 phases reflects the extent of DNA synthesis. As a result, the synthesis of a different amount of genetic material can alter the standard timeline of pronuclear appearance. In conclusion, euploid status might play a role in determining the morphokinetic patterns of embryos.[24]

CONCLUSION

This study concludes that expansion grade could be a potential factor for accurately predicting the ploidy status of embryos. Moreover, only the time to pronuclear fading and the synchrony of the third cell cycle in morphokinetic parameters

are significantly associated with ploidy status.

STRENGHT AND LIMITATION

This study evaluates, envies, and strengthens the previous studies that morphology embryo and morphokinetic parameters impact embryo euploid status. However, only 124 embryos were analysed; further studies should be performed using bigger samples to establish an accurate non-invasive methods predictive model for embryo selection.

CONFLICT of INTEREST

The author declare that they have no conflict of interest.

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