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

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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 euploid blastocyst influence the live birth rate (LBR) following single frozen-thawed embryo transfer (FET) cycles?

Methods: Women who undergone first preimplantation genetic testing for aneuploidy (PGT-A) and following received single FET cycles from June 2017 to September 2019 were divided into three age groups (<30, 30-34 and ≥35 years). The primary outcome measure was LBR. Outcomes were compared to determine the association among different blastocyst quality (Good, Average and Poor), inner cell mass (ICM) grade (A and B), and trophectoderm (TE) grade (A, B and C) and LBR.

Results: In the youngest group (<30 years, n=86), LBR were compared between cycles with various blastocyst quality (72.22% for good quality, 54.55% for average quality and 34.78% for poor quality; *P*=0.019), ICM grade (70.59% for grade A and 42.03% for grade B; *P*=0.035) and TE grade (85.71% for grade A,57.58% for grade B and 34.78% for grade C; *P*=0.015). Similarly, in the 30-34 years group, LBR ranged from 50.00% for good quality to 45.45% for poor quality (*P*=0.870), from 35.29% for ICM grade A to 51.22% for ICM grade B (*P*=0.291), from 60.00% for TE grade A to 45.45% for TE grade C (*P*=0.634). Likewise, in the oldest group (≥35years, n=47), LBR were also comparable between these subgroups, but no significant differences were seen between blastocyst morphologic parameters and LBR (*P*>0.05).

Conclusion: LBR was associated with morphologic parameters of euploid blastocysts, especially in 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, a significant number of women 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].So women received ART treatment are rapidly aging simultaneously. Studies have also shown that the high incidence of embryo aneuploidy is one of the important factors affecting in vitro fertilization embryo transfer (IVF-ET) success [3, 4], which manifested as about 50% of embryos occurring errors during gametogenesis and early mitotic divisions throughout their preimplantation development. To our knowledge, blastocysts morphology alone cannot accurately evaluate ploidy status, because more than half of embryos with high morphological scores were eventually screened as aneuploidy[5].

As the advances of molecular biology and its relative techniques, and the aim of ART is always to give birth to a healthy baby, since then the third-generation IVF technology PGT-A was introduced, which allows patients to avoid the transfer of aneuploid embryos and thus improve implantation rate and

decrease the number of transferred embryos and 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, repeated implantation failure, recurrent spontaneous abortion and severe teratozoospermia[8].

Routine morphologic assessment has been a relatively primary method of embryos selection in IVF. Most of the previous studies have focused on the relationship between embryo quality and pregnancy outcomes, which did not exclude the influence of embryo aneuploidy. Until now, there are few studies discussed the impact of euploid embryos transfer after PGT-A on pregnancy outcomes. In a retrospective cohort study by Minasi et al. [5], the embryos with good quality have a statistically significantly higher implantation failure (IR) than those with poor quality. Nevertheless, Anderson et al. demonstrated that as long as single euploid embryo is transferred, the higher occurrence of implantation and live birth are attained irrespective of embryo quality[9]. Thus, the quality of euploid embryos for clinical outcomes are still needs further explore. On the basic of previous researches, we investigated whether the morphologic parameters of euploid blastocysts associated with LBR in single FET cycles, 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 was performed at the Reproductive Medical Center of the Third Affiliated Hospital of Zhengzhou University between June 2017 and September 2019. Women who undergone first autologous PGT-A due to advanced age (≥38years) or had a history of more than two unsuccessful IVF-ET cycles or recurrent spontaneous abortion and then followed by single euploid FET cycles were enrolled. Finally, only 232 cycles were found to be eligible for inclusion in the data analysis, as shown in Figure 1. 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 underwent a standard ovarian stimulation, trigger of oocyte maturation, oocyte retrieval, fertilization, embryo culture and transfer. 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[10]. 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; and (6) a hatched blastocyst, with the blastocyst completely escaping from the zona. 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. The ICM was graded as follows: (A) tightly packed, with many cells; (B)loosely gathered, with several cells; and (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. 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 based on the time of blastulation. 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[11]. 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)[12]. The vitrified-warmed procedure has been described in detailed previously[13].

