Live Birth Rate and Neonatal Outcomes of Different Quantities and Qualities of Frozen Transferred Blastocyst in Patients Requiring Whole Embryo Freezing Stratified by Age

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Abstract

Background: Multiple pregnancies are associated with significant complications and health risks for both the mothers and infants. Single blastocyst transfer (SBT) is a logical and effective measure to reduce the incidence of multiple pregnancy with assisted reproductive technology (ART). Whether it is suitable for everyone undergoing SBT was inconclusive, in view of the consideration of embryo quality and patients’ age.

Objective: To explore live birth rate (LBR) and neonatal outcomes of different quantities and qualities of blastocysts in patients stratified by age, using a cutoff of 35 years, who required whole embryo freezing and underwent a subsequent frozen-thawed transfer (FET) cycle.

Methods: A total of 3362 patients were divided into five groups: group A (n=1569) received a single good-quality blastocyst; group B (n=1113) received two good-quality blastocysts; group C (n=313) received one good- and one average-quality blastocyst; group D (n=222) received two average-quality blastocysts; and group E (n=145) received one average-quality blastocyst.

Results: For patients have good-quality blastocysts, irrespective of age, the LBR of double blastocyst transfer (DBT) were about 50-65% and multiple pregnancy rate (MPR) were 40-60%; however, the LBR of single blastocyst transfer (SBT) were 40-55% and MPR were 3.5-6.3%. For patients who only had average-quality blastocysts, the MPR of double average-quality blastocysts transfer was as high as 30-50%. Moreover, about 70-90% of preterm births resulted from multiple pregnancies, and about 85-95% of low birth weight babies come from multiple pregnancies. The neonatal outcomes (gestational age, birth weight and birth height) of SBT were significantly lower than those of DBT regardless of age, and this statistical difference disappeared if the patients were sub-grouped by singleton or twin. There is no significant difference in neonatal outcomes between single good-quality blastocyst and single average-quality blastocyst transfer.

Conclusions: SBT is a preferable option for patients regardless of age when good-quality blastocysts are available. For patients who only had average-quality blastocysts, patients should be informed that DBT was associated with higher multiple pregnancy and adverse neonatal outcomes when compared with SBT regardless of age, suggesting that the practice of SBT is also feasible for these patients.

Introduction

Since the birth of the world’s first baby with the help of in vitro fertilization-embryo transfer (IVF-ET) in 1978 [1], this technology has become an effective procedure for infertile patients and is widely used around the world. Two or more embryos were transferred to increase the chance of pregnancy, as a result, leading to a high risk of multiple pregnancy which is considered the most common adverse event associated with IVF-ET technology [2]. Multiple pregnancies are associated with significant complications and health risks for both the mothers and infants [3]. Therefore, clinicians are gradually shifting from the initial goal of obtaining pregnancy to attaining the birth of a single healthy baby. Obviously, decreasing the number of transferred embryos, specifically, employing the practice of single embryo transfer, is a logical and effective measure to reduce the incidence of multiple pregnancy with assisted reproductive technology (ART) [4, 5].

With the improvement of laboratory environments and culture conditions, embryo culture can be extended to the blastocyst stage. Blastocyst culture has the advantage of self-selection of viable embryos that attain a greater implantation rate and pregnancy outcomes. A previous study showed a significantly higher pregnancy rate in patients undergoing single blastocyst transfer (SBT) versus single cleavage stage embryo [6]. Another study indicated that selective SBT (eSBT) significantly reduced the risk of multiple pregnancy without compromising the pregnancy rate compared with double blastocyst transfer (DBT) [7, 8].

A clear definition of selective SBT was given in a study [9], namely, a single blastocyst was transferred and at least one blastocyst was available for cryopreservation. However, the detailed grading of transferred embryos for eSBT and DBT in these studies was not clearly described [7, 10, 11]. The quality of the blastocysts transferred in the DBT group met the conditions of one of three scenarios; the two blastocysts may have been both of good-quality, of average-quality or a combination of one good- and one average-quality blastocyst. As far as we know, there have been no relevant studies stratified by age that compare the live birth rate and neonatal outcomes derived from the three cases of DBT with single good-quality blastocyst transfer. The purpose of this study was to evaluate the pregnancy and neonatal outcomes of different numbers and grades of frozen blastocysts in patients stratified by age who required whole embryo freezing and underwent a subsequent frozen-thawed transfer cycle. Ultimately, this information will provide strong evidence for clinicians who select the number of transferred embryos based on maternal age and embryo quality in clinical practice.

