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The effect of transferring a low-quality embryo along with a high-quality embryo on the pregnancy outcome

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Abstract

Background Previous evidence suggests that low-quality embryos may send negative signals to the endometrium and affect the receptivity of the endometrium. This study aimed to evaluate the influence of transferring an additional low-quality embryo with a high-quality embryo on the pregnancy outcome.

Methods A total of 1506 fresh embryo transfer cycles between January 2018 and June 2020 were included. The patients were separated into two groups: a single embryo transfer group (SET, patients receiving a single high-quality embryo) and a double embryo transfer group (DET, patients receiving a high-quality embryo and a low-quality embryo). Main outcome measures including multiple pregnancy rate and live birth rate were discussed.

Overall, in the primary analysis, patients who receive an additional low-quality embryo improved the live birth by 8.7% and multiple pregnancy rate by 10.0%. In women aged less than 35 years, compared with SET, DET increased the birth rate by 6.0% but resulted in a 13.5% increase in multiples. Women of 35 years above, adding a low-quality embryo increased the live birth rate by only 2.2% but increased multiples by 14.7%. In patients with one cycle of ET, the same results were obtained. In patients with multiple cycles of ET and adding a low-quality embryo, the live birth rate was similar to SET but with a 14.7% increase in multiples.

Conclusions Compared to DET, we prefer to transfer a high-quality embryo. Nevertheless, in women 35 years or older or in patients with multiple cycles of embryo transfer, adding a low-quality embryo did not significantly improve live birth but increased the multiple rate.

Keywords Embryo quality, Live birth rate, Multiple pregnancy rate

Introduction

Since the first IVF baby was born in China, assisted reproductive technology (ART) has become a routine way for the treatment of infertile couples [1]. There are many factors that affect the success rate of IVF-ET, such as embryo quality and patient-specific parameters, among them embryo quality is a crucial factor influencing the success of pregnancy [2]. The ultimate goal of ART is the birth of a healthy offspring being born at full-term gestation [3]. Although SET is the recommended approach during IVF treatment to achieve the ultimate goal, DET still holds a dominant position [4, 5], especially for patients with a poor prognosis and fewer high-quality embryos available. To improve the success rate of treatment, the transfer of a low-quality embryo plus a high-quality embryo is still generally considered by patients and professionals [6].

Evidence suggests that endometrial stromal cells can distinguish between high-quality and low-quality embryos and select abnormal embryos to prevent

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them from implanting [7, 8]. The existence of abnormal embryos may trigger this selection by the endometrium, which may lead to failure of implantation in high-quality embryos [9]. This phenomenon is known as embryo-endometrial "crosstalk" [10]. Due to the high incidence of abnormal chromosomes in the process of human reproduction, this phenomenon may effectively protect women against the risk of abnormal pregnancy [9, 11]. Although protective, this phenomenon could be potentially harmful to patients with a poor prognosis [12]. It is likely that the low-quality embryo might send aberrant signals to the endometrium, resulting in a rejection response and detrimental reproductive outcomes of the co-transferred high-quality embryo. Is the signal transmission between high-quality embryos and the endometrium truly disrupted by low-quality embryos?

Transferring a low-quality embryo and a high-quality embryo together is a common problem in IVF. The mechanism underlying embryo-endometrial "crosstalk," however, remains unclear. Despite previous retrospective research have discussed this issue, the sample sizes of those studies were small. Furthermore, previous studies did not stratify patient by age or the ET cycle rank. Additionally, there is no discussion on the rate of preterm birth. Therefore, whether we should transfer a low-quality embryo along with a high-quality embryo needs to be re-evaluated. We designed this study with the overarching objective of exploring the question of whether low-quality embryos have an adverse impact on high-quality embryos when transferred together.

Materials and methods

Study design and patients

This study retrospectively analyzed fresh embryo transfers on day 3 after fertilization that were conducted at the Reproductive Center of The First Affiliated Hospital of Zhengzhou University from January 2018 until June 2020. The study set two groups: 732 double embryo transfers (DETs) with one high-quality embryo plus one low-quality embryo and 774 single embryo transfers (SETs) with a high-quality embryo. The exclusion criteria were as follows: (a) maternal age or paternal age > 45 years, (b) endometrial thickness ≤ 7 mm, (c) excluded cycles include with missing clinical data or patients lost to follow-up, (d) cycles with donor oocytes or preimplantation genetic diagnosis (PGD).