Endometrial preparation

Embryos that were screened by NGS to be euploid were transferred in FET cycles. In general, women with regular ovulatory cycles underwent natural FET cycles and the artificial cycles were applied for women with irregular menses, ovulation dysfunction or thinner endometrial thickness. After five days ovulation and/or when endometrial thickness was ≥7 mm, which were all monitored by vaginal ultrasound, only single selective frozen-thawed euploid blastocyst was transferred and then all patients provided for conventional luteal support.

Outcome measures and statistical analysis

All statistical results were calculated with SPSS 25.0 statistical software (IBM, United States). Using Empower Stats software to draw the smooth curve fitting diagram between maternal age and live birth rate and to analysis the threshold effect. LBR after the transfer of euploid embryos are we mainly discussed measure in this study. The secondary outcome measures are pregnancy rate (PR) and early spontaneous abortion rate (SAB). The LBR was defined as the number of live births divided by the sum of embryos transferred cycles included in the cohort. The PR was defined as the percentage of the intrauterine gestational sac with fetal heartbeat by all transferred embryos at 4 weeks after blastocyst transfer. The SAB 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 Chi-square (X^2) and 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. The odds ratio (OR) 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 232 cycles followed by FET met the study inclusion criteria. The total LBR is 48.48% (112/231). The association between women's age and LBR was presented in Figure 2, the LBR reached highest in women aged 30 years and then declined gradually with women's age, although this difference is not statistically significant after adjusting for age, BMI, FSH, AMH, Endometrial thickness on transfer day and type of infertility (odds ratio [OR] 1.0; 95% confidence interval [CI], 0.9~1.0, P=0.253).

According to this result, all cycles were categorized into three groups:<30 years old (n=88), 30-34 years old (n=99), ≥35 years old (n=47). 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 BMI, FSH, endometrial thickness on transfer day, infertility diagnosis, day of TE biopsy, blastocysts with no results and euploidy rate among the three age groups. The number of blastocysts biopsied and euploid blastocysts were highest in the youngest age group and decreased gradually with women's age. The proportion of secondary infertility was highest in the oldest age group (P<0.001).

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

Characteristic	<30 (n=86)	30-34 (n=99)	≥35 (n=47)	*P* value
Age(years)	26.79±2.09	31.52±1.27	38.74±3.23	<0.001
BMI(kg/m^2)	24.30±2.94	24.07±2.92	24.20±3.02	0.639
FSH(IU/L)	6.37±2.53	6.52±2.02	7.14±2.47	0.598
AMH(ng/ml)	5.35±3.97	5.36±3.95	4.99±3.83	0.866
Endometrial thickness on transfer day(mm)	9.22±1.37	8.85±1.69	8.97±1.65	0.273
Type of infertility, n (%)				<0.001
Primary	44(51.16)	29(29.29)	4(8.51)	
Secondary	42(48.84)	70(70.71)	43(91.49)	
Infertility diagnosis, n (%)				0.587
Female factor	24(27.91)	40(40.40)	17(36.17)	
Male factor	28(32.56)	31(31.31)	13(27.66)	
Combined factor	25(29.07)	18(18.18)	12(25.53)	
Unexplained	9(10.47)	10(10.10)	5(10.64)	
FET endometrial preparation, n (%)				0.697
Natural cycles	34(39.53)	63(63.63)	32(68.09)	
Artificial cycles	52(60.47)	36(36.37)	15(31.91)	
Day of TE biopsy, n (%)				0.379
Day 5	45(52.33)	62(62.62)	28(59.57)	
Day 6	41(47.67)	37(37.38)	19(40.42)	
Blastocysts biopsied	4.55±2.09	4.52±2.15	3.49±1.86	0.010
Euploid blastocysts	2.12±1.46	2.22±1.28	1.57±0.80	0.001
Blastocysts with no results	0.06±0.44	0.08±0.34	0.06±0.32	0.916
Euploidy rates	182(46.91)	213(49.53)	74(46.54)	0.696

The effects of women's age on the pregnancy rate and spontaneous abortion rate were also evaluated in Figure 3, there was no significant difference in the PR (*P*=0.911) and SAR between different age groups (*P*=0.365).

