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ARTICLE

The correlation between morphology and implantation of euploid human blastocysts

BIOGRAPHY

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KEY MESSAGE

The results suggest that blastocyst morphologic grading, particularly inner cell mass grade but also composite grade, is predictive of ongoing pregnancy/live birth after single euploid frozen embryo transfers. This large study provides a new framework to establish an individualized prognosis for implantation of screened embryos based on composite morphologic grading.

ABSTRACT

Research question: Does the composite morphology score or a particular developmental component (expansion stage, inner cell mass [ICM] or trophectoderm [TE]) of euploid blastocysts undergoing single frozen embryo transfer (FET) impact ongoing pregnancy/live birth (OP/LB) rates?

Design: Retrospective cohort study including a total of 2236 embryos from 1629 patients who underwent single euploid FET between 2012 and 2017.

Results: Embryos with an ICM grade of A compared with C had a higher OP/LB rate (55.6% versus 32.3%, $P < 0.001$). Blastocysts with a TE grade of A or B compared with C had a higher likelihood of OP/LB (A versus C: odds ratio [OR] 1.6, 99% confidence interval [CI] 1.1–2.3, B versus C: OR 1.5, 99% CI 1.1–2.1), and blastocysts with a developmental stage of 4 or 5 compared with 6 had higher odds of OP/LB (4 versus 6: OR 1.6, 99% CI 1.2–2.2, 5 versus 6: OR 1.6, 99% CI 1.2–2.3).

Conclusions: Among euploid embryos, ICM morphology is the best predictor of sustained implantation; however, a composite score may provide additional guidance. While there is a known benefit in genomic screening prior to embryo selection, morphology provides individualized, prognostic information about implantation potential.

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KEYWORDS

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Inner cell mass
Morphology
Single-embryo transfer

INTRODUCTION

The primary goal of IVF is to select the highest-quality embryo for transfer to achieve a healthy singleton pregnancy. Embryo quality has traditionally been based on morphologic characteristics; however, the assessment and selection of the optimal embryo for transfer has been modified as a result of advancements in culture conditions, cryopreservation technique and genomic screening. As the use of preimplantation genetic testing (PGT) continues to rise, and the use of a single-embryo transfer (SET) strategy among patients with more than one euploid embryo becomes increasingly common, it is necessary to understand the influence of euploid embryo morphology on IVF outcomes.

For nearly 20 years, laboratory assessment and grading of embryo morphology has been the primary method of embryo selection. Gardner developed a three-component morphologic scoring system for embryos that includes an assessment of blastocyst expansion, and the development of the inner cell mass (ICM) and trophectoderm (TE) (*Gardner, 1999*). Since the implementation of this scoring system, there have been conflicting data as to the value of embryo morphologic grade in predicting pregnancy outcomes. Moreover, there is debate about which component of the composite score has the greatest impact on IVF outcomes. Despite being subjective and not standardized in the industry, the grading system remains an integral aspect of the embryo selection process, particularly for unscreened embryos.

PGT has revolutionized the process of embryo selection. Advancements in PGT technology from cleavage-stage biopsy and the use of fluorescence in-situ hybridization to TE biopsy and comprehensive chromosomal screening have increased the detection of chromosomal abnormalities (*Lee et al., 2015*), resulting in improved implantation and live birth rates and decreased early pregnancy losses from IVF (*Forman et al., 2013; Scott et al., 2013*). Moreover, PGT and blastocyst transfer have allowed for the increasing use of SET, thus minimizing the multiple gestation rate without impairing pregnancy rates (*Forman et al., 2014; Gardner et al., 2000*).

Many euploid embryos fail to implant despite the increased use of comprehensive chromosomal screening. Morphologic assessment of euploid embryos may improve the embryo selection process and impact IVF outcomes. Studies have yet to fully evaluate the influence of embryo morphology on implantation rates of euploid embryos. Previous research has been limited by the analysis of the separate components of the grade, or by categorization of several embryos into 'excellent, good, average, or poor' quality based on composite grades (*Capalbo et al., 2014; Irani et al., 2017*). This study aimed to determine whether the post-warming composite grade and/or a particular developmental component of the grade (i.e. expansion stage, ICM or TE) of euploid embryos undergoing single frozen embryo transfer (FET) is associated with improved IVF outcomes, particularly ongoing pregnancy/live birth (OP/LB) rates.

