Human Reproduction Open, pp. 1–8, 2021
https://doi.org/10.1093/hropen/hoab036

human reproduction open

ORIGINAL ARTICLE

Blastocyst quality and perinatal outcomes in women undergoing single blastocyst transfer in frozen cycles

Kai-Lun Hu[1,2,†], Xiaoying Zheng[1,†], Sarah Hunt[3], Xiaohong Li[4], Rong Li [1]*, and Ben W. Mol[3]

[1]Department of Obstetrics and Gynaecology, Center for Reproductive Medicine, Peking University Third Hospital, Beijing, People's Republic of China [2]Key Laboratory of Reproductive Genetics (Ministry of Education), Department of Reproductive Endocrinology, Women's Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, People's Republic of China [3]Department of Obstetrics and Gynaecology, Monash University, Clayton, VIC, Australia [4]Department of Obstetrics and Gynecology, Centre for Reproductive Medicine, West China Second University Hospital of Sichuan University, Chengdu, People's Republic of China

*Correspondence address. Department of Obstetrics and Gynecology, Peking University Third Hospital, No. 49 HuaYuan North Road, HaiDian District, Beijing 100191, People's Republic of China. Tel/Fax: +86-10-82265080; E-mail: roseli001@sina.com
https://orcid.org/0000-0003-0305-5579

Submitted on May 19, 2021; resubmitted on August 10, 2021; editorial decision on October 14, 2021

STUDY QUESTION: Is the morphological grading system for blastocysts associated with perinatal outcomes in women undergoing frozen–thawed single blastocyst transfer (SBT)?

SUMMARY ANSWER: Preferential transfer of a blastocyst based on their inner cell mass (ICM) and trophectoderm (TE) grading appears to be supported by observed differences in perinatal outcomes.

WHAT IS KNOWN ALREADY: The transfer of a morphologically good quality blastocyst is associated with a higher chance of implantation and pregnancy as compared to transfer of a poor quality blastocyst. However, to date, the association of the morphological parameters of the blastocyst with perinatal outcomes after blastocyst transfer remains unknown.

STUDY DESIGN, SIZE, DURATION: This retrospective cohort study started with 27 336 frozen–thawed SBT cycles from January 2013 to December 2019.

PARTICIPANTS/MATERIALS, SETTING, METHODS: There were 7469 women with singleton deliveries in Peking University Third Hospital eligible for analysis. Multivariate logistic regression was used to test the risk of factors with the expression of crude odds ratios (ORs) and adjusted OR with 95% CIs.

MAIN RESULTS AND THE ROLE OF CHANCE: Transfer of a blastocyst with a low overall grading was associated with a higher chance of female baby (48% vs 42%, adjusted OR = 1.26 (1.13, 1.39)) and a higher rate of caesarian section (C-section; 71% vs 68%, adjusted OR = 1.15 (1.02, 1.29)). Compared with Grade A ICM blastocyst transfer, Grade B ICM and Grade C ICM blastocyst transfers were associated with a lower chance of a female baby (adjusted OR = 0.83 (0.73, 0.95), 0.63 (0.50, 0.79), respectively) and a higher risk of large for gestational age (LGA; adjusted OR = 1.23 (1.05, 1.45), 1.47 (1.12, 1.92), respectively); Grade C ICM blastocyst transfer was also associated with an increased risk of macrosomia (adjusted OR = 1.66 (1.20, 2.30)). Compared with Grade A TE blastocyst transfer, there was an increased risk of small for gestational age with Grade C TE blastocyst transfer (adjusted OR = 1.74 (1.05, 2.88)). Both Grade B TE and Grade C TE blastocyst transfer had a higher chance of female baby (adjusted OR = 1.30 (1.11, 1.53), 1.88 (1.57, 2.26), respectively) and a lower risk of gestational diabetes mellitus (adjusted OR = 0.74 (0.59, 0.94), 0.67 (0.50, 0.88), respectively) than Grade A TE blastocyst transfer.

LIMITATIONS, REASONS FOR CAUTION: The main limitations of this study were its retrospective nature and the relative subjectivity of blastocyst scoring. The follow-up was conducted through a phone call and some patients may not have reported their obstetrical and neonatal outcomes, leading to a relatively lower rate of several obstetrical outcomes. Due to the missing information in our dataset, we were not able to separate out iatrogenic preterm birth nor adjust for obstetric complications in previous pregnancies as a confounder in the mutivariate analysis. Because the days of blastocyst culture in total were unclear in our dataset, analysis of the association between the time to reach blastocyst expansion and perinatal outcomes was not performed.

[†]The first two authors contributed equally to this study.

WIDER IMPLICATIONS OF THE FINDINGS: Transfer of a blastocyst with a low overall grading is associated with a higher rate of C-section and a higher chance of a female baby. The association between ICM grading and LGA would suggest that Grade A ICM blastocysts should be transferred preferentially to Grade B/C ICM blastocysts. Our results support the use of current morphological systems for embryo prioritization.

STUDY FUNDING/COMPETING INTEREST(S): This study was supported by the National Key Research and Development Program of China (2018YFC1004100 to R.L.), the National Science Fund for Distinguished Young Scholars (81925013 to R.L.) and a Zhejiang University Scholarship for Outstanding Doctoral Candidates (to K.-L.H.). All authors have read the journal's authorship agreement and policy on disclosure of potential conflicts of interest, and have no conflicts of interest to declare.

