

Original Research

Day 3 Embryo Morphology is a Significant Predictor of Blastocyst Euploidy

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Abstract

Background: This retrospective cohort study aims to determine the relationship between morphologic grading of day 1 or 3 embryos and euploid blastocyst rate in the preimplantation genetic testing cycle. **Methods:** 2001 two pronucleus (2PN) embryos were obtained from 219 patients in our *in vitro* fertilization center on day 1. Embryo morphologic grading was conducted on day 1 and day 3. A blastocyst trophectoderm biopsy was conducted on day 5 or day 6, followed by aneuploid screening using next generation sequencing platform. Chi-square test, Student's *t*-tests and Mann-Whitney *U* tests were used to compare categorical and continuous variables between the cohorts. Logistic regression analysis for euploidy was conducted to determine the association of embryo morphological grading with blastocyst euploidy. **Results:** 811 blastocysts selected from 2001 2PN embryos were appropriate for biopsy and DNA from all biopsies were successfully amplified for aneuploidy screening. The day 1 pronuclear pattern showed a weak, non-statistically significant association with euploid blastocyst ($p > 0.05$). In contrast, day 3 cleavage-stage embryo scoring, which included blastomere number ($p < 0.01$, odds ratio (OR) = 1.156), symmetry ($p < 0.01$, OR = 0.710) and fragmentation ($p < 0.01$, OR = 0.624) all showed significant association with euploid blastocyst. 8 cell or ≥ 12 cell embryos were measured with the highest euploid rate, while increased blastomere size differences and fragmentation decreased the euploid embryo rate. **Conclusions:** Day 3, but not day 1, embryo morphology was a significant predictor for euploid blastocysts. Day 3 morphology provides individualized, visualized, and prognostic information concerning the euploid nature of a blastocyst. Blastomeres are assigned properties when selecting day 3 embryos for implantation or blastocyst culturing; thus, the morphology of day 3 embryos provides a guide for selecting euploid embryos and improving *in vitro* fertilization outcomes.

Keywords: embryo morphology; PGT; euploid blastocyst

1. Introduction

In past 40 years, *in vitro* fertilization (IVF) success rates have remarkably improved [1–3]. However, analysis of embryonic developmental potential remains a significant challenge [4–6]. Chromosomal abnormality is the most common cause for miscarriage, and selection of euploid embryos for implantation can markedly improve IVF outcomes [7,8]. Morphologic assessment is the primary method for embryo selection, the factors used in assessment include oocyte, zygote, cleavage-stage embryo, and blastocyst. However, this approach cannot evaluate the status of the embryonic genome [9–12]. Time-lapse microscopy (TLM) can monitor embryo development over a 24 hour window without removing the embryo from the incubator. Due to recently acquired knowledge of embryo development dynamics, TLM technology has the potential to become a priority method for embryo selection. However, concerns are that the needed equipment is expensive and there is potential harm from 24 hour monitoring of the embryo, thus the use of TLM remains controversial. Moreover, the cost of equipment and consumables will increase the economic burden on patients seeking IVF [13–16].

Preimplantation genetic testing for aneuploidy (PGT-A) reduces the time to pregnancy and avoids the transfer of aneuploidy embryos [17–20]. Early on, IVF centers used blastomere biopsy on day 3 to identify euploid embryos [21]. Within the development of blastocysts in culture, vitrification, trophectoderm (TE) biopsy, and next generation sequencing (NGS) are widely used for aneuploid testing [22,23]. Although PGT-A has many advantages for patients, damage to the trophectoderm (TE) from embryo biopsy is still unclear [24], and some studies considered that TE plays a crucial role during embryo development [10,25]. Several studies have demonstrated that blastocyst grading can predict euploid blastocysts, but the association is weak or moderate [26,27]. Liu *et al.* [28] have studied the correlation between day 3 morphologic grading and pregnancy outcomes in preimplantation genetic testing (PGT) cycles that underwent blastomere biopsy and found that poor/fair-quality embryos have a substantially reduced likelihood of retaining viability after biopsy. However, studies that have explored the potential correlation between day 3 morphologic grading and euploid blastocysts which have undergone trophectoderm (TE) biopsy remains still limited.



