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ASSISTED REPRODUCTION TECHNOLOGIES

The impact of euploid blastocyst morphology and maternal age on pregnancy and neonatal outcomes in natural cycle frozen embryo transfers

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Abstract

Purpose To evaluate whether morphology impacts the pregnancy and neonatal outcomes of euploid blastocysts, and whether maternal age still affects outcomes when top-graded, euploid blastocysts are used.

Methods This retrospective cohort study included all natural-cycle frozen embryo transfers (NC-FET) using an autologous, euploid blastocyst from June 2016 to June 2020 ($n=610$). There were five groups based on embryo grade: AA, AB, BA, BB, and "any C". For analysis of only AA-graded embryos, there were three maternal age groups: < 35, 35–39, and 40 + years. The main outcomes measured were clinical pregnancy and live birth rates, while the secondary outcomes included neonatal outcomes such as gestational age at delivery and birthweight. Multivariable logistic regression models were performed to adjust for confounders.

Results Euploid blastocysts with poorer morphology had lower odds of pregnancy and live birth; specifically, embryos with inner cell mass (ICM) graded as "C" had statistically significant decreased odds of pregnancy (aOR 0.33, $p=0.04$) and live birth (aOR 0.32, $p=0.03$) compared with ICM grade "A". The differences in pregnancy rate between trophectoderm grades were not statistically significant. Even in cycles that transferred a top-graded (AA) euploid embryo, maternal age at transfer was independently associated with outcomes. Embryo grade and maternal age, however, did not significantly impact neonatal outcomes such as prematurity and birthweight.

Conclusion The morphology of euploid blastocysts and maternal age at NC-FET both independently impact pregnancy outcomes. Neonatal outcomes were similar across embryo morphology and maternal age groups, suggesting that lower morphology euploid embryos not be discounted as viable options for transfer.

Keywords Embryo morphology · Maternal age · Euploid blastocysts · Pregnancy outcomes · Neonatal outcomes · Frozen embryo transfer (FET)

Introduction

Advances in laboratory and clinical techniques have allowed single embryo transfer to become more common practice, lending more importance to the process of embryo selection. One parameter that is often used to inform such decision-making is embryo morphology [1–6]. Blastocyst grading is based on the morphology of the inner cell mass (ICM) and trophectoderm (TE), as well as the expansion of the blastocyst cavity. A commonly utilized method follows the grading system described by Gardner and Schoolcraft in 1999, with grades ranging from "A" for best quality to "D" for worst [1, 7]. Multiple studies have since demonstrated blastocyst morphology to be a good predictor of live birth rates following both fresh and frozen embryo transfers [1–6]. Alfarawati

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et al. further investigated the potential of morphology as a means to predict ploidy status but found a weak association between morphology and aneuploidy; almost half of the top-grade blastocysts in their study were aneuploid, while over a third of the low-grade blastocysts were euploid [8].

With the growing use of preimplantation genetic testing (PGT) to assess ploidy, there has been debate about whether embryo morphology plays a significant role in predicting pregnancy outcomes. Capalbo et al. examined 215 FET cycles performed with euploid blastocysts and reported that embryo morphology was not predictive of implantation potential [9]. A more recent 2019 study by Viñals Gonzalez et al. that examined 179 FET cycles of euploid blastocysts similarly found that embryo morphology did not significantly affect implantation, clinical miscarriage, or live birth rates [10]. Irani et al.'s study of 417 FET of euploid blastocysts, however, suggested that blastocyst morphology could prove valuable as an adjunct to PGT for embryo selection [11]. Importantly, none of these (nor any prior) studies examined the impact morphology had on neonatal outcomes. Furthermore, the majority of prior studies did not account for the FET protocol utilized, which could confound outcomes [11–19]. Thus, our original study aims to evaluate whether embryo morphologic grading affects pregnancy and neonatal outcomes when euploid blastocysts are used in NC-FET.