MATERIALS AND METHODS

This single-centre, retrospective, cohort analysis included infertility patients undergoing autologous IVF cycles and subsequent single euploid FET from February 2012 to November 2017. Patients aged 22 to 46 years who had their blastocysts screened for aneuploidy by PGT prior to single-embryo transfer (SET) were identified in an electronic medical record database and included in the study. Patients who underwent unmedicated/natural cycle endometrial preparation prior to FET, or had an endometrial thickness less than 7 mm at time of transfer, were excluded from the study. This study was approved by the Western Institutional Review Board (Study # 1167398, approved 12 July 2018).

Clinical protocols

Ovarian stimulation

Patients underwent ovarian stimulation for IVF as previously described (*Rodriguez-Purata et al., 2016*). Oocyte maturation was induced with recombinant or purified human chorionic gonadotrophin (HCG) alone (Ovidrel®, EMD Serono, Rockland, MA, USA; Novarel®, Ferring Pharmaceuticals, Parsippany, NJ, USA; or Pregnyl®, Schering-Plough, Kenilworth, NJ, USA) or with a 'dual trigger' combination of 40 IU of leuprolide acetate (Lupron®, AbbVie Laboratories, Chicago, IL, USA) and 1000 IU of HCG (Novarel, Ferring Pharmaceuticals or Pregnyl®, Schering-Plough). Patients underwent ultrasound-guided vaginal oocyte retrieval 36 h after surge. Oocytes were inseminated by intracytoplasmic sperm injection in preparation for planned PGT.

Laboratory procedures and embryo assessment

Embryo culture

Embryos were cultured to the blastocyst stage. Following vaginal oocyte retrieval, embryos were cultured in Quinn's Advantage™ Cleavage Medium (CooperSurgical, Trumbull, CT, USA) until Day 3. Media supplementation consisted of 5% human serum albumin with 100 mg/ml HAS-Solution™ (Vitrolife, Goteborg, Sweden) on Day 0, and 10% synthetic serum substitute (SSS) with 6% protein components consisting of 84% pharmaceutical grade human serum albumin (50 mg/ml) (SSS, Irvine Scientific, Santa Ana, CA, USA) from Day 1 to Day 6 development. Low-oxygen conditions were maintained at 5% oxygen, 5.5% carbon dioxide, and balanced with nitrogen (Panasonic Sterisonic GxP incubator, Sanyo North America, Wood Dale, IL, USA) and Nunclon 60 mm dishes with 10 microdrops of 50 µl drops for up to one embryo per drop under 100% paraffin oil (Ovooil™, Vitrolife). On Day 3 after fertilization, the embryos were transferred from Quinn's Advantage Cleavage Medium (zero glucose, pyruvate-dominant) to glucose-rich G-2.5™ Vitrolife Blastocyst Media and supplement protein (10% SSS, Irvine Scientific). On Day 3 of embryo development, all embryos underwent assisted hatching to facilitate TE herniation by creating a 25–30 µm opening in the zona pellucida with a 200–300 µs pulse from a ZILOS-tk laser (Hamilton Thorne Biosciences, Beverly, MA, USA).

Embryo grading

Prior to embryo biopsy, blastocysts were graded based on a modification of the Gardner system, which accounts for the degree of blastocoel expansion and ICM and TE development. The degree of re-expansion was defined as follows: 1 = early blastocyst – cavity beginning to form, 2 = early blastocyst – cavity is less than 50% of the volume of the embryo, 3 = full blastocyst – cavity completely fills the embryo, 4 = expanded blastocyst – cavity volume larger than

that of the full blastocyst, zona pellucida thinning, 5 = hatching blastocyst – TE is herniating through the zona, 6 = hatched blastocyst – blastocyst completely escaped from the zona. Stage 6 blastocysts hatched either spontaneously or from being pulled from the zona pellucida during biopsy. The ICM grading was determined as follows: A = many cells – tightly compacted, B = some cells – tightly compacted or organizing, C = some cells – disorganized, D = few cells – disorganized. TE was graded as follows: A = many cells forming a cohesive epithelium, B = moderate cells forming a loose epithelium, C = some cells forming a loose epithelium, D = very few cells. For this study, embryos with expansion stage <4, or ICM or TE grades of D, were excluded because these embryos were rarely biopsied and transferred.