Table 1. Demographics and IVF-PGT cycle characteristics of the 219 patients in 3 groups.

	PGT-M		PGT-SR		PGT-A		Total		<i>p</i> -value
	54 patients		91 patients		74 patients		219 patients		
	519 2PN embryos		976 2PN embryos		506 2PN embryos		2001 2PN embryos		
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	
Age (years)	31.2 ± 4.7	21, 41	31.2 ± 3.6	24, 40	37.4 ± 5.2	27, 45	33.3 ± 5.3	21, 45	<0.01
BMI (kg/m ²)	21.1 ± 2.8	16.0, 27.7	21.7 ± 2.8	16.2, 29.2	22.0 ± 2.4	16.9, 30.1	21.7 ± 2.7	16.0, 30.1	NS
FSH (IU/L)	5.7 ± 1.6	0.4, 9.6	5.8 ± 1.7	3.2, 12.6	6.5 ± 3.0	2.9, 14.2	6.0 ± 2.2	0.4, 16.1	NS
LH (IU/L)	3.5 ± 1.8	0.4, 9.6	4.0 ± 2.4	0.5, 14.5	3.3 ± 1.5	1.0, 7.5	3.6 ± 2.0	0.4, 14.5	NS
AMH (ng/mL)	3.8 ± 3.2	0.8, 16.3	4.5 ± 3.0	0.5, 17.5	2.9 ± 2.8	0.1, 14.6	3.8 ± 3.1	0.1, 17.5	<0.01
Oocytes retrieved	16.3 ± 7.5	4, 40	16.4 ± 8.5	2, 43	11.0 ± 8.7	2, 41	14.5 ± 8.7	1, 43	<0.01
Mature oocytes	13.2 ± 6.0	3, 27	13.2 ± 7.1	2, 42	8.8 ± 7.2	2, 37	11.7 ± 7.2	2, 42	<0.01
2PN embryos	11.0 ± 5.9	1, 27	11.0 ± 6.0	2, 34	7.2 ± 6.0	1, 31	10.1 ± 6.5	1, 34	<0.01
TE biopsy	4.1 ± 3.3	1, 14	4.2 ± 3.3	1, 20	2.8 ± 3.3	1, 16	3.7 ± 3.4	1, 20	<0.01
Euploid blastocyst	1.8 ± 1.7	0, 7	1.5 ± 1.6	0, 8	1.9 ± 1.9	0, 6	1.7 ± 1.8	0, 8	NS

IVF-PGT, *in vitro* fertilization preimplantation genetic testing; PGT-M, preimplantation genetic testing for monogenic; PGT-SR, preimplantation genetic testing for structural rearrangements; PGT-A, preimplantation genetic testing for aneuploidy; 2PN, two pronucleus; BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; AMH, anti-Müllerian hormone; TE, trophectoderm; SD, standard deviation; NS, no significance.

A total of 219 women who have at least one blastocyst available for biopsy participated in this study. 811 biopsied blastocysts obtained from 2001 embryos underwent aneuploidy screening using a NGS platform, and on days 1 and 3 morphologic grading was recorded. We observed that morphologic grading on day 3, but not day 1, was a significant predictor for euploid embryo identification.