Materials and methods

Patients

Our retrospective cohort study included all NC-FET cycles performed at Stanford Fertility and Reproductive Health Center in which a single autologous, euploid blastocyst was transferred between June 2016 and June 2020. Prior to embryo transfer, patients were confirmed to have normal uterine cavity via hysteroscopy, hysterosalpingogram, or saline infusion sonogram. All demographic, fertility, pregnancy, and neonatal information were collected from medical records. Demographics and clinical characteristics included maternal age at retrieval and at FET, number of prior embryo transfers, gravidity and parity, BMI, smoking status, race/ethnicity, and infertility diagnosis. Cycle characteristics included serum estradiol, luteinizing hormone (LH), progesterone, and endometrial thickness at the time of trigger. The Stanford University Institutional Review Board approved the study protocol.

NC-FET protocol

Our standard protocol for NC-FET starts with a baseline ultrasound on cycle days 2–5, followed by ultrasound monitoring 3–4 days prior to expected ovulation. After the dominant follicle is > 15 mm in size, patients had their serum estradiol, LH, and progesterone checked and subsequently underwent daily ultrasound monitoring until either the dominant follicle was ≥ 18 mm or a positive LH surge was noted (defined as LH ≥ 20 mIU/mL). Ovulation was then triggered or reinforced with recombinant hCG (250 mcg Ovidrel, EMD Serono). If a LH surge was detected, FET was performed 6 days later, and if not, FET was performed 7 days later [20]. In general, we would only proceed with FET if endometrial thickness was ≥ 7 mm, but if the patient had a history of endometrial thicknesses below this threshold, exceptions were made if the current cycle was a personal best; of note, our study included only 7 such cases, all of which transferred blastocysts graded BB or better. Patients were instructed to start progesterone supplementation (Crinone 8% gel daily vaginally or Endometrin 100 mg twice a day vaginally) 3 days after spontaneous LH surge or 4 days after hCG trigger. Serum βhCG was obtained 9 days after FET, and clinical pregnancy was confirmed on transvaginal ultrasound at 6–7 weeks gestational age for the presence of a viable intrauterine pregnancy. Serum estradiol, LH, progesterone, and βhCG levels were assayed with the Roche Cobas E411 analyzer (Roche Diagnostics).

All embryos transferred were blastocysts (typically frozen on day 5 or 6) derived from autologous oocytes and confirmed to be euploid by PGT. Prior to freezing, blastocysts were graded from AA to DD based on the inner cell mass (the first grade) and trophectoderm morphology (the second grade); the survival rate of blastocyst thawing within our laboratory is 95–97%. Our clinical practice is to biopsy embryos with grade CC or higher for PGT.

Study outcomes

The primary outcomes studied were clinical pregnancy (presence of fetal cardiac activity) and live birth (live infant born after 24 weeks of gestation). Secondary outcomes included clinical miscarriage (pregnancy loss prior to 20 weeks of gestation), biochemical miscarriage (rise and fall in βhCG without evidence of a clinical pregnancy), ectopic pregnancy, cesarean delivery, gestational age at delivery, birthweight, and sex assigned at birth.

Statistical analysis

The study cohort was divided into five groups based on embryo grade: AA ($n = 197$), AB ($n = 124$), BA ($n = 63$), BB ($n = 177$), and "any C" (including AC, CA, BC, CB, and CC; $n = 49$). Study data were managed using the Stanford REDCap electronic data tool [21] and analyzed by a biostatistician who was not part of the data collection. Patient and cycle characteristics for the different embryo

grade groups were compared using absolute standardized differences (ASD), which measure the difference in means or proportions between two groups in units of standard deviations [22]. Since we compared multiple groups, ASD was calculated by taking the average of the pairwise comparisons. ASD values of 0.2, 0.5, and 0.8 correspond to small, moderate, and large differences, respectively. We performed a secondary analysis including only AA euploid embryos to compare pregnancy and neonatal outcomes between three maternal age groups at transfer: < 35 ($n = 70$), 35–39 ($n = 97$), and 40 + ($n = 30$) years.

