Overall Blastocyst Quality, Trophectoderm Grade, and Inner Cell Mass Grade Predict Pregnancy Outcome in Euploid Blastocyst Transfer Cycles

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Abstract

Background: Despite recent advances that have improved the pregnancy success rates that can be achieved via *in vitro* fertilization (IVF) therapy, it is not yet clear which blastocyst morphological parameters best predict the outcomes of single blastocyst transfer. In addition, most of the previous studies did not exclude the effect of embryo aneuploidy on blastocysts transfer. Thus, the present study investigated the predictive value of various parameters on the pregnancy outcomes achieved via the transfer of frozen euploid blastocysts.

Methods: The study retrospectively analyzed 914 single euploid blastocyst transfer cycles that were performed at the Peking University Third Hospital Reproductive Medical Center between June 2011 and May 2016. The expansion, trophectoderm (TE), and inner cell mass (ICM) quality of the blastocysts were assessed based on blastocyst parameters, and used to differentiate between "excellent", "good", "average", and "poor"-quality embryos. The relationship between these embryo grades and the achieved pregnancy outcomes was then analyzed via the Chi-square and logistic regression tests.

Results: For embryo grades of excellent, good, average and poor, the clinical pregnancy rates were 65.0%, 59.3%, 50.3% and 33.3%, respectively; and the live-birth rates were 50.0%, 49.7%, 42.3% and 25.0%, respectively. Both the clinical pregnancy rate ($\chi^2 = 21.28$, $P = 0.001$) and live-birth rate ($\chi^2 = 13.50$, $P < 0.001$) increased with the overall blastocyst grade. Both rates were significantly higher after the transfer of a blastocyst that exhibited either an A-grade or B-grade TE, and similarly, an A-grade ICM, than after the transfer of a blastocyst that exhibited a C-grade TE and/or ICM. The degree of blastocyst expansion had no apparent effect on the clinical pregnancy or live-birth rate. All odds ratio were adjusted for patient age, body mass index, length (years) of infertility history, and infertility type.

Conclusions: A higher overall euploid blastocyst quality is shown to correlate most strongly with optimal pregnancy outcomes. The study thus supports the use of the described TE and ICM morphological grades to augment current embryo selection criteria.

Key words: Blastocyst Inner Cell Mass; Embryo Transfer; Fertilization *In vitro* Genetic Testing; Trophoblasts

INTRODUCTION

Good embryo quality is essential to achieve a successful pregnancy using *in vitro* fertilization (IVF) therapy, with either fresh or frozen embryos. Current IVF practices support the transfer of a single fresh blastocyst as the method most likely to achieve a healthy pregnancy and safe delivery, since it facilitates pregnancy rates comparable to those that can be achieved via double fresh-embryo transfer, but does not necessarily incur the risks associated with a multiple pregnancy, for example, premature birth.[1,2] Establishing criteria to select blastocysts that exhibit optimal development potential is essential for successful IVF therapy.[3]

Currently, evaluating blastocyst morphology and preimplantation genetic screening/diagnosis (PGS/PGD) are the two methods used to select healthy embryos for implantation. As recommended by Gardner and Schoolcraft,[4] these methods evaluate the degree of blastocyst expansion, the consistency of the inner cell mass (ICM), and the cohesiveness

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of the trophectoderm (TE). Till date, it is not clear which of these three parameters are the most crucial to predict the outcome of single blastocyst transfer,[3,5,6] since various research groups have reported each to be the most closely correlated with optimal pregnancy outcomes.[3,5-12] Most of these studies did not, however, assess embryonic ploidy before transplantation, although previous research has shown embryos with an abnormal number of chromosomes (aneuploidy) to exhibit an increased risk of undergoing developmental arrest, implantation failure, and spontaneous abortion.[13] This limitation may underlie the lack of consensus as to which morphological parameters best predict IVF outcomes.