2. Materials and Methods

2.1 Study Design

219 PGT cycles which come from 219 patients were performed between 2017 and 2019 at our IVF center included in this retrospective cohort study. Patients in PGT cycle who have obtained at least one biopsied blastocyst and agreed to participate this study were included. Patients did not obtained any biopsied blastocysts and refused to participate this study were excluded. Total 2001 two pronucleus (2PN) embryos formed after intracytoplasmic sperm injection (ICSI) and all the 2PN embryos were assigned for blastocyst culturing. At last, 811 biopsied blastocysts were obtained from 219 women. All patients who participated in this study were divided into 3 groups, PGT-A, preimplantation genetic testing for monogenic (PGT-M) and preimplantation genetic testing for structural rearrangements (PGT-SR), based on the results of genetic counselling. PGT-A patients underwent at least two spontaneous miscarriages or at least once miscarriage indicating abnormalities in chromosomal structure. The mean age of patients assigned to PGT-A was 38 years old and had implantation failure ≥ 3 times. PGT-M patients were assigned based on both members of the couple are the recessive monogenetic disorder gene carriers, or one of them is dominant monogenetic disorder patient. PGT-SR patients, or their husband, exhibits a chromo-

somal rearrangement. Principal information gathered during the PGT cycle is presented in Table 1. Morphologic assessments were performed from day 1 to day 6 after fertilization and all embryos underwent non-selective culture until day 5 or day 6. Blastocyst quality graded ≥ 3 BB were biopsied prior to vitrification. Blastocyst quality graded < 3 BB did not considered biopsying and vitrification.

2.2 Embryo Culture

All were oocytes placed in Quinn's Advantage Fertilization Medium (ART-1020, Origio, Pasadena, CA, USA) supplemented with 5% human serum albumin (HSA) (90165, Irvine Scientific, Santa Ana, CA, USA) under oil (10029 Ovoil, Vitrolife, Gothenburg, Sweden). Intracytoplasmic sperm injection (ICSI) was conducted approximately 4 hours after oocyte retrieval and after this, oocytes were returned to the incubator for culture. Embryos were cultured up to the blastocyst stage in 6% CO₂ and 5% O₂. All embryos were transferred into G1 medium (10128, Vitrolife, Gothenburg, Sweden) from day 1 to day 3, and subsequently transferred into G2 medium (10132, Vitrolife, Gothenburg, Sweden) from day 3 to day 6.

2.3 Morphologic Assessment

All embryo assessment followed the Istanbul consensus or Gardner's system for grading human blastocysts. Zygote assessment was performed at 17 ± 1 hour post-ICSI. The morphological parameters for zygote scoring on day 1 were number of pronuclei and pronuclear pattern and based on these criteria only 2 pronuclei zygotes was considered for further culture. The pronuclear pattern was classified into 4 categories, Z1, Z2, Z3, and Z4 based on pronuclear appearance. Day 3 cleavage-stage embryo scoring was per-

formed at 68 ± 1 hour post-ICSI, and morphological parameters assessed included blastomere number, degree of fragmentation, and size and shape of blastomeres. Degree of fragmentation is the percentage of the volume of the embryo occupied by chromosomal fragments, specifically, (1) <10% fragmentation; (2) 20–30% fragmentation; (3) 20–30% fragmentation, (4) >50% fragmentation. Symmetry is defined as the size and shape of the blastomeres within cleavage-stage embryo, specifically, (1) even division; (2) <20% difference; (3) 20–50% difference; (4) >50% difference. Day 5 and day 6 morphological evaluation of blastocysts included the stage (early, expanding, expanded, hatching or hatched) as well as the quality of the inner cell mass (ICM) and trophoctoderm (TE). All embryonic grading was individually recorded and reviewed in real-time by two senior embryologists [28–31].

2.4 TE Biopsy and Aneuploidy Testing

Blastocyst quality was assessed prior to TE biopsy. Only blastocyst quality of $\geq 3\text{BB}$ were considered for TE biopsy. All biopsy procedures were performed on the heated stage of a Nikon IX-70 microscope (CIE, Nikon, Chiyoda, Tokyo, Japan) equipped with micromanipulation tools. Detailed procedures have been outlined in a previous report from our group [12]. For TE biopsies, blastocysts were transferred into microcentrifuge tubes containing 2 mL phosphate buffered saline (PBS), and this was followed by multiple displacement amplification (MDA) using REPLI-g Single Cell kit (10534, Qiagen, Hilden, North Rhine-Westphalia, Germany). The biopsies were stored at -20°C for one week if MDA was not performed. Preimplantation genetic testing for aneuploidy was performed using the MiSeq NGS platform (Illumina) following the manufacturer's protocol. Details of this analysis are outlined in **Supplementary Fig. 1**.

