A deep dive into the morphokinetics and ploidy of low-quality blastocysts

Molly M. Quinn, M.D.,[a] Philip Marsh, M.S.,[b] Salustiano Ribeiro, M.S.,[b] Rhodel K. Simbulan, M.S.,[b] and Mitchell P. Rosen, M.D.[b]

[a] Department of Obstetrics and Gynecology, University of Southern California, Los Angeles, California; and [b] Department of Obstetrics, Gynecology and Reproductive Sciences, University of California San Francisco, San Francisco, California.

Objective: To describe morphokinetic parameters and ploidy among low-quality blastocysts not meeting the criteria for clinical use.
Design: Prospective cohort study.
Setting: Academic medical center.
Patient(s): Two hundred patients undergoing in vitro fertilization between February 2018 and November 2019.
Intervention(s): All embryos were cultured in a time-lapse incubator. All expanded blastocysts underwent preimplantation genetic testing for aneuploidy using next-generation sequencing.
Main Outcome Measure(s): Static blastocyst morphology grading; morphokinetic parameters, including time to each cell division (2-cell formation to 8-cell formation); time to morula formation; time to the start of blastulation; time to blastocyst formation; and preimplantation genetic testing for aneuploidy results.
Result(s): A total of 1,306 embryos progressed to the expanded blastocyst stage; of these, 935 embryos met the criteria for clinical use and were designated as high quality, whereas 371 embryos were graded as low quality and did not meet the criteria for use. In morphokinetic evaluation, low-quality embryos developed more quickly to 5-cell formation (t5) 48.4 [42.4–48.7) vs 50.2 [46.3–50.1] hours, but progressed more slowly thereafter with tM 91.5 [85.9–92.3] vs 88.3 [82.1–88.3] and tB 114.0 [106.4–113.9] vs 106.9 [101.3–107.4] hours. Among the low-quality embryos, 75.5% were aneuploid, 22.4% were euploid, and 2.2% had undetermined chromosome copy number results. Morphokinetic parameters did not differ between the euploid and aneuploid low-quality embryos.
Conclusion(s): Morphokinetic analysis did not distinguish between euploid and aneuploid low-quality embryos. (Fertil Steril Rep® 2022;3:231–6. ©2022 by American Society for Reproductive Medicine.)
Key Words: IVF, embryo morphokinetics, aneuploidy, embryo quality, time-lapse imaging

In vitro fertilization has progressed to a stage at which the focus is no longer on the ability to achieve a pregnancy but on the time to singleton pregnancy and live birth. Improvements in the embryology laboratory have allowed for blastocyst culture and other methods aimed at selecting the single best embryo for transfer. One of these methods is trophectoderm (TE) biopsy at the blastocyst stage for preimplantation genetic testing for aneuploidy (PGT-A). A downside of PGT-A is the need for a "freeze all" cycle with an attendant delay in transfer and a requirement for vitrification and subsequent warming of a selected embryo. Time-lapse imaging (TLI) has been explored as a noninvasive mechanism for identifying an embryo that is most likely to result in successful implantation via continuous image acquisition and study of individual embryo morphokinetics. Multiple algorithms have been designed to use embryo morphokinetics to predict embryos with a higher probability of live birth upon transfer; however, none of these have demonstrated an association with ploidy that is strong enough to supplant the clinical application of PGT-A (1).

Historically, standard static embryo morphology gradings have been used to select embryos for transfer. In practices performing a high volume of PGT-A, it is common to define a criterion for biopsy that excludes embryos with poor-quality TE or inner cell mass (ICM) grading from undergoing TE biopsy for PGT-A. In this setting, low-quality embryos are discarded (2). However, studies have shown that low-quality embryos can result in successful pregnancies (3). We sought to describe the morphokinetics of low-quality embryos and any relationship to embryonic ploidy in an attempt to elucidate features that could predict euploid status from a low-quality embryo. Our hypothesis is that morphokinetic parameters predict blastocyst quality as measured by static morphology; however, we predict that

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Reprint requests: Molly M. Quinn, M.D., Department of Obstetrics and Gynecology, Keck School of Medicine at the University of Southern California, 2020 Zonal Ave, IRD 534, Los Angeles, California 90033 (E-mail: Molly.Quinn@med.usc.edu).