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 72.22% for good quality, 54.55% for average quality and 34.78% for poor quality (*P*=0.019). Nevertheless, the blastocyst quality did not affect LBR in the other two age groups. In women aged 30-34 years old, LBRs was 50% for good quality, 51.52% for average quality and 45.45% for poor quality (*P*=0.870). Similarly, the oldest patients (≥35 years) had comparable LBR, ranging from 57.14% to 42.11% (*P*=0.671).

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:70.59% vs. 42.03% (*P*=0.035) in women younger than 30 years old. In the same way, 51.22% vs. 35.29% (*P*=0.291) in women aged 30-34 years old and 62.50% vs. 46.15% (*P*=0.261) in women aged ≥35 years.

Likewise, the effect of different TE grades were also had a relationship with LBR in youngest women (<30 years old), which ranged from 85.71% to 34.78% (*P*=0.015). But in women aged 30 years or older, TE grade did not influence LBR, which ranged from 60% to 45.45% in 30-34 age group (*P*=0.634) and 54.17% to 42.11% in patients aged more than 35 years old (*P*=0.821).

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

Age	<30 (n=86)	30-34 (n=99)	≥35 (n=47)
Embryo quality			
Good (n=47)	72.22	50.00	42.86
Average (n=76)	54.55	51.52	57.14
Poor (n=109)	34.78	45.45	42.11
P value	0.019	0.870	0.671
ICM grade			
A (n=42)	70.59	35.29	62.50
B (n=190)	42.03	51.22	46.15
P value	0.035	0.291	0.261
TE grade			
A (n=26)	85.71	60.00	50.00
B (n=97)	57.58	47.50	54.17
C (n=109)	34.78	45.45	42.11
P value	0.015	0.634	0.821

Discussion

This study determined the correlation between euploid blastocyst morphology and LBR following FET cycles. We found that the gradually decline with age in LBR in women older than 30 years old. In the light of this, we further indicated that the LBR was associated with morphologic parameters of euploid blastocyst in women aged < 30 years, but in women aged 30 years or older, the LBR do not influence by euploid blastocyst morphology.

In general, the maternal age is one of key factors determining the possibility of pregnancy outcome either in ART conception or spontaneous conception[14]. Most importantly, the reason for age-related decline in reproductive ability is contributed to the decrease of functional ovarian reserve and the increase of aneuploidy with advancing women's age. So it seems reasonable that we speculated the effect of maternal age on pregnancy outcome is eliminated after PGT-A. It is in line with our expectation, the PR and SAR were comparable between three age groups. But from the association between women's age and LBR, we can see the LBR are highest in younger women and then start to gradually decline in women older than 30 years of age, although the difference is not statistically significant. 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 this condition, we assume that the women's age maybe important confounding factors affecting LBR. Given that we divided all patients into three age group to investigated whether the euploid blastocysts morphologic parameters influence 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 reason 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[15, 16]. Irian et al.[17] confirmed that good quality euploid embryos were associated with a higher IR 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[18]. 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. Suggesting 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. However, there was no significant difference in the LBR of euploid blastocyst regardless of their morphology quality in older women. 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[19]. Emphasizing that the poor quality euploid embryos can also develop well. So, poor quality embryos should not be discarded, which can reduce the transfer cycles and economic burden of patients. Perhaps morphological grading alone may not be reliable, 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 [20, 21], 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[22, 23]. 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. At the same time, some researchers noted that the degree of blastocoel expansion to be a strong predictor of successful embryo implantation. It should be noted that these studies did not confirm that the blastocysts being transferred were euploid. The very small sample size of euploid blastocysts with blastocoel expansion grades 5 and 6, so we did not investigate the impact of blastocoel expansion on LBR. We found that in the younger population, embryos with a better grading of ICM and TE are associated with a higher LBR compared with embryos with lower morphology grading. Therefore, we think both the inner cell mass and the trophectoderm are good predictors for evaluating the LBR. Conversely, the ICM and TE grade were not correlated with the live birth in women more than 30 years, this is conform to our main results. In agreement with our findings, Capalbo et al.[24] also determined that none of the morphologic parameters provides additional valuable information for PGT-A cycles to select the best developmental embryos for transfer. This may be due to we only included small sample size, therefore this conclusion may not represent the general population.