PGS can be used to screen embryo biopsies for aneuploidy before transplantation, and thus promote better pregnancy outcomes from IVF therapy.[14,15] While embryo biopsies can be performed at the zygote (via the removal of 1–2 polar bodies), cleavage (via the removal of 1–2 blastomeres from a 6–8-cell embryo), and/or blastocyst (via the removal of 5–10 TE cells) stage of development,[16] PGS is currently predominantly conducted using blastocysts, since they exhibit both a low-mosaic rate and high developmental potential, and facilitate ICM evaluation.[17,18] Specific indications for PGS include infertility at an advanced maternal age (>35 years), previous unsuccessful IVF treatments (i.e. more than two failed IVF cycles), and a history of more than two spontaneous miscarriages.[19] However, many clinicians have begun offering PGS to all patients undergoing IVF therapy, since it can lead to improved pregnancy outcomes.[20]

Till date, only a very limited number of studies have investigated the effect of transferring only euploid embryos after PGS on pregnancy outcomes. By excluding the confounding effects of aneuploidy, these studies have provided some valuable insights into the role of blastocyst morphology in predicting pregnancy outcomes.[19,21] However, they each only studied a relatively small number of patients, enrolled their participant from different institutions, and produced conflicting results. Thus, the present study aimed to further investigate the correlation between euploid embryo (blastocyst) morphology and IVF outcomes using a large patient cohort. In total, the study retrospectively analyzed the outcomes of 914 cycles of single frozen euploid embryo transfer that were performed at the Peking University Third Hospital Reproductive Medical Center.

METHODS

Ethical approval

The study was conducted in accordance with the *Declaration of Helsinki* and was approved by the Ethics Committee of Peking University Third Hospital (No. 2017SZ-065). Since the study was retrospective and analyzed patient data anonymously, informed patient consent was not required.

Patients

The present study retrospectively analyzed 914 single blastocyst frozen embryo transfer (FET) cycles that were conducted after PGS/PGD, at the Peking University Third Hospital Reproductive Medical Center between June 2011 and May 2016. Among the 914 cycles, the PGS/PGD tests were 166 and 748 cycles, respectively. Patients underwent PGS/PGD therapy had specific indications. All of the tests had signed informed consent according to the regulations of our reproductive center.

Ovarian stimulation

Controlled ovarian hyperstimulation was achieved using a gonadotropin-releasing hormone agonist or antagonist, as previously described.[22] Ovarian follicle development was monitored by analyzing serum estradiol levels and transvaginal ultrasonographic measurements. When at least one follicle reached a mean diameter of 18 mm, and the serum estradiol concentration exceeded 500 pg/ml, 10,000 units of urinary human chorionic gonadotropin (Serono, Aubonne, Switzerland) was administered, and ultrasonography-guided oocyte retrieval subsequently performed. Luteal support (60 mg of progesterone) was initiated on the day after oocyte retrieval.[22]

Embryo culture and blastocyst grading

On the day of ovum retrieval, intracytoplasmic sperm injection fertilization was performed. On day 5 of *in vitro* cultivation, resulting blastocysts were evaluated morphologically.[4] Initially, they were assigned a numeric score (between 1 and 6) based on their degree of expansion, and hatching status: (1) early blastocyst (i.e., where the blastocoel formed less than half of the volume of the embryo); (2) blastocyst, (where the blastocoel formed more than half of the volume of the embryo); (3) full blastocyst, (where the blastocoel completely filled the embryo); (4) expanded blastocyst, (where the blastocoel volume was larger than that of the early embryo, and the zona had begun to thin); (5) hatching blastocyst, (where the TE had begun to herniate though the zona); and (6) hatched blastocyst, (where the blastocyst had completely escaped from the zona). For blastocysts graded 3–6 (i.e., full blastocysts onward), the development of the ICM and TE were then assessed. The ICM grade was determined as follows: A, tightly packed, with many cells; B, loosely grouped, with several cells; and C, very few cells. The TE grades consisted of A, many cells, forming a cohesive epithelium; B, few cells, forming a loose epithelium; and C, very few large cells.[4]

To facilitate comparisons between the results of the present and previous studies by Capalbo *et al.*,[19] and Irani *et al.*,[21] the blastocysts were assigned a three-character score according to their exhibited degree of blastocyst expansion, and their ICM and TE grades, respectively, immediately prior to being biopsied. These scores differentiated between "excellent" (≥3AA), "good" (3–6AB, 3–6BA, 1–2AA), "average" (3–6BB, 3–6AC, 3–6CA, 1–2AB, 1–2BA), and "poor" (1–6BC, 1–6CB, 1–6CC, 1–2BB) grade blastocysts.

Blastocyst biopsy and diagnosis

Euploid embryos were identified via 24-chromosome PGS using either an array-based comparative genomic hybridization or an array-based single nucleotide-polymorphism method, as previously described.[23] Selected embryos were vitrified, warmed, and transplanted as previously described.[24]

Clinical outcomes

The outcomes of the analyzed cycles were assessed regarding the achieved clinical pregnancy (i.e., where a fetal heartbeat was detected 5 weeks after embryo transfer via ultrasound), and live-birth rates.