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morphokinetic parameters will have a more limited impact on embryonic ploidy.

MATERIALS AND METHODS

Trial Design and Study Population

This is a secondary analysis of a sibling oocyte study of 2 different culture media systems designed to study early embryonic development within a time-lapse incubator (4). In the primary study, embryo quality, morphokinetic parameters, and aneuploidy rates from TE biopsy were similar between sibling embryos cultured in distinct media systems from the time of gamete isolation. For this study, we focused on the static morphology, morphokinetics, and ploidy of high-quality embryos vs. those of poor-quality embryos that were graded insufficient for clinical use. Individuals planning in vitro fertilization with the intent of blastocyst culture and PGT-A were offered enrollment in the study before their treatment cycle between February 2018 and November 2019. During this time frame, 631 patients were eligible to participate and 200 patients consented to participation. The inclusion and exclusion criteria have been described previously (4). One hundred seventy-six individuals completed the study.

The study was approved by the institutional review board of the University of California San Francisco (IRB #17-22331) and registered on clinicaltrials.gov (NCT 03503877). Written informed consent was obtained from all study subjects before participation.

Ovarian Hyperstimulation and Laboratory Procedures

Ovarian stimulation was performed as described previously (4). Oocyte retrieval was performed according to clinic standard 36 hours after ovulatory trigger. A semen sample was obtained by masturbation within 1 hour of oocyte retrieval. The method of fertilization—via conventional insemination vs. intracytoplasmic sperm injection—was determined by the patient's primary physician.

At 16–18 hours, fertilization was evaluated by the existence of 2 pronuclei. All embryos were cultured in the EmbryoScope+ time-lapse incubator (Vitrolife A/S, Viby J, Denmark). Once placed in the EmbryoScope+ time-lapse incubator, embryos were cultured at 37 °C with 6.5% CO_2 and 5.0% O_2 for up to 6 days without media exchange.

The EmbryoScope+ incubation chamber contains a built-in microscope and camera, allowing for continuous monitoring of embryonic development. An image acquisition software was used to obtain high-contrast images every 10 minutes from several focal planes to create time-lapse videos. Conventional embryonic assessment was made through observations at prespecified time points. Cleavage-stage embryos were assessed for cell number, symmetry, percentage fragmentation, evidence of multinucleation, and progression of compaction. Blastocysts were evaluated to assess for blastocele volume and expansion, ICM development, and TE organization.

Additional morphokinetic parameters were assessed with time-lapse videos, including time to pronuclear fading or syngamy, time to 2–8 cells, time to morula, time to start of blastulation, time to blastocyst, and time to expanded blastocyst. Cleavage anomalies were recorded.

Embryonic biopsy for preimplantation genetic testing was performed at the blastocyst stage in all embryos reaching full blastocyst. On the day of the biopsy, 5–10 TE cells were gently aspirated. Biopsied cells were washed and cryopreserved before being sent for testing. Biopsied TE cells were analyzed for all 24 chromosomes by the testing laboratory (PacGenomics, Agoura Hills, CA) using a next-generation sequencing–based assay.

Predictors

The primary predictors or exposure variables were morphokinetic parameters assessing time to specific developmental endpoints from TLI (as delineated earlier). The age of the oocyte from which an embryo was derived was dichotomized to <35 years or ≥ 35 years for stratified analysis.

Outcomes

The primary outcome was static blastocyst embryo morphology. High-quality embryos meeting the criteria for clinical use were defined as blastocysts with expansion grade 3–6 according to Gardner criteria and at least a B grading for ICM and TE (5). Expanded blastocysts with C grading for either t TE or ICM were defined as low-quality embryos and were deemed unsuitable for clinical use. A secondary outcome was embryonic ploidy determined by TE biopsy with PGT-A. A subanalysis included the type of aneuploidy (simple, segmental, or complex).