Multivariable logistic regression models were performed to assess associations in pregnancy outcomes with maternal age at time of embryo transfer, nulliparity, endometrial lining thickness, inner cell mass grade (A-C), and trophectoderm grade (A-C). Due to some variation in embryo grade with respect to race, diminished ovarian reserve (DOR), and male factor infertility, we assessed whether there was a relationship between these factors and pregnancy outcomes. These factors were not included in the logistic regression after univariate analysis showed no significant relationship with pregnancy outcomes. We utilized generalized estimating equations (GEE) to account for correlation between patients with repeat cycles. We calculated adjusted odds ratios (aOR) and 95% confidence intervals (CIs) to evaluate the relative odds for live birth and clinical pregnancy. Due to few live births with prematurity and/or low birthweight, comparisons between embryo morphology groups were made using descriptive statistics. Analyses were performed using the R statistical software version 3.6.2, and GEE analyses were performed using library geepack [23–26]. All statistical tests were two-sided and performed at the 0.05 significance level.

Results

Participant and cycle characteristics

A total of 431 women underwent a total of 610 NC-FET cycles between June 2016 and June 2020 at our institution. Cycles were divided into five groups based on embryo grade: AA ($n = 197$), AB ($n = 124$), BA ($n = 63$), BB ($n = 177$), and "any C" ($n = 49$). The maternal age at transfer ranged from 25 to 48 years (mean of 36.2), and the maternal age at retrieval ranged from 25 to 48 years (mean of 35.7). The respective mean age data for each embryo grade cohort is detailed in Table 1, ranging from mean age at transfer of 35.4 years (AA) to 37.3 years ("any C") and mean maternal age at retrieval from 35.0 years (AA) to 36.8 years ("any C"). The overall cohort's mean BMI was 25.0 kg/m^2, with similar mean BMI among the five embryo grade groups. Other than participants' race/ethnicity, there were no substantial differences between the embryo grade groups with respect to BMI, nulliparity, number of prior embryo transfer cycles, smoking status, or infertility diagnoses (Table 1).

Cycle characteristics on the day of ovulation trigger—which included serum estradiol, LH, and progesterone—were similar between the embryo grade groups (Table 1). There was a moderate difference in the endometrial thickness on the day of hCG trigger, ranging from a mean thickness of 8.5 mm for the "any C" group to 9.5 mm for the AB group (ASD = 0.33).

Pregnancy outcomes comparing euploid embryo grades

The "any C" group had a clinical pregnancy rate of 40.8%, compared to 60.5% for BB, 61.9% for BA, 68.5% for AB, and 67.5% for AA (ASD = 0.26). Similarly, the "any C" group had a live birth rate of 40.8%, which was substantially lower compared to 56.5% for BB, 55.6% for BA, 67.5% for AB, and 64.5% for AA (ASD = 0.26). This indicates a notable drop-off in the incidence of clinical pregnancy and live birth when an euploid embryo had a "C" morphologic grade. The incidences of biochemical miscarriage, ectopic pregnancy, and clinical miscarriage were not substantially different between the groups. The "any C" and AA groups had a higher rate of Cesarean delivery (40% and 37%, respectively) compared to the other groups (30% for BB, 20% for BA, 24% for AB; ASD = 0.28) (Table 2).

After accounting for patients undergoing repeat cycles using GEE methodology in a multivariable logistic regression, the pregnancy outcome patterns persisted (Table 3). Cycles that transferred euploid embryos with "any C" grade trended towards lower odds of clinical pregnancy and live birth. Specifically, the cycles with ICM graded "C" had a statistically significant decreased odds of clinical pregnancy (aOR 0.33; 95% CI 0.12, 0.93; $p = 0.04$) and live birth (aOR 0.32; 95% CI 0.11, 0.91; $p = 0.03$) when compared with cycles with ICM graded "A". The differences when comparing trophectoderm grades were not statistically significant. Of note, 114 patients had repeat cycles; of those with repeat cycles, 67 patients transferred embryos of varying grades, 38 (56.3%) of whom achieved pregnancy with a lower quality embryo than previously transferred.