Statistical analysis

All data analyses were performed using SPSS software version 19.0 (IBM, New York, USA). All continuous data were tested for normality. Those found to be normally distributed were presented as the mean ± standard deviation (SD), and were subjected to a Student's t-test. Those data that were not normally distributed were presented as the mean (interquartile range), and were analyzed using a Mann-Whitney U-test. Categorical variables were expressed as a frequency (percentage), and analyzed using a Chi-square test. The relationships between pregnancy outcomes and morphological parameters were analyzed via a multivariate logistic regression analysis. A value of $P < 0.05$ was considered to indicate statistical significance.

RESULTS

The indications for PGS are listed in Table 1. The majority of PGS cycles had more than twice previous unsuccessful IVF treatments, and more than one indication might exist in one cycle. The blastocysts resulting from the 914 analyzed FET cycles were each classed as being either excellent, good, average, or poor quality. The number of transferred embryos in each group and the overall grade of each transferred embryo are listed in Table 2. No significant difference in patient age, body mass index (BMI), or length (years) of infertility history was observed between the four categories. Similarly, patient infertility types were not significantly different between any two groups of blastocysts, except the good and poor categories ($\chi^2 = 5.49$, $P = 0.020$; Table 3).

The clinical pregnancy rate achieved via transplantation of the excellent-quality embryos was significantly higher than that achieved using either the average-quality (65.0% vs. 50.3%; $P = 0.012$; adjusted odds ratio [OR], 1.9; 95% confidence interval [CI], 1.1–3.1) or poor-quality embryos (65.0% vs. 33.3%; $P < 0.001$; adjusted OR, 3.8; 95% CI, 2.1–7.2), and comparable to that achieved using the good-quality embryos (65.0% vs. 59.3%; $P = 0.300$; adjusted OR, 1.3; 95% CI, 0.8–2.3). Similarly, the clinical pregnancy rate achieved via transplantation of the good-quality embryos was significantly higher than that achieved using the average-quality (59.3% vs. 50.3%; $P = 0.039$; adjusted OR, 1.4; 95% CI, 1.0–2.0) or poor-quality embryos (59.3% vs. 33.3%; $P < 0.001$; adjusted OR, 2.9; 95% CI, 1.8–4.9). Finally, the clinical pregnancy rate achieved using the average-quality embryos was significantly higher than that achieved using the poor-quality embryos (50.3% vs. 33.3%; $P = 0.002$; adjusted OR, 2.0; 95% CI, 1.3–3.2; Table 4). Thus, transplanting a blastocyst of higher quality was shown to promote a higher clinical pregnancy rate ($\chi^2 = 21.28$, $P = 0.001$; Figure 1).

The live birth resulting from the transfer of the excellent-quality embryos was significantly higher than that achieved using the poor-quality embryos (50.0% vs. 25.0%; $P = 0.001$; adjusted OR, 3.1; 95% CI, 1.6–5.8) and comparable to achieved using the good- (50.0% vs. 49.7%; $P = 0.870$; adjusted OR, 1.1; 95% CI, 0.6–1.8) and average-quality embryos (50.0% vs. 42.3%; $P = 0.160$; adjusted OR, 1.4; 95% CI, 0.9–2.3). Similarly, the transfer of good-quality embryos resulted in a significantly higher live-birth rate than was achieved via the transfer of the poor-quality embryos (49.7% vs. 25.0%; $P < 0.001$; adjusted OR, 2.9; 95% CI, 1.7–5.0), and comparable to that achieved via the transfer of the average-quality embryos (49.7% vs. 42.3%; $P = 0.078$; adjusted OR, 1.3; 95% CI, 0.9–1.9). Finally, the live-birth rate achieved via

Table 1: PGS indications and the number of cycles in each indication

Indications for PGS	Number of cycles
Embryonic chromosomal abnormalities	2
Advanced maternal age (>35 years)	2
Male chromosomal abnormalities	6
Female chromosomal abnormalities	14
Recurrent pregnancy loss (≥2)	49
Previous unsuccessful IVF treatments	99

IVF: *In vitro* fertilization; PGS: Preimplantation genetic screening.