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 and we do not analysis the association between blastocyst development rate and LBR. 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 general, 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; IR: Implantation rate; ART: Assisted reproductive technology; IVF: In vitro fertilization; PCR: Polymerase Chain Reaction; AFC: Antral follicle count; BMI: Body mass index; FSH: Follicle stimulating hormone; PR: pregnancy rate; SAR: spontaneous abortion rate.

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 analysed 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 and KHJ 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

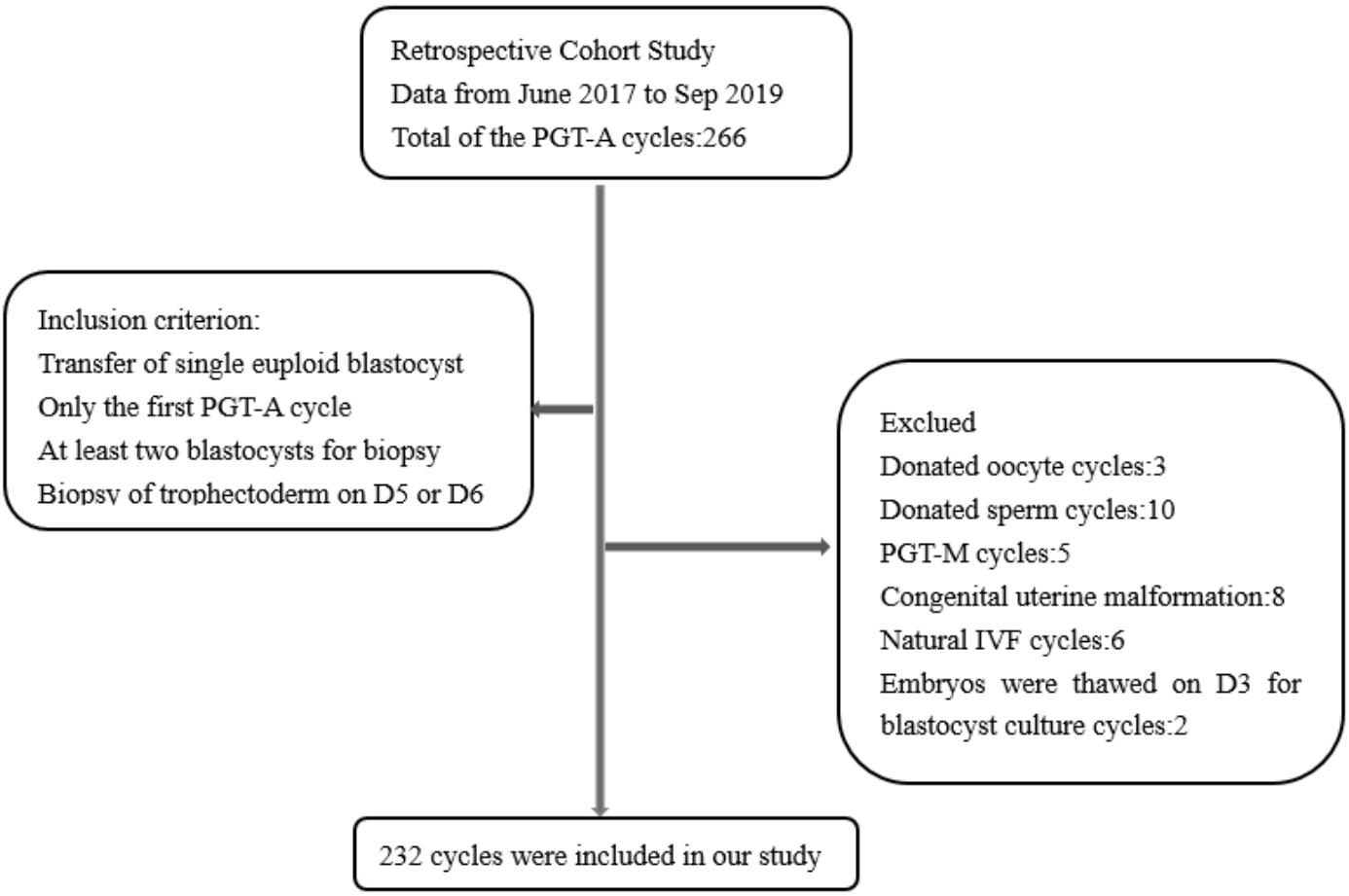

Figure 1

Data selection progress for analysis cycles utilizing preimplantation genetic testing for aneuploidy screening.