Material And Methods

Study population and grouping

This was a retrospective, single-center study of patients undergoing frozen embryo transfer (FET) from January 2016 to October 2018 at the Department of Reproductive Medicine Center in the Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, China. Inclusion criteria: 1) women were 20 to 42 years of age, 2) basal FSH < 10mlU/ml, 3) the first cycle of IVF/ICSI, 4) the first FET cycle after whole embryo freezing, 5) transferred one or two day 5 blastocysts. Exclusion criteria: 1) oocyte donation and cycles with preimplantation genetic testing (PGT), 2) known uterine anomalies confirmed by 3-dimensional sonography and/or hysteroscopy, including endometrial polyps, septal uterine cavity, submucosal fibroid, etc., 4) the presence of hydrosalpinx not corrected surgically prior to FET, 5) uncontrolled endocrine and/or immune disorders or other systemic diseases, including hypertension, diabetes, thyroid disease, hyperprolactinemia, antiphospholipid syndrome, systemic lupus erythematosus, etc. Each patient has signed a informed consent on obtaining and analyzing their clinical data prior to the initiation of IVF/ICSI treatment.
These women, aged 20–42 years old, were divided into five groups depending on the quantity and quality of day 5 blastocyst: group A (n = 1569) received one good-quality blastocyst; group B (n = 1113) received two good-quality blastocysts; group C (n = 313) received one good and one average-quality blastocysts; group D (n = 222) received two average-quality blastocysts; and group E (n = 145) received one average-quality blastocyst.

**Ovarian Stimulation**

Patients in this study were given a long protocol treatment of down-regulation with gonadotrophin releasing hormone (GnRH) agonist (Triptorelin; Diphereline, Ipsen, France) or GnRH antagonist (Cetrorelix; Cetrotide, Merck, Germany) protocol for ovarian stimulation. Individually tailored doses of recombinant human follitropin (r-hFSH; Gonal F, Merck Serono, Switzerland or Puregon, MSD, Netherlands) were administered and then adjusted dosage based on the follicular development indicated by ultrasound monitoring and serum estradiol levels. Urine human chorionic gonadotrophin (u-HCG; Lizhu Group Co., China) or recombinant HCG (r-HCG; Merck Serono) was administered to induce oocyte maturation when at least three follicles reached a mean diameter of 17 mm. Oocyte retrieval was performed 36–38 h after HCG injection and oocytes were incubated in incubators for insemination by conventional IVF or ICSI determined by sperm quality.

**Embryo Grading, Vitrification, And Warming**

Blastocysts were graded and scored using the Garden criteria according to blastocyst expansion, inner cell mass (ICM) development and trophectoderm (TE) appearance. Patients in our study were only transferred day 5 blastocyst. Blastocysts graded 4 and over with ICM A or B and TE A or B were considered as good-quality embryo. Those blastocysts that presented as grade 4 and over with an ICM C or TE C, and all blastocysts that graded 3 were regarded as average-quality embryos.

All available blastocysts were cryopreserved by vitrification method according to manufacturer's instruction. Vitrified blastocysts were thawed by a rapid thawing method on the morning of embryo transfer. The number and stage of transferred embryos was determined by clinicians and the couples, giving priority to clinical factors including patients’age, blastocysts qualities and quantities.

**Frozen-thawed Cycle And Embryo Transfer**

Endometrial preparation for FET included natural cycle (NC) program and hormone replacement therapy (HRT) program have been described previously. In short, NC was suitable for patients with a regular menstrual cycle and ovulation. One or two blastocysts were transferred on the sixth day after ovulation. HRT was applicable for patients with irregular menstrual cycles or poor endometrium development in NC. The endometrium preparation of HRT was used with daily oral estradiol valerate tablets (Progynova, Bayer, Germany) since the second to fourth day after menstruation, and embryo transfer was performed on the sixth day of progesterone injection. All patients received luteal support with progesterone after embryo transfer. If transvaginal ultrasound showed gestational sac and embryonic heartbeat 4–6 weeks after embryo transfer, luteal support was continued until 10 weeks of gestational age.