Table 2: The number of transferred embryos in specific morphological category

Excellent (n = 80)		Good (n = 189)		Average (n = 549)		Poor (n = 96)	
Grade	n	Grade	n	Grade	n	Grade	n
3AA	1	3AB	2	3BB	3	4BC	11
4AA	2	4AB	4	4BB	30	5BC	63
5AA	70	5AB	102	5BB	408	6BC	6
6AA	7	6AB	12	6BB	104	4CB	2
		4BA	2	5AC	4	5CB	13
		5BA	50	3AC	0	6CB	1
		6BA	17	4AC	0	1BC	0
		3BA	0	6AC	0	2BC	0
		1AA	0	3CA	0	3BC	0
		2AA	0	4CA	0	1CB	0
				5CA	0	2CB	0
				6CA	0	3CB	0
				1AB	0	1CC	0
				2AB	0	2CC	0
				1BA	0	3CC	0
				2BA	0	4CC	0
						5CC	0
						6CC	0
						1BB	0
						2BB	0

The number of each category is presented. The morphological characteristics are depicted in the order of the degree of blastocyst expansion, ICM grade, and TE grade. All of them were evaluated based on Gardner and Schoolcraft's criteria, for the degree of blastocyst expansion ranging from 1 to 6, the quality of ICM and TE ranging from A to C. No blastocysts Graded 1–2 was transferred. TE: Trophectoderm; ICM: Inner cell mass.

Table 3: Patient characteristics associated with embryos from different grades

Patient characteristic	Excellent (n = 80)	Good (n = 189)	Average (n = 549)	Poor (n = 96)	P1	P2	P3	P4	P5	P6
Age (years)	31.29 ± 4.29	31.13 ± 4.37	31.00 (28.00, 34.00)	31.68 ± 5.11	0.78	0.75	0.59	0.77	0.59	0.36
BMI (kg/m²)	22.22 ± 3.05	21.48 (19.99, 23.80)	21.48 (19.87, 23.81)	22.32 ± 3.14	0.82	0.58	0.84	0.71	0.70	0.44
Years of infertility	3.00 (1.00, 4.75)	3.00 (1.00, 4.50)	3.00 (2.00, 5.00)	3.00 (2.00, 5.00)	0.76	0.25	0.19	0.25	0.22	0.63
Type of infertility, n										
Primary infertility	35	71	230	50	0.34	0.75	0.27	0.30	0.02	0.06
Secondary infertility	45	118	319	46						

Data were present as mean ± SD or mean (interquartile range). *P*1: *P* value between excellent and good; *P*2: *P* value between excellent and average; *P*3: *P* value between excellent and poor; *P*4: *P* value between good and average; *P*5: *P* value between good and poor. For the age between different groups, the *t* value of *P*1, *P*3, and *P*5 are 0.28, −0.54, and−0.54, respectively; the Mann-Whitney *U* value of *P*2, *P*4, and *P*6 are 2.15, 5.12, and 2.48, respectively. For BMI between different groups, the *t* value of *P*3 is−0.21; the Mann-Whitney *U* value of *P*1, *P*2, *P*4, *P*5, and *P*6 are 7.42, 2.11, 5.09, 8.82, and 2.51, respectively. For the years of infertility between different groups, the Mann-Whitney *U* value of *P*1, *P*2, *P*3, *P*4, *P*5, and *P*6 are 7382.50, 20,250.00, 3405.00, 49,021.50, 8281.00, and 25,540.00, respectively. For the type of infertility between different groups, the χ^2 value of *P*1, *P*2, *P*3, *P*4, *P*5, and *P*6 are 0.90, 0.10, 1.21, 1.09, 5.49, and 3.45, respectively. *P*6: *P* value between average and poor. BMI: Body mass index; SD: Standard deviation.

Table 4: Relationship between outcome and blastocyst morphological parameters among different morphological groups

Morphological parameter	Grade	Number of cycles	Clinical pregnancy rate (%)	P	OR	95% CI	Live birth rate (%)	P	OR	95% CI
Blastocyst	Excellent*	80	65.0	<0.001	3.8	2.1–7.2	50.0	0.001	3.1	1.6–5.8
	Good*	189	59.3	<0.001	2.9	1.8–4.9	49.7	<0.001	2.9	1.7–5.0
	Average*	549	50.3	0.002	2.0	1.3–3.2	42.3	0.001	2.2	1.3–3.6
	Poor	96	33.3		vs poor		25.0		vs poor	
ICM	A†	204	61.3	0.015	4.3	1.3–14.2	50.0	0.029	4.3	1.2–15.6
	B	694	49.3	0.051	3.1	0.9–10.0	41.1	0.064	3.3	0.9–12.0
	C	16	25.0		vs C		12.5		vs C	
TE	A‡	149	62.4	0.001	2.6	1.4–4.6	49.7	0.006	2.4	1.3–4.3
	B‡	681	51.1	0.013	1.8	1.1–2.9	42.9	0.011	2.0	1.2–3.2
	C	84	35.7		vs C		27.4		vs C	
Expansion degree	6	147	57.1	0.311	1.3	0.8–2.3	46.3	0.132	1.6	0.9–2.8
	5	710	51.0	0.091	1.7	0.9–3.2	42.4	0.055	1.9	0.9–3.6
	4 + 3	57	43.9		vs 4+3		31.6		vs 4+3	