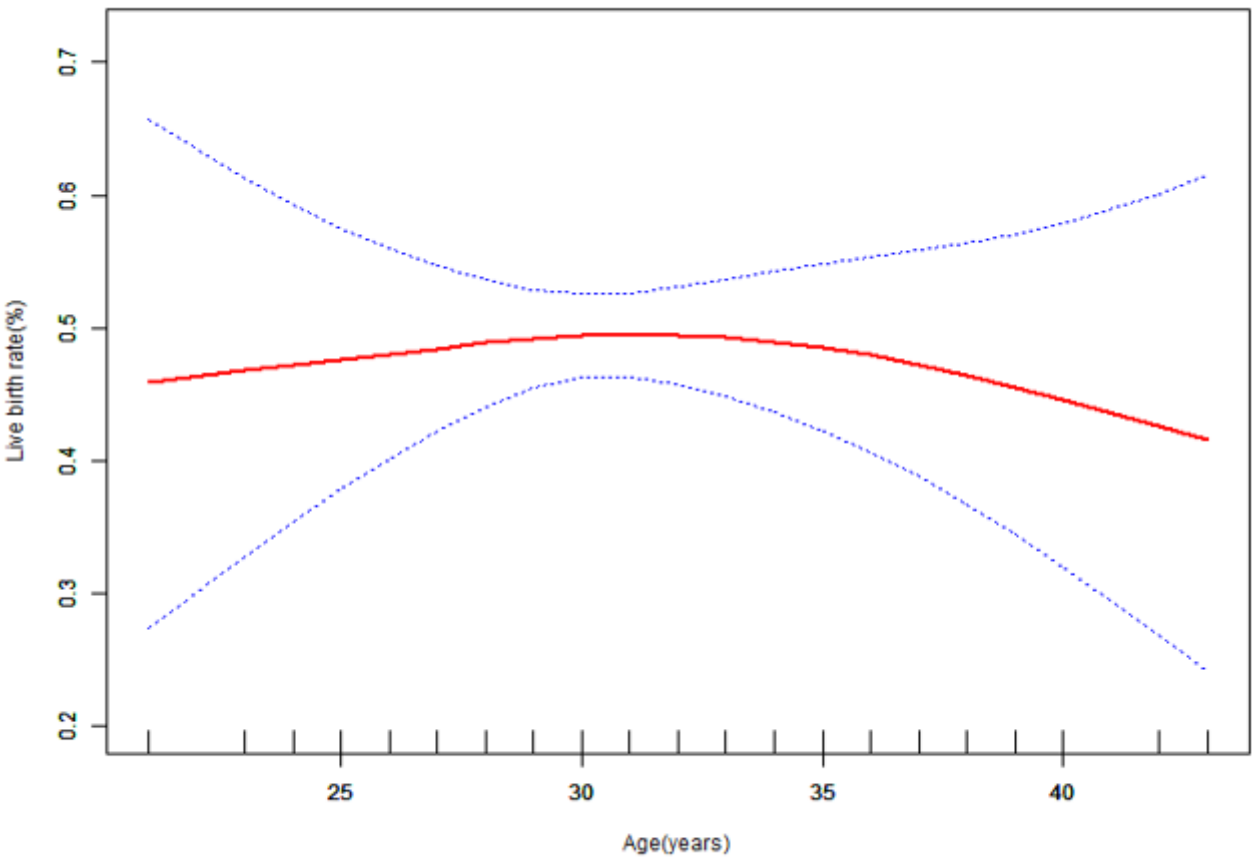

Figure 2

Live birth rates according to women's age.