Pregnancy outcomes comparing maternal age

Our secondary analysis that included only the top-graded (AA) embryos demonstrated moderate ASD differences in the rates of clinical pregnancy and live birth between the age groups (Table 4). The 40 + age group had a lower rate of clinical pregnancy at 50%, compared to 63.9% for the 35–39 group and 78.6% for the < 35 group (ASD = 0.44). The 40 + age group also had a lower live birth rate of

Table 1 Patient demographics, baseline clinical and cycle characteristics

	AA	AB	BA	BB	Any C	ASD[a]
Clinical characteristics	***N* = 145**	***N* = 92**	***N* = 42**	***N* = 120**	***N* = 32**	
Age at FET (years)	35.4 ± 3.9	36.5 ± 3.4	35.8 ± 3.9	36.9 ± 3.9	37.3 ± 3.9	0.26
Age at retrieval (years)	35.0 ± 4.0	36.0 ± 3.2	35.0 ± 4.2	36.1 ± 3.9	36.8 ± 4.1	0.25
Number of prior transfers	1.3 ± 0.6	1.5 ± 0.8	1.2 ± 0.5	1.5 ± 0.9	1.5 ± 0.9	0.22
Maternal BMI (kg/m^2)	25.7 ± 5.7)	25.0 ± 5.2	23.3 ± 3.4	24.9 ± 4.5	24.2 ± 4.2	0.25
Nulliparous	97 (66.9)	57 (62.0)	25 (59.5)	66 (55.0)	16 (50.0)	0.17
Never smoker	138 (95.2)	88 (95.7)	41 (97.6)	113 (94.2)	31 (96.9)	0.09
Race/ethnicity[b]:	50 (34.5)	35 (38.0)	12 (28.6)	49 (40.8)	14 (43.8)	0.32
White	77 (53.1)	48 (52.2)	26 (61.9)	62 (51.7)	15 (46.9)	
Asian American	4 (2.8)	5 (5.4)	0 (0.0)	5 (4.2)	2 (6.2)	
Hispanic/Latino	5 (3.4)	2 (2.2)	0 (0.0)	1 (0.8)	0 (0.0)	
African American	10 (6.9)	3 (3.3)	1 (2.4)	6 (5.0)	1 (3.1)	
Other	3 (2.1)	4 (4.3)	3 (7.1)	2 (1.7)	2 (6.2)	
Unknown						
Infertility Diagnosis[b]:	41 (28.3)	21 (22.8)	6 (14.3)	30 (25.0)	3 (9.4)	0.25
Male factor						
DOR	21 (14.5)	19 (20.7)	7 (16.7)	33 (27.5)	12 (37.5)	0.27
Ovulatory dysfunction	11 (7.6)	4 (4.3)	1 (2.4)	4 (3.3)	1 (3.1)	0.11
RPL	26 (17.9)	11 (12.0)	6 (14.3)	15 (12.5)	9 (28.1)	0.19
Endometriosis	6 (4.1)	4 (4.3)	3 (7.1)	5 (4.2)	0 (0.0)	0.17
Uterine	11 (7.6)	3 (3.3)	2 (4.8)	2 (1.7)	2 (6.2)	0.15
Tubal	6 (4.1)	3 (3.3)	3 (7.1)	6 (5.0)	1 (3.1)	0.09
Single gene disorder	6 (4.1)	8 (8.7)	3 (7.1)	7 (5.8)	3 (9.4)	0.11
Unexplained	29 (20.0)	27 (29.3)	13 (31.0)	27 (22.5)	4 (12.5)	0.23
Other	8 (5.5)	6 (6.5)	4 (9.5)	6 (5.0)	0 (0.0)	0.21
Cycle characteristics	***N* = 197**	***N* = 124**	***N* = 63**	***N* = 177**	***N* = 49**	
Estradiol at trigger (p/mL)	342.3 ± 340.6	360.9 ± 485.0	290.1 ± 131.7	298.9 ± 317.4	341.6 ± 354.4	0.11
Progesterone at trigger (ng/ML)	0.54 ± 0.36	0.58 ± 0.96	0.52 ± 0.26	0.57 (0.40)	0.55 ± 0.46	0.06
LH at trigger (mIU/mL)	28.4 ± 24.3	25.4 ± 22.8	30.4 ± 28.2	28.2 (25.8)	25.6 ± 20.6	0.10
Endometrial lining thickness (mm)	9.1 ± 1.5	9.2 ± 1.6	9.5 ± 1.7	9.0 (1.3)	8.5 ± 0.9	0.33