*The clinical pregnancy rate and live-birth rate of the excellent, good and average group are significantly different from the poor group. †The clinical pregnancy rate and live-birth rate of embryos in ICM Grade A is significantly different from Grade C; ‡The clinical pregnancy rate and live-birth rate of embryos in TE Grade A and B are significantly different from Grade C; Clinical pregnancy rate: The *P* value between excellent- and good-quality group is 0.300; adjusted *OR*: 1.3; 95% *CI*: 0.8–2.3; *OR* average-quality group 0.012; adjusted *OR*: 1.9; 95% *CI*: 1.1–3.1; *OR* between good- and average-quality group 0.039; adjusted *OR*: 1.4; 95% *CI*: 1.0–2.0. The *P* value between ICM Graded A and B is 0.064; adjusted *OR*: 1.4; 95% *CI*: 0.9–1.9. The *P* value between TE Graded A and B is 0.079; adjusted *OR*: 1.4; 95% *CI*: 0.9–2.1. The *P* value of expansion degree between 5 and 6 is 0.270; adjusted *OR*: 1.23; 95% *CI*: 0.9–1.8. Live-birth rate: The *P* value between excellent- and good-quality group is 0.870; adjusted *OR*: 1.1; 95% *CI*: 0.6–1.8; *OR* average-quality group 0.160; adjusted *OR*: 1.4; 95% *CI*: 0.9–2.3; *OR* between good- and average-quality group 0.078; adjusted *OR*: 1.3; 95% *CI*: 0.9–1.9. The *P* value between ICM Graded A and B is 0.154; adjusted *OR*: 1.3; 95% *CI*: 0.9–1.8. The *P* value between TE Graded A and B is 0.325; adjusted *OR*: 1.2; 95% *CI*: 0.8–1.8. The *P* value of expansion degree between 5 and 6 is 0.496; adjusted *OR*: 1.14; 95% *CI*: 0.8–1.6. All of the blastocysts were evaluated based on Gardner and Schoolcraft's criteria, for the degree of blastocyst expansion ranging from 1 to 6, the quality of ICM and TE ranging from A to C. *OR*: Odds ratio; *CI*: Confidence interval; TE: Trophectoderm; ICM: Inner cell mass; vs: Versus.

the transfer of the average-quality embryos was significantly higher than that achieved via the transfer of the poor-quality embryos (42.3% vs. 25.0%; *P* = 0.001; adjusted *OR*, 2.2; 95% *CI*, 1.3–3.6; Table 4). Thus, overall the live-birth rate increased significantly with blastocyst quality (χ^2 = 13.50, *P* < 0.001; Figure 2). All *OR*s were adjusted for patient age, BMI, length (years) of infertility history, and infertility type.

It was also observed that blastocysts with an A-grade ICM yielded a clinical pregnancy and live-birth rate that was significantly higher than that achieved using blastocysts with a C-grade ICM (61.3% vs. 25.0%, and 50.0% vs. 12.5%; *P* = 0.015, and *P* = 0.029; adjusted *OR*, 4.3 and 4.3; 95% *CI*, 1.3–14.2, and 1.2–15.6, respectively) and comparable to that achieved using blastocysts with a B-grade ICM (61.3% vs. 49.3%, and 50.0% vs. 41.1%; *P* = 0.064, and *P* = 0.154; adjusted *OR*, 1.4, and 1.3; 95% *CI*, 0.9–1.9, and 0.9–1.8, respectively). There was no significant difference in the clinical pregnancy or live-birth rates achieved using blastocysts with a B- compared to a C-grade ICM [49.3% vs. 25.0%, and 41.1% vs. 12.5%; *P* = 0.051, and *P* = 0.064; adjusted *OR*, 3.1, and 3.3; 95% *CI*, 0.9–10.0, and 0.9–12.0, respectively, Table 4]. All *OR*s were adjusted for TE grade, patient age, BMI, length (years) of infertility history, and infertility type.