All blastocyst grading and biopsies were performed by one of five embryologists on the morning of Day 5 or Day 6, regardless of the exact time of vaginal oocyte retrieval on the morning of Day 0. These five embryologists received extensive biopsy training in order to standardize their technique and to minimize inter-observer variability in embryo grading. After training was completed, each embryologist underwent annual competency training and examination to ensure quality control.

Embryo biopsy

TE biopsy was performed on Day 5 or Day 6 based on morphologic assessment (>3BC) and hatching rate. Embryo biopsy was carried out under oil in Falcon 1006 Petri dishes (Becton Dickinson, Franklin Lakes, NJ, USA) in 10 µl drops of Enhance WG–Vitrolife HTF/HEPES. Using an Olympus IX70 microscope with Narishige micromanipulators (East Meadow, NY, USA), the blastocyst was secured with a thick-walled, blunt glass-holding pipette (internal diameter 20–30 µm), stabilizing the TE at the 3 o'clock position. Four to seven TE cells were drawn into the lumen of a sharp, thin-walled biopsy pipette (internal diameter 30 µm) and removed from the blastocyst via the use of 500 µs of near-infrared pulsations and gentle traction. The TE cells were analysed by quantitative PCR (qPCR) (*Treff et al., 2012*) or next-generation sequencing (NGS) (*Fiorentino et al., 2014*). All biopsy samples were placed in hypotonic wash buffer and submitted for analysis, and all embryos were vitrified after biopsy. PGT results were reported as euploid or aneuploid based on qPCR platform, or reported as euploid, aneuploid or mosaic based on NGS.

Embryo selection

Euploid embryos with the best grades were selected for transfer. In cases of elective sex selection, the highest-graded embryo of the desired sex was selected for transfer. Embryos biopsied on Day 5 were preferentially selected over embryos of all grades biopsied on Day 6 (*Slifkin et al., 2016*). Among embryos biopsied on the same day of development, ICM grade was prioritized in embryo selection, followed by expansion grade, and then TE grade.

Cryopreservation-rewarming technique

The cryopreservation-rewarming technique has been previously described (*Rodriguez-Purata et al., 2016*). After rewarming, embryo survival was determined according to the appearance of the blastomeres, zona pellucida, and the ability of the blastocoel to re-expand. After rewarming, embryos were re-graded based on the Gardner system as described above. Only embryos that survived rewarming and subsequently re-expanded were included in the study.

FET cycles

FET was performed after synthetic preparation of the endometrium. Patients were started on oral estradiol (Estrace®, Teva Pharmaceuticals, Sellersville, PA, USA) 2 mg twice daily for up to 1 week followed by three times daily. The endometrium was assessed weekly until a thickness of ≥7 mm was observed. Progesterone supplementation was then added with either 50 mg of intramuscular (IM) progesterone (Progesterone injection®, Watson Pharma Inc., Parsippany, NY, USA) or a combination of 100 mg oral progesterone (Endometrin®, Ferring Pharmaceuticals, Parsippany, NJ, USA) and 200 mg vaginal progesterone (Prometrium®, AbbVie Laboratories, Chicago, IL, USA) twice daily (PO/PV). The endometrial pattern was recorded as being in one of three categories as previously described by *Grunfeld et al. (1991)*: (i) late proliferative, (ii) early secretory, (iii) mid-late secretory. Embryo warming and transfer was performed after 5 days of progesterone supplementation. Embryo transfer was performed with a Wallace catheter under abdominal ultrasound guidance approximately 4–6 h following warming.

Outcome measures

The primary outcome of interest was the ongoing pregnancy or live birth (OP/LB) rate. An ongoing pregnancy was defined as a viable intrauterine gestation at the time of discharge from the practice, which occurred no earlier than 8 weeks of gestation. A live birth was considered the delivery of a live-born infant after 24 weeks' gestation. Secondary outcomes included the clinical pregnancy rate, early pregnancy loss (EPL) rate and clinical pregnancy loss (CPL) rate. Clinical pregnancy was confirmed by the sonographic evidence of fetal cardiac activity. EPL was defined as a pregnancy loss occurring prior to the detection of an intrauterine gestational sac on ultrasound. CPL was defined as a loss following the detection of an intrauterine gestational sac on ultrasound.