Statistical Analysis

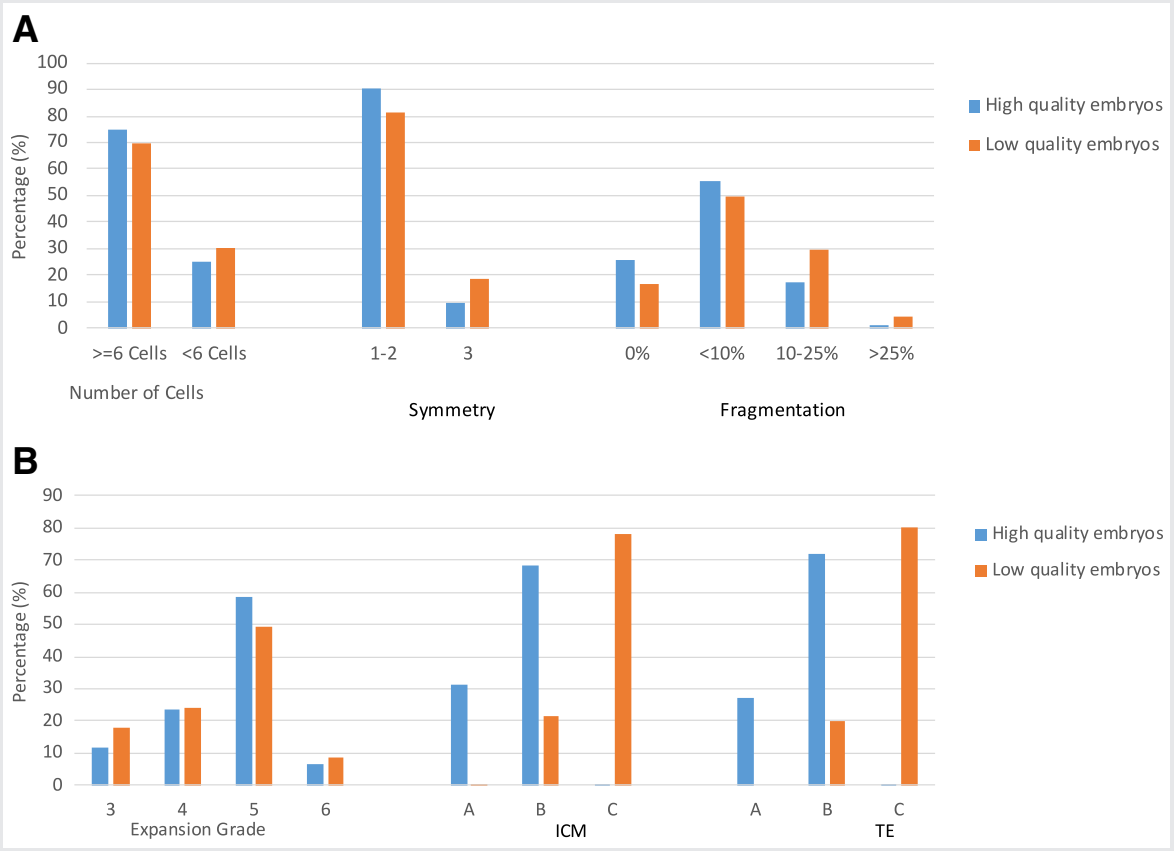
Outcomes were assessed for normality of distribution. Mean, standard deviation, medians, and interquartile ranges are reported. Chi-square was used as appropriate. Morphokinetic evaluations were compared using Wilcoxon rank sum testing with Bonferroni correction to adjust for multiple comparisons. All analyses were performed in the statistical software package R version 3.6.3 (R Core Team, 2020).

RESULTS

Baseline characteristics and stimulation parameters of the enrolled patients were reported previously (4). One hundred seventy patients contributed embryos to the study. The median number of oocytes collected was 13.5 (10.0–19.75). The median number of normally fertilized oocytes (2PN) per patient was 10.5 (7–15) and that of blastocysts formed was 7 (4–11). Blastulation rate (blastocysts/2PN) per patient was 71% (55%–85%).