TRIAL REGISTRATION NUMBER: N/A.

Key word: single blastocyst transfer / embryo quality / inner cell mass / trophectoderm / perinatal outcome

WHAT DOES THIS MEAN FOR PATIENTS?

Because the transfer of a blastocyst with an advanced degree of inner cell mass and trophectoderm is associated with high live birth rates and low risks of adverse perinatal outcomes, transfer of a good quality blastocyst is preferential.

Introduction

Culturing embryos to the blastocyst stage has been used as an embryo selection tool and blastocyst transfer has become increasingly popular in the last decade because of its potential to increase the success rate of pregnancy in a complete IVF cycle (Ginström Ernstad *et al.*, 2019; Spangmose *et al.*, 2020). However, emerging evidence has suggested that blastocyst transfer in frozen cycles can be associated with increased risks of preterm delivery (PTD), perinatal mortality, placenta previa, placental abruption and large for gestational age (LGA), compared with cleavage-stage embryo transfer in frozen cycles (Ginström Ernstad *et al.*, 2016; Martins *et al.*, 2016; De Vos *et al.*, 2018). Therefore, selecting a blastocyst to transfer with a lower rate of adverse perinatal outcomes is preferable, to reduce perinatal morbidity.

Considerable evidence generated over the last two decades has demonstrated that morphological grading of embryos correlates with cycle outcome. The transfer of morphologically good quality blastocysts is associated with higher implantation and pregnancy rates as compared to poor quality blastocyst transfer (Balaban *et al.*, 2000; Gardner *et al.*, 2000). In addition, the morphological sub-parameters describing the blastocyst expansion grade, inner cell mass (ICM) and trophectoderm (TE) demonstrate a correlation with pregnancy rate (Ahlström *et al.*, 2013; Boynukalin *et al.*, 2020). However, to date, few studies have addressed the association of the morphological parameters of the embryo with the perinatal outcomes after blastocyst transfer, and the studies which have addressed this have been limited by small sample sizes or selective reporting of outcomes (Oron *et al.*, 2014; Bakkensen *et al.*, 2019; Chen *et al.*, 2020; Li *et al.*, 2020; Zhang *et al.*, 2020). In addition, some of these studies have included both cleavage embryo transfer and blastocyst transfer, with different evaluation scores used in embryo quality. To further investigate the association of blastocyst quality and perinatal outcomes, we conducted a retrospective cohort study with a large sample size in a single centre to analyse a range of perinatal outcomes in women undergoing single frozen blastocyst transfer cycles.

Materials and methods

Ethical approval

This study was approved by the Ethics Committee of Peking University Third Hospital.

Participants

This study was a retrospective cohort including a total of 27 336 single frozen–thawed blastocyst transfer cycles in Peking University Third Hospital from January 2013 to December 2019. Repeated cycles in the same patient, cycles involving pre-implantation genetic testing, failed cycles, embryos that had been vitrified at the cleavage stage, twin deliveries and neonatal death as well as those lost to follow-up were excluded. Cycles, where blastocyst grading, was not recorded were also excluded.

IVF procedures

The ovarian stimulation protocol and IVF-ET procedures were as previously described (Hu *et al.*, 2020). Fertilization was performed by conventional IVF or by ICSI. Embryos were vitrified on Days 5–6 of embryo culture. The procedure of embryo vitrification and thaw in our centre was described in previous studies (Zheng *et al.*, 2017; Hu *et al.*, 2020). Grading of blastocyst morphology was performed before vitrification on Day 5 or Day 6 of embryo culture according to the Gardner and Schoolcraft's system which has been used in our centre since 2008 (Gardner *et al.*, 2000, 2004). The detailed scoring system is included in Supplementary Table SI. Blastocysts were graded according to three separate quality scores: the development stage status of the blastocyst (1–6), the grade of ICM (A, B, C), and the grade of TE (A, B, C). Blastocysts with stage status higher than 2, ICM grade higher than C and TE grade higher than C (≥3BB) were considered as good quality and blastocysts with grading lower than 3BB were considered as poor quality (Gardner *et al.*, 2004; Oron *et al.*, 2014). In most cases, lower grade blastocysts were only used when no higher grades ones were available

Table I Characteristics of women with good or poor quality blastocyst transfer.

