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Original Paper

Effect of Low-Quality Embryo on Adverse Obstetric and Perinatal Outcomes

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Abstract

Background: *This retrospective study focused on whether low-quality embryos have the risk on perinatal and obstetric outcomes.* ***Methods:*** *This study enrolled 600 women undergoing fresh embryos transfer (ET) cycles between June 2019 and December 2022. The patients were stratified into two groups, high-quality embryo group and low-quality embryo group. In both groups, the perinatal and obstetric outcomes were the primary outcomes. Moreover, we conducted a multi-variable logistic regression analysis, where additional possible confounding factors were controlled, to determine how diverse embryo qualities affected the primary outcomes.* ***Results:*** *The results showed that compared with the low-quality group, the high-quality group showed increased clinical pregnancy (63.33% vs 26.33%) as well as a higher number of live birth rates (52.67% vs 18.33%) ($P < 0.001$). There were no statistically significant differences in unfavorable perinatal and obstetric outcomes between high- and low-quality groups ($p > 0.05$). Similarly, the transfer of blastocysts developing from high-quality embryos led to increased clinical pregnancy rates (84.50% vs 48.05%, $p<0.001$) and live birth rates (74.64% vs 38.96%, $p<0.001$). Transfer of blastocysts developing from low-quality embryos did not impact the unfavorable perinatal or obstetric outcomes. The logistic regression analysis showed that low-quality could not increase the unfavorable perinatal or obstetric outcomes.* ***Conclusion:*** *In summary, low-quality ET does not increase the risk of unfavorable perinatal or obstetric outcomes. Overall, compared to low-quality embryos, the transfer of high-quality embryos increases the clinical pregnancy and live birth rates.*

Keywords

Embryo quality, obstetric outcome, perinatal outcome

1. Introduction

In vitro fertilization-embryo transfer (IVF-ET) technology has gained popularity, and most couples see assisted reproductive technology (ART) as safe and reliable (Kaplan, Levy-Toledano, Davies, Roy, Howles, & Lass, 2021). This technology can improve the multiple pregnancy and live birth rates while eliminating unfavorable perinatal or obstetric outcomes. ART primarily aims to give birth to a robust baby without any maternal complications (Zhu, Lin, Gao, Wang, Wang, & Wang, 2020). Among the factors that can increase the IVF-ET success rate, embryo quality is the main factor with a prominent role in successful pregnancy (Shen, Long, Gao, Guo, Xie, Chen, Cong, Wang, Li, Si, Zhao, Lyu, Kuang, & Wang, 2020; Rhenman, Berglund, Brodin, Olovsson, Milton, Hadziosmanovic, & Holte, 2015). In most cases, clinically morphological scoring methods are employed to assess the quality of embryos, but these methods fail to completely and accurately reflect the embryo's developmental potential (Lagalla, Barberi, Orlando, Sciajno, Bonu, & Borini, 2015).

In the fertility treatment cycle, the clinical pregnancy rate of high-quality embryo transfer (ET) increases compared with low-quality ET; however, irrespective of the degree of any amelioration by a controlled ovulation stimulation program or embryo culture system, high-quality embryos acquired from every other cycle are limited (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011). In IVF-ET treatment, most patients may have low-quality embryos, while some may have high-quality embryos. So what should be the planting potential for such low-quality embryos? Should they be discarded, retained, or cultivated as blastocysts for quality selection? There is no unified guide or tool exists on this matter. Oron et al. reported that the low-quality embryo showed a comparable possibility to reach live birth to the high-quality one while achieving clinical pregnancy (Roberts, McGowan, Mark Hirst, Vail, Rutherford, Lieberman, & Brison, 2011). The transplantation of low-quality embryos may or may not increase the miscarriage rate, birth defect rate, low birth weight infants. Other adverse obstetric outcomes are a common concern of patients and medical workers.

This paper reviews the impact of fresh low-quality embryo transfer on pregnancy outcome to lay a certain foundation for the clinical application of low-quality embryos in ART.