Statistical methods

Demographic and cycle characteristics between outcomes were compared using univariable mixed-effect logistic regression models with a random intercept term to account for patients who contributed more than one embryo. Mixed models were used in lieu of a generalized estimating equation (GEE) approach as the number of observations per subject was unbalanced. In the presence of unbalanced data, a GEE model is not recommended and standard error estimates will be biased downward (*Fitzmaurice et al., 2008*). To assess the impact of each grade on clinical outcomes, a mixed-effect logistic regression model was fitted for each clinical outcome (OP/LB, clinical pregnancy, EPL and CPL rates) with expansion, ICM and TE grade set as categorical predictors. An additional random effect was added to account for the embryologist performing the embryo biopsy and grading. Models were also adjusted for covariates including day of embryo biopsy, endometrial pattern and thickness at time of transfer, age, body mass index (BMI), gravidity, parity, number of previous euploid embryo transfers, type of endometrial preparation (IM versus PO/PV progesterone), PGT platform used, and triggering signal type (HCG only versus dual trigger). The likelihood of clinical outcomes was presented as odds ratios (OR) with 99% confidence intervals (CI). All hypothesis tests were two-sided and evaluated at

the 0.01 significance level. An OR table based on the composite grade was then developed to determine the likelihood of each clinical outcome. Holding all other covariates constant, the OR of each clinical outcome was determined by comparing the composite grade to a 6AA embryo, which was considered the best possible score. All analyses were conducted using SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Study population and cycle characteristics

A total of 2236 embryos from 1629 subjects who underwent single euploid embryo transfer were included for analysis. Among the 1629 subjects, 1160 (71.2%) contributed only one embryo and the remaining 469 (28.8%) contributed between two and six embryos. The demographic and cycle characteristics of patients who underwent embryo transfer are listed in TABLE 1. The likelihood of OP/LB was not affected by age, BMI, gravidity, parity, type of endometrial preparation (IM versus PO/PV progesterone), type of trigger signal (HCG only versus dual trigger), or type of PGT platform used (qPCR versus NGS). There was also no difference in OP/LB based on IVF stimulation cycle parameters, including number of oocytes retrieved or fertilized, number of good-quality blastocysts or number of euploid blastocysts obtained. OP/LB was more likely with a thicker endometrium (9.6 ± 2.0 versus 9.2 ± 1.8 mm, $P < 0.0001$), an early secretory (type 2) rather than mid-late secretory (type 3) endometrium at the time of transfer (15.2% [$n = 174$] versus 11.0% [$n = 120$], $P = 0.004$), and after fewer previous FET cycles (0.4 ± 0.7, 0.5 ± 10.0, $P = 0.0003$). Embryos with adequate development to allow Day 5 biopsy as compared with Day 6 had a higher OP/LB rate (75.2% versus 65.6%, $P < 0.001$) and a higher clinical pregnancy rate (75.0% versus 65.6%, $P < 0.001$).

Individual morphology components and IVF outcomes

The blastocyst ICM grade was the greatest predictor of pregnancy outcomes when evaluated independently from TE, expansion grades and other covariates (TABLE 2). Embryos with an ICM grade of A compared with C had a two-fold increased odds of OP/LB (OR 2.2, 99% CI 1.3–3.8) and a two-fold greater likelihood of clinical pregnancy (OR 2.5, 99% CI 1.5–4.3), as well as a higher OP/LB rate (55.6% versus 32.3%, $P < 0.001$) and clinical pregnancy rate (60.5% versus 34.6%, $P < 0.001$). Embryos with an ICM grade of A compared with B also had a greater odds of OP/LB (OR 1.5, 99% CI 1.1–2.0) and clinical pregnancy (OR 1.4, 99% CI 1.1–1.9). An EPL was approximately four times more likely with an ICM grade of C as compared with A (OR 4.2, 99%CI 2.1–8.2), and almost three times as likely with an ICM grade of C as compared with B (OR 2.7, 99% CI 1.3–5.5). ICM grade was not associated with the likelihood of CPL.