	Poor quality blastocyst (n = 1937)	Good quality blastocyst (n = 5532)	P-value[a]
Female age (years)	32.2 (4.1)	32.0 (4.0)	0.06
Male age (years)	33.6 (5.1)	33.5 (5.0)	0.26
Duration of infertility (years)	4 (2–6)	4 (2–6)	0.09
Female BMI (kg/m^2)	22.4 (3.4)	22.6 (3.7)	0.16
Male BMI (kg/m^2)	25.5 (3.8)	25.4 (4.0)	0.75
Primary infertility	1268 (65%)	3509 (63%)	0.11
Parity	97 (6%)	280 (5%)	0.68
Reason for IVF			
Ovulatory dysfunction	304 (17%)	864 (16%)	0.94
Adenoendometriosis	120 (6%)	324 (6%)	0.59
Tube factor	768 (40%)	2273 (41%)	0.27
Male factor	846 (44%)	2182 (39%)	<0.01
Endometrium thickness (mm)	10.2 (1.7)	10.2 (1.7)	0.86
Fertilization method			<0.05
IVF	1071 (55%)	3237 (59%)	
ICSI	817 (42%)	2188 (39%)	
Unclear	49 (3%)	107 (2%)	
2PN	1889 (98%)	5428 (98%)	0.11
Assisted hatching	1684 (87%)	4678 (85%)	0.01
Endometrium preparation			0.91
Natural cycle	1051 (54%)	2975 (54%)	
Hormone replaced cycle	658 (34%)	1889 (34%)	
Stimulated cycle	228 (12%)	668 (12%)	

[a]χ^2, non-parametric test, or *t*-test as appropriate. Data are expressed with mean (SD) or median (IQR) as appropriate.

and women were fully informed of the risks associated with the transfer of a lower grade blastocyst and provided their consent before the embryo transfer. The blastocyst transfers were performed on Day 5 after ovulation in natural or ovarian stimulated cycles, or after 7 days of progesterone supplementation in artificial hormonal replacement cycles. The warmed blastocysts were cultured for 30–120 min to evaluate their quality and blastocysts with good survival signs (less than half of the blastomeres showing signs of damage) were transferred.

Outcome definitions

The outcomes in this study included: PTD, defined as a baby born before 37 weeks of gestation; very PTD (VPTD), defined as a baby born before 34 weeks of gestation; newborn sex; low birth weight (LBW), defined as birth weight less than 2500 g at any gestational week; macrosomia, defined as birth weight greater than 4000 g at any gestational week; small for gestational age (SGA) and LGA, defined using reference birth weight percentiles as less than 10th percentile and greater than 90th percentile, respectively, using two different birth weight references (Dai *et al.*, 2014; Yao *et al.*, 2018); gestational hypertensive disease (GHD), defined as the development of blood pressure greater than 140/90 mmHg in pregnancy with or without proteinuria or other signs of preeclampsia; gestational diabetes mellitus (GDM), defined as carbohydrate intolerance of variable severity with onset or first recognition during pregnancy; and preterm premature rupture of membrane (PPROM), defined as rupture of the amniotic membranes before the onset of the preterm labour. All participants were followed up ~3 months post-delivery by a trained nurse through phone call and the follow-up content was well described in a previous study (Hu *et al.*, 2020).

Statistical analyses

Independent-sample *t*-test, nonparametric test or Chi-square test were used to compare variables between groups as appropriate. Multivariate logistic regression was used to test the risk of factors with the expression of crude odds ratios (ORs) and adjusted OR with 95% CIs. The following variables were considered as potential confounders, including female age, female BMI, infertility duration, primary or secondary infertility, parity, reason for IVF, assisted hatching and blastocyst expansion. Due to the missing information of previous pregnancy complications, they were not included in the multivariate logistic regression analysis. All statistical procedures were run in Stata 15.1 (StataCorp LLC, TX, USA). $P < 0.05$ was considered to indicate statistically significant differences. There was no missing data of blastocyst quality, newborn sex, PPROM, GDM, GHD, VPTD or PTD. Less than 1.0% of birth weight data were missing (LBW, SGA, LGA) in the final dataset. Covariables including female BMI (1.4%), male BMI (12.9%), male age (0.2%), infertility duration (1.8%), fertilization method (3.6%) and endometrium preparation (0.1%) contained missing observations. All missing data were considered as missing values in the analysis. Because spontaneous PTD was not recorded in the dataset as separate to iatrogenic PTD, iatrogenic PTD was not analysed.

Results

Characteristics of women eligible for analysis

A total of 7469 women were eligible for the final analysis of the association between blastocyst scores and perinatal outcomes (Supplementary Fig. S1).

According to the definition of good quality and poor quality blastocysts as described above, 1937 women underwent transfer of a poor quality blastocyst and 5532 women underwent good quality blastocyst transfer, then had successful pregnancies resulting in a singleton live birth. Cycles resulting in the transfer of a poor quality embryo were more likely in couples with male factor infertility and where ICSI was utilized ($P < 0.01$, $P < 0.05$) as compared to cycles where good quality embryo transfer resulted. No statistically significant difference was found in female age, male age, female BMI, male BMI, endometrium thickness, primary infertility, parity or endometrium preparation between poor and good quality blastocyst transfers (Table I).

Perinatal outcomes in women with good or poor quality blastocyst transfer

Transfer of a poor quality blastocyst was associated with a higher rate of C-section (71% vs 68%, adjusted OR = 1.15 (1.02, 1.29)) and a higher

Table II Perinatal outcomes in women with good or poor quality blastocyst transfer.