2. Materials and Methods

Altogether, our retrospective study enrolled 600 fresh IVF-ET cycles conducted at the Center for Reproductive Medicine of The First Affiliated Hospital of Zhengzhou University, China, between June 2019 and December 2022. They were classified as high-quality ET group (300 cases, including 229 high-quality cleavage embryos and 71 blastocysts developing from high-quality cleavage embryo) and low-quality embryo group (300 cases, including 223 low-quality cleavage embryos and 77 blastocysts developing from low-quality cleavage embryo) according to embryo type. To minimize the selection bias due to the retrospective research, the Propensity Score Matching (PSM) method was used, and low- and high-quality ET groups were matched using the following matching criteria: patient age,

infertility type, infertility ay 3, high-quality embryos scored with Grade I or II was defined with 7, 8 or 9 blastomeres with equal-sized symmetrical cells with no fragmentation or less than 20% of fragmentation. Low-quality embryos were Grade III, embryos were within the developmental stage, and or showing retarded (slower) cleavage, equal-sized symmetrical cells with less than 20% fragmentation or 4-5 cells. The quality of the blastocysts was evaluated according to the Gardner grade (Van den Abbeel, Balaban, Ziebe, Lundin, Cuesta, Klein, Helmgaard, & Arce, 2013), taking into account the level of expansion and the development of the inner cell mass (ICM) and the trophectoderm (TEduration, acquired count of oocytes, fertilization number, embryo transfer number, method of fertilization, and the transferred embryo period. The matching tolerance is 0.02. The software automatically uses logistic regression to fit the independent variable and the dependent variable, calculate the ps value of each sample, and complete the pairing.

2.1 Ovarian Stimulation

In the fresh IVF-ET cycles, controlled ovarian stimulation (COS) was performed with human menopausal gonadotropin HMG, recombinant FSH. The dose of gonadotropins and stimulus protocol were determined on an individual basis according to the female's age, hormone level, and ovarian reserve. When ultrasound detection found more than three follicles with a diameter of ≥16 mm, the patient was given 5000–10000u HCG (Serrano, Switzerland). After 36 h, the oocytes were retrieved under the guidance of vaginal ultrasound. Luteal support was commenced on the day after oocyte retrieval, using 60mg of progesterone progesterone intramuscular injection (Xianju Pharmacy, Zhejiang, China). The retrieved oocytes were cultured in an environment of 6% CO2, 5% O2, and 89% N2. Intracytoplasmic sperm injection (ICSI) or IVF was adopted for oocyte fertilization.

2.2 Embryo Quality

Embryo quality was evaluated based on the embryo cleavage stage classification system, Embryo morphology at Day 3 is graded according to: number , multi-nucleation, size and symmetry, diopter and the cellular fragmentation of the blastomeres (Puissant, Van Rysselberge, Barlow, Deweze, & Leroy, 1987; Baczkowski, Kurzawa, & Głabowski, 2004). In addition, embryos were classified according to their cleavage stage in relation to the day of embryo transfer (Day2: 4-cell, Day3: 8-cell). On D). Based on this classification, we defined blastocyst grading was good, fair, or poor quality. A good grade was assigned for ICM grade A and TE grade A or B (AA or AB blastocysts). A fair grade was assigned for ICM grade B and TE grade A, B, or C (BA, BB, or BC blastocysts). A poor grade was assigned for any ICM grade C (CC or CB blastocysts). Embryos were defined as high-quality embryos group at grades I/II cleavage stages, and low-quality ones at grades III. All cleavage embryos were graded by three experienced embryologists.

2.3 Embryo Culture

The day of fertilization is defined as Day 0. Embryos were cultured individually in G1 PLUS (Vitrolife) medium until day 3. Subsequently, cleavage embryo were transferred to fresh G2 PLUS (Vitrolife)

medium for further culture up to day 5 or day 6. Finally cultured to blastocyst stage, and the culture environment was 37◦C, 6% CO2, 5% O2, and 89% N2.

2.4 Clinical Outcomes

Cycle outcome was evaluated as clinical pregnancy, which included miscarriage, ectopic pregnancy, and live birth. Live birth was deemed as the primary pregnancy outcome in this study, and it referred to the birth of a live baby after 23 gestational weeks. Perinatal outcomes were obtained according to telephone follow-up, including unadjusted birth weight, very low birth weight (VLBW, <1500 g), low birth weight (LBW, <2500 g), preterm birth (PTD <37 weeks), birth defect number and maternal complications, ingcluding diabetes, placental abruption, pregnancy induced hypertension, etc.