Pregnancy outcomes were also associated with blastocyst TE grade and the degree of re-expansion. Blastocysts with a TE grade of A or B as compared with a grade of C had a statistically higher likelihood of OP/LB (A versus C: OR 1.6, 99% CI 1.1–2.3; B versus C: OR 1.5, 99% CI 1.1–2.1) and clinical pregnancy (A versus C: OR 1.5, 99% CI 1.1–2.2, B versus C: OR 1.5, 99% CI 1.1–2.2). Although all embryos underwent assisted hatching, blastocysts with an expansion grade of 4 or 5 as compared with 6 had higher odds of OP/LB (4 versus 6: OR 1.6, 99% CI 1.2–2.2; 5 versus 6: OR 1.6, 99% CI 1.2–2.3). The odds of achieving a clinical pregnancy were also higher among blastocysts with an expansion grade 4 or 5 as compared with 6 (4

TABLE 1 DEMOGRAPHICS AND CYCLE CHARACTERISTICS FOR 1629 SUBJECTS

	All single euploid embryo transfers ($n = 2236$)		Ongoing pregnancy/live birth ($n = 1143$)		No ongoing pregnancy/live birth ($n = 1093$)		*P*-value
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	
Age (years)	35.8 ± 4.2	21.2, 45.5	35.7 ± 4.3	21.5, 45.5	36 ± 4.1	21.2, 44.5	NS
BMI (kg/m^2)	23.6 ± 4.3	14.9, 43.0	23.5 ± 4.1	14.9, 40.2	23.8 ± 4.4	15.7, 43.0	NS
Gravidity	1.2 ± 1.4	0, 8	1.1 ± 1.4	0, 8	1.3 ± 1.4	0, 7	NS
Parity	0.4 ± 0.7	0, 5	0.4 ± 0.7	0, 5	0.5 ± 0.8	0, 5	NS
Endometrial thickness at time of transfer (mm)	9.4 ± 1.9	7, 20.7	9.6 ± 2	7, 20.7	9.2 ± 1.8	7, 19.7	<0.0001
Previous PGT euploid transfers	0.5 ± 0.9	0, 8	0.4 ± 0.7	0, 4	0.5 ± 1	0, 8	0.0003
Number of oocytes retrieved	15.6 ± 8.4	1, 61	15.6 ± 8.3	2, 56	15.6 ± 8.4	1, 61	NS
Number of fertilized oocytes	10.0 ± 5.8	0, 45	10.1 ± 6.0	0, 45	10.0 ± 5.7	0, 37	NS
Number of Day 5 blastocysts	7.2 ± 4.6	0, 33	2.7 ± 2.8	0, 20	2.6 ± 2.7	0, 16	NS
Number of euploid blastocysts	3.5 ± 2.6	1, 21	3.5 ± 2.6	1, 21	3.5 ± 2.6	1, 18	NS
	No.	%	No.	%	No.	%	
Lining type 3 at time of transfer	1942	86.9	969	84.8	973	89.0	0.004
Day 5 TE biopsy	1585	70.9	859	75.2	726	66.4	<0.0001
Intramuscular progesterone (ng/ml)	1567	70.1	823	72.0	744	68.1	NS
	No./No. obs.	%	No./No. obs.	%	No./No. obs.	%	
PCR/PCR + NGS	1119/2171	51.5	572/1114	51.3	547/1057	51.8	NS
HCG/HCG + dual trigger	816/2058	39.7	406/1051	38.6	410/1007	40.7	NS

HCG = human chorionic gonadotrophin; NGS = next-generation sequencing; PGT = preimplantation genetic testing; TE = trophectoderm.