Similarly, blastocysts that exhibited an A-grade TE yielded a significantly higher clinical pregnancy and live-birth rate than those that exhibited a C-grade TE (62.4% vs. 35.7%, and 49.7% vs. 27.4%; *P* = 0.001, and *P* = 0.006; adjusted
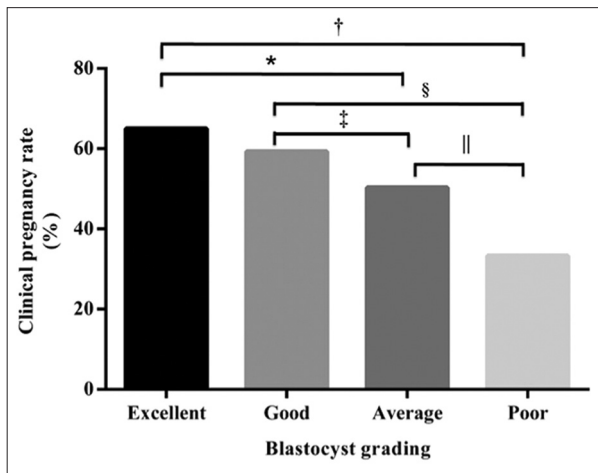
Figure 1: Clinical pregnancy rates from FET using euploid blastocysts of different grades. The clinical pregnancy rate has an increasing trend with better blastocyst grades based on Chi-square test ($\chi^2 = 21.28$, $P = 0.001$). $*\chi^2 = 6.07$, $P = 0.014$; $^\dagger\chi^2 = 17.54$, $P < 0.001$; $^\ddagger\chi^2 = 4.55$, $P = 0.033$; $^\S\chi^2 = 17.12$, $P < 0.001$. $^\|\chi^2 = 9.40$, $P = 0.002$. FET: Frozen embryo transfer. Excellent ($n = 80$); Good ($n = 189$); Average ($n = 549$); Poor ($n = 96$).

Figure 2: Live-birth rates from FET using euploid blastocysts of different grades. The live-birth rate has an increasing trend with better blastocyst grades based on Chi-square test ($\chi^2 = 13.50$, $P < 0.001$). $*\chi^2 = 11.79$, $P = 0.001$; $^\dagger\chi^2 = 16.06$, $P < 0.001$; $^\ddagger\chi^2 = 10.17$, $P = 0.001$. FET: Frozen embryo transfer. Excellent ($n = 80$); Good ($n = 189$); Average ($n = 549$); Poor ($n = 96$).

OR, 2.6, and 2.4; 95% CI, 1.4–4.6, and 1.3–4.3, respectively), and comparable rates to those achieved using blastocysts that exhibited a B-grade TE (62.4% vs. 51.1% and 49.7% vs. 42.9%; $P = 0.079$, and $P = 0.325$; adjusted OR, 1.4, and 1.2; 95% CI, 0.9–2.1, and 0.8–1.8, respectively). The clinical pregnancy and live-birth rates achieved using blastocysts with a B-grade TE were significantly higher than those achieved using blastocysts with a C-grade TE (51.1% vs. 35.7%, and 42.9% vs. 27.4%; $P = 0.013$, and $P = 0.011$; adjusted OR, 1.8, and 2.0; 95% CI, 1.1–2.9, and 1.2–3.2, respectively, Table 4); All ORs were adjusted for the ICM grade, patient age, BMI, length (years) of infertility history, and infertility type.

Finally, the degree of blastocyst expansion was found to have no effect on either the clinical pregnancy or live-birth rate [Table 4].