**Statistical analysis**

The statistical analysis was performed using the Statistical Package for Social Science (SPSS) version 22.0. The baseline characteristic was expressed as the mean ± SD (standard deviation) and analyzed using one-way analysis of variance (ANOVA) or Student’s t-test. Categorical variables were described as frequencies and percentages, and compared using chi-square test or Fisher’s exact test. P < 0.05 was considered statistically significant. Live birth was defined as the delivery of any viable neonate who was 28 weeks of gestation or older. Clinical pregnancy (PR) was defined as the present of gestational sac on ultrasound at 6–8 weeks of gestation, low birth weight was defined as the birth weight less than 2500 g and very low birth weight less than 1500 g.

**Results**

A total of 3362 patients who met the inclusion criteria were included in the study from January 2016 to October 2018. A total of 22 pregnant patients were lost to follow-up in this study. The clinical and neonatal outcomes were analyzed according to age, using a cutoff of 35 years. The baseline characteristics of the patients are presented in Table 1. When patients in the same age category, no significant differences were found in terms of age, body mass index (BMI), basal follicle stimulating hormone (FSH), anti-mullerian hormone (AMH), infertility duration, type of infertility, the type of endometrial preparation and endometrium thickness (all values of P>0.05).
The clinical outcomes of the patients in groups A-D stratified by 35 years of age are shown in Table 2. There were significant differences in the rates of implantation, clinical pregnancy, live birth and multiple pregnancy among the groups A-D when patients were in the same age category. For women under 35 years old, the live birth rate (LBR) in group B and C was significantly higher than group A, but the LBR in group A was acceptable (54.2%) and the multiple pregnancy rate (MPR, 3.5%) was significantly lower than group B (62.4%) and C (49.7%). There was no significant difference in the LBR between group A, B and C and D, but MPR in group A was significantly lower than group B (62.4%) and C (49.7%). Meanwhile, we compared the clinical outcomes of patients with two average-quality blastocysts (group D) versus one average-quality blastocyst (group E). The results showed that, for patients under 35 years old, the LBR in group D was higher than group E, but the multiple pregnancy rate (MPR, 3.5%) was significantly lower than group B and C (49.7%). There was no significant difference in the LBR between group A and group C or D, but MPR in group A was significantly lower than group C and D. Meanwhile, we compared the clinical outcomes of patients with two average-quality blastocysts (group D) versus one average-quality blastocyst (group E). The results showed that, for patients under 35 years old, the LBR in group D was higher than group E, but the multiple pregnancy rate (MPR, 3.5%) was significantly lower than group B (62.4%) and C (49.7%).
Table 2: The clinical outcomes of the patients in groups A-D stratified by 35 years of age