Ovarian stimulation

In the fresh cycles, according to the female's age, hormone level and ovarian reserve choose the corresponding ovulation induction plan (including super-long scheme, long plan and antagonist prescription case). Controlled ovarian stimulation (COS) was performed with human menopausal gonadotropin HMG, recombinant FSH. When at least one follicle reached 16 mm in diameter as determined by ultrasound, recombinant human chorionic gonadotropin (hCG) was administered. After 36 h, the oocytes were retrieved under the guidance of vaginal ultrasound. Luteal support was commenced on the day after oocyte retrieval, using 60 mg of 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.

Embryo morphological assessment

Assessment of embryo morphology was performed daily. Pronuclear formation was scored 17–18 h after fertilization. After fertilization, 2 normally fertilized PN embryos were scored by their morphological appearance and developmental stage. Embryo morphology at day 3 after fertilization was graded according to number, multinucleation, size and symmetry, diopter, and the cellular fragmentation of the blastomeres. On day 3, high-quality embryos scored as grade I or II; low-quality embryos scored as grade III. Grade I: embryos had 8 cells, with even, regular, spherical blastomeres, and with no fragmentation or less than 5% fragmentation. Grade II: embryos had ≥ 6 cells with regular, spherical blastomeres, and less than 15% fragmentation. Grade III: embryos had ≥ 4 cells with uneven shaped blastomeres, and more than 15% fragmentation.

Outcome measures

The primary outcome of this study was live birth, defined as one or more new-born alive after 23 weeks of gestation and survived more than 28 days. Clinical pregnancy defined as a positive fetal heartbeat by transvaginal ultrasound. Secondary outcomes were multiple gestation, which was defined as more than one sac with a fetal pole on ultrasound scan divided by the total number of clinical pregnancies.

Statistical analysis

Data were analyzed using SPSS 23. Normality of continuous variables was examined using the Shapiro–Wilk test. Continuous variables were analyzed using *T* tests and multivariable logistic regression was performed to explore the effect of SET and DET on pregnancy outcomes after controlling for potential confounders, including maternal age, BMI, the ET cycle rank, method of fertilization, days of gonadotropins, ovarian stimulation protocol, total gonadotropin dose, number of oocytes retrieved, and endometrial thickness. $P < 0.05$ considered the difference to be statistically significant.

Results

In this study, a total of 1506 fresh IVF embryo transfer cycles were included from January 2018 until June 2020. There were 774 SETs with one high-quality embryo and 732 DETs with one high-quality embryo and a second lower-quality embryo. Patients with a DET were 2.54 years younger than those who received SET ($P<0.001$). Patients with DET with a lower body mass index ($P<0.001$) also had higher serum estradiol levels on the day of trigger ($P<0.001$) and lower total gonadotropin doses ($P<0.01$). Patients with single embryo transfer in this primary analysis had fewer oocytes retrieved ($P<0.001$) and a higher normal cleavage rate ($P<0.01$) (Table 1).

Table 2 summarizes the pregnancy outcomes in SET and DET. The live birth rate in the DET group was 8.7% higher than that in the SET group (33.2% vs. 24.5%, $P<0.001$), and the same trend was observed with the

Table 1 Baseline demographics and treatment characteristics between single high-quality embryo transfer and transfer of a second lower-quality embryo with a high-quality embryo