TABLE 2 PREGNANCY OUTCOMES BASED ON BLASTOCYST EXPANSION, ICM AND TE GRADE

		Ongoing pregnancy/live birth			Clinical pregnancy			EPL			CPL		
		No./No. obs. (%)	Adjusted OR (99% CI)	*P*-value	No./No. obs. (%)	Adjusted OR (99% CI)	*P*-value	No./No. obs. (%)	Adjusted OR (99% CI)	*P*-value	No./No. obs. (%)	Adjusted OR (99% CI)	*P*-value
Expansion grade	4	491/909 (54.0)	1.61 (1.20, 2.17)	<0.001	539/909 (59.3)	1.67 (1.24, 2.25)	<0.001	107/681 (15.7)	0.75 (0.47, 1.19)	0.26	83/681 (12.2)	0.82 (0.48, 1.38)	NS
	5	341/607 (56.2)	1.63 (1.18, 2.26)		375/607 (61.8)	1.75 (1.26, 2.43)		84/491 (17.1)	0.82 (0.50, 1.35)		66/491 (13.4)	0.94 (0.54, 1.63)	
	6	311/720 (43.2)	Ref		344/720 (47.8)	Ref		95/472 (20.1)	Ref		66/472 (14.0)	Ref	
ICM grade	A	857/1541 (55.6)	2.22 (1.29, 3.84)	<0.001	933/1541 (60.5)	2.49 (1.45, 4.26)	<0.001	169/1181 (14.3)	0.24 (0.12, 0.47)	<0.001	155/1181 (13.1)	1.63 (0.58, 4.60)	NS
	B	245/568 (43.1)	1.51 (0.85, 2.68)		281/568 (49.5)	1.75 (0.99, 3.07)		84/380 (22.1)	0.38 (0.18, 0.77)		51/380 (13.4)	1.56 (0.53, 4.64)	
	C	41/127 (32.3)	Ref		44/127 (34.6)	Ref		33/83 (39.8)	Ref		9/83 (10.8)	Ref	
TE grade	A	427/767 (55.7)	1.56 (1.08, 2.26)	0.002	461/767 (60.1)	1.51 (1.05, 2.18)	0.002	97/588 (16.5)	0.89 (0.51, 1.57)	NS	64/588 (10.9)	0.66 (0.35, 1.26)	NS
	B	531/1006 (52.8)	1.53 (1.10, 2.13)		589/1006 (58.5)	1.54 (1.11, 2.15)		125/763 (16.4)	0.83 (0.49, 1.38)		107/763 (14.0)	0.89 (0.51, 1.56)	
	C	185/463 (40)	Ref		208/463 (44.9)	Ref		64/293 (21.8)	Ref		44/293 (15.0)	Ref	

CI = confidence interval; CPL = clinical pregnancy loss; EPL = early pregnancy loss; ICM = inner cell mass; NS = not statistically significant; OR = odds ratio; TE = trophectoderm.

versus 6: OR 1.7, 99% CI 1.2–2.3, 5 versus 6: OR 1.8, 99% CI 1.3–2.4). Blastocyst TE and expansion grades were not associated with the likelihood of EPL or CPL.

Composite grade and IVF outcomes

When evaluating the composite score (as compared with a reference 6AA blastocyst) there was a significantly higher likelihood of OP/LB as well as clinical pregnancy among blastocysts with grades 4AA and 4AB or 5AA and 5AB (OR 1.6–1.8, 99% CI in TABLE 3 and Supplementary TABLE 1). Blastocysts with grades 6AC, 6BA, 6BB, 6BC, 6CA, 6CB, 6CC, 5CC and 4CC were significantly less likely to result in an OP/LB or clinical pregnancy than the reference 6AA blastocyst (OR 0.27–0.70, 99% CI in TABLE 3 and Supplementary TABLE 1). Most blastocysts with a poorly developed ICM (grade of C), regardless of TE grade or expansion score, had a significantly lower odds of OP/LB (OR 0.29–0.47, 99% CI in TABLE 3 and Supplementary TABLE 1) and clinical pregnancy (OR 0.27–0.47, 99% CI in TABLE 3 and Supplementary TABLE 1) and higher odds of EPL (OR 2.9–4.7, 99% CI in TABLE 3 and Supplementary TABLE 1) than the reference 6AA blastocyst. All other embryos had similar odds of CPL and EPL as the reference embryos. A ranking of all embryos based on the composite grade and day of blastocyst biopsy is described in Supplementary TABLE 2. These data demonstrate the significance of all three components of the grade on pregnancy outcomes.

DISCUSSION

The study results suggest that morphology of biopsied blastocysts post-warming is correlated with live birth and thus should be considered in embryo selection. Previous studies that have analysed the predictive value of embryo morphology have been limited by a lack of comprehensive chromosomal screening or by the transfer of more than one embryo (*Ahlstrom et al., 2013; Chen et al., 2014; Desai et al., 2016; Du et al., 2016; Hill et al., 2013; Subira et al., 2016; Thompson et al., 2013*). Moreover, previous research has focused on the contribution of separate components of the grade on pregnancy outcomes rather than assessing the three-part grade as a whole. This study is the first to evaluate the association between each composite grade and pregnancy outcomes after single euploid FET.