2.5 Statistical Analysis

Student's *t*-tests or Mann-Whitney *U* tests were conducted to assess statistically significant differences. Continuous variables are shown as mean $\pm$ standard deviation (SD). Binary logistic analysis was conducted to investigate embryo euploidy and embryonic morphology by defining the binary response parameter as either euploid (1) or aneuploid (2). Categorical variables are provided with 95% confidence interval (CI). Univariate analysis was conducted to compare euploid embryo rate in different groups. $p < 0.05$ was considered statistically significant and statistical analyses were performed using SPSS 19 (IBM SPSS, Chicago, IL, USA).

3. Results

3.1 Demographics and IVF Cycle Characteristics in the Study Population

From January, 2017 to December, 2019, data from 2001 2PN embryos which originated from 219 patients, in

219 PGT cycle, were included in this study. The 219 patients and 2001 2PN embryos were divided into 3 groups based on data gathered. Specifically, 54 patients and 519 embryos were placed in the PGT-M group, 91 patients and 976 embryos were placed in the PGT-SR group, and 74 patients and 506 embryos were placed in the PGT-SR group. IVF-PGT cycle information and patient demographics are outlined in Table 1.

3.2 Assessment of Morphology Effects on Blastocyst Euploidy by Logistic Regression Analysis

We assessed the effects of different morphological factors on blastocyst euploidy using logistical regression analysis, and measured euploid blastocyst development at day 5 or day 6. In day 3 blastocysts, blastomere number, symmetry grading, and fragmentation grading were included in the logistic regression analysis. The results outlined in Table 2 indicate that blastomere number had the strongest association with blastocyst euploidy (odds ratio (OR) = 1.156, 95% CI = 1.103–1.121, $p < 0.01$). This was followed by association with blastomere symmetry (OR = 0.710, 95% CI = 0.591–0.852, $p < 0.01$), and blastomere fragmentation (OR = 0.624, 95% CI = 0.504–0.774, $p < 0.01$). However, neither day 1 pronucleus (PN) pattern ($p > 0.05$) or PGT indication (data not shown) failed to show any association with blastocyst euploidy.

Table 2. Logistic regression analysis of the indicated variables associated with euploid blastocyst in 219 patient PGT cycles.

Parameter	OR	95% CI	<i>p</i> value
PN pattern	0.976	0.832–1.145	NS
Blastomere number	1.156	1.103–1.121	<0.01
Blastomere symmetry	0.710	0.591–0.852	<0.01
Fragmentation	0.624	0.504–0.774	<0.01

PGT, preimplantation genetic testing; PN, pronucleus; OR, odds ratio; CI, confidence interval; NS, no significance.

3.3 Univariate Analysis of Blastomere Number, Symmetry, and Fragmentation Analysis in Different PGT Groups

We next analyzed the association between blastomere number and euploid blastocysts in the three PGT groups. Embryos were divided into 9 sub-groups based on blastomere number in each group (Fig. 1A). In the PGT-M group, 9 day 3 blastomere embryos obtained the highest rate of euploidy, followed by ≥ 12 and 8 blastomere embryos, specifically, 0.364, 0.346, and 0.310, respectively (Fig. 1A and Table 3). In the PGT-SR group, ≥ 12 day 3 blastomeres had the highest euploidy rate, followed by 8 and 7 blastomere embryos, 0.220, 0.188, and 0.177, respectively (Fig. 1A, Table 3). In the PGT-A group, 8 blastomere day 3 embryos also displayed the highest euploidy rate, followed 9 and ≥ 12 blastomere embryos, 0.293, 0.262, and 0.242, respectively (Fig. 1A, Table 3). Finally, we an-

alyzed the total data of all three groups and found that day 3 ≥ 12 blastomere embryo represented highest euploid rate followed 8 and 9 blastomere embryos 0.260, 0.248, and 0.241 (Fig. 1A, Table 3). In regards to embryo symmetry we noted that as the blastomere difference increased, the euploid rate decreased (Fig. 1B, Table 3). Similar results were obtained from fragmentation analysis (Fig. 1C, Table 3); specifically, embryos within fragmentation $< 10\%$ displayed the highest rate of euploidy. Further, as increasing fragmentation was observed, the euploid rate commensurately decreased. All euploid rates for different embryos from the three PGT groups and combined group scoring is provided in Table 3.

