Does Blastocyst Morphology Affect Live Birth Rate After Transfer of Single Euploid Embryo?

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Research Article

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Abstract

Background

The aim of this study was to investigate whether the morphologic parameters of blastocyst influence the live birth rate (LBR) of euploid embryos transferred in subsequent single frozen-thawed embryo transfer (FET) cycles?

Methods

Women who received first preimplantation genetic testing for aneuploidy (PGT-A) and following underwent frozen-thawed single euploid blastocyst transfer cycles from June 2017 to May 2020 were divided into three age groups (< 30, 30–34 and ≥ 35 years). The primary outcome measure was LBR. Outcomes were compared between different blastocyst quality, inner cell mass (ICM) grade, trophectoderm (TE) grade and day of TE biopsy within the same age group.

Results

In the youngest group (< 30 years, n = 100), LBR were compared between cycles with various blastocyst quality (66.67% for good quality, 65.52% for average quality and 36.36% for poor quality; *P* = 0.013), ICM grade (61.11% for grade A and 51.22% for grade B; *P* = 0.466), TE grade (68.75% for grade A,65.00% for grade B and 36.30% for grade C; *P* = 0.012) and day of TE biopsy (65.38% for Day 5 and 39.58% for Day 6; *P* = 0.010). Similarly, in the 30–34 years group(n = 121) and the oldest group (≥ 35years, n = 58), LBR were also comparable between these subgroups, but no significant differences were seen between blastocyst morphologic grading and LBR (*P* > 0.05). Moreover, good quality (adjusted odds ratio [aOR] 3.30; 95% confidence interval [CI], 1.09 ~ 9.99; *P* = 0.035) and average quality (aOR 3.71; 95%CI, 1.25 ~ 11.01; *P* = 0.018) embryos were still yielded a significantly higher LBR than poor-quality embryos, TE grade B embryos were also associated with a statistically significantly higher LBR compared with TE grade C embryos (aOR 3.69;95%CI, 1.37 ~ 9.95; *P* = 0.010) after adjusting for the potential confounding factors.

Conclusion

Blastocyst quality and trophectoderm grading is a useful predictor of LBR in single frozen-thawed euploid embryo transfer cycles among women < 30 years old. However, these differences were not found in women older than 30 years.

Background

The development of assisted reproductive technology (ART) has greatly helped many infertile couples realize their wishes to be parents in the past few decades, while embryos suffer developmental arrest and early spontaneous abortion are still difficult problems in the treatment of infertility. In addition, with the increase of work pressure and the acceleration of life rhythm, more females choose to have children late. Nevertheless, they will face a lower conception chance and higher miscarriage rates with age gradually increase[1]. This condition is ascribed to the rapid increase of aneuploidy rates in older women [2]. Furthermore, it has been demonstrated that the high incidence of embryo aneuploidy is one of the important causes of in vitro fertilization embryo transfer (IVF-ET) failure [3, 4], which manifested as about 50% of embryos occurring errors during gametogenesis and early mitotic divisions throughout their preimplantation development. It is clear that the number of people who received ART treatment are rapidly aging simultaneously.

To our knowledge, conventional morphologic assessment has been a widely used method to select the embryos with the best development potential. However, it cannot accurately evaluate ploidy status, because more than half of embryos with high morphological scores were eventually screened as aneuploidy[5]. The great significance of ART is to achieve a healthy live birth and as the advances of molecular biology and its relative techniques, the third-generation IVF technology has been introduced. Since then, the benefits of PGT-A is to overcome this high aneuploidy rate through transfer of euploid embryos, which increases the likelihood of improving implantation potential and decreasing miscarriage rate from IVF therapy[6]. The current technology now used for PGT-A is based on next generation sequencing (NGS), which is done using polymerase chain reaction (PCR) amplification and then the results of sequencing are distinguished from normal and abnormal amounts of DNA[7].Today PGT-A is mainly applied to the following specific population, such as advanced maternal age (AMA), repeated implantation failure (RIF), recurrent spontaneous abortion (RSA) and severe teratozoospermia[8].