Data are presented as mean ± SD or *n* (%)

ASD absolute standardized difference; *FET* frozen embryo transfer; *BMI* body mass index; *DOR* diminished ovarian reserve; *RPL* recurrent pregnancy loss

[a]Absolute standardized difference: 0.2, small difference; 0.5, medium difference; 0.8 + , large difference

[b]These variables allowed for multiple selections per patient if applicable

43.3%, compared to 60.8% for the 35–39 group and 78.6% for the < 35 group (ASD = 0.51). The 40 + age group had a higher incidence of miscarriage at 13.3%, compared to 4.8% for the 35–39 group and 0% for the < 35 age group (ASD = 0.39). The 40 + age group also had a higher incidence of cesarean delivery at 53.8%, compared to 39% for the 35–39 age group and 30.9% for the < 35 age group (ASD = 0.38). Of note, the mean maternal age at FET for every cohort was within 8 months of mean maternal age at oocyte retrieval. Thus, overall, the age at which participants underwent FET was not substantially remote from when they underwent oocyte retrieval.

GEE adjusted regression demonstrated maternal age at transfer to be independently associated with outcomes (Table 3). Compared to the < 35 reference group, the 40 + age group had a significantly decreased odds of clinical pregnancy (aOR 0.54, 95% CI 0.31, 0.96; $p = 0.04$) and live birth (aOR 0.48; 95% CI 0.27, 0.85; $p = 0.01$); the 35–39 age group also had a statistically significant decreased odds of clinical pregnancy (aOR 0.53, 95% CI 0.34, 0.82; $p < 0.01$) and live birth (aOR 0.54, 95% CI 0.35, 0.84; $p < 0.01$).

Neonatal outcomes comparing euploid embryo grade

The median gestational ages were similar among the different embryo grades, ranging from 273.0 days [interquartile range, IQR, 269.5, 277.5] for "any C" neonates to 275.0 days [IQR 267.0, 279.3] for AA neonates (ASD = 0.17) (Table 2). Thus, there were similar incidences of prematurity among

Table 2 Comparison of pregnancy outcomes and neonatal outcomes among different euploid blastocyst grades