|                      | 35 years of age | ≥35 years of age |
|----------------------|-----------------|------------------|
|                      | Group A         | Group B          | Group C          | Group D          | Group E          | \( p \# \)      | Group A         |
| Cycles (n)           | 1425            | 844              | 206              | 183              | 120              | 144             |
| Implantation rate    | 63.72\(^b\,c\)  | 60.37(1019/1688) | 53.64(221/412)  | 45.90(168/366)  | 46.67(56/120)    | 0.000           | 55.56\(^b\,c\)  |
| (908/1425)           |                 |                  |                 |                 |                  |                 | (80/144)        |
| Pregnancy rate       | 63.72\(^b\,c\)  | 75.59(638/844)   | 72.33(149/206)  | 62.30(114/183)  | 46.67\(^d\) (56/120) | 0.000           | 55.56\(^b\,c\)  |
| (908/1425)           |                 |                  |                 |                 |                  |                 | (80/144)        |
| Live birth           | 54.25\(^b\,c\)  | 64.57(545/844)   | 64.08(132/206)  | 48.63(89/183)   | 36.67\(^d\) (44/120) | 0.000           | 42.36\(^b\) (61/144) |
| (773/1425)           |                 |                  |                 |                 |                  |                 |                  |
| Singleton            | 52.70\(^b\,c\)  | 33.05(279/844)   | 38.84(80/206)   | 25.13(46/183)   | 36.67\(^d\) (44/120) | 0.000           | 40.97           |
| (751/1425)           |                 |                  |                 |                 |                  |                 | (59/144)        |
| Twin                 | 1.55\(^a\,b\,c\) | 31.52(266/844)   | 25.24(52/206)   | 23.50(43/183)   | 0.0\(^d\) (0/120) | 0.000           | 1.39\(^a\,b\)  |
| (22/1425)            |                 |                  |                 |                 |                  |                 | (2/144)         |
| Multiple pregnancy rate | 3.52\(^a\,b\,c\) | 62.38(398/638) | 49.66(74/149) | 50.0(57/114) | 0.0\(^d\) (0/56) | 0.000           | 6.25\(^a\,b\,c\) |
| (32/908)             |                 |                  |                 |                 |                  |                 | (5/80)          |
| Monozygotic twins rate | 3.52           | 2.66(17/638)    | 1.34(2/149)     | 2.63(3/114)     | 0.0              | 0.462           | 6.25            |
| (32/908)             |                 |                  |                 |                 |                  |                 | (5/80)          |
| Early abortion rate  | 11.67\(^a\)    | 8.31(53/638)     | 8.72(13/149)    | 15.79(18/114)   | 21.43(12/56)     | 0.037           | 20.0            |
| (106/908)            |                 |                  |                 |                 |                  |                 | (16/80)         |
| Abortion rate        | 13.33           | 11.44(73/638)    | 9.40(14/149)    | 19.30(22/114)   | 21.43(12/56)     | 0.067           | 21.25           |
| (121/908)            |                 |                  |                 |                 |                  |                 | (17/80)         |
| Ectopic pregnancy rate | 0.88           | 1.25(8/638)     | 1.34(2/149)     | 0.0(0/114)      | 0.0              | 0.597           | 2.50            |
| (8/908)              |                 |                  |                 |                 |                  |                 | (2/80)          |

\(^a\) comparison among groups A-D. \( P < 0.05 \) was considered statistically significant.

\(^b\) \( p \) value < .05 compared to the group B.

\(^c\) \( p \) value < .05 compared to the group C.

\(^d\) \( p \) value < .05 compared to the group D.

\(^e\) \( p \) value < .05 between group D and E.

Comparisons of neonatal outcomes of patients among groups A-E stratified by 35 years of age are presented in Table 3. The results showed that 70–90% of preterm births resulted from multiple pregnancies, and about 85–95% of low birth weight babies come from multiple pregnancies. When patients were in the same age category, the gestational age, birth weight and birth height of group A were significantly higher than those of group B, C or D, but this statistical difference disappeared if the patients were sub-grouped by singleton or twin birth. Meanwhile, there was no significant difference in terms of the gestational age, birth weight and birth height between the groups A and group E. Finally, the neonatal outcomes were compared between group D and group E, and the results showed that the gestational age, birth weight and birth height of patients in group D were significantly lower than those of group E when patients were in the same age category.
Table 3
The neonatal outcomes of patients among groups A-E stratified by 35 years of age.