Several studies have focused on the relationship between embryo quality and pregnancy outcomes, which did not exclude the influence of embryo aneuploidy. Considering that the increased risk of aneuploidy is the main resistance for decline in embryo development potential, it would be reasonable to assume that as long as single euploid embryo is transferred, the higher occurrence of implantation and live birth are attained irrespective of embryo quality. However, in a retrospective cohort study by Minasi et. al.[5], the embryos with good quality have a statistically significantly higher implantation rate than those with poor quality. Similarly, a recently study we published suggested that blastocyst morphologic grading was associated with implantation rate for euploid embryo transfers after adjustment for potential confounders among people under 35 years old[9]. In contrast, Anderson et al. concluded that good quality and poor quality euploid blastocysts have similar pregnancy outcomes[10]. Thus, the impact of euploid embryos quality on clinical outcomes has not been determined and are still needs further exploration. Therefore, on the basic of our previous research, the current study was conducted to identify whether the morphologic parameters of blastocysts allow for further optimization of live birth rates in transfers of known euploid embryos in single FET cycles once embryos were implanted successfully, which is helpful

for doctors to select embryos to transfer and provide consultation for patients who received PGT-A for pregnancy in clinical practice.

Material And Methods

Study design and population

This retrospective cohort study included women who undergone first autologous PGT-A and then followed by frozen-thawed single euploid embryo transfer cycles at the Reproductive Medical Center of the Third Affiliated Hospital of Zhengzhou University between June 2017 and May 2020.We excluded donation cycles, women with uterine malformation and frozen oocyte cycles. This study was performed in accordance with the Code of Ethics in the Declaration of Helsinki and was approved by the Ethics Review Committee of our hospital (protocol number 2021-WZ-010).

Ovarian stimulation protocol

Each female patient usually underwent a GnRH antagonist ovarian stimulation protocol. The Gonadotropin (Gonal-F, Merck Serono, Switzerland) started injection from the second or third day of the menstrual cycle, dosage (150-300IU) was adjusted based on patient's age, basal antral follicle count (AFC), body mass index (BMI), basal follicle stimulating hormone (FSH)and ovarian reserve. The response to stimulation was assessed by performing transvaginal ultrasounds and measuring serum estradiol levels. GnRH antagonist (Cetrotide, Merck Serono, Switzerland) 0.25mg was usually injected for pituitary suppression when follicle diameter is 12 ~ 14 mm or Gonadotropin has been used for 5 ~ 6 days. GnRH agonist 0.2 mg (Dophereline, Ipsen Pharma Biotech, France) was used to trigger the final oocyte maturation. Untrasound guided oocyte retrieval was performed 33-36 hours after the trigger.

Laboratory protocols

Blastocyst evaluation was performed prior to embryo biopsy. Blastocysts were graded according to the Gardner and Schoolcraft grading system, and the score was dependent on blastocyst expansion, ICM development and trophectoderm TE appearance[11]. The degree of expansion included the following six grades:(1) a nonexpanded embryo with the blastocele filling <50%; (2) the blastocele filling >50% of the embryo; (3) a full blastocyst with a blastocoele filling the embryo; (4) an expanded blastocyst with a blastocoele volume larger than that of the full blastocyst, with a thinning zona; (5) a hatching blastocyst with the TE starting to herniate through the zona; (6) a hatched blastocyst, with the blastocyst completely escaping from the zona. The ICM was graded as follows: (A) tightly packed, with many cells; (B)loosely gathered, with several cells; (C) very few cells. The three TE grades were: (A) many cells forming a cohesive epithelium, (B) few cells establishing a loose epithelium and (C) very few large cells. In our center, for blastocysts with an expansion score ≥4, the development of the ICM and TE was then evaluated and the ICM grade should at least B, because the ICM grade C isn’t really used often. The quality of the blastocyst was grouped into three categories based on ICM and TE scoring: good quality: AA, AB and BA; average quality: BB; and poor quality: AC and BC. Embryo grading was performed by the