2.5 Statistical Analysis

SPSS version 23.0 was used for statistical analyses to check normality assumptions and to ensure adequate variability. Continuous variables were expressed as means ±SD, and the basic characteristics and perinatal outcomes were compared by ANOVA, whereas the chi-square test was performed to analyze categorical variables that were expressed as a percentage. The relationship of embryo quality with perinatal and obstetric outcomes was evaluated by logistic regression after adjusting all the confounders, such as maternal age, embryo quality, method of fertilization, basal follicle-stimulating hormone (FSH) level, infertility duration, stage of embryo development, obtained oocyte counts, cleavage oocyte counts, and fertilized oocyte counts. A difference of $P<0.05$ stood for statistical significance.

3. Results

In the current study, 600 fresh ET cycles (300 high-quality, 300 low-quality) were enrolled, leading to 269 pregnancies and 213 births. Table 1 presents the demographic and therapeutic features of the enrolled patients. The high-quality ET group had remarkably increased average basic LH level, obtained oocyte number, cleavage oocyte number, and fertilized oocyte number compared with the low-quality group ($p<0.05$). But the high-quality ET group with lower basic FSH level ($P<0.001$). In addition, there were no statistically significant differences in maternal BMI, maternal age, infertility duration, endometrial thickness, transferred embryo number, method of fertilization, type of infertility, and stage of embryo development between the two groups ($P>0.05$).

Table 1. Basic information of Patients with High or Low-quality Embryo Transfers

	Transfer high-quality embryo (n=300)	Transfer low-quality embryo (n=300)	*P* value
Maternal age(y)	32.60±5.43	32.25±5.56	0.244
Maternal BMI(kg/m^2)	23.41±3.29	22.90±3.17	0.091
Duration of infertility(y)	4.22±3.00	4.38±3.37	0.498

Number of oocytes retrieved	11.91±6.75	8.47±5.13	0.000
Number of fertilized oocytes	8.83±5.93	4.79±3.50	0.000
Number of cleavage oocytes	8.63±5.76	4.53±3.35	0.000
Number of transfer embryos	1.28±0.45	1.36±0.48	0.054
Basal FSH(IU/L)	6.77±2.36	7.74±3.17	0.000
Basal LH(IU/L)	6.22±8.47	5.07±3.90	0.004
Type of infertility, n (%)			0.624
Primary infertility	146(48.67)	152(50.67)	
Second infertility	154(51.33)	148(49.33)	
method of fertilization			0.133
IVF	190(63.33)	172(57.33)	
ICSI	110(36.67)	128(42.67)	
Stage of embryo development			0.570
Cleavage-stage embryo	229(76.33)	223(74.33)	
Blastocyst	71(23.67)	77(25.67)	
Endometrial thickness(mm)	11.89±2.87	11.92±2.63	0.785

Abb: BMI, body mass index; ICSI, intracytoplasmic sperm injection. Results were expressed in mean±SD and proportion (%) in the above Table.

Table 2 summarizes the obstetric and perinatal outcomes for high-and low-quality ET groups. The High-quality ET group had a significantly increased clinical pregnancy rate (63.33% vs. 26.33%, p <0.001) and live birth rate (52.67% vs. 18.33%, p<0.001) compared to the low-quality ET group. After achieving pregnancy, the low-quality ET group showed an increased miscarriage rate (25.31% vs. 15.26%, p = 0.052) and ectopic pregnancy rate (5.06% vs. 1.57%, p = 0.102). Besides, differences in perinatal outcomes and perinatal outcomes were not statistically significant between the two groups (p>0.05). One birth defect in the high-quality embryo transfer group was congenital diaphragmatic hypoplasia.