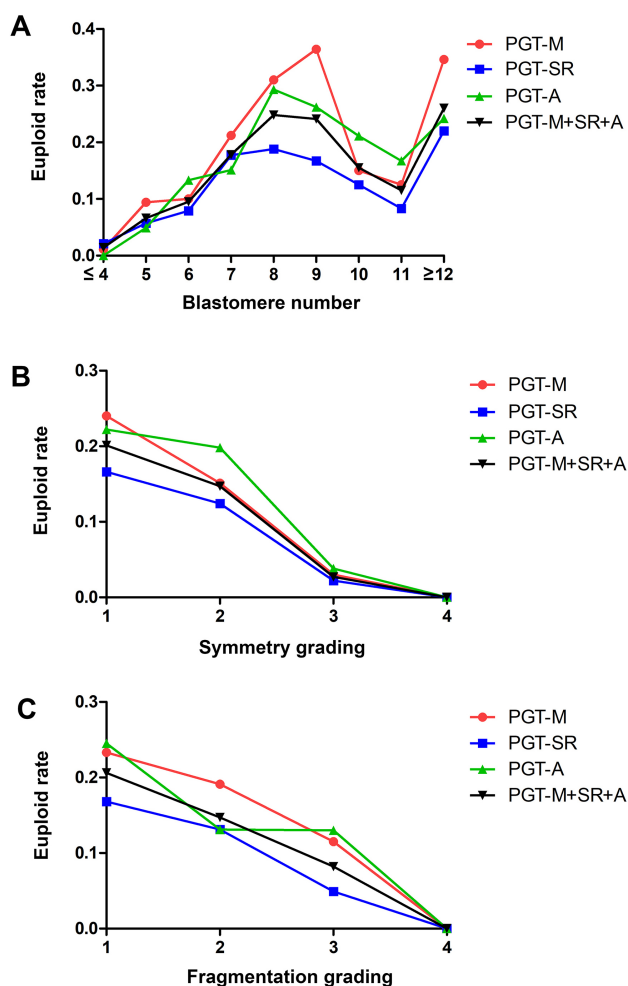


Fig. 1. Univariate analysis of day 3 morphological grading factors in PGT embryos. Univariate analysis of day 3 embryo blastomere number (A), symmetry grading (B) and fragmentation grading (C) in PGT-M (red), PGT-SR (blue), PGT-A (green) and PGT total (black).

4. Discussion

Embryo selection and euploid embryo transplantation are critical steps for improving pregnancy rates following

IVF. In this study, 811 biopsied blastocysts, which were obtained from 2001 2PN embryos in 219 patients, were included in our analysis. As outlined, patients were divided into 3 groups, PGT-M, PGT-SR and PGT-A based on clinical indications. We found several euploid embryo predictors within IVF preimplantation genetic testing (PGT) morphologic data. Day 5 or day 6 blastocysts with quality $\geq 3BB$ underwent TE biopsy followed by MDA and NGS-based euploid screening of DNA isolated from these blastocysts. After statistical analysis, results showed that day 3 embryonic blastomere number ($OR = 1.315, p < 0.01$), blastomere fragmentation ($OR = 0.900, p < 0.01$) and blastomere symmetry ($OR = 0.621, p < 0.01$) are predictive of euploid blastocysts, with blastomere number being the strongest factor associated with blastocyst euploidy. However, a 2PN pattern in day 1 embryos did not exhibit a statistically significant association with blastocyst euploidy. A previous study by Tesarik *et al.* [32] demonstrated similar results; however, these findings were opposite to those reported by Faramarzi *et al.* [31,33]. We speculate that because the 2PN pattern was evaluated by using different methods and different investigators, these differences may account for these disparate results.