same team of four highly trained embryologists and each with five years of experience, which minimized the difference in human judgment. Then the embryos were biopsied on day 5 or day 6 when the expansion degree of blastocyst cavity is ³ 4. The zona pellucida was perforated by use of a Saturn laser system (Research Instruments, Singapore) to opening of 6–9 mm, and a biopsy pipette was used to aspirate 3–5 herniated TE cells. Then the washed TE cells were placed in 0.2mL PCR tubes containing 5μL phosphate-buffered saline solution (PBS). All selected embryos were screened for 24 chromosome aneuploidy with NGS, as described in Zimmerman et al[12]. Finally, three different outcomes were considered after the PGT-A testing: euploid and aneuploid and mosaic. After the biopsy, the blastocyst were vitrificated using Cryotop® (Kitazato Corporation, Shizuoka, Japan)[13]. The vitrified-warmed procedure has been described in detailed previously[14].

Endometrial preparation

Only embryos that were screened by NGS to be euploid were transferred in FET cycles. In general, women with regular ovulatory cycles underwent natural cycles, using transvaginal ultrasonography and urine luteinizing hormone (LH) test to monitor the development of the dominant follicle and endometrial thickness from the 10th day of the menstrual cycle until ovulation. While artificial cycles were applied for women with irregular menses who received estradiol valerate (4~8 mg daily started on the 3rd day of the menstrual cycle) for 12 days. When endometrial thickness ≥7mm, all patients will provide for conventional luteal support and continue until 7 weeks of gestation. When on the 5th day after ovulation or the 6th day of progesterone administration, single selective frozen-thawed euploid blastocyst was transferred.

Outcome measures and statistical analysis

All statistical results were calculated with SPSS 25.0 statistical software (IBM, United States). LBR after the transfer of euploid embryos are we mainly discussed measure in this study. The secondary outcome measures are pregnancy rate and early spontaneous abortion rate. The LBR was defined as the number of live births divided by the sum of embryos transferred cycles included in the cohort. The pregnancy rate was defined as the percentage of the intrauterine gestational sac with fetal heartbeat by all transferred embryos at 4 weeks after blastocyst transfer. The early spontaneous abortion rate was considered to be the proportion of clinical pregnancies (a fetal heartbeat was seen on scan) that did not progress in the first-trimester spontaneous abortion.

All cycles were divided into three groups according to the women's age (<30 ,30-34 and ≥35 years). The outcomes measure, embryos data and the baseline demographic characteristics were all compared among the three age groups. Categorical variables were compared with the Pearson chi-square (X^2) or Fisher's exact tests. Continuous variables were tested for normality, and they were expressed as mean ± standard deviation, and parametric data were compared using the analysis of variance (ANOVA) test. In order to further explore the association between morphologic parameters and LBR in women <30 years old, multivariable logistic regression analyses were performed. The adjusted odds ratio (aOR) with 95%

confidence interval (CI) were calculated and controlled for confounding factors. $P<0.05$ was considered to be statistically significant.

Results

Finally, a total of 279 single frozen-thawed euploid embryo transfer cycles met the study inclusion criteria. Of the 279 patients who underwent PGT-A, 33 (11.83%) had AMA, 75 (26.88%) had severe teratozoospermia, 15 (5.38%) had AMA and severe teratozoospermia, 84 (30.11%) had RSA, 10 (3.58%) had AMA and RSA, 48 (17.20%) had RIF, 8 (2.87%) had AMA and RIF, 6 (2.15%) couples performed PGT-A as unexplained infertility. The total LBR is 51.61% (144/279). All cycles were categorized into three groups based on female age :<30 years old group (n = 100), 30–34 years old group (n = 121), ≥ 35 years old group (n = 58). The Characteristic and embryo ploidy data of women who underwent PGT-A cycles are listed in Table 1. No statistically significant differences were seen in female BMI, basal FSH, endometrial thickness on transfer day, FET endometrial preparation protocol and day of TE biopsy among the three age groups ($P > 0.05$). The aneuploidy rates of biopsied blastocysts were lowest in the youngest age group and increased gradually with women's age, although this difference is not statistically significant ($P > 0.05$). The number of good quality embryos, euploid embryo, and AMH in < 30 years old group and 30–34 years old group were significantly higher than that in ≥ 35 years old group ($P < 0.05$). The proportion of secondary infertility increase steadily with age, reaching > 80% in women ≥ 35 years old ($P < 0.05$).