The study shows that blastocysts with a composite grade >4AB have superior pregnancy outcomes compared with a 6AA embryo. This categorization of embryos differs from previously published data by *Irani et al. (2017)* and *Capalbo et al. (2014)* in which 'excellent' embryos were only considered ≥3AA. Similar to the findings of *Irani et al. (2017)*, this large study demonstrates that ICM grade is a better predictor of implantation than other components of embryo grading. As compared with embryos with an ICM grade of C, embryos with an ICM grade of A have a two-fold higher likelihood of ongoing pregnancy or live birth, a two-fold higher likelihood of clinical pregnancy, and a significantly lower risk of early miscarriage. Conversely, embryos with an ICM of C were more likely to result in EPL. These findings are in contrast to several previous studies that demonstrated a lack of added predictability of the ICM grade as compared with the TE grade or degree of expansion (*Ahlstrom et al., 2013; Hill et al., 2013; Thompson et al., 2013*). The present study does demonstrate the value of TE and expansion grade in determining outcomes, consistent with other previously published data (*Ahlstrom et al., 2013; Du et al., 2016; Hill et al., 2013*) as a higher likelihood of ongoing pregnancy or live birth was found in blastocysts with TE grade of A or B as compared with C, and embryos with an expansion stage of 4 or 5 as compared with 6. In this study, the likelihood of CPL was not affected by morphology, while the likelihood of EPL was associated with blastocyst grade, most notably among

TABLE 3 ODDS OF IVF OUTCOMES BASED ON COMPOSITE MORPHOLOGIC GRADE COMPARED WITH 6AA EMBRYO

		Adjusted OR [99% CI]	Adjusted OR [99% CI]	Adjusted OR [99% CI]	
Ongoing pregnancy or live birth					
	ICM	A	B	C	
Expansion grade					TE
4		1.61[a] [1.20, 2.17]	1.09 [0.73, 1.63]	0.73 [0.39, 1.34]	A
		1.58[a] [1.07, 2.33]	1.07 [0.68, 1.69]	0.71 [0.37, 1.36]	B
		1.03 [0.67, 1.60]	0.70 [0.43, 1.15]	0.46[a] [0.24, 0.91]	C
5		1.63[a] [1.18, 2.26]	1.11 [0.71, 1.73]	0.74 [0.39, 1.39]	A
		1.60[a] [1.02, 2.52]	1.08 [0.64, 1.85]	0.72 [0.36, 1.45]	B
		1.05 [0.62, 1.76]	0.71 [0.40, 1.27]	0.47[a] [0.23, 0.98]	C
6		Reference	0.68[a] [0.51, 0.91]	0.45[a] [0.26, 0.78]	A
		0.98 [0.73, 1.30]	0.66[a] [0.45, 0.98]	0.44[a] [0.24, 0.81]	B
		0.64[a] [0.44, 0.92]	0.43[a] [0.28, 0.68]	0.29[a] [0.15, 0.55]	C
Clinical pregnancy					
	ICM	A	B	C	
Expansion grade					TE
4		1.67[a] [1.24, 2.25]	1.17 [0.79, 1.75]	0.67 [0.37, 1.23]	A
		1.71[a] [1.15, 2.53]	1.20 [0.76, 1.90]	0.69 [0.36, 1.31]	B
		1.11 [0.72, 1.72]	0.78 [0.48, 1.27]	0.45[a] [0.23, 0.86]	C
5		1.75[a] [1.26, 2.42]	1.23 [0.78, 1.92]	0.70 [0.38, 1.31]	A
		1.78[a] [1.13, 2.82]	1.25 [0.73, 2.14]	0.72 [0.36, 1.44]	B
		1.16 [0.69, 1.95]	0.81 [0.45, 1.46]	0.47[a] [0.22, 0.96]	C
6		Reference	0.70[a] [0.53, 0.94]	0.40[a] [0.23, 0.69]	A
		1.02 [0.76, 1.37]	0.72 [0.48, 1.06]	0.41[a] [0.22, 0.75]	B
		0.66[a] [0.46, 0.95]	0.46[a] [0.30, 0.72]	0.27[a] [0.14, 0.50]	C
Early pregnancy loss					
	ICM	A	B	C	
Expansion grade					TE
4		0.75 [0.46, 1.19]	1.17 [0.62, 2.19]	3.1[a] [1.40, 6.83]	A
		0.69 [0.38, 1.26]	1.08 [0.53, 2.19]	2.87[a] [1.23, 6.72]	B
		0.84 [0.43, 1.65]	1.31 [0.61, 2.82]	3.48[a] [1.43, 8.46]	C
5		0.82 [0.50, 1.35]	1.28 [0.64, 2.55]	3.40[a] [1.50, 7.75]	A
		0.76 [0.38, 1.52]	1.19 [0.52, 2.69]	3.16[a] [1.25, 7.99]	B
		0.92 [0.41, 2.06]	1.44 [0.58, 3.58]	3.82[a] [1.41, 10.41]	C
6		Reference	1.57[a] [1.00, 2.44]	4.16[a] [2.11, 8.19]	A
		0.93 [0.59, 1.45]	1.45 [0.8, 2.64]	3.86[a] [1.76, 8.44]	B
		1.12 [0.64, 1.98]	1.76 [0.88, 3.50]	4.67[a] [2.02, 10.83]	C
Clinical pregnancy loss					
	ICM	A	B	C	
Expansion grade					TE
4		0.82 [0.48, 1.38]	0.78 [0.38, 1.59]	0.50 [0.16, 1.57]	A
		1.10 [0.56, 2.15]	1.05 [0.47, 2.35]	0.67 [0.2, 2.23]	B
		1.23 [0.58, 2.64]	1.18 [0.49, 2.84]	0.76 [0.22, 2.59]	C
5		0.94 [0.54, 1.63]	0.90 [0.42, 1.95]	0.58 [0.18, 1.84]	A
		1.26 [0.58, 2.72]	1.21 [0.48, 3.03]	0.77 [0.22, 2.74]	B
		1.42 [0.58, 3.47]	1.36 [0.49, 3.79]	0.87 [0.23, 3.28]	C
6		Reference	0.96 [0.58, 1.58]	0.61 [0.22, 1.73]	A
		1.34 [0.82, 2.2]	1.29 [0.65, 2.53]	0.82 [0.27, 2.56]	B
		1.51 [0.8, 2.87]	1.45 [0.66, 3.18]	0.93 [0.28, 3.02]	C