	AA	AB	BA	BB	Any C	ASD [a]
Pregnancy outcomes	***N* = 197**	***N* = 124**	***N* = 63**	***N* = 177**	***N* = 49**	
Clinical pregnancy	133 (67.5)	85 (68.5)	39 (61.9)	107 (60.5)	20 (40.8)	0.26
Biochemical miscarriage	7 (3.6)	7 (5.7)	0 (0.0)	15 (8.5)	5 (10.2)	0.24
Ectopic pregnancy	3 (1.5)	0 (0.0)	0 (0.0)	1 (0.6)	0 (0.0)	0.09
Clinical miscarriage	6 (4.5)	2 (2.4)	4 (10.3)	7 (6.5)	0 (0.0)	0.25
Live birth	127 (64.5)	83 (66.9)	35 (55.6)	100 (56.5)	20 (40.8)	0.26
Neonatal outcomes	***N* = 127**	***N* = 83**	***N* = 35**	***N* = 100**	***N* = 20**	
Cesarean delivery	47 (37)	20 (24)	7 (20)	30 (30)	8 (40)	0.28
Gestational age at delivery (days) [median IQR [b]]	275.0 [267.0, 279.2]	275.0 [270.7, 280.0]	275.0 [269.3, 280.0]	274.5 [270.3, 281]	273.0 [269.5, 277.5]	0.17
Preterm delivery	13 (10.2)	4 (4.8)	2 (5.7)	4 (4.0)	1 (5.0)	0.18
Birthweight (grams) [median IQR [b]]	3200 [2896, 3590]	3280 [3085, 3600]	3307 [3070, 3685]	3317 [3118, 3680]	3450 [3319, 3650]	0.20
Neonate sex	48 (37.8)	44 (53.0)	10 (28.6)	34 (34.0)	13 (65.0)	0.44
-Female	77 (60.6)	36 (43.4)	24 (68.6)	60 (60.0)	6 (30.0)	
-Male	2 (1.6)	3 (3.6)	1 (2.9)	6 (6.0)	1 (5.0)	
-Ambiguous or unknown						

Data are presented as mean ± SD, *n* (%), or median[interquartile range]

[a]Absolute standardized difference: 0.2, small difference; 0.5, medium difference; 0.8 +, large difference

[b]*IQR* interquartile range. Because these variables were skewed, median and IQR were utilized instead of mean and standard deviation

the groups. The median neonatal birthweights were also similar among the different grades, ranging from 3200 g [IQR 2896, 3590] for AA neonates to 3450 [IQR 3319, 3650] for "any C" neonates.

The secondary analysis comparing different maternal age groups (< 35, 35–39, and 40 + year) showed comparable median gestational ages (ASD = 0.07), prematurity rates (ASD = 0.21), median birthweights (ASD = 0.23), and neonate sex distribution (ASD = 0.23) (Table 4).

Discussion

Our present study is the first to our knowledge to examine the impact of euploid blastocyst morphology on both pregnancy and neonatal outcomes in NC-FET, with the goal of informing the process of embryo selection. We demonstrated that higher graded blastocysts are associated with better pregnancy outcomes with regard to both clinical pregnancy and live birth rates. Also of note, the ICM grade specifically conveys a more significant impact than the TE grade. In addition, maternal age continues to play a significant role in pregnancy outcomes even when accounting for the embryo quality and ploidy; the odds of clinical pregnancy and live birth markedly decrease with advanced age. Nonetheless, our data reassuringly suggests that blastocyst morphology and maternal age are not associated with neonatal outcomes such as prematurity or lower birthweight.

It has been widely recognized that human oocytes retrieved for assisted reproduction have a high prevalence of aneuploidy, and multiple studies have demonstrated that the produced embryos are often affected by chromosome abnormalities [27–31]. Prior to PGT, the embryo selection process relied heavily on embryo morphology [1–6]. With the introduction and now rapidly growing use of PGT, clinicians can better evaluate ploidy status. The question that naturally follows is whether embryo morphology still plays a role in predicting outcomes of euploid blastocysts. Thus far, only a handful of studies have investigated such question. Capalbo et al. first examined the outcomes of euploid blastocysts in 2014 when investigating the correlation between blastocyst morphology and ploidy status. Their additional analysis of 215 FET cycles performed with euploid blastocysts found that embryo morphology was not predictive of implantation potential, though they were limited by a small sample size of lower quality embryos [9]. Similarly, Viñals Gonzalez et al. examined 179 FET cycles of euploid blastocysts and found that embryo morphology did not significantly affect implantation, clinical miscarriage, and live birth rates [10]. However, their study only included women with advanced maternal age (AMA), thereby limiting generalizability. Irani et al.'s 2016 study of 417 FET cycles included all infertility diagnoses and