Table 1
Characteristic and embryo ploidy data of women who underwent PGT-A cycles.

Characteristic	< 30	30–34	≥ 35	*P* value		
	(n = 100)	(n = 121)	(n = 58)	*P*1	*P*2	*P*3
Female age (years)[a]	26.74±2.16	31.64±1.35	38.05±2.95	< 0.001	< 0.001	< 0.001
Male age (years) [a]	27.97±2.55	32.39±2.77	38.16±3.94	< 0.001	< 0.001	< 0.001
Female BMI (kg/m^2) [a]	24.25±3.14	24.09±3.25	23.95±2.88	1	1	1
Basal FSH (IU/L) [a]	6.33±2.17	6.20±2.20	6.99±2.89	1	0.265	0.106
AMH (pmol/L) [a]	33.89±23.63	30.17±22.67	21.54±13.00	0.554	< 0.001	0.005
Endometrial thickness on transfer day(mm) [a]	9.20±1.36	9.02±1.69	9.22±1.67	1	1	1
Type of infertility, n (%) [b]				0.001	< 0.001	0.038
Primary infertility	47(49.00)	31(25.62)	7(12.07)			
Secondary infertility	53(53.00)	90(74.38)	51(87.93)			
FET endometrial preparation protocol, n (%) [b]				0.371	0.904	0.377
Natural cycles	49(49.00)	52(42.98)	29(50.00)			
Artificial cycles	51(51.00)	69(57.02)	29(50.00)			

Note: BMI: body mass index; FSH: follicle stimulation hormone; AMH: anti-Müllerian hormone; FET: frozen-thawed embryo transfer; TE: trophectoderm; *P*1 value comparing < 30 versus 30–34 age groups, *P*2 value comparing < 30 versus ≥ 35 age groups, *P*3 value comparing 30–34 versus ≥ 35 age groups.

[a] One-way ANOVA.

[b] Pearson chi-square test.

Characteristic	< 30	30–34	≥ 35	*P* value		
Day of TE biopsy, n (%) [b]				0.320	0.652	0.190
Day 5	52(52.00)	71(58.68)	28(48.28)			
Day 6	48(48.00)	50(41.32)	30(51.72)			
Number of good quality embryos [a]	4.58±2.09	4.53±2.15	3.49±1.86	1	0.016	0.017
Euploid embryos [a]	2.25±1.24	2.12±1.45	1.57±0.80	0.944	0.019	0.001
Aneuploidy rates [b]	208/419(49.64)	246/494(51.82)	102/185(55.14)	0.963	0.213	0.215
Note: BMI: body mass index; FSH: follicle stimulation hormone; AMH: anti-Müllerian hormone; FET: frozen-thawed embryo transfer; TE: trophectoderm; *P*1 value comparing < 30 versus 30–34 age groups, *P*2 value comparing < 30 versus ≥ 35 age groups, *P*3 value comparing 30–34 versus ≥ 35 age groups.						
[a] One-way ANOVA.						
[b] Pearson chi-square test.						

The likelihood of pregnancy rate($P = 0.410$), spontaneous abortion rate ($P = 0.885$) as well as live birth rate ($P = 0.687$) were not affected by women's age between different age groups, which evaluated in Fig. 1.