There were 935 high-quality blastocysts and 371 low-quality blastocysts that did not meet the criteria for clinical use. The distribution of static morphology grading at the cleavage stage and the final blastocyst grading is depicted in Figure 1. Full automatic annotations were possible for 864 high-quality and 328 low-quality blastocysts. Time-lapse imaging revealed a shorter time to 5 cells in embryos that subsequently became low-quality blastocysts: 48.4 (42.4–48.7) hours vs. 50.2 (46.3–50.1) hours; $P = .02$

FIGURE 1



(**A**) Cleavage-stage embryo grading among high- and low-quality embryos. (**B**) Blastocyst stage final embryo grading among high- and low-quality embryos (Gardner criteria). ICM = inner cell mass. TE = trophectoderm.

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(Table 1). Embryos that would become low-quality blastocysts reached all subsequent morphokinetic milestones at a slower pace. The time to blastocyst formation was 114.0 (106.4–113.9) hours vs 106.9 (101.3–107.4) hours, $P<.0001$ (Table 1). A similar pattern was observed when restricting the morphokinetic analysis to euploid embryos graded low vs. high quality (Supplemental Table 1, available online).

Among low-quality blastocysts, 280 were aneuploid, 83 were euploid, and 8 had an indeterminate result. Morphokinetic parameters did not differ between euploid and aneuploid low-quality blastocysts (Table 2). High-quality embryos were more likely to be euploid (41.5% vs. 22.4%, $P<.001$). This was true in a subgroup analysis of embryos derived from women aged $\leq$ 35 years and >35 years (Table 3). Complex aneuploidy was more frequently identified in poor-quality embryos, particularly in women aged >35 years (Supplemental Table 2).

DISCUSSION

In this secondary analysis of a large sibling oocytes study using TLI and TE biopsy for PGT-A to evaluate the developmental competence of blastocysts, we demonstrated that nearly a quarter of low-quality blastocysts were euploid; however, TLI was unable to distinguish euploid vs. aneuploid low-quality embryos.

Multiple prior publications have explored the use of morphokinetic timings gleamed from TLI to predict ploidy status among blastocysts. A recent review on the topic concluded that although morphokinetic parameters from TLI may relate to ploidy status, the predictive value was inadequate to replace PGT-A for aneuploidy screening (6). This was especially true when kinetic risk models developed at different centers were adopted before internal validation (7). Minasi et al. (8) explored the relationship among standard morphology, morphokinetic development, and embryonic ploidy as determined by TE biopsy with PGT-A via array comparative genomic hybridization in 928 blastocysts. Euploid embryos demonstrated a shorter time to start blastulation, expansion, and hatching than that demonstrated by aneuploid embryos. Notably, standard morphology was poorly predictive of ploidy with C gradings for ICM among 17.1% of euploid blastocysts and C gradings for TE among 26.6% of euploid blastocysts (8). Similarly, Capalbo et al (3) found a 25.5% euploidy rate among poor-quality blastocysts, a rate of 30.1% when ICM was "C," and a rate of 23.4% with a TE "C" score. Of note, this study included only 153 total poor-quality blastocysts from 2

TABLE 1

Morphokinetic parameters for high-quality and low-quality embryos in time-lapse imaging.