Table 2.Obstetric and Perinatal Outcomes of High- and Low- quality Embryo-transfer Group

	Transfer high-quality embryo (n=300)	Transfer low-quality embryo (n=300)	*P* value
Clinical pregnancy, n (%)	190/300(63.33)	79/300(26.33)	0.000
Live birth, n (%)	158/300(52.67)	55/300(18.33)	0.000
Other outcome variables	n=190	n=55	
Miscarriage, n (%)	29/190(15.26)	20/79(25.31)	0.052
Ectopic pregnancy n (%)	3/190(1.57)	4/79(5.06)	0.102

Preterm delivery, n (%)	17/190 (8.95)	2/79(2.53)	0.061
Birthweight, (g)	3269.36±615.91	3239.14±572.03	0.647
LBW < 2500g, n (%)	18/190(9.47)	5/55(9.10)	0.401
VLBW < 1500g, n (%)	2/190(1.05)	1/79(1.23)	0.879
Number of birth defect	1	0	/
Maternal complications,n (%)	6/190(3.15)	4/79(5.06)	0.452

Data are presented as a proportion (%).

Table3 summarizes neonatal and perinatal outcomes in the blastocysts developing from high-and low-quality cleavage embryo groups. The blastocysts developing from the high-quality cleavage embryo group had a remarkably increased clinical pregnancy rate (84.50% vs.48.05%, p<0.001) and live birth rate (74.64% vs. 38.96%, p<0.001) compared with the blastocysts developing from low-quality ET group. Differences in preterm delivery and miscarriage rate showed no statistical significance (p>0.05). In addition, differences in all perinatal outcomes were not statistically significant between the two groups (p>0.05). The birth defect was not seen in any group.

Table 3. Obstetric and Perinatal Outcomes of Blastocysts Developing from High-or Low-quality Cleavage-stage Embryo

	Transfer blastocysts developing from high-quality cleavege embyro (n=71)	Transfer blastocysts developing from low-quality cleavege embyro (n=77)	*P* value
Clinical pregnancy, n (%)	60/71(84.50)	37/77(48.05)	0.000
Live birth, n (%)	53/71(74.64)	30/77(38.96)	0.000
Other outcome variables	n=60	n=37	
Miscarriage, n (%)	7/60(11.66)	5/37(13.51)	0.788
Ectopic pregnancy n (%)	0	1/37(2.70)	0.201
Preterm delivery, n (%)	3/60(5.00)	1/37 (2.70)	0.580
Birthweight, (g)	3321.69±485.31	3308.07.39±594.55	0.788
LBW < 2500g, n (%)	1/60(1.66)	3/29(6.90)	0.100
Maternal complications, n (%)	2/60(3.33)	3/37(8.11)	0.302

Data are presented as a proportion (%).

Logistic regression analysis is shown in Table 4. Relative to the high-quality ET group, the low-quality ET group had reduced clinical pregnancy rate (OR = 0.191, 95%CI: 0.122–0.301) and live birth rate (OR = 0.202, 95%CI: 0.126–0.324), when all the possible confounders were adjusted, including maternal age, embryo quality, method of fertilization, basal FSH level, infertility duration, stage of

embryo development, obtained oocyte number, cleavage oocyte number, and fertilized oocyte number. Meanwhile, differences in miscarriage rate, preterm delivery rate, ectopic pregnancy rate, LBW and maternal complications were not statistically significant between the two groups ($p>0.05$).

Table 4. Logistic Regression Analysis for Obstetric and Perinatal Outcomes with High and Low-quality Embryo

	OR(95%CI)	*P* value	Adjusted OR (95%CI)	Adjusted *P* value
High-quality embryo	1.0		1.0	
Low-quality embryo				
Clinical pregnancy, n (%)	0.207(0.146,0.293)	0.000	0.191(0.122,0.301)	0.000
Miscarriage, n (%)	0.054(0.989,3.580)	0.054	1.831(0.863,3.888)	0.115
Preterm delivery, n (%)	0.264(0.060,1.172)	0.080	0.504(0.101,2.508)	0.402
Live birth, n (%)	0.202(0.139,0.292)	0.000	0.202(0.126,0.324)	0.000
Ectopic pregnancy n (%)	0.122(0.727,15.211)	0.122	1.478(0.227,9.631)	0.683
LBW < 2500g, n (%)	0.646(0.231,1.804)	0.404	0.573(0.180,1.825)	0.346
Maternal complications, n (%)	0.611(0.168,2.229)	0.456	1.001(0.174,5.771)	0.999

Obstetric and perinatal outcomes were adjusted for confounders such as maternal age, embryo quality, method of fertilization, basal FSH level, infertility duration ,stage of embryo development, obtained oocyte number, cleavage oocyte number, and fertilized oocyte number. A high-quality embryo transfer group is the reference group.