As shown in Table 2, the primary focus of our analysis was the LBR of different morphologic parameters related to euploid blastocysts quality between all age group. In the youngest age group (< 30 years), the prevalence of live birth was 66.67% for good quality, 65.52% for average quality and 36.36% for poor quality ($P = 0.013$). Nevertheless, the blastocyst quality did not affect LBR in the other two age groups. In women aged 30–34 years old, LBR was 62.07% for good quality, 50.00% for average quality and 50.00% for poor quality ($P = 0.525$). Similarly, the oldest patients (≥ 35 years) had comparable LBR, ranging from 56.00–36.00% ($P = 0.394$).

When cycles were stratified according to the ICM grade before the day of TE biopsy, cycles in which ICM were graded A were comparable with cycles in which ICM were graded B:61.11% vs. 51.22 % ($P = 0.446$) in women younger than 30 years old. In the same way, 42.86% vs. 55.00% ($P = 0.311$) in women aged 30–34 years old and 63.64% vs. 42.55% ($P = 0.207$) in women aged ≥ 35 years.

Likewise, the effect of different TE grades was also had a relationship with LBR in youngest women (< 30 years old), which ranged from 68.75–36.36% ($P = 0.012$). But in women aged 30 years or older, TE grade did not influence LBR, which ranged from 77.78–47.27% in 30–34 age group ($P = 0.070$) and 60.00–36.00% in patients aged more than 35 years old ($P = 0.389$).

The day of TE biopsy was also associated with LBR in < 30 years old group, which was shown as 65.38% for women whose embryos were biopsied on Day 5 and 39.58% for Day 6 in ($P = 0.010$). However, no statistically significant differences were seen in the impact of the LBR on the other two age groups ($P > 0.05$).

Table 2
Live birth rate in women of different age groups.

Age	< 30	30–34	≥35
	(n = 100)	(n = 121)	(n = 58)
Embryo quality			
Good	18/27 (66.67)	18/29 (62.07)	4/8 (50.00)
Average	19/29 (65.52)	22/44 (50.00)	14/25 (56.00)
Poor	16/44 (36.36)	24/48 (50.00)	9/25 (36.00)
P value	0.013 [a]	0.525 [a]	0.394 [b]
ICM grade			
A	11/18 (61.11)	9/21 (42.86)	7/11 (63.64)
B	42/82 (51.22)	55/100 (55.00)	20/47 (42.55)
P value	0.446 [a]	0.311 [a]	0.207
TE grade			
A	11/16 (68.75)	14/18 (77.78)	3/5 (60.00)
B	26/40 (65.00)	26/55 (47.27)	15/28 (53.57)
C	16/44 (36.36)	24/48 (50.00)	9/25 (36.00)
P value	0.012 [a]	0.070 [a]	0.389 [b]
Day of TE biopsy			
Day 5	34/52 (65.38)	41/71 (57.75)	16/28 (57.14)
Day 6	19/48 (39.58)	23/50 (46.00)	11/30 (36.67)
P value	0.010 [a]	0.202 [a]	0.118 [a]
Note: ICM: inner cell mass; TE: trophectoderm.			
[a] Pearson chi-square test.			
[b] Fisher's exact test.			

When we discovered that embryo quality, ICM grade and day of TE biopsy are associated with the LBR in women under 30 years old, we then performed multivariate logistic regression analysis to investigate the prevalence of LBR. After adjusting for female age, male age, female BMI, basal FSH, AMH, endometrial

thickness on transfer day, type of infertility and day of TE biopsy, good quality (aOR 3.30; 95% CI, 1.09 ~ 9.99; *P* = 0.035) and average quality (aOR 3.71;95%CI, 1.25 ~ 11.01; *P* = 0.018) embryos still yielded a statistically significantly higher LBR compared with poor quality. Furthermore, TE grade B embryos were also corrected with higher LBR than TE grade C embryos (aOR 3.69;95%CI, 1.37 ~ 9.95; *P* = 0.010). However, after adjusting for embryo quality, ICM grade, TE grade and all other confounders mentioned above, the differences in LBR between Day 5 and Day 6 were not statistically significant (aOR 2.50; 95%CI, 0.93 ~ 6.77; *P* = 0.071).