CI = confidence interval; ICM = inner cell mass; OR = odds ratio; TE = trophectoderm.

[a] If 99% CI interval does not cross one.

blastocysts with an ICM grade of C. While it remains to be determined why some euploid embryos fail to implant, these findings suggest that morphology may represent a contributing factor. In a recent editorial, *Forman (2017)* suggested that not all euploid blastocysts have equal potential of implantation, and embryo morphology may be a differentiator. He reported an increased implantation rate among highly graded euploid blastocysts (4AA, 5AA and 6AA) as compared with euploid blastocysts with lesser grades (80.9% versus 56.3%, respectively). These findings are consistent with the results of the present study.

The study is limited by the subjective nature of morphologic grading, even though inter-observer variability is minimal in our practice. Additionally, given that all embryos underwent assisted hatching, the predictive value of the expansion grade on pregnancy outcomes may be limited. The varying ability of patients to produce high-quality euploid embryos may decrease the applicability of these findings. Moreover, embryonic ploidy was determined based on one of two different PGT platforms including qPCR, which is relatively insensitive for detection of mosaicism or segmental imbalances that might contribute to lower implantation rates. Future studies with a larger sample size of embryos only undergoing PGT by NGS may further elucidate the role of morphology in predicting IVF outcomes.

Due to its retrospective design, the study is also limited by selection bias. While the statistical model controlled for many possible confounding variables, only patients with a blastocyst eligible for TE biopsy were included. Many patients with poor ovarian response or blastulation were excluded from the analyses, as were patients with an endometrial thickness <7 mm. Strengths of the study include a large sample size and analyses of only euploid SET. As singleton deliveries have become a priority in the field, and as implementation of a PGT/SET strategy continues to rise, the findings of this study are relevant to current and future IVF practice. This study might provide guidance for optimal selection of supernumerary euploid embryos to maximize the likelihood of clinical pregnancy after FET.

This large study is the first to propose a data-driven system for selection of the optimal euploid blastocyst based on morphology. The importance of ICM grade in selecting an optimal embryo for transfer after comprehensive chromosomal screening is demonstrated. However, a composite score, rather than a separate analysis of individual components, improves embryo assessment and selection in the setting of PGT and could help maximize the ability to achieve a healthy singleton pregnancy. Our findings suggest that genomic and morphologic criteria offer complementary information that may optimize outcomes following single euploid FET.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2018.10.007.

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