	Poor quality blastocyst[a] (n = 1937)	Good quality blastocyst[a] (n = 5532)	P-value[b]	Crude OR (95% CI)	Adjusted[c] OR (95% CI)
Gestational week	38.3 (1.8)	38.3 (1.7)	0.80	NA	NA
PTD	186 (10%)	530 (10%)	0.98	1.00 (0.84, 1.19)	1.02 (0.85, 1.22)
VPTD	46 (2%)	116 (2%)	0.47	1.14 (0.80, 1.60)	1.16 (0.82, 1.64)
Sex of newborn (female/male)			<0.001	**1.25 (1.13, 1.39)**	**1.26 (1.13, 1.39)**
Male	1010 (52%)	3192 (58%)			
Female	927 (48%)	2339 (42%)			
Birth weight g	3366 (527)	3377 (518)	0.41	NA	NA
C-section	1316 (71%)	3662 (68%)	0.03	**1.14 (1.02, 1.28)**	**1.15 (1.02, 1.29)**
LBW	84 (4%)	226 (4%)	0.64	1.06 (0.82, 1.37)	1.08 (0.84, 1.40)
Macrosomia	216 (11%)	611 (11%)	0.90	1.01 (0.86, 1.18)	1.03 (0.88, 1.22)
SGA	85 (4%)	197 (4%)	0.10	1.24 (0.96, 1.61)	1.27 (0.98, 1.65)
LGA	418 (22%)	1181 (22%)	0.84	1.01 (0.89, 1.15)	1.04 (0.92, 1.18)
PPROM	22 (1%)	83 (2%)	0.24	0.75 (0.47, 1.21)	0.70 (0.43, 1.14)
GDM	188 (10%)	605 (11%)	0.13	0.88 (0.74, 1.04)	0.87 (0.73, 1.04)
GHD	76 (4%)	202 (4%)	0.59	1.08 (0.82, 1.41)	1.07 (0.81, 1.40)

GDM, gestational diabetes mellitus; GHD, gestational hypertensive disease; LBW, low birth weight; LGA, large for gestational age; OR, odds ratio; PPROM, preterm premature rupture of membrane; PTD, preterm delivery; SGA, small for gestational age; VPTD, very preterm delivery. The values in bold are statistically significant.
[a]Blastocysts with stage status higher than 2, ICM grade higher than C and TE grade higher than C (≥3BB) were considered as being of good quality and blastocyst with grading lower than 3BB were considered as poor quality.
[b]χ^2 or *t*-test as appropriate. Data are expressed with mean (SD) or number (percent) as appropriate.
[c]Adjusted with female age, female BMI, primary infertility, parity, reason for IVF and assisted hatching.

chance of female baby (48% vs 42%, adjusted OR = 1.26 (1.13, 1.39)) as compared to good quality blastocyst transfer. There was a trend towards an increased risk of SGA after transfer of a poor quality blastocyst (adjusted OR = 1.27 (0.98, 1.65)). There was no significant difference in the gestational week at delivery, PTD, VPTD, birth weight, LBW, LGA, PPROM, GDM or GHD between the poor quality blastocyst and the good quality blastocyst transfer groups (Table II).

ICM quality and perinatal outcomes

Compared with Grade A ICM blastocyst transfer, there was a trend towards an increased rate of PTD and C-section in Grade C ICM blastocyst transfer (PTD, adjusted OR = 1.23 (0.86, 1.79); C-section, adjusted OR = 1.22 (0.95, 1.57)). Grade B ICM and Grade C ICM blastocyst transfer were associated with a lower chance of female baby (adjusted OR = 0.83 (0.73, 0.95), 0.63 (0.50, 0.79), respectively) and a higher risk of LGA (adjusted OR = 1.23 (1.05, 1.45), 1.47 (1.12, 1.92), respectively) than Grade A ICM blastocyst transfer. Grade C ICM blastocyst transfer was also associated with macrosomia (adjusted OR = 1.66 (1.20, 2.30)). The associations of ICM grading with other perinatal outcomes were not significant and the effect sizes are demonstrated in Table III.

TE quality and perinatal outcomes

Compared with Grade A TE blastocyst transfer, Grade C TE blastocyst transfer was associated with a higher risk of SGA (adjusted OR = 1.74 (1.05, 2.88)). There was also a trend towards an increased risk of C-section in Grade C TE blastocyst transfer (adjusted OR = 1.14 (0.94, 1.39)). Grade B TE and Grade C TE blastocyst transfer had a higher chance of female baby (adjusted OR = 1.30 (1.11, 1.53), 1.88 (1.57, 2.26), respectively) and a lower risk of GDM (adjusted OR = 0.74 (0.59, 0.94), 0.67 (0.50, 0.88), respectively) than Grade A TE blastocyst transfer. The association of TE quality with other perinatal outcomes was not significant (Table IV).

Discussion

In this study, we demonstrated that the transfer of morphologically poor quality blastocysts was associated with a higher rate of C-section and a higher chance of a female baby. Grade B ICM and Grade C ICM blastocyst transfers were associated with lower chances of a female baby and a higher risk of LGA. Grade C ICM blastocyst transfer increased the risk of macrosomia. Grade B TE and Grade C TE blastocyst transfer had a higher chance of female baby and a lower risk of GDM. Grade C TE blastocyst transfer was associated with an increased chance of SGA.