	High quality			Low quality				
Parameter	n	Mean ± SD	Median (IQR)	n	Mean ± SD	Median (IQR)	*P* value (Wilcoxon rank sum)	*P* value (Bonferroni corrected)
tPNf[a]	864	24.0 ± 3.6	23.6 (21.8–24.0)	328	24.5 ± 3.9	24.3 (22.0–24.5)	.028	.396
t2[b]	864	26.5 ± 3.7	26.1 (24.3–26.5)	328	27.1 ± 4.1	26.9 (24.6–27.1)	.011	.158
t3[c]	864	37.5 ± 4.7	37.5 (34.9–37.5)	328	37.2 ± 5.7	37.4 (34.2–37.2)	.477	1
t4[d]	864	38.5 ± 4.6	38.2 (35.8–38.5)	328	39.3 ± 5.5	39.0 (35.6–39.3)	.048	.668
t5[e]	864	50.1 ± 7.0	50.2 (46.3–50.1)	328	48.7 ± 8.5	48.4 (42.4–48.7)	.001	.020
t6[f]	864	52.2 ± 6.5	51.9 (48.5–52.2)	328	52.8 ± 8.6	52.4 (47.4–52.8)	.563	1
t7[g]	864	54.2 ± 7.4	53.3 (49.6–54.2)	328	56.0 ± 9.0	54.7 (49.9–56.0)	.003	.0370
t8[h]	864	57.7 ± 8.9	56.1 (51.6–57.7)	328	60.3 ± 10.1	59.0 (53.0–60.3)	<.001	<.001
tM[i]	864	88.3 ± 9.5	88.3 (82.1–88.3)	328	92.3 ± 10.0	91.5 (85.9–92.3)	<.001	<.001
tSB[j]	864	99.0 ± 8.4	98.4 (93.4–99.0)	328	103.3 ± 9.1	102.7 (97.2–103.3)	<.001	<.001
tB[k]	847	107.4 ± 9.0	106.9 (101.3–107.4)	312	113.9 ± 10.5	114.0 (106.4–113.9)	<.001	<.001
cc2[l]	864	11.0 ± 2.3	11.3 (10.6–11.0)	328	10.1 ± 4.3	11.2 (10.0–10.1)	.097	1
cc3[m]	864	11.6 ± 4.9	12.2 (10.9–11.6)	328	9.4 ± 6.6	11.3 (1.3–9.4)	<.001	<.001
db[n]	847	8.5 ± 3.6	7.8 (6.1–8.5)	312	11.0 ± 5.6	9.8 (7.0–11.0)	<.001	<.001

Note: All times are presented in hours; tSB notation required for inclusion. IQR = interquartile range.
[a] From insemination to pronuclear fading.
[b] Two-cell formation.
[c] Three-cell formation.
[d] Four-cell formation.
[e] Five-cell formation.
[f] Six-cell formation.
[g] Seven-cell formation.
[h] Eight-cell formation.
[i] Morula formation.
[j] Appearance of blastocele/start of blastulation.
[k] Formation of blastocyst.
[l] Duration of second cell cycle.
[m] Duration of third cell cycle.
[n] Duration of blastulation (tb–tSB).

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TABLE 2

Morphokinetic parameters for euploid and aneuploid low-quality embryos in time-lapse imaging.

	Euploid			Aneuploid				
Parameter	n	Mean ± SD	Median (IQR)	n	Mean ± SD	Median (IQR)	*P* value (Wilcoxon rank sum)	*P* value (Bonferroni corrected)
tPNf[a]	73	24.7 ± 3.5	24.7 (22.2–24.7)	247	24.4 ± 4.1	24.2 (21.9–24.4)	.571	1
t2[b]	73	27.3 ± 3.6	27.4 (24.9–27.3)	247	27.0 ± 4.3	26.8 (24.4–27.0)	.463	1
t3[c]	73	37.5 ± 5.1	37.8 (34.4–37.5)	247	37.0 ± 5.8	37.0 (34.1–37.0)	.453	1
t4[d]	73	39.5 ± 5.3	39.3 (36.8–39.5)	247	39.1 ± 5.5	38.9 (35.5–39.1)	.654	1
t5[e]	73	48.2 ± 7.7	49.0 (41.9–48.2)	247	48.7 ± 8.6	48.1 (42.7–48.7)	.845	1
t6[f]	73	52.5 ± 7.5	52.6 (47.5–52.5)	247	52.6 ± 8.7	51.8 (47.4–52.6)	.630	1
t7[g]	73	55.9 ± 8.6	55.5 (51.2–55.9)	247	55.7 ± 9.0	54.5 (49.7–55.7)	.721	1
t8[h]	73	60.6 ± 10.0	58.6 (53.3–60.6)	247	60.1 ± 10.2	59.1 (52.4–60.1)	.813	1
tM[i]	73	93.5 ± 10.2	94.1 (87.8–93.5)	247	91.6 ± 9.7	91.1 (85.8–91.6)	.132	1
tSB[j]	73	103.6 ± 9.5	102.6 (97.9–103.6)	247	102.9 ± 8.8	102.6 (96.8–102.9)	.656	1
tB[k]	71	113.7 ± 9.8	113.5 (106.9–113.7)	233	113.7 ± 10.6	113.9 (106.2–113.7)	.959	1
cc2[l]	73	10.2 ± 4.7	11.3 (10.0–10.2)	247	10.1 ± 4.3	11.2 (9.9–10.1)	.559	1
cc3[m]	73	8.7 ± 6.2	11.1 (1.0–8.7)	247	9.5 ± 6.6	11.4 (1.4–9.5)	.349	1
db[n]	71	10.6 ± 5.4	9.6 (6.6–10.6)	233	11.1 ± 5.7	10.0 (7.2–11.1)	.380	1