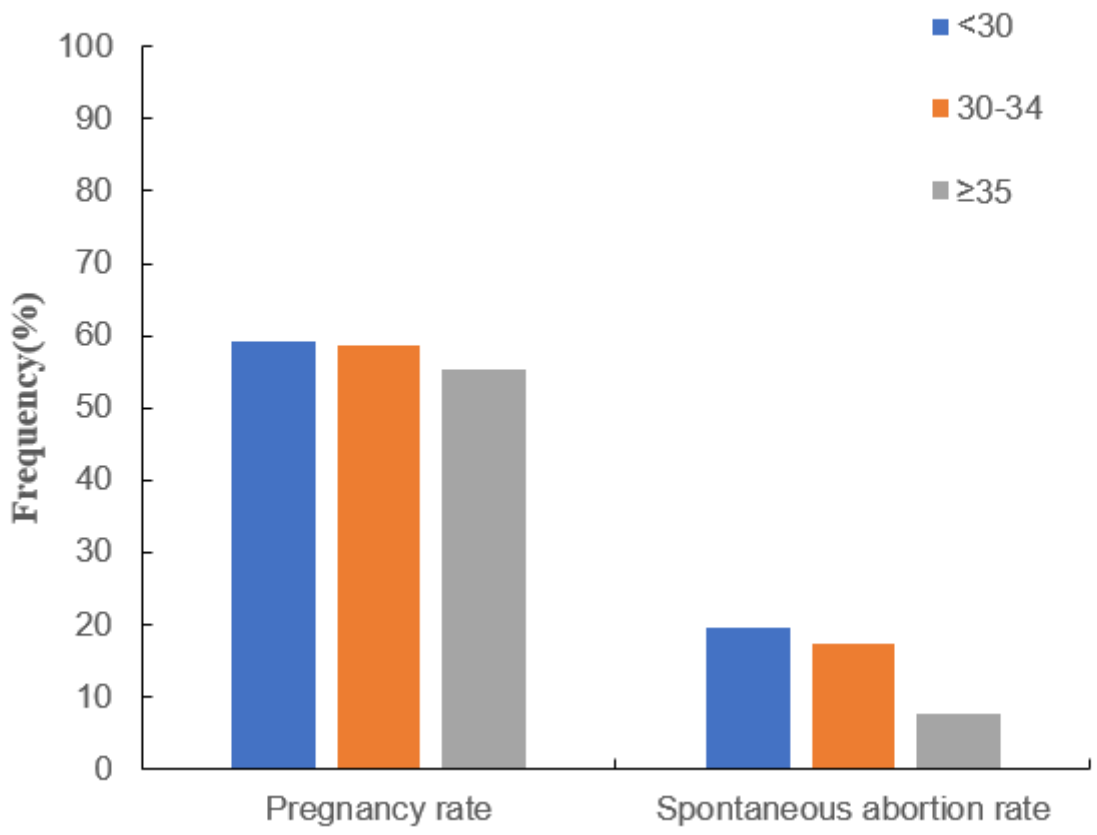

Figure 3

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