A previous study has suggested that low sperm motility in IVF is associated with an increase in female/male ratio (Arikawa *et al.*, 2016). In addition, single poor-quality embryo transfers in frozen cycles have a higher chance of resulting in a female infant (Oron *et al.*, 2014; Lou *et al.*, 2020). Consistent with these studies, our study shows poor quality blastocyst transfer can result in a higher rate of female live birth than the transfer of a good quality blastocyst transfer as defined by conventional embryo morphological grading. The increased ratio of female/male babies after poor quality blastocyst transfers can potentially be explained by a higher surviving ability of a female embryo within the maternal body. Our study demonstrated an association between

Table III Perinatal outcomes in women with different ICM grades.

Perinatal outcomes	Grade A (n = 1356)	Grade B (n = 5704)	Grade C (n = 409)
PTD	125 (9%)	546 (10%)	45 (11%)
(Crude OR)	(Reference)	1.04 (0.85, 1.28)	1.22 (0.85, 1.75)
(Adjusted OR)	(Reference)	1.05 (0.85, 1.32)	1.23 (0.86, 1.79)
VPTD	29 (2%)	125 (2%)	8 (2%)
(Crude OR)	(Reference)	1.03 (0.68, 1.54)	0.91 (0.41, 2.01)
(Adjusted OR)	(Reference)	0.97 (0.63, 1.50)	0.89 (0.40, 1.96)
Female newborn	612 (45%)	2509 (44%)	145 (35%)
(Crude OR)	(Reference)	0.95 (0.85, 1.08)	**0.67 (0.53, 0.84)**
(Adjusted OR)	(Reference)	**0.83 (0.73, 0.95)**	**0.63 (0.50, 0.79)**
C-section	875 (66%)	3824 (69%)	279 (71%)
(Crude OR)	(Reference)	**1.15 (1.01, 1.31)**	1.25 (0.98, 1.60)
(Adjusted OR)	(Reference)	1.11 (0.97, 1.27)	1.22 (0.95, 1.57)
LBW	53 (4%)	241 (4%)	16 (4%)
(Crude OR)	(Reference)	1.08 (0.80, 1.47)	0.99 (0.56, 1.76)
(Adjusted OR)	(Reference)	1.06 (0.76, 1.46)	0.98 (0.55, 1.74)
Macrosomia	137 (10%)	627 (11%)	63 (15%)
(Crude OR)	(Reference)	1.10 (0.90, 1.33)	**1.61 (1.17, 2.22)**
(Adjusted OR)	(Reference)	1.12 (0.91, 1.38)	**1.66 (1.20, 2.30)**
SGA	44 (3%)	224 (4%)	14 (3%)
(Crude OR)	(Reference)	1.22 (0.88, 1.69)	1.05 (0.57, 1.93)
(Adjusted OR)	(Reference)	1.09 (0.77, 1.55)	1.02 (0.55, 1.88)
LGA	254 (19%)	1242 (22%)	103 (25%)
(Crude OR)	(Reference)	**1.21 (1.04, 1.40)**	**1.45 (1.11, 1.88)**
(Adjusted OR)	(Reference)	**1.23 (1.05, 1.45)**	**1.47 (1.12, 1.92)**
PPROM	16 (1%)	83 (1%)	6 (1%)
(Crude OR)	(Reference)	1.24 (0.72, 2.12)	1.25 (0.48, 3.21)
(Adjusted OR)	(Reference)	1.43 (0.81, 2.52)	1.38 (0.53, 3.57)
GDM	136 (10%)	614 (11%)	43 (11%)
(Crude OR)	(Reference)	1.08 (0.89, 1.32)	1.05 (0.73, 1.51)
(Adjusted OR)	(Reference)	1.15 (0.93, 1.42)	1.07 (0.73, 1.55)
GHD	45 (3%)	215 (4%)	18 (4%)
(Crude OR)	(Reference)	1.14 (0.82, 1.58)	1.34 (0.77, 2.33)
(Adjusted OR)	(Reference)	1.11 (0.78, 1.58)	1.32 (0.74, 2.35)

Adjusted with female age, female BMI, primary infertility, parity, reason for IVF and assisted hatching, blastocyst development stage status and TE quality. The values in bold are statistically significant.

GDM, gestational diabetes mellitus; GHD, gestational hypertensive disease; LBW, low birth weight; LGA, large for gestational age; OR, odds ratio; PPROM, preterm premature rupture of membrane; PTD, preterm delivery; SGA, small for gestational age; TE, trophectoderm; VPTD, very preterm delivery.

poor quality blastocyst transfer and increased risk of C-section compared with good quality blastocyst transfer. This is contradictory to previous reports which showed no difference in C-section delivery with regard to the quality of transferred embryo (Oron *et al.*, 2014). That study, however, included Day 2, Day 3 and Day 5 embryos and used an alternative definition for determining embryos as poor or good quality. In addition, the study included only a small number of poor quality embryo transfer cycles (Oron *et al.*, 2014). These differences may explain the variation in association with C-section seen between this study and our study. Considering that a large proportion of C-section deliveries in ART conceived pregnancies in China are performed electively (Yang *et al.*, 2014), this result should be confirmed by future studies stratifying C-section delivery by indication. A recent study showed a similar rate of SGA and LGA and a similar birth weight in singletons derived from single good and/or poor quality blastocyst transfers (Huang *et al.*, 2020). However, another study from the same research group demonstrates that singletons from the poor-quality blastocyst group have lower birth weight, a higher rate of SGA, and a lower rate of LGA than those from the excellent-quality blastocyst group (Zhang *et al.*, 2020). The inconsistency can be attributed to the difference in the definition of good or poor quality embryos by Zhang *et al.*

Although previous studies indicate comparable perinatal outcomes in comparisons of Day 7 vs Day 5 and Day 7 vs Day 6 embryos

Table IV Perinatal outcomes in women with different TE grades.