Noe: All times are presented in hours, tSB notation required for inclusion. IQR = interquartile range.
[a] From insemination to pronuclear fading.
[b] Two-cell formation.
[c] Three-cell formation.
[d] Four-cell formation.
[e] Five-cell formation.
[f] Six-cell formation.
[g] Seven-cell formation.
[h] Eight-cell formation.
[i] Morula formation.
[j] Appearance of blastocele/start of blastulation.
[k] Formation of blastocyst.
[l] Duration of second cell cycle.
[m] Duration of third cell cycle.
[n] Duration of blastulation (tb–tSB).

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TABLE 3

Ploidy status of high-quality and low-quality embryos by age ≤35 years or > 35 years.

Group	Characteristics	High quality n	High quality %	Low quality n	Low quality %	*P* value (chi-square)
All	Euploid	388	41.5	83	22.4	<.001
	Aneuploid (all)	530	56.7	280	75.5	
	Undetermined	17	1.8	8	2.2	
Age (y) ≤35 years	Euploid	146	48.8	42	32.6	.008
	Aneuploid	147	49.2	84	65.1	
	Undetermined	6	2.0	3	2.3	
Age (y) >35 years	Euploid	242	38.1	41	16.9	<.001
	Aneuploid	383	60.2	196	81.0	
	Undetermined	11	1.7	5	2.1	

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centers. This finding that poor-quality blastocysts have potential for euploid status is important when many clinics exclude embryos for biopsy on the basis of static morphology grading (2).

Although we demonstrate a shorter time to 5-cell formation in embryos that subsequently became low-quality embryos, the significance of this finding is uncertain. Notably, 5-cell formation is before embryonic genome activation. Prior research limited by the assessment of embryonic ploidy at the cleavage stage by array comparative genomic hybridization reported some ability to distinguish between aneuploid and euploid embryos on the basis of morphokinetic development to the 4-cell stage (9). Specifically, the investigators demonstrated a greater standard deviation in time to early cell divisions among embryos with meiotic errors (10) and an increased risk for falling outside optimal ranges proposed for 5-cell formation to 2-cell formation and duration of third cell cycle (9). Although this differential was demonstrated in the time to 5-cell formation between high and low-quality embryos within our study, it was not seen when comparing the time to 5-cell formation between low-quality euploid and aneuploid blastocysts.

A significant limitation of our study is that low-quality euploid embryos were not transferred. As a result, we are unable to describe the reproductive potential of these low-quality blastocysts. In the study by Capalbo et al (3), poor-quality euploid embryos were eligible for transfer, and 7 of 13 (53.8%) of these resulted in ongoing implantation. This outcome was not different from euploid embryos that were graded as average quality (3). These data raise the question of whether embryology laboratories should consider poor-quality embryos suitable for clinical use either for transfer untested or when PGT-A is planned. This question reveals the tension between an approach that favors high levels of embryo selection designed to minimize time to pregnancy and an alternative that maximizes cumulative live birth per retrieval cycle. Future investigations should evaluate the reproductive potential of low-quality euploid blastocysts in larger cohorts.

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