Table 3 Adjusted odds ratios (aORs) for clinical pregnancy and live birth

Adjusted Odds Ratio (aOR)[a] for clinical pregnancy		95% CI	*P*-value
Inner cell mass grade = A	Ref		
B	0.79	(0.54, 1.15)	0.22
C	0.33	(0.12, 0.93)	0.04*
Inner cell mass grade = B	Ref		
C	0.44	(0.17, 1.14)	0.09
Trophectoderm grade = A	Ref		
B	1.07	(0.72, 1.57)	0.74
C	0.63	(0.25, 1.59)	0.32
Trophectoderm grade = B	Ref		
C	0.55	(0.22, 1.35)	0.19
Age at transfer < 35	Ref		
35–39	0.53	(0.34, 0.82)	< 0.01*
40 +	0.54	(0.31, 0.96)	0.04*
Nulliparity	1.36	(0.95, 1.94)	0.09
Adjusted Odds Ratio (aOR)[a] for live birth		95% CI	*P*-value
Inner cell mass grade = A	Ref		
B	0.70	(0.48, 1.02)	0.06
C	0.32	(0.11, 0.91)	0.03*
Inner cell mass grade = B	Ref		
C	0.48	(0.18, 1.26)	0.14
Trophectoderm grade = A	Ref		
B	1.20	(0.81, 1.77)	0.36
C	0.84	(0.33, 2.16)	0.72
Trophectoderm grade = B	Ref		
C	0.66	(0.27, 1.61)	0.36
Age at transfer < 35	Ref		
35–39	0.54	(0.35, 0.84)	< 0.01*
40 +	0.48	(0.27, 0.85)	0.01*
Nulliparity	1.19	(0.84, 1.69)	0.33

[a]Outcome adjusted for the following confounding variables: maternal age at FET, maternal BMI, nulliparity, race/ethnicity, infertility diagnosis of diminished ovarian reserve (DOR), male factor infertility, inner cell mass grade (A–C), and trophectoderm grade (A–C)

*Statistically significant at the 0.05 level

had a robust sample size of lower quality embryos; their findings were consistent with those of our study, suggesting that blastocyst morphologic grading was useful in predicting ongoing pregnancy rates [5]. Furthermore, they also found ICM grade to be of more significant utility when compared to trophectoderm grade. Nonetheless, the evidence regarding which component of the blastocyst grading is more consequential remains mixed. Although our study is consistent with Irani et al. among other studies [5, 32–34], multiple studies have found TE to be more significant, though these studies did not use PGT screened embryos [3, 4, 35, 36]. This range of conclusions highlights the need for further investigation.

Even after controlling for the morphologic grade of euploid blastocysts, our data suggests that maternal age still significantly impacts pregnancy outcomes. We continue to see a significant decline in the odds of clinical pregnancy and live birth with advancing maternal age even after euploid blastocysts of the best grade (AA) are transferred. It has long been known that aging decreases female fertility due to diminished oocyte yield and increased aneuploidy risk, and the decreased reproductive potential of older women has largely been attributed to this increased rate of aneuploidy [27, 28, 30, 31, 37–41]. However, when looking at studies that have only included euploid blastocysts—thereby removing the postulated main cause of age-related decline—the evidence has been mixed on whether maternal age continues to affect reproductive outcomes. Scott et al.'s 2012 study found a significant decline in the implantation rates of euploid embryos in the ≥ 35 cohort [29], while Harton et al.'s 2013 study of euploid embryos found comparable ongoing pregnancy rates among different age groups (< 35, 35–37, 38–40, and 41–42 years) [31]. Neither study reported embryo morphology, and both included cleavage-stage embryos. More contemporary, Irani et al.'s 2019 study found maternal age only significantly impacts the quantity of euploid embryos achieved rather than implantation potential [42], while Reig et al.'s 2020 study found implantation rates to negatively correlate with age among euploid embryo transfers [43]. Thus, further investigation is warranted, and our data suggest that there are potentially age-related factors beyond aneuploidy and morphologic quality that impact reproductive potential.