Perinatal outcomes	Grade A (n = 818)	Grade B (n = 5116)	Grade C (n = 1525)
PTD	88 (11%)	497 (10%)	141 (9%)
(Crude OR)	(Reference)	0.87 (0.68, 1.11)	0.85 (0.64, 1.12)
(Adjusted OR)	(Reference)	0.86 (0.67, 1.11)	0.84 (0.63, 1.14)
VPTD	23 (3%)	101 (2%)	38 (2%)
(Crude OR)	(Reference)	0.69 (0.44, 1.10)	0.88 (0.52, 1.49)
(Adjusted OR)	(Reference)	0.72 (0.45, 1.19)	0.94 (0.54, 1.64)
Female newborn	311 (38%)	2174 (43%)	781 (51%)
(Crude OR)	(Reference)	**1.20 (1.03, 1.40)**	**1.71 (1.44, 2.03)**
(Adjusted OR)	(Reference)	**1.30 (1.11, 1.53)**	**1.88 (1.57, 2.26)**
C-section	535 (67%)	3409 (69%)	1034 (71%)
(Crude OR)	(Reference)	1.08 (0.92, 1.26)	1.20 (0.99, 1.44)
(Adjusted OR)	(Reference)	1.02 (0.87, 1.21)	1.14 (0.94, 1.39)
LBW	41 (5%)	201 (4%)	68 (5%)
(Crude OR)	(Reference)	0.77 (0.55, 1.09)	0.89 (0.60, 1.32)
(Adjusted OR)	(Reference)	0.76 (0.53, 1.09)	0.89 (0.58, 1.35)
Macrosomia	80 (10%)	594 (12%)	153 (10%)
(Crude OR)	(Reference)	1.21 (0.95, 1.55)	1.03 (0.76, 1.37)
(Adjusted OR)	(Reference)	1.10 (0.85, 1.43)	0.95 (0.70, 1.27)
SGA	23 (3%)	188 (4%)	71 (5%)
(Crude OR)	(Reference)	1.31 (0.85, 2.04)	**1.69 (1.05, 2.72)**
(Adjusted OR)	(Reference)	1.33 (0.84, 2.12)	**1.74 (1.05, 2.88)**
LGA	166 (21%)	1118 (22%)	315 (21%)
(Crude OR)	(Reference)	1.09 (0.91, 1.31)	1.02 (0.83, 1.26)
(Adjusted OR)	(Reference)	1.02 (0.84, 1.23)	0.96 (0.77, 1.20)
PPROM	11 (1%)	78 (1%)	16 (1%)
(Crude OR)	(Reference)	1.13 (0.60, 2.14)	0.78 (0.36, 1.68)
(Adjusted OR)	(Reference)	1.06 (0.55, 2.04)	0.63 (0.28, 1.42)
GDM	108 (13%)	540 (10%)	145 (9%)
(Crude OR)	(Reference)	**0.77 (0.62, 0.97)**	**0.69 (0.53, 0.90)**
(Adjusted OR)	(Reference)	**0.74 (0.59, 0.94)**	**0.67 (0.50, 0.88)**
GHD	31 (4%)	189 (4%)	58 (4%)
(Crude OR)	(Reference)	0.97 (0.66, 1.43)	1.00 (0.64, 1.56)
(Adjusted OR)	(Reference)	0.94 (0.62, 1.42)	0.97 (0.60, 1.57)

Adjusted with female age, female BMI, primary infertility, parity, reason for IVF and assisted hatching, blastocyst development stage status and ICM quality. The values in bold are statistically significant.

GDM, gestational diabetes mellitus; GHD, gestational hypertensive disease; ICM, inner cell mass; LBW, low birth weight; LGA, large for gestational age; OR, odds ratio; PPROM, preterm premature rupture of membrane; PTD, preterm delivery; SGA, small for gestational age; TE, trophectoderm; VPTD, very preterm delivery.

(Hiraoka *et al.*, 2009; Huang *et al.*, 2020), the number of Day 6 or Day 7 blastocyst was not large. Because the expansion degree can affect the morphological degree to some extent, we included the expansion degree as a covariate in the mutivariate analysis of the association between morphological degree and perinatal outcomes. Blastocyst expansion was significantly related to the total days of blastocyst culture. Given that the total days of blastocyst culture was poorly recorded in our dataset, the analysis of the association between blastocyst expansion and perinatal outcomes was not performed.