|                  | 35 years of age | ≥ 35 years of age | P   |
|------------------|-----------------|-------------------|-----|
|                  | Group A | Group B | Group C | Group D | Group E |       | Group A | Group B | Group C | Group D | Group E |       |
| Cycles (n)       | 1425 | 844 | 206 | 183 | 120 | 144 | 269 | 107 | 39 | 25 | | |
| Preterm birth (<37 weeks) | 11.77<sup>a,b,c</sup> (91/773) | 39.45 (215/545) | 33.33 (44/132) | 42.70 (38/89) | 4.55<sup>d</sup> (2/44) | 0.000 | 8.20<sup>b</sup> (5/61) | 35.0 | 25.0 | 16.67 | 16.67 | 0.001 |
| Singleton (%)    | 8.67<sup>a</sup> (67/773) | 3.30 | 6.82 | 3.37 | 4.55 | 0.001 | 4.92 | 5.62 | 7.69 | 0.0 | 0.0 | 0.755 |
| Twin (%)         | 3.10<sup>a,b,c</sup> (24/773) | 36.15 (197/545) | 26.51 (35/132) | 39.33 (35/89) | 0.0<sup>d</sup> (0/44) | 0.000 | 3.28<sup>b</sup> (2/61) | 29.38 | 17.31 | 16.67 | 16.67 | 0.000 |
| Gestational age (weeks) | 38.70±1.94<sup>a,b,c</sup> | 37.37±2.52 | 37.59±2.52 | 36.86±3.33 | 39.18±1.91<sup>d</sup> | 0.000 | 38.72±1.23<sup>a,b,c</sup> | 37.61±2.33 | 37.66±2.89 | 37.00±3.43 | 38.32±0.86<sup>d</sup> | 0.022 |
| Singleton (%)    | 38.80±1.83 | 38.94±1.71 | 38.51±2.18 | 39.00±1.58 | 39.18±1.91 | 0.325 | 38.81±1.56 | 38.59±2.14 | 38.76±1.97 | 38.52±1.71 | 38.32±0.86 | 0.951 |
| Twin (%)         | 35.32±1.47 | 36.04±2.32 | 36.02±2.37 | 35.15±3.01 | / | 0.095<sup>#</sup> | 36.20±0.40 | 35.98±1.63 | 35.06±3.21 | 34.29±4.99 | / | 0.330<sup>#</sup> |
| Birth height (mm) | 49.51±2.3<sup>a,b,c</sup> | 47.72±3.21 | 48.23±2.41 | 46.61±4.26 | 49.89±2.22<sup>d</sup> | 0.000 | 49.77±1.85<sup>a,b,c</sup> | 48.03±2.70 | 47.95±3.75 | 48.31±5.70 | 49.75±0.96<sup>d</sup> | 0.000 |
| Singleton (%)    | 49.68±2.27 | 49.82±1.98 | 49.88±1.73 | 49.53±1.44 | 49.88±2.22 | 0.838 | 49.93±1.76 | 49.50±2.39 | 49.77±2.41 | 50.00±2.00 | 49.75±0.96 | 0.868 |
| Twin (%)         | 46.66±2.40 | 46.72±2.95 | 46.97±2.20 | 45.50±4.32 | / | 0.113<sup>#</sup> | 46.75±0.35 | 46.90±1.91 | 45.70±3.70 | 45.83±6.83 | / | 0.155<sup>#</sup> |
| Birth weight (kg) | 3.16±0.54<sup>a,b,c</sup> | 2.73±0.61 | 2.79±0.62 | 2.57±0.66 | 3.19±0.43<sup>d</sup> | 0.000 | 3.12±0.51<sup>a,b,c</sup> | 2.76±0.60 | 2.81±0.75 | 2.55±0.88 | 3.30±0.29<sup>d</sup> | 0.000 |
| Singleton (%)    | 3.22±0.50 | 3.27±0.51 | 3.24±0.52 | 3.17±0.33 | 3.19±0.43 | 0.583 | 3.36±0.49 | 3.22±0.50 | 3.24±0.49 | 3.26±0.45 | 3.30±0.29 | 0.666 |
| Twin (%)         | 2.34±0.43 | 2.48±0.45 | 2.44±0.42 | 2.31±0.54 | / | 0.113<sup>#</sup> | 2.55±0.25 | 2.43±0.34 | 2.24±0.52 | 2.00±0.82 | / | 0.142<sup>#</sup> |
| Still birth (%)  | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | / | 0.0 | 0.0 | 0.0 | 0.0 | / | |
| Congenital anomalies (%) | 1.64 | 1.60 | 1.09 | 1.52 | 2.27 | 0.958 | 0.0 | 0.0 | 0.0 | 0.0 | / | |
| Sex ratio(male/female) | 1.30 | 1.43 | 1.10 | 1.46 | 0.83 | 0.445 | 1.43 | 1.22 | 1.16 | 0.63 | 1.00 | 0.632 |
| Low birth weight (<2500 g) in singleton (%) | 7.04<sup>a,b,c</sup> | 27.25 | 28.26 | 34.09 | 4.55<sup>d</sup> | 0.000 | 6.34<sup>b</sup> | 29.03 | 27.94 | 13.33 | 0.0 | 0.002 |
| Singleton (%)    | 4.02 | 4.44 | 4.35 | 4.55 | 4.55 | 0.977 | 3.17 | 2.76 | 1.47 | 0.0 | 0.0 | 0.833 |

<sup>a</sup>comparison among groups A-D. <sup>P</sup><0.05 was considered statistically significant.