4. Discussion

In human-assisted reproductive technology for infertility treatment, embryo quality is the main factor affecting pregnancy outcomes. However, several novel methods have evolved to evaluate embryo development potential, such as a real-time observation system of embryo dynamics and metabolite detection within the culture medium. Morphological scoring remains the primary means to evaluate embryo quality and select transplanted embryos (Simopoulou, Sfakianoudis, Tsioulou, Rapani, Maziotis, Giannelou, Grigoriadis, Pantou, Nikolettos, Vlahos, Pantos, & Koutsilieris, 2019; Ma, Mochel, Pham, Yoo, Cho, & Digman, 2019). As embryogenesis represents the dynamic process, assessment of embryo morphology at a specific time point cannot cover the dynamic development process of the embryo, which reduces the objectivity and repeatability of embryo evaluation (Lagalla, Barberi, Orlando, Sciajno, Bonu, & Borini, 2015; Azzarello, Hoest, & Mikkelsen, 2012). Moreover, the embryo itself has plasticity and reparability, which can regulate a few abnormal chromosome cells, so that the low-quality embryo can continue to develop into a blastocyst. Therefore, it is challenging for

embryologists to judge embryos' developmental potential accurately only by morphological observation (Ma, Mochel, Pham, Yoo, Cho, & Digman, 2019; Azzarello, Hoest, & Mikkelsen, 2012).

The current study focused on exploring the commonly observed problems in the IVF-ET field. It remains undefined whether the low quality of cleavage ET increases the risk of perinatal and obstetric outcomes. In this research, for minimizing confounders, we performed 1:1 propensity score matching and classified all cases as high-quality ET group (n = 300) and low-quality ET group (n = 300). However, once the clinical pregnancy was achieved, differences in miscarriage rate, ectopic pregnancy rate, and preterm delivery rate showed no statistically significant difference between the two groups. Both groups also had no difference in the perinatal outcome, including birth weight, LBW, VLBW, the number of birth defects and maternal complications. Therefore, this study documented that the low-quality ET group had no increased risk of perinatal or obstetric outcome relative to the high-quality ET group. Our findings may increase the confidence of women with low-quality embryos. Similar to the following literature reports, a Canadian study compared 386 singletons during high-quality ET cycles with 54 matched low-quality ET cycles and reported that embryo quality showed no association with the perinatal outcomes, regardless of the small sample size (Oron, Son, Buckett, Tulandi, & Holzer, 2014). Zhu and colleagues compared 2586 singletons during double cleavage ET cycles between high-(n = 2487) and low-quality (n = 99) ET cycles, their results showed that transfer of poor-quality embryos did not increase the risk of adverse perinatal outcomes; however, the quality of cleavage stage embryos significantly affected the ongoing pregnancies (Zhu, Lian, Li, Chen, Liu, & Qiao, 2014). To our knowledge, the correlation of embryo quality in fresh cycles with perinatal outcomes remains unknown. However, some researchers contradicted the results by suggesting that embryo quality is significantly related to perinatal outcomes. Huang et al. compared 1854 singletons during high-quality ET cycles, and 549 matched low-quality ET cycles. They demonstrated that compared with the high-quality embryo group, the neonatal prognosis is worse and has a higher incidence of PTB and LBW, irrespective of blastocyst embryo transfer and the cleavage stage (Huang, Tao, Zhang, Yang, Wu, Kuang, & Wang, 2020).