Our cohort study is unique in several aspects. It is the first to study the effect euploid blastocyst morphology has on both pregnancy and neonatal outcomes. Additionally, we studied all completed cycles using euploid blastocysts over the course of four years, thereby minimizing selection bias and allowing for a robust sample size. The inclusion of only NC-FET cycles (the most common protocol for FET at our clinic) also minimized potential confounding of pregnancy and neonatal outcomes by different endometrial preparations. Furthermore, we gathered all fertility, pregnancy, and neonatal data from medical records, minimizing recall bias.

Our study has some limitations. Although we had a relatively large sample size compared to prior studies, the number of cycles that transferred grade C blastocysts was small (49 out of 610). The majority of patients in our cohort were Caucasian and Asian, potentially limiting generalizability to other ethnic groups. Thus, further studies are needed to confirm our findings and to understand the mechanisms behind these differences in outcomes.

PGT utilization is on the rise and now used in over 40% of all cycles in the USA, prompting investigation into the utility of morphologic grades during embryo selection [44]. Our findings suggest that even when euploidy is

Table 4 Comparison of pregnancy and neonatal outcomes of grade AA euploid blastocysts among different maternal age groups (< 35, 35–39, 40 + years)

Age groups	< 35	35–39	40 +	ASD[a]
Pregnancy outcomes	***N* = 70**	***N* = 97**	***N* = 30**	
Clinical pregnancy	55 (78.6)	62 (63.9)	15 (50.0)	0.44
Biochemical miscarriage	2 (2.9)	3 (3.1)	2 (6.7)	0.12
Ectopic pregnancy	2 (2.9)	0 (0.0)	1 (3.3)	0.18
Clinical miscarriage	0 (0.0)	3 (4.8)	2 (13.3)	0.39
Live birth	55 (78.6)	59 (60.8)	13 (43.3)	0.51
Neonatal outcomes	***N* = 55**	***N* = 59**	***N* = 13**	
Cesarean delivery	17 (30.9)	23 (39.0)	7 (53.8)	0.38
Gestational age at delivery (days) [median IQR [b]]	275.0 [267.0, 279.0]	275.0 [268.3, 280.0]	276.0 [270.0, 278.0]	0.07
Preterm delivery	5 (9.1)	6 (10.2)	2 (15.4)	0.21
Birthweight (grams) [median IQR [b]]	3141 [2892, 3520]	3197 [2898, 3728]	3401 [3174, 3545]	0.23
Neonate sex	24 (43.6)	19 (32.2)	5 (38.5)	0.23
-Female	30 (54.5)	39 (66.1)	8 (61.5)	
-Male	1 (1.8)	1 (1.7)	0 (0.0)	
-Ambiguous or unknown				

Data are presented as mean ± SD, *n* (%), or median [interquartile range]

[a]Absolute standardized difference: 0.2, small difference; 0.5, medium difference; 0.8 +, large difference

[b]*IQR* interquartile range. Because these variables were skewed, median and IQR were utilized instead of mean and standard deviation

confirmed, embryo grade—particularly the ICM grade—is still associated with pregnancy outcomes, and age independently remains a significant factor in the odds of success. Reassuringly, morphologic grade and maternal age are not associated with neonatal outcomes such as preterm delivery and birthweight, suggesting that lower grade euploid embryos still could be viable options for transfer. The results from our study may not only help guide embryo selection, but also facilitate patient counseling during this critical shared-decision-making process.

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Author contribution W.Y.Z., J.K.J, B.B, and A.A.M. contributed substantially to study design, supervision of study protocol and interpretation of data; W.Y.Z., J.K.J., and B.B. were responsible for data collection; W.Y.Z. and R.M.G. were responsible for data analysis. The first draft of the manuscript was written by W.Y.Z. All co-authors revised the manuscript and approved the final version.

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Declarations

Competing interests The authors declare no competing interests.

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