Consistent with another recent study, our study shows a significant association between Grade B and Grade C ICM and LGA, although that study did not reach a significance due to the small sample size (Bakkensen *et al.*, 2019). Our study also demonstrated some associations between morphological grading and perinatal outcome, not previously described: Grade C ICM blastocyst transfer was associated with increased macrosomia and Grade B and C TE blastocyst transfer displayed lower GDM risk compared with Grade A TE blastocyst transfer. The mechanisms underlying the association between ICM grading and feotal growth and TE grading and gestational diabetes are unknown and require further investigation. The observed correlation between ICM and LGA or between TE and SGA might be attributed to the vital role of ICM and TE during implantation and placentation. The associations between ICM grading and LGA and macrosomia and between TE and SGA would suggest that

blastocysts with Grade A ICM blastocysts should be transferred preferentially to Grade B/C ICM blastocysts and Grade A/B TE blastocysts should be transferred preferentially to Grade C TE blastocysts. These results support the use of current morphological grading systems for embryo prioritization.

To the best of our knowledge, this is the largest cohort investigating blastocyst quality and perinatal outcomes. Previous studies have addressed relatively few perinatal outcomes while a variety of perinatal outcomes are explored in our study. We investigated the association of the morphological parameters of the blastocyst with perinatal outcomes in addition to global grading of blastocysts as good or poor quality. Furthermore, our data are relatively complete with very few missing data.

The main limitations of this study are its retrospective nature and the relative subjectivity of blastocyst scoring. The follow-up was conducted through a phone call in our centre and some patients may not have reported their obstetrical and neonatal outcomes, leading to a relatively lower rate of several obstetrical outcomes, such as GHD and PPROM. The blastocyst morphology was evaluated before vitrification and the re-expansion data after warming was lacking. It is possible that this may have influenced our results, although a recent study has suggested that the pre-vitrification features of a blastocyst rather than post-warming features of the survived blastocyst should be used to select the competent embryos (Cimadomo *et al.*, 2018). Due to the missing information in our dataset, we were not able to separate out iatrogenic preterm birth nor adjust for obstetric complications in previous pregnancies as a confounder in the mutivariate analysis. Blastocyst expansion was significantly related to the days of blastocyst culture in total and given that this was unclear in our dataset, the analysis of the association between blastocyst expansion and perinatal outcomes was not performed.

In conclusion, Grade C ICM blastocysts should be deferred for transfer if blastocysts of Grade A or Grade B ICM are available. Transfer of a blastocyst with grading lower than 3BB is associated with a higher rate of C-section delivery and a higher chance of a female baby.

Supplementary data

Supplementary data are available at *Human Reproduction Open* online.

Data availability

All generated data are incorporated into the article and its online supplementary material. The original data of individual participants underlying this article will be shared on reasonable request to the corresponding author.

Acknowledgements

We thank Miss. Lixue Chen in Peking University Third Hospital for helping us to collect the data.

Authors' roles

K.-L.H. and B.W.M. conceived the idea. K.-L.H. reviewed the literature, designed the study, conducted the analysis, designed the figures and tables and wrote the manuscript. X.Z., S.H., X.L., R.L. and B.W.M. revised the manuscript. All authors participated in the discussion of analysis and interpretation of data in this article.

Funding

This study was supported by the National Key Research and Development Program of China (2018YFC1004100 to R.L.), the National Science Fund for Distinguished Young Scholars (81925013 to R.L.) and a Zhejiang University Scholarship for Outstanding Doctoral Candidates (to K.-L.H.).

Conflict of interest

All authors declare no conflict of interest.

References

Ahlström A, Westin C, Wikland M, Hardarson T. Prediction of live birth in frozen-thawed single blastocyst transfer cycles by pre-freeze and post-thaw morphology. *Hum Reprod* 2013;**28**:1199–1209.

Arikawa M, Jwa SC, Kuwahara A, Irahara M, Saito H. Effect of semen quality on human sex ratio in in vitro fertilization and intracytoplasmic sperm injection: an analysis of 27,158 singleton infants born after fresh single-embryo transfer. *Fertil Steril* 2016;**105**:897–904.

Bakkensen JB, Brady P, Carusi D, Romanski P, Thomas AM, Racowsky C. Association between blastocyst morphology and pregnancy and perinatal outcomes following fresh and cryopreserved embryo transfer. *J Assist Reprod Genet* 2019;**36**:2315–2324.

Balaban B, Urman B, Sertac A, Alatas C, Aksoy S, Mercan R. Blastocyst quality affects the success of blastocyst-stage embryo transfer. *Fertil Steril* 2000;**74**:282–287.

Boynukalin FK, Gultomruk M, Cavkaytar S, Turgut E, Findikli N, Serdarogullari M, Coban O, Yarkiner Z, Rubio C, Bahceci M. Parameters impacting the live birth rate per transfer after frozen single euploid blastocyst transfer. *PLoS One* 2020;**15**:e0227619.

Chen S, Du H, Liu J, Liu H, Li L, He Y. Live birth rate and neonatal outcomes of different quantities and qualities of frozen transferred blastocyst in patients requiring whole embryo freezing stratified by age. *BMC Pregnancy Childbirth* 2020;**20**:655.

Cimadomo D, Capalbo A, Levi-Setti PE, Soscia D, Orlando G, Albani E, Parini V, Stoppa M, Dovere L, Tacconi L *et al.* Associations of blastocyst features, trophectoderm biopsy and other laboratory practice with post-warming behavior and implantation. *Hum Reprod* 2018;**33**:1992–2001.