Table 3
Adjusted odds ratios of live birth rate by different morphologic grading of euploid blastocyst graded

Parameter	Category	aOR (95%CI)	*P* value
Embryo quality	Good	3.30 (1.09 ~ 9.99)	0.035
	Average	3.71 (1.25 ~ 11.01)	0.018
	Poor	Reference	
ICM grade	A	1.43 (0.45 ~ 4.57)	0.544
	B	Reference	
TE grade	A	3.12 (0.85 ~ 11.47)	0.087
	B	3.69 (1.37 ~ 9.95)	0.010
	C	Reference	
Day of TE biopsy	Day 5	2.50 (0.93 ~ 6.77)	0.071
	Day 6	Reference	
Note: ICM: inner cell mass; TE: trophectoderm; aOR: adjusted odds ratio; CI: confidence interval.			
P values are adjusted for female age, male age, female BMI, basal FSH, AMH, endometrial thickness on transfer day, type of infertility and day of TE biopsy. The *P* value of ICM, TE and day of TE biopsy groups were also adjusted for the other morphologic grading characteristics: ICM, TE and embryo quality.			

Discussion

This study determined the correlation between blastocyst morphology and LBR after transfer of frozen-thawed single euploid embryo. We found that euploid embryos graded as good and average are powerful predictor for LBR than euploid embryos graded as poor in women aged < 30 years. In the light of this, we further indicated that the TE grade has the great influence on LBR. However, the LBR do not affect by euploid blastocyst morphology in women aged 30 years or older.

In general, the maternal age is one of key factors determining the possibility of pregnancy outcome either in ART conception or spontaneous conception[15]. Most importantly, the reason for age-related decline in

reproductive ability is contributed to the decline of ovarian reserve function and the increase of aneuploidy with advancing women's age. And from our data, we can see the aneuploidy rate are certainly highest in older women and then start to gradually decline with women's age, although the difference is not statistically significant. Hence, it seems logical that we speculated the effect of maternal age on pregnancy outcome is eliminated after PGT-A. It is in line with our expectation, the pregnancy rate, spontaneous abortion rate and live birth rate were comparable between three age groups. While a literature reported that in women ≤ 35 years the chance of conception increased higher than those older 35 years old after transfer of euploid embryos[3]. In addition, we previously pointed out that if multiple embryos are euploid, morphology should be the main criterion used to select an embryo for transfer in younger women[9]. In this condition, we assume that whether blastocyst grading can foretell LBR in the frozen-thawed embryo transfer of single euploid embryo once they succeed in implantation. Given that we divided all patients into three age group to investigated whether the euploid blastocysts morphologic parameters predict the LBR in the same age groups.

To our surprise, we found that the effect of morphological parameters of euploid blastocysts is not same in different age group. There is reasonable to believe that best quality embryos have highest implantation potential and further development competence. Researches have been studied that traditional morphologic assessment has been still a guiding principle for embryo selection even among the euploid blastocysts[16, 17]. Irian et al.[18] confirmed that good quality euploid embryos were associated with a higher implantation rate and LBR than poor quality euploid embryos. In their another study, they also concluded that better morphologic scores embryos yield a higher ongoing pregnancy rate compared with lower morphologic grading euploid blastocysts[19]. Consistent with their results, we also reported that the euploid embryos with higher morphologic scores had a statistically significantly LBR than those transfer lower morphologic scores, especially for women who younger than 30 years old after adjusting for day of TE biopsy and other possible related influencing factor. However, there was no significant difference in the LBR of euploid blastocyst regardless of their morphology quality in older women. The reason for this increased prevalence of LBR may be that high quality euploid embryos of young patients have good development potential and thus result in a favorable outcome. It is suggested that morphological assessment of blastocyst may still a valuable reference when selecting embryos for transfer in the patients under 30 years who underwent PGT-A cycles. In a recent retrospectively analysis, the authors also concluded that LBR is not affected by embryo quality once PGT-A has been performed, the mean female age was 38.6±5.2 years[20], emphasizing that the poor quality euploid embryos can also develop well. So, poor quality embryos should not be ignored, which can reduce the transfer cycles and economic burden of patients. Perhaps morphological grading alone may not be reliable due to the difference in sample size between the three age groups, we might combine time-lapse microscopy, metabonomics and protein profiles to comprehensively evaluate the quality of embryos and screen out the embryos with the most developmental potential, and thus promote successful IVF treatment outcomes.