Maternal age is a critical factor for blastocyst euploidy. This factor can affect the blastocyst euploid rate [34]. Advanced age patients in IVF treatment usually obtained lower quality embryos and clinical pregnancy rate and higher miscarriage rate than the younger patients [35]. Average percent euploid embryos increased from $\sim 60\%$ to $\sim 75\%$ between maternal ages 22 and 28, dipping to $\sim 60\%$ by age 35, followed by a steady decline to $\sim 40\%$ by age 40 until reaching $\sim 10\%$ by age 45 [36–38]. In our study, the maternal age distribution in these 3 groups is significantly different and results showed that PGT-A group being the oldest (Table 1). Otherwise, chromosome abnormality is also a factor can not be ignored in blastocyst euploidy. Such as Balanced translocations, Robertsonian translocations, insertions, and inversions are abnormalities that change the natural order of chromosomal segments. The carriers of above abnormalities are typically asymptomatic but more easily produce chromosomal copy number abnormal gamete. This result will make infertility problems, increase the possibility of miscarriage, fetal anomalies and affect offspring's intelligence [39–41]. On the other hand, most of patients in PGT-M group do not have the infertility, and their blastocyst have relative high euploid rate. So, we analyzed the results in different group to avoid the impact of different group clinical indications on the final results (Table 1).

We examined the effect of blastomere number on predicting embryonic euploidy in three groups. 9 blastomere embryos received the highest euploidy rate in the PGT-M group, but 8 blastomere embryos received the highest euploidy rate in the PGT-SR and PGT-A groups. It is commonly viewed that 7–9 blastomere embryos are considered

Table 3. Embryo euploid rate at different cleavage-stage embryo grading in 3 PGT groups.

	PGT-M			PGT-SR			PGT-A			PGT-M+SR+A		
Blastomere number	Embryo number	Euploid number	Euploid rate	Embryo number	Euploid number	Euploid rate	Embryo number	Euploid number	Euploid rate	Embryo number	Euploid number	Euploid rate
≤4	87	1	0.011	143	3	0.021	59	0	0.000	289	4	0.014
5	53	5	0.094	88	5	0.057	41	2	0.049	182	12	0.066
6	60	6	0.100	126	10	0.079	45	6	0.133	231	22	0.095
7	66	14	0.212	147	26	0.177	73	11	0.151	286	51	0.178
8	155	48	0.310	303	57	0.188	188	55	0.293	646	160	0.248
9	44	16	0.364	84	14	0.167	42	11	0.262	170	41	0.241
10	20	3	0.150	32	4	0.125	19	4	0.211	71	11	0.155
11	8	1	0.125	12	1	0.083	6	1	0.167	26	3	0.115
≥12	26	9	0.346	41	9	0.220	33	8	0.242	100	26	0.260
Symmetry	Embryo number	Euploid number	Euploid rate	Embryo number	Euploid number	Euploid rate	Embryo number	Euploid number	Euploid rate	Embryo number	Euploid number	Euploid rate
1	257	60	0.233	459	77	0.168	282	69	0.245	998	206	0.206
2	173	33	0.191	328	43	0.131	153	20	0.131	654	96	0.147
3	87	10	0.115	185	9	0.049	69	9	0.130	341	28	0.082
4	2	0	0.000	4	0	0.000	2	0	0.000	8	0	0.000
Fragmentation	Embryo number	Euploid number	Euploid rate	Embryo number	Euploid number	Euploid rate	Embryo number	Euploid number	Euploid rate	Embryo number	Euploid number	Euploid rate
1	350	84	0.240	585	97	0.166	342	76	0.222	1277	257	0.201
2	93	14	0.151	234	29	0.124	101	20	0.198	428	63	0.147
3	67	2	0.030	136	3	0.022	53	2	0.038	256	7	0.027
4	9	0	0.000	21	0	0.000	10	0	0.000	40	0	0.000