<sup>b</sup>p value < .05 compared to the group B.

<sup>c</sup>p value < .05 compared to the group C.

<sup>d</sup>p value < .05 compared to group D and E.
Table 1: Pregnancy and neonatal outcomes sub-grouped by age.

|                      | <35 years of age | ≥35 years of age |
|----------------------|------------------|------------------|
| Twin                | 3.02<sup>a,b,c</sup> | 3.17<sup>a,b</sup> |
|                     | (24/795)         | (2/63)           |
| Singleton           | 0.75<sup>a,c</sup> | 2.76            |
|                     | (6/795)          | (0/63)           |
| Twin                | 0.12<sup>a,b,c</sup> | 0.000           |
|                     | (1/795)          | (0/63)           |

<sup>a</sup> comparison among groups A-D. <sup>b</sup> p value < .05 compared to the group B. <sup>c</sup> p value < .05 compared to the group C.<sup>d</sup> p value < .05 between group D and E.

Discussion

The suggestion on the number of transferred embryos is usually affected by the patient's age in clinical practice. Doctors tend to provide the recommendation of transferring two embryos for older patients to increase the clinical pregnancy. It has been reported that the live birth rate are similar for the transfer of one or two blastocysts [14]. However, it is not certain whether the above conclusions are applied to older patients. There are two studies to explore the effect of the number of blastocyst transfer on pregnancy outcomes sub-grouped by age. Eum and colleagues found that the live birth or ongoing pregnancy rate of eSBT and DBT were equivalent, but eSBT had a lower risk of multiple pregnancy, regardless of age, for both fresh and vitriied-warmed cycles [7]. Similarly, another investigation reported a comparable pregnancy rate and a significantly reduced multiple pregnancy rate of eSBT compared to that of DBT in patients 35-39 years of age [15]. But, it is worth emphasizing that transferred blastocyst quality has not been mentioned in detail in these studies, which can not be ignored for the embryo quality also is a determinant factor of success in ART cycles. Therefore, this study is the first to explore the pregnancy and neonatal outcomes associated with different quantity and quality of blastocysts transferred in patients undergoing FET cycles after whole embryo freezing stratified by age. Our results showed that, SBT is a preferable strategy for patients irrespective of age when good-quality blastocysts are available. And for patients who only had average-quality blastocysts, we should be caution about the suggestion of transferring two embryos because of DBT was associated with higher multiple pregnancy and adverse neonatal outcomes when compared with SBT even for older patients.

A woman's age is considered the most important factor that influences fertility potential, and this potential significantly decreases after the age of 35 years [16]. Extending embryo culture to the blastocyst stage has the advantage of natural selection of the most viable, genetically competent embryos, which is particularly important for advanced maternal age [17]. A recent prospective study reported a higher ongoing pregnancy rate following blastocyst transfer than cleavage embryo transfer for women 35 years of age and older, whereas the difference was not significant in younger women [18]. An increased risk of adverse obstetrical and neonatal complications associated with multiple pregnancy was observed, especially for patients of advanced maternal age [19]. Our results suggested that SBT also appeared to be a promising option that did not compromise the live birth rate for women over the age of 35 years with available good-quality blastocysts. Moreover, recent studies also indicated that the practice of selective SBT was feasible and resulted in reduced multiple pregnancy rates in women aged 40-43 without compromising cumulative live birth rate compared with DBT [9,10], which suggested maternal age was not a significant predictor for live birth and the competence of the oocytes developing into good-quality blastocysts is more important than maternal age [10]. Additionally, another study reported that maternal age has no effect on pregnancy rates when fully expanded blastocysts are achieved [20]. Therefore, we believe that attaining more, good-quality blastocysts through the improvement of the stimulation protocol and culture environment is crucial for older women.