Variable	SET High-quality embryo ($n=774$)	DET High-quality embryo + low-quality embryo ($n=732$)	*P* value
Maternal age (years)	35.39 ± 5.26	32.85 ± 5.57	< 0.001
Body mass index (kg/m^2)	23.68 ± 3.09	22.93 ± 3.11	< 0.001
Days of gonadotropins (day)	12.53 ± 2.78	12.50 ± 2.64	0.843
Total gonadotropin dose (IU)	3189 ± 1046.39	2992.65 ± 1631.03	0.006
Estradiol day of trigger (pg/mL)	1845.32 ± 1330.67	2439.79 ± 1694.02	0.001
Progesterone day of trigger (ng/mL)	1.01 ± 5.84	0.93 ± 2.09	0.725
Endometrial thickness (mm)	11.53 ± 2.69	11.60 ± 2.83	0.633
Ovarian stimulation protocol			0.060
Super-long scheme	470 (60.7)	410 (56.0)	
Long scheme	253 (32.7)	253 (34.6)	
Antagonist prescription case	51 (6.6)	69 (9.4)	
ET cycle rank	1.48 ± 0.80	1.55 ± 0.92	0.166
Number of oocytes retrieved	6.68 ± 4.40	8.85 ± 4.65	< 0.001
MII rate (%)	78.57 ± 23.79	78.52 ± 19.25	0.964
Method of fertilization (%)			< 0.001
IVF	561 (72.5)	458 (62.6)	
ICSI	213 (27.5)	274 (37.4)	
Normal fertilization rate (%)			0.209
IVF	57.91 ± 25.38	59.79 ± 21.51	
ICSI	69.98 ± 27.78	70.52 ± 24.15	
Normal cleavage rate (%)	99.38 ± 3.27	98.60 ± 5.13	0.001

ICSI, intracytoplasmic sperm injection. See text for explanation of abbreviation. The results are expressed as the mean ± SD and proportion (%). All *P* values less than 0.05 were considered statistically significant

Table 2 Clinical outcomes between transfer of a high-quality embryo and transfer of a second lower-quality embryo with a high-quality embryo

	SET High-quality embryo ($n=774$)	DET High-quality embryo + low-quality embryo ($n=732$)	*P* value
Clinical pregnancy rate, *n* (%)	33.3 (258/774)	42.3 (310/732)	< 0.001
Miscarriage rate, *n* (%)	26.4 (68/258)	19.0 (59/310)	0.037
Preterm delivery rate, *n* (%)	7.8 (20/258)	6.8 (21/310)	0.654
Multiple pregnancy rate, *n* (%)	0.8 (2/258)	14.8 (46/310)	< 0.001
Live birth rate, *n* (%)	24.5 (190/774)	33.2 (243/732)	< 0.001

Data are presented as a proportion (%). All *P* values less than 0.05 were considered statistically significant

clinical pregnancy rate between the two groups (42.3% vs. 33.3%, $P<0.001$). However, the multiple pregnancy rate increased from 0.8% in SET to 14.8% in DET with a second lower-quality embryo. When a pregnancy was achieved, transfer using SET resulted in a higher miscarriage rate (26.4% vs. 19.0%, $P=0.037$). In addition, there were no differences in any of the perinatal outcomes between the two groups (Table 2).

Given that advanced age may affect pregnancy outcomes [13], we stratified our analysis into women under 35 and over 35 years old. In women under 35 years old, adding a low-quality embryo increased the live birth rate by 6% (41.6% vs. 35.6%, $P=0.084$) and the multiple gestation rate from 1.4 to 14.9% ($P<0.001$). In women 35 years of age or older, we noticed a similar trend in the live birth (15.6% vs. 17.8%, $P=0.671$). However, the multiple pregnancy rate increased from 0 to 14.7% ($P<0.001$). In addition, there were no differences in miscarriage rate and perinatal rate between the two groups (Table 3).

In patients with 1 cycle of ET, a significant difference was found between SET and DET in the clinical pregnancy rate (35.5% vs. 49.4%, $P<0.001$) and the live birth rate (25.5% vs. 39.1%, $P<0.001$). However, multiple pregnancy rate increased from 0.5% in SET to 15.1% in DET. In patients with multiple cycles of ET, except for the significant difference in the multiple pregnancy rate (1.3% vs. 14.1%, $P=0.003$) between the two groups (Table 4).

While other parameters are not significantly different between SET and DET, logistic regression analysis showed that compared with SET, DET increased the multiple pregnancy rate (OR = 0.047, 95% CI: 0.011–0.199) after adjustment for known risk factors, including maternal age, maternal BMI, number of cycles, method of fertilization, days of gonadotropins, total gonadotropin dose, number of oocytes retrieved, and endometrial thickness (Table 5).