The traditional blastocyst grading system including three morphologic parameters: the degree of blastocoel expansion, the consistency of ICM and TE. Until now, there are conflicting data regarding

which parameter are the most indicator to predict the outcome of blastocyst transfer. Some researchers have reported that ICM morphology can statistically significantly predict LBR [21, 22], because ICM is differentiated into fetal, so ICM grade should theoretically be the most important morphologic feature influencing transfer outcomes. While recent publications in human have shown that TE quality should be corrected with viability[23, 24]. This may due to TE become into the placenta, and healthy trophectoderm is required to have the capacity to invade the endometrium to initiate the complex process of implantation and to maintain normal pregnancy progress. We also found that in the younger population, embryos with TE grade B are associated with a higher LBR compared with embryos with lower morphology grading. At the same time, some researchers noted that the degree of blastocoel expansion to be a strong predictor of successful embryo implantation. Because of the very small sample size of euploid blastocysts with blastocoel expansion grades 5 and 6, we did not investigate the impact of blastocoel expansion on LBR. It should be noted that these studies did not confirm that the blastocysts being transferred were euploid. In contrast, Capalbo et al.[25] determined that none of the morphologic parameters provides additional valuable information for PGT-A cycles to select the best developmental embryos for transfer. Conform to our main results, the ICM and TE grade were not correlated with the LBR in women more than 30 years. This may be due to we only included small sample size, therefore this conclusion may not represent the general population.

In addition, there is also an ongoing debate on the live birth rate of the timing of the blastulation of blastocyst. In a retrospective cohort study they reported that live birth rates were significantly higher with Day 6 compared with Day 5 blastocysts, regardless of embryo quality [26]. Irani et al. previously demonstrated that Day 5 blastocysts yielded a significantly higher LBR than Day 6 embryos of similarly graded euploid blastocysts[18].However, our data showed that the timing of the blastulation of a euploid embryo does not influences the LBR. The possible explanation for our results is that, morphologic grading may be a better indicator of euploid embryo development than the speed of blastulation in predicting LBR.

The strength of our research are as follows. First, all embryos and cycles were performed at a single reproductive medical center. Second, embryo scoring was conducted by the same team of four highly trained embryologists and each with five years of experience. Third, we only transfer single euploid embryo that underwent first autologous PGT-A treatment, this may eliminate factors which we have known can influence our outcomes. The present study also has some limitations. First, its retrospective nature that cannot be neglected. Second, if more than one euploid embryo is available for transfer, blastocysts with good quality are usually preferred when we selected blastocysts. Thus, this may cause selection bias. Third, the number of cases in each embryo quality category were relatively small. Large prospective or sample size analysis are required to validate our current findings in the future studies.

Conclusion

In conclusion, this study provides guidance for reproductive medical center worker that the common morphologic parameters of blastocysts assessment should be also used to help in the selection of embryos in PGT-A cycles, especially in women younger than 30 years. Furthermore, in clinical practice, we can provide consulting services for relatively older patients, if they have no good quality euploid embryos for transfer, poor quality euploid embryo are also an option, because they will produce similar LBR.