DISCUSSION

The use of embryos with a high developmental potential is essential for successful IVF cycles.[25] Although embryos are highly dynamic, and able to repair and compensate for structural damage or cell-number abnormalities they should maintain correct TE, epiblast, and primitive endoderm formation to enable implantation and term delivery.[25] Schoolcraft *et al.*[4] previously recommended assessing blastocyst expansion, and ICM and TE grades as the best way to select embryos with a normal morphological structure, cell number, and cell distribution as viable candidates for transfer. The results of the present study are consistent with this method, since the revealed an association between embryo grading and pregnancy outcomes in single blastocyst FET cycles, and also showed the ICM and TE grade to be important for predicting the achieved clinical-pregnancy and live-birth rates.
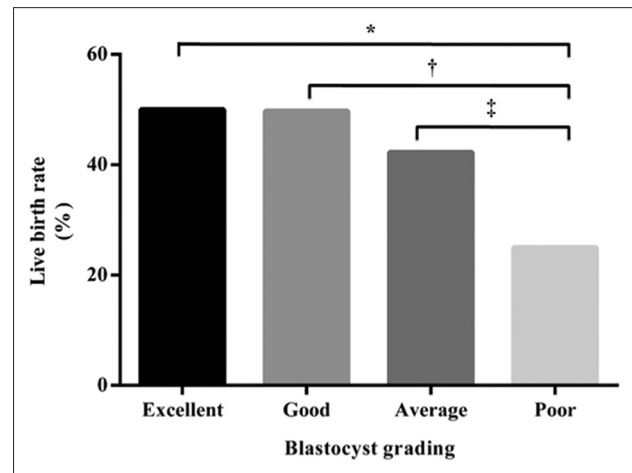
Till date, there previous studies have produced conflicting results regarding which blastocyst morphology parameter is most closely related to pregnancy outcomes. Various research groups have suggested that the TE grade best predicts IVF success for fresh or frozen-thawed single-blastocysts,[7-9] while others report the ICM grade to be a strong predictor of successful embryo implantation and live birth,[3,6,10] and some recommend assessing the degree of blastocyst expansion to predict pregnancy outcomes.[5,11,12] Notably, many of these previous studies did not confirm that the blastocysts being transferred were euploid, despite the fact that aneuploidy has been shown to be the most common cause of pregnancy failure, via its impacts on ICM and TE morphology, and overall blastocyst development.[13] Given that, a considerable fraction of aneuploid embryos can achieve a high morphology score, and that the shape of some euploid embryos can appear poor, it is clear from previous research that optimal morphology alone is not sufficient to exclude aneuploidy.[13]

During PGS, biopsied TE cells are comprehensively screened to confirm the embryo's chromosome number, and thus accurately analyze the embryonic genetic status.[26] Only 5–10 TE cells are biopsied, and the low rate of chromosomal mosaicism that occurs in blastocysts means that the TE and ICM cell karyotype are consistent; thus, very accurate results can be generated while only incurring minimal impacts on embryonic viability.[18,27-29] For women receiving IVF therapy, especially those with specific genetic indications, PGS can be used to avoid the transfer of morphologically normal aneuploid embryos, and thus, promote a higher clinical and ongoing pregnancy rate, and a reduced rate of spontaneous abortion.[14,15] Nevertheless, while PGS can be used to reduce the IVF failure rate by excluding aneuploid embryos, further study is needed to assess the predictive role of blastocyst morphology parameters on pregnancy outcomes for euploid embryos.

Capalbo et al.,[19] previously analyzed the outcome of 215 single euploid blastocyst transfer cycles, and reported the implantation potential of embryos with different qualities to be similar to the extent that none of the three blastocyst morphology parameters described by the present study were predictive of embryo euploidy. Thus, they suggested that all euploid embryos be considered to be equally viable regardless of their morphology. In contrast, Irani et al.,[21] studied 477 PGS-confirmed single euploid blastocyst FET cycles using the same embryo grading method as in the current study, and revealed that the overall blastocyst quality and ICM grades were the most effective predictors of achieving an ongoing pregnancy. They thus suggested that these two parameters be used to aid the selection of high-quality euploid blastocysts for IVF transfer. The results of the present study were consistent with their recommendations, since the achieved pregnancy outcomes were shown to be positively correlated with the overall blastocyst quality, such that higher quality blastocysts, and/or those that exhibited a higher TE and/or ICM grade, yielded significantly higher clinical-pregnancy and live-birth rates. It is possible that the discrepancies between the results presented by Capalbo et al.,[19] and both this and the previous study by Irani et al.,[21] may arise from the low number (13) of poor-quality embryos that were analyzed in the study by Capalbo et al.,[19] and/or the fact that that study analyzed IVF therapies performed at two separate healthcare centers, thus increasing the risk of confounding heterogeneity (e.g. in the utilized patient clinical management and/or blastocyst classification methods). In contrast, the present study used the same embryo grouping method as Irani et al.,[21] and produced the same results regarding the association of overall embryo quality with improved pregnancy outcomes. Notably, the results of the present study also suggested that the TE grade is closely correlated with pregnancy outcome. The reason for this discrepancy is not clear; however, the two studies did use different sample sizes, and where the present study excluded the potential effects of embryo-embryo interactions by only analyzing single-embryo IVF cases, the study by Irani et al.,[21] analyzed cycles in which either one, or two embryos of the same overall quality, were transferred.