Discussion

In recent years, more and more studies have shown that embryo morphology cannot completely reflect the developmental potential of human embryos. Morphological assessment correlates with operator proficiency and subjectivity and only to a certain extent reflects the quality of the embryo [14]. Some research found that the transfer of a low-quality embryo may reduce the clinical pregnancy rate and live birth rate compared with a high-quality embryo. When pregnancy was achieved, there were no differences between transfer of a low-quality and

Table 3 SET compared to DET analyzed separately in patients older than and younger than 35 years

	< 35 year			≥ 35 year		
	SET High-quality embryo ($n=326$)	DET High-quality embryo + low-quality embryo ($n=473$)	*P* value	SET High-quality embryo ($n=448$)	DET High-quality embryo + low-quality embryo ($n=259$)	*P* value
Clinical pregnancy rate, *n* (%)	45.4 (148/326)	51.2 (242/473)	0.109	24.6 (110/448)	26.3 (68/259)	0.616
Miscarriage rate, *n* (%)	21.6 (32/148)	15.3 (37/242)	0.112	32.7 (36/110)	32.4 (22/68)	0.959
Preterm delivery rate, *n* (%)	9.5 (14/148)	6.6 (16/242)	0.306	5.5 (6/110)	7.4 (5/68)	0.751
Multiple pregnancy rate, *n* (%)	1.4 (2/148)	14.9 (36/242)	0.000	0	14.7 (10/68)	0.000
Live birth rate, *n* (%)	35.6 (116/326)	41.6 (197/473)	0.084	16.5 (74/448)	17.8 (46/259)	0.671

Data are presented as a proportion (%). All *P* values less than 0.05 were considered statistically significant

Table 4 SET compared to DET analyzed separately in patients in the ET cycle rank

	1 cycle of ET			≥ 2 cycles of ET		
	SET High-quality embryo ($n=513$)	DET High-quality embryo + low-quality embryo ($n=470$)	*P* value	SET High-quality embryo ($n=261$)	DET High-quality embryo + low-quality embryo ($n=262$)	*P* value
Clinical pregnancy rate, *n* (%)	35.5 (182/513)	49.4 (232/470)	0.000	29.1 (76/261)	29.7 (78/262)	0.870
Miscarriage rate, *n* (%)	28.0 (51/182)	17.2 (40/232)	0.009	22.4 (17/76)	24.4 (19/78)	0.770
Preterm delivery rate, *n* (%)	8.8 (16/182)	6.9 (16/232)	0.146	5.3 (4/76)	11.5 (9/78)	0.161
Multiple pregnancy rate, *n* (%)	0.5 (1/182)	35/232 (15.1)	0.000	1.3 (1/76)	14.1 (11/78)	0.003
Live birth rate, *n* (%)	25.5 (131/513)	39.1 (184/470)	0.000	22.6 (59/261)	22.5 (59/262)	0.981

Data are presented as a proportion (%). All *P* values less than 0.05 were considered statistically significant

Table 5 Logistic regression analysis for pregnancy outcomes with single embryo transfer and double embryo transfer

	OR (95% CI)	*P* value	Adjusted OR (95% CI)	Adjusted *P* value
SET with a high-quality embryo	1.0		1.0	
DET with a high-quality embryo + a low-quality embryo				
Clinical pregnancy, *n* (%)	0.681 (0.552, 0.839)	0.000	0.826 (0.654, 1.042)	0.107
Multiple rate, *n* (%)	0.045 (0.011, 0.187)	0.000	0.047 (0.011, 0.199)	0.000
Miscarriage rate, *n* (%)	1.523 (1.024, 2.263)	0.038	1.180 (0.771, 1.804)	0.446
Preterm delivery rate, *n* (%)	1.156 (0.612, 2.184)	0.654	1.207 (0.606, 2.402)	0.592
Live birth rate, *n* (%)	0.655 (0.523, 0.820)	0.000	0.828 (0.654, 1.042)	0.139

Pregnancy outcomes were adjusted for confounders such as maternal age, maternal BMI, number of cycles, method of fertilization, days of gonadotropins, total gonadotropin dose, number of oocytes retrieved, ovarian stimulation protocol, and endometrial thickness. The SET with a high-quality embryo group is the reference group. All *P* values less than 0.05 were considered statistically significant

a high-quality embryo in terms of adverse obstetric outcomes or neonatal complications [15, 16]. Another study demonstrated a strong association between embryo quality and endometrial response, and transferring embryos of different qualities created different implantation responses in the endometrium [17]. High-quality embryos have a positive implantation response in the endometrium, and low-quality embryos have a negative implantation response. Low-quality embryos may potentially send negative crosstalk. High-quality embryos send the opposite signal [18, 19]. Therefore, we wondered whether a low-quality embryo had an adverse effect on a high-quality embryo.