Abbreviations

LBR
Live birth rate; FET:Frozen-thawed embryo transfer; NGS:Next generation sequencing; PGT-A:Preimplantation genetic testing for aneuploidy; ICM:Inner cell mass; TE:Trophectoderm; ART:Assisted reproductive technology; IVF:In vitro fertilization; PCR:Polymerase Chain Reaction; AMA:Advanced maternal age; RIF:Repeated implantation failure, RSA:Recurrent spontaneous abortion; AFC:Antral follicle count; BMI:Body mass index; FSH:Follicle stimulating hormone.

Declarations

Ethics approval and consent to participate

Administrative permissions were obtained from the Ethics Committee of the

Third Affiliated Hospital of Zhengzhou University to access the medical files

described in the study. All methods were carried out in accordance with the Declaration of Helsinki. And the study was approved by the Ethics Review Committee of the Third Affiliated Hospital of Zhengzhou University following reference number (2021-WZ-010).

All participants are exempted from informed consent to participate in this study by the Ethics Review Committee of the Third Affiliated Hospital of Zhengzhou University.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

LH and GYC proposed the design ideas, LN, RBN and ZYC acquired and analyzed the data, DYL, KHJ and ZYJ prepared all tables and figures, LN wrote the manuscript, LH revised the manuscript. All authors read and approved the final manuscript.

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Figures

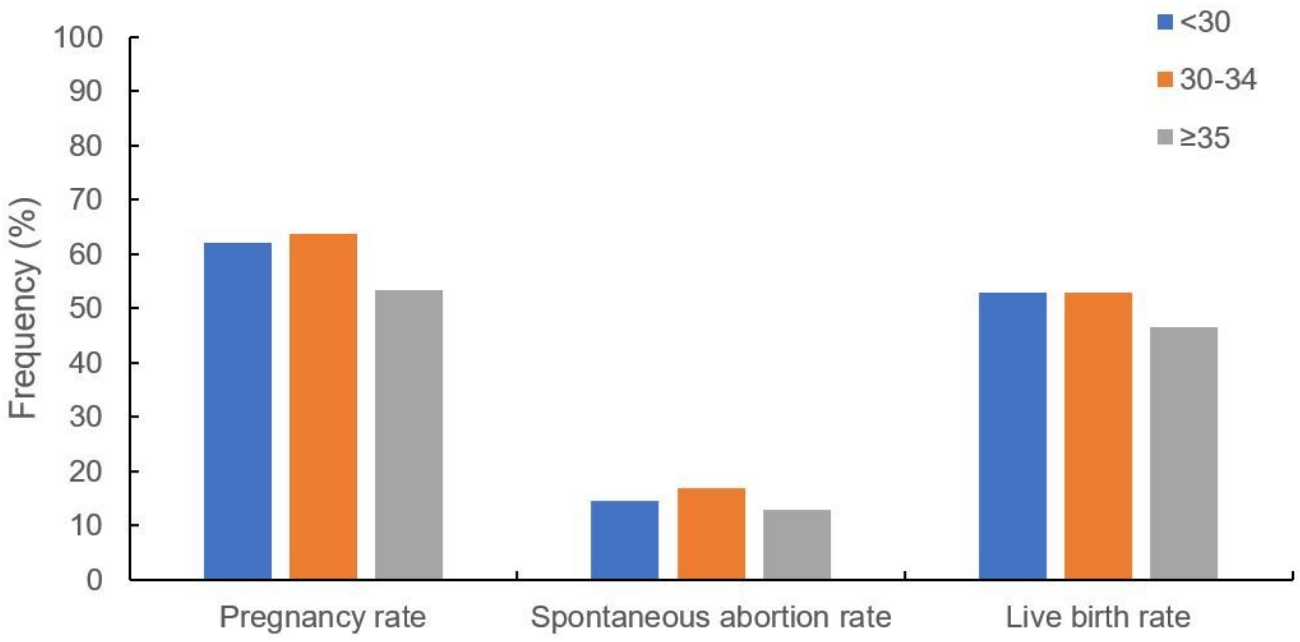

Figure 1

The association between pregnancy rate/spontaneous abortion rate/live birth rate and women's age.