The mechanism underlying the demonstrated correlations between embryonic TE and ICM grades and pregnancy outcomes is not clear; however, the critical role of the TE in mediating correct embryo implantation is well established. High-grade TE cells are likely to secrete human chorionic gonadotropin more readily, and thus more effectively/quickly initiate maternal-fetal communications.[8] The transcriptional expression profile of biopsied TE cells could predict implantation rate and pregnancy outcome.[8] Furthermore, the ICM has been shown to promote TE cell proliferation, possibly by secreting Fgf4.[30] For example, murine TE cells that are located closest to the ICM divide faster than distantly located TE cells.[31] Interestingly, TE cells isolated from intact human blastocysts have been shown to be capable of developing into blastocysts with a NANOG-positive ICM.[32] Together, these data suggest that various interactions between the TE and ICM are critical to facilitate normal embryogenesis, and thus, both structures should be of high quality to ensure optimal development.

While some previous studies have suggested that the degree of blastocyst expansion may be predictive of the overall IVF reproductive outcome, others instead report that it only reflects embryos implantation potential,[5,33] and some suggest that it is not correlated with either the ongoing pregnancy or spontaneous abortion rate.[21] Again, many of these studies failed to exclude aneuploidy during their analyses. The results of the present study suggest that blastocyst expansion does not predict IVF outcome. The degree of achieved blastocyst expansion is dependent on TE functionality, including the success of adjuvant blastomere compaction, and tight-junction formation, and the capacity of various cellular mechanisms to pump water and other ions in and out of the cell.[34] It is possible that the degree of blastocyst expansion does not predict the pregnancy outcome because at the analyzed stage of development, this parameter does not yet affect embryonic development. It may be that euploid embryos with a high-quality ICM and TE may continue to expand after TE biopsy.[21]

The present study excluded aneuploidy via PGS/PGD, and thus only considered the predictive value of blastocyst morphology parameters on pregnancy outcomes; however, it was conducted retrospectively, and therefore, additional prospective studies with larger cohorts are needed to confirm the present results. While the described morphological parameters are descriptive and convenient, embryos can often only be observed at various time points due to logistical issues, despite the fact that embryonic morphology changes within hours. This can lead to a high rate of variability during morphology evaluations, and emphasizes the need to develop more reliable blastocyst quality assessment methods.[6,26,35] Furthermore, this study did not compare the development potential between aneuploid and euploid blastocysts, which would be more interesting and meaningful.

In summary, the overall blastocyst quality, and the TE and ICM grades were shown by the present study to be closely and positively associated with patient pregnancy outcomes during single euploid blastocyst transfer cycles. Thus, TE and ICM morphological grades would likely be effective supplementary parameters to consider during embryo selection.

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Conflicts of interest

There are no conflicts of interest.

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囊胚总体质量及TE与ICM评级对整倍体胚胎移植妊娠结局的预测作用

摘要

背景： 近年来随着助孕技术的改善，尽管体外受精后的妊娠率有所提高，但关于囊胚移植过程中哪一个形态学参数能够更好地预测妊娠结局尚存争议，且既往较多研究未排除非整倍体囊胚移植的影响。因此，本文对囊胚形态学参数在冻融周期整倍体囊胚移植妊娠结局中的预测作用进行研究。

方法： 回顾性分析2011年6月至2013年5月在北京大学第三医院生殖医学中心完成的914例整倍体单倍体囊胚移植周期。根据囊胚参数来评估囊胚扩张程度，滋养外胚层（trophectoderm, TE）和内细胞团（inner cell mass, ICM）质量，并分为"优秀"，"良好"，"一般"和"较差"质量的胚胎。通过卡方检验和Logistic回归检测胚胎分级与妊娠结局之间的关系。

结果： 临床妊娠率（$\chi^2 = 21.28, P = 0.001$）和活产率（$\chi^2 = 13.50, P < 0.001$）随囊胚总体评级的升高呈上升趋势。移植TE评级为A级或B级的囊胚，或移植ICM评级为A级的囊胚，其临床妊娠率和活产率均显著高于TE或ICM评级为C级的囊胚。囊胚扩张程度对临床妊娠率或活产率无显著影响。所有优势比（ORs）根据患者年龄，体重指数，不孕年限和不孕类型等进行调整。

结论： 整倍体囊胚的总体评级较高与最佳妊娠结局相关。使用TE和ICM形态评级可来改善当前的胚胎选择标准。