In this research, our findings indicated that transfer of a low-quality embryo along with a high-quality embryo did not have a detrimental effect on high-quality embryos. Conversely, the live birth rate in the DET with a high-quality embryo plus a low-quality embryo group was 8.7% higher than that in the SET with a high-quality embryo group (33.2% vs. 24.5%, $P<0.001$), and the same trend was observed with the clinical pregnancy rate between the two groups (42.3% vs. 33.3%, $P<0.001$). However, the multiple pregnancy rate increased from 0.8% in SET to 14.8% in DET with a second lower-quality embryo. Although adding a low-quality embryo increases the live birth rate and clinical pregnancy rate, the risk of two embryo implantation leads to adverse obstetric outcomes associated with multiple pregnancies. We did not find significant differences in the preterm delivery rate ($P>0.05$). There is no article to report whether high-quality embryos can promote low-quality embryos to a certain extent. After adjusting for confounding factors, compared with SET, DET significantly increases the multiple pregnancy rate.

Advanced age and the ET cycle rank might have certain effects on pregnancy outcomes. Therefore, we stratified our patients in terms of age and ET cycle rank. Regardless of age, DET group led to a higher live birth. Moreover, in women less than 35 years old, we observed a slight improvement of 6% in the live birth rate at the expense of an increase in the multiple rate from 1.4 to14.9%. Women over the age of 35 benefitted the least from the DET because their live birth rate only increased by 2.2%, and the multiple pregnancy rate increased from 0 to 14.7%. Similar results were observed in patients treated with one cycle of ET and multiple cycles of ET. Compared with 1 cycle of ET, the live birth rate after more than one cycle of ET did not significantly increase, but the risk of multiple pregnancy rates increased significantly.

Several research assessed this issue. In the study by Dobson et al., compared with the transfer of one high-quality fresh embryo, the double embryo transfer group with the addition of a low-quality embryo had a higher multiple birth rate and no difference in the live birth rate [20]. In another analysis, Dobson et al. compared high-quality embryo transfers versus high-quality embryo transfers together with a low-quality embryo in a mixture of embryos and blastocysts. They found that a low-quality embryo did not negatively affect a high-quality embryo [20]. However, some researchers reached the opposite conclusion. Wintner et al. showed that after the transfer of a low-quality embryo along with a high-quality embryo, the clinical pregnancy rate and live birth rate were similar when compared with the transfer of two high-quality embryos [21].

Our study was potentially limited by its retrospective study. There may be inherent confounding factors and bias. Firstly, we did not take into account the possible impact of male factors on pregnancy outcomes. Secondly, our data were collected and entered by telephone interviewers, and there was some subjective influence.

In conclusion, this study found that adding a low-quality embryo does not reduce the live birth rate compared with the transfer of a single high-quality embryo. However, transferring double embryos with a second low-quality embryo increased the clinical pregnancy rate and

live birth rate. In addition, in women older than 35 years or in patients with multiple cycles of ET, physicians should consider the benefits and risks of DET.

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Authors' contributions
Xianju Hang contributed to the conception and design of this study. Xinle Lu performed the data analysis. Xinle Lu and Ludan Chao supervised the project administration and assisted in writing the manuscript. Xue Jiang and Xiao Wang assisted in the data analysis. All authors revised the manuscript thoroughly for important intellectual content and finally approved the version to be published.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request and we provide the raw data in a supplementary file.

Declarations

Ethics approval and consent to participate
We provide it in a supplementary file that the institutional and/or licensing committee approve the experiments. And the datasets used and/or analyzed during the current study are just only a part of another experimental article, so we provide this statement identifying.

Consent for publication
We declare that we all voluntarily assign copyright of our published original research papers to the journal.

Competing interests
The authors declare that the research was conducted without pecuniary relationships that could be construed as a potential conflict of interest.

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