the best choice for transplantation; however, in this study embryos with ≥ 12 blastomeres proved to be a better choice when compared to 7 or 9 blastomere embryos. Compaction is a critical morphological event of day 3 embryos. It typically occurs on ≥ 10 blastomere embryo and then the embryo develop into morula stage [42]. Previous study have demonstrated that, day 3 morula stage embryo have better development competence than day 3 cleavage stage embryo [43]. In all case, morula stage embryo associate with higher pregnancy and implantation rate than cleavage stage embryo [44,45]. So, the result that embryo ≥ 12 blastomere have higher euploid rate is reliable. The influence of symmetry and fragmentation is not similar in each group. Our results showed that, symmetry has the strongest influence in PGT-M group (Fig. 1B), but fragmentation has the strongest influence in PGT-A group (Fig. 1C). The underlying mechanism need further study.

Recently, a variety of technologies for euploid blastocyst screening have been developed. Preimplantation genetic testing for aneuploidy is an attractive technology that has the potential to increase IVF success rates [17]. However, the association of TE or blastomere biopsy and impairment of embryonic development remains controversial, as well as biopsy increasing the time exposure out of incubator [18,19]. Day 5 or day 6 blastocyst scoring has been

confirmed as a feasible method for embryo selection, but blastocyst scoring cannot be applied to embryos only culture for 3 days, and approximately 40% of embryos fail to reach blastocyst stage. Alternatively, day 3 embryo scoring systems involve more variables than the blastocyst scoring system, so day 3 embryo assessment likely offers more morphologic information than either day 5 or day 6 embryos.

Time-lapse microscopy is a novel technology for optimizing embryo selection. Many studies have demonstrated that some parameters of embryo kinetics are useful for embryo selection, but a gold standard in the field is still lacking [46–48]. Potential embryo damage from exposure to camera lamplight every 5 mins remains unclear [13–15]. Recent attention has focused on developing non-invasive approaches for PGT such as analysis of cell-free DNA in blastocoelic fluid or culture medium [49]. Although noninvasive methods decrease embryo impairment, the accuracy and specificity of these noninvasive approaches require improvement [50,51].

There are a number of limitations in this study. Additional data is required to confirm the benefit by applying the conclusion of our study for the IVF cycle. We would further assess the impact of day 3 embryo morphology on the rates of clinical pregnancy, implantation, miscarriage, and live birth. We found as showed in Table 1 that the eu-

ploid embryo number per patient in PGT-M group is less than PGT-A group. The main reason for this finding may be the limited sample size.

5. Conclusions

In conclusion, this study confirmed that day 3 embryonic blastomere number, symmetry, and fragmentation are statistically significant predictors of euploid blastocysts. In contrast, the 2PN pattern in day 1 embryos was not associated with blastocyst euploidy. 8 blastomere or blastomere number ≥ 12 , even division embryos with fragmentation ≤ 10 in day 3 embryos represent a best choice for either blastocyst culture or day 3 implantation. And we first report the euploid rate of blastocyst which derived from day 3 moular stage embryo. The impact of blastomere symmetry is greater than fragmentation and gives a good suggestion for embryo selection. We believe that this study will provide a rapid, efficiency and cost-effective method for selecting high developmental potentiality embryo to improve outcomes of IVF.

Availability of Data and Materials

The data that support the findings of this study are available from Guangzhou Women and Children's Medical Center but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Guangzhou Women and Children's Medical Center.

Author Contributions

YL designed the research study. YL, ZO and ZC performed the research. ZO provided help and advice on data collection. YL, ZO and ZC analyzed the data. YL and ZO wrote the first draft of the paper. ZC provided critical reviews and interpretation of the results. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by Reproductive Medical Ethics Committee of Guangzhou Women and Children's Medical Center (Approval number: 2023-152A01) and written informed consent was obtained from all subjects.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/j.ceog5011230>.

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