To increase the odds of a successful pregnancy for patients without good-quality blastocysts, two blastocysts were usually transferred to patients in our reproductive center. However, our study indicated that for these women with DBT, the multiple pregnancy rate was as high as 50% in patients under 35 years of
age and 31.3% in patients aged 35 years and over. A previous study has highlighted that the multiple birth rate is 28% in women aged 38-40 years when two embryos are transferred [21], suggesting that advanced maternal age does not protect against multiple pregnancy. For advanced women who only has average-quality blastocyst, our results showed that the clinical PR and LBR observed in patients with SBT was similar to that of DBT, and no multiple pregnancies occurred with SBT. Because these results were obtained based on the small sample size of patients, it is difficult to advocate a routine policy of single blastocyst transfer in patients over the age of 35 years without good-quality blastocysts. However, couples with only average-quality blastocysts should be informed that DBT can obtained multiple pregnancy rate of 30-50%, which leads to higher perinatal morbidity and mortality rates than those associated with single embryo transfer [4,9]. Additionally, Our results were in accordance with the guidelines of the American Society for Reproductive Medicine, which recommends that eSBT should be performed for patients under 35 years old with good prognosis and should also be considered in women aged 35-42 if they have good-quality euploid blastocysts available for transfer [22].

It is well known that multiple pregnancies are associated with higher risk of neonatal and perinatal complications [10]. Our results are consisted with aforementioned conclusion, showed that 70-90% of preterm births resulted from multiple pregnancies, and about 85-95% of low birth weight babies come from multiple pregnancies. However, whether the embryos quality affects the neonatal outcomes is still controversial. Two previously published studies found that singletons derived from poor-quality embryos were not at a higher risk of adverse neonatal outcomes and embryo quality was not correlated with pregnancy complications [23,24]. Our results are in line with these studies describing that there was no difference in the birthweight of newborns between group A and E. But, a recent study suggested the transfer of a poor-quality blastocyst was associated with lower mean birthweight when compared with the transfer of an excellent-quality blastocyst during FET cycles [25]. The differences in terms of the study population and embryo development degree may be account for the inconsistent results.

Our study has some limitations that need to be taken into consideration. The retrospective nature of this study is a major limitation; however, it is important to note that there were no differences with regard to baseline characteristics of the patients among the groups A-E stratified by age, suggesting that these five cohorts comprised similar populations in this study. The large variation in the number of cases among these groups was another weakness of the study, especially, a very small sample in patients with only average-quality blastocyst for the preferential selection of good-quality blastocysts in our study. However, it is worth mentioning that the sample size of patients in this study was larger than those of other similar studies, so the results from the present study are valuable for guiding clinical practice and encouraging single blastocyst transfer for patients undergoing ART when the expanded blastocyst is obtained, especially for patients of advanced maternal age.

In conclusion, our results suggested that when good-quality blastocysts are available, SBT should be incorporated into daily practice because of reduced risk of multiple pregnancies without significantly affecting the live birth rate. For patients who only have average-quality blastocysts, DBT was associated with higher multiple pregnancies and adverse neonatal outcomes when compared with SBT, suggesting that the practice of SBT is also preferable option for these patients regardless of age.

**Abbreviations**

AMH: anti-mullerian hormone  
ANOVA: one-way analysis of variance  
ART: assisted reproductive technique  
BMI: body mass index  
DBT: double blastocyst transfer  
FET: frozen embryo transfer  
FSH: follicle stimulating hormone  
GnRH: gonadotrophin releasing hormone  
HCG: human chorionic gonadotrophin  
HRT: hormone replacement therapy  
ICM: inner cell mass  
ICSI: intracytoplasmic sperm injection  
IVF-ET: in vitro fertilization and embryo transfer  
LBR: live birth rate  
MPR: multiple pregnancy rate  
NC: natural cycle  
PGT: preimplantation genetic testing
Declarations

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Availability of data and materials