Dai L, Deng C, Li Y, Zhu J, Mu Y, Deng Y, Mao M, Wang Y, Li Q, Ma S *et al.* Birth weight reference percentiles for Chinese. *PLoS One* 2014;**9**:e104779.

De Vos A, Santos-Ribeiro S, Van Landuyt L, Van de Velde H, Tournaye H, Verheyen G. Birthweight of singletons born after cleavage-stage or blastocyst transfer in fresh and warming cycles. *Hum Reprod* 2018;**33**:196–201.

Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. *Fertil Steril* 2000;**73**:1155–1158.

Gardner DK, Surrey E, Minjarez D, Leitz A, Stevens J, Schoolcraft WB. Single blastocyst transfer: a prospective randomized trial. *Fertil Steril* 2004;**81**:551–555.

Ginström Ernstad E, Bergh C, Khatibi A, Källén KB, Westlander G, Nilsson S, Wennerholm UB. Neonatal and maternal outcome after blastocyst transfer: a population-based registry study. *Am J Obstet Gynecol* 2016;**214**:378.e1–378.e10.

Ginström Ernstad E, Spangmose AL, Opdahl S, Henningsen AA, Romundstad LB, Tiitinen A, Gissler M, Wennerholm UB, Pinborg A, Bergh C *et al*. Perinatal and maternal outcome after vitrification of blastocysts: a Nordic study in singletons from the CoNARTaS group. *Hum Reprod* 2019;**34**:2282–2289.

Hiraoka K, Hiraoka K, Miyazaki M, Fukunaga E, Horiuchi T, Kusuda T, Okano S, Kinutani M, Kinutani K. Perinatal outcomes following transfer of human blastocysts vitrified at day 5, 6 and 7. *J Exp Clin Assist Reprod* 2009;**6**:4.

Hu KL, Liu FT, Xu H, Li R, Qiao J. Association of serum anti-Müllerian hormone and other factors with cumulative live birth rate following IVF. *Reprod Biomed Online* 2020;**40**:675–683.

Hu KL, Liu FT, Xu H, Li R, Qiao J. High anti-Müllerian hormone levels are associated with preterm delivery in patients with polycystic ovary syndrome. *Fertil Steril* 2020;**113**:444–452.e1.

Huang J, Tao Y, Zhang J, Yang X, Wu J, Kuang Y, Wang Y. Poor embryo quality is associated with a higher risk of low birthweight in vitrified-warmed single embryo transfer cycles. *Front Physiol* 2020; **11**:415.

Huang J, Yang X, Wu J, Kuang Y, Wang Y. Impact of day 7 blastocyst transfer on obstetric and perinatal outcome of singletons born after vitrified-warmed embryo transfer. *Front Physiol* 2020;**11**:74.

Li M, Yin M, Wu L, Yan Z, Lyu Q, Yan Z, Li B. Pregnancy and neonatal outcomes of morphologically grade CC blastocysts: are they of clinical value? *Arch Gynecol Obstet* 2020;**302**:1511–1521.

Lou H, Li N, Zhang X, Sun L, Wang X, Hao D, Cui S. Does the sex ratio of singleton births after frozen single blastocyst transfer differ in relation to blastocyst development? *Reprod Biol Endocrinol* 2020;**18**:72.

Martins WP, Nastri CO, Rienzi L, van der Poel SZ, Gracia CR, Racowsky C. Obstetrical and perinatal outcomes following blastocyst transfer compared to cleavage transfer: a systematic review and meta-analysis. *Hum Reprod* 2016;**31**:2561–2569.

Oron G, Son WY, Buckett W, Tulandi T, Holzer H. The association between embryo quality and perinatal outcome of singletons born after single embryo transfers: a pilot study. *Hum Reprod* 2014;**29**: 1444–1451.

Spangmose AL, Ginström Ernstad E, Malchau S, Forman J, Tiitinen A, Gissler M, Opdahl S, Romundstad LB, Bergh C, Wennerholm UB *et al*. Obstetric and perinatal risks in 4601 singletons and 884 twins conceived after fresh blastocyst transfers: a Nordic study from the CoNARTaS group. *Hum Reprod* 2020;**35**:805–815.

Yang X, Li Y, Li C, Zhang W. Current overview of pregnancy complications and live-birth outcome of assisted reproductive technology in mainland China. *Fertil Steril* 2014;**101**:385–391.

Yao F, Miao H, Li B, Wu Y, Zhao Q. New birthweight percentiles by sex and gestational age in Southern China and its comparison with the INTERGROWTH-21st Standard. *Sci Rep* 2018;**8**:7567.

Zhang J, Huang J, Liu H, Wang B, Yang X, Shen X, Mao X, Wang Y, Kuang Y. The impact of embryo quality on singleton birthweight in vitrified-thawed single blastocyst transfer cycles. *Hum Reprod* 2020; **35**:308–316.

Zheng X, Chen Y, Yan J, Wu Y, Zhuang X, Lin S, Zhu J, Lian Y, Qiao J, Liu P. Effect of repeated cryopreservation on human embryo developmental potential. *Reprod Biomed Online* 2017;**35**:627–632.