Although Huang et al. did not attempt to examine the relationship of embryo quality with pregnancy outcomes, our findings demonstrated that the high-quality ET group had increased clinical pregnancy and live birth rates by almost twice compared with the low-quality ET group (63.33% and 26.33% vs.52.67% and 18.33%, respectively). Such result in the high-quality ET group was possibly associated with the increased obtained oocyte number, number of fertilized oocytes, and number of cleavage oocytes. Similar results were observed in the blastocysts transfer group. ET quality has been suggested to have an important impact on live birth and clinical pregnancy rates, regardless of blastocyst or cleavage-stage (Van den Abbeel, Balaban, Ziebe, Lundin, Cuesta, Klein, Helmgaard, & Arce, 2013; Zhu, Lin, Gao, Wang, Wang, & Wang, 2020; Spitzer, Haidbauer, Corn, Stadler, Wirleitner, & Zech, 2012).

Studies have reported that certain low-quality ET cycles reach the blastocyst stage, achieving successful pregnancies and delivering healthy babies (Sallem, Santulli, Barraud-Lange, Le Foll, Ferreux, Maignien, Bourdon, Chapron, de Ziegler, & Wolf, 2018; Shaw-Jackson, Bertrand, Becker, Colin, Beaudoin-Chabot, Rozenberg, & Autin, 2013). Our results corroborate the above results. By analyzing blastocysts that developed from cleavage embryos with diverse qualities, blastocysts developed from low-quality cleavage embryos still could achieve relatively high live birth and clinical pregnancy rates in our study. Differences in the miscarriage rate and preterm delivery in the two groups were not statistically significant ($p>0.05$). A similar conclusion was reached regarding the perinatal outcome. Our sample size was limited, and no birth defects were noticed in the two groups. However, blastocyst culture of low-quality cleavage embryos could screen out embryos with implantation potential, increase embryo utilization efficiency, and reduce embryo waste (Martins, Nastri, Rienzi, van der Poel, Gracia, & Racowsky, 2016; Guerif, Frapsauce, Chavez, Cadoret, & Royere, 2011).

This study emphasized questioning the commonly encountered issues by physicians in the clinical management of IVF. How can we deal with low-quality embryos? During the embryo culture period, several factors are associated with the low quality of the embryo, which includes factors specific to patients like maternal BMI and age, and low ovarian reserve; these factors may predict unfavorable perinatal and obstetric outcomes (Dobson, Lao, Michael, Varghese, & Jayaprakasan, 2018; Wennberg, Opdahl, Bergh, Aaris Henningsen, Gissler, Romundstad, Pinborg, Tiitinen, Skjærven, & Wennerholm, 2016). Embryo quality is suggested to be closely related to pregnancy outcomes, high-quality embryos can result in better pregnancy outcomes (Ma, Mochel, Pham, Yoo, Cho, & Digman, 2019; Zhu, Lin, Gao, Wang, Wang, & Wang, 2020; Lou, Li, Guan, Zhang, Hao, & Cui, 2021). Our logistic regression analysis showed that low-quality ET decreased live birth and clinical pregnancy rates when additional confounders were adjusted compared to high-quality embryos.

An obvious advantage is that this study was conducted at a large reproductive center, and embryos were rated by the same well-trained embryologists, which could avoid heterogeneity in embryo quality standards among the centers. The embryo culture was performed in the same medium, eliminating the possible bias or effect of different media on newborn birth weight (Bick, Nielsen, & Knudsen, 2021; Gu, Deng, Gao, Wang, Ding, Xu, & Zhou, 2016). Nonetheless, certain limitations exist in this study. First, this was a retrospective study with a small sample size. Second, embryo scoring was relatively subjective. Third, the rarity of low-quality ET cycles restricted our research results. Also, this study did not consider the effect of different ovarian stimulation programs on pregnancy outcomes.

Overall, in the fresh embryo transfer cycle, in the absence of any high-quality embryo, transferring a low-quality cleavage embryo or its developed blastocyst can grow in a healthy baby, which can be used and should not be given up. Although we conclude that low-quality ET does not increase the risk of unfavorable perinatal or obstetric outcomes, ample scope exists to comprehensively understand the relationship of embryo quality with the unfavorable perinatal or obstetric outcome. The association of embryo quality with unfavorable perinatal or obstetric outcomes needs a larger sample size to verify.

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