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

Authors’ contributions

YXH designed research. SPC, YXH, HZD, JQL, HYL and LL analyzed data. HYZ and SPC wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the ethics committee of the Third Affiliated Hospital of Guangzhou Medical University. Since this is a retrospective investigation, patients were not asked to participate in this study. Each patient has signed an informed consent on obtaining and analyzing their clinical data prior to the initiation of IVF/ICSI-ET treatment.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. Lancet. 1978;2(8085):366.
2. Kupka MS, et al. Assisted reproductive technology in Europe, 2010: results generated from European registers by ESHRE. Hum Reprod. 2014;29(10):2099 – 113.
3. Tobias T, et al. Promoting the use of elective single embryo transfer in clinical practice. Fertil Res Pract. 2016;2:1.
4. Sullivan EA, et al. Single embryo transfer reduces the risk of perinatal mortality, a population study. Hum Reprod. 2012;27(12):3609-15.
5. Practice Committee of Society for Assisted Reproductive T, Practice Committee of American Society for Reproductive M. Elective single-embryo transfer. Fertil Steril. 2012;97(4):835 – 42.
6. Papanikolaou EG, et al. In vitro fertilization with single blastocyst-stage versus single cleavage-stage embryos. N Engl J Med. 2006;354(11):1139-46.
7. Eum JH, et al. Clinical outcomes of single versus double blastocyst transfer in fresh and vitrified-warmed cycles. Clin Exp Reprod Med. 2016;43(3):164 – 68.
8. Criniti A, et al. Elective single blastocyst transfer reduces twin rates without compromising pregnancy rates. Fertil Steril. 2005;84(6):1613-9.
9. Tannus S, Son WY, Dahan MH. Elective single blastocyst transfer in advanced maternal age. J Assist Reprod Genet. 2017;34(6):741-8.
10. Tannus S, et al. Cumulative live birth rate following elective single blastocyst transfer compared with double blastocyst transfer in women aged 40 years and over. Reprod Biomed Online. 2017;35(6):733-8.
11. He QH, et al. Clinical outcomes of frozen-thawed single blastocyst transfer in patients requiring whole embryo freezing. Syst Biol Reprod Med. 2016;62(2):133-8.
12. Gardner DK, et al. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. Fertil Steril. 2000;73(6):1155-8.
13. He Y, et al. Delayed frozen embryo transfer failed to improve live birth rate and neonatal outcomes in patients requiring whole embryo freezing. Reprod Biol Endocrinol. 2020;18(1):1.
14. Racca A, et al. Single and double embryo transfer provide similar live birth rates in frozen cycles. Gynecol Endocrinol. 2020;undefined:1–5.
15. Mullin CM, et al. Comparison of pregnancy outcomes in elective single blastocyst transfer versus double blastocyst transfer stratified by age. Fertil Steril. 2010;93(6):1837-43.
16. DeCherney AH. Berkowitz GS. Female fecundity and age. N Engl J Med. 1982;306(7):424-6.
17. Harton GL, et al. Diminished effect of maternal age on implantation after preimplantation genetic diagnosis with array comparative genomic hybridization. Fertil Steril. 2013;100(6):1695 – 703.
18. Fernández-Shaw S, et al. Ongoing and cumulative pregnancy rate after cleavage-stage versus blastocyst-stage embryo transfer using vitrification for cryopreservation: impact of age on the results. J Assist Reprod Genet. 2015;32(2):177 – 84.
19. Waldenström U, et al. Advanced maternal age increases the risk of very preterm birth, irrespective of parity: a population-based register study. Bjog. 2017;124(8):1235-44.
20. Shapiro BS, et al. Influence of patient age on the growth and transfer of blastocyst-stage embryos. Fertil Steril. 2002;77(4):700-5.
21. Sunderam S, et al. Assisted Reproductive Technology Surveillance-United States, 2013. MMWR Surveill Summ. 2015;64(11):1–25.
22. Practice Committee of the American Society for Reproductive Medicine. Electronic address Aao, Practice Committee of the Society for Assisted Reproductive T. Guidance on the limits to the number of embryos to transfer: a committee opinion. Fertil Steril. 2017;107(4):901-3.
23. Oron G, et al. The association between embryo quality and perinatal outcome of singletons born after single embryo transfers: a pilot study. Hum Reprod. 2014;29(7):1444-51.
24. Zhu J, et al. Does IVF cleavage stage embryo quality affect pregnancy complications and neonatal outcomes in singleton gestations after double embryo transfers? J. Assist Reprod Genet. 2014;31(12):1635-41.
25. Zhang J, et al. The impact of embryo quality on singleton birthweight in vitrified-thawed single blastocyst transfer cycles. Hum Reprod. 2020;35(2):308 – 16.
26. He YX, et al. Clinical outcomes of different quantity and quality of frozen blastocyst transfer in patients requiring whole embryo freezing stratified by age. IFFS 2019: International Federation of Fertility Societies Shanghai World Congress; 2019 Apr 11–15; Shanghai, China. Global Reproductive Health: p e30.