Poor-quality embryos compared with available embryos at cleavage stage have similar outcomes in vitrified-thawed single blastocyst transfer

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Abstract

Background

It was known that in patients with good prognosis, day 5 good-quality blastocysts formed by available cleavage embryos (AEs) or poor-quality cleavage embryos (PQEs) did not affect clinical outcomes. However, the clinical outcomes of day 5/6 expanded blastocysts cultured in patients who faced with PQEs was unclear.

Methods

Retrospective cohort study was conducted among women aged ≤ 38 who underwent vitrified-warmed single blastocyst transfers (SBTs) which originated from AEs (n = 382), PQEs with AEs (n = 99), or only PQEs without AEs (n = 101).

Results

The PQEs compared to the AEs showed lower CPR (38.38% in PQEs with AEs; 36.63% in PQEs without AEs; versus 57.07% in AEs) and lower LBR (28.28%; 29.70%; versus 44.50%), but there was no statistical difference in CPR (adjusted OR 0.78, 95% CI 0.46–1.31; adjusted OR 0.82, 95% CI 0.47–1.42) and LBR (adjusted OR 0.91, 95% CI 0.53–1.59; adjusted OR 0.94, 95% CI 0.50–1.78) after controlling for confounding variables. Better outcomes were associated with thicker endometrial thickness (CPR: adjusted OR 1.18, 95% CI 1.05–1.33; LBR: adjusted OR: 1.16, 95% CI 1.03–1.30) and blastocyst expansion on day 5 (CPR: adjusted OR 2.52 95% CI 1.62–3.94; LBR: adjusted OR: 2.28, 95% CI 1.43–3.65). ICM score C (CPR: adjusted OR 0.08, 95% CI 0.01–0.67; LBR: adjusted OR: 0.10, 95% CI 0.01–0.76) were associated with worse outcomes.

Conclusions

Once the blastocyst was available, endometrial thickness, ICM grade, and the day of blastocyst expansion were the factors affecting the outcome, while the quality of cleavage embryos (PQEs or AEs) had no effect on subsequent clinical outcomes.

Introduction

Morphological score of cleavage embryos was based on the fact that the possibility of successful implantation varies among different embryos, and this could be predicted to some extent according to the embryonic development. Many studies supported a strong association between embryo quality and the chances for successful implantation and eventual live birth (1–5).
The efficiency of prolong culture strategy had already been demonstrated, especially in good-prognosis populations, when numerous good-quality embryos were available at cleavage stage (6–7). Although it was recognized by blastomeres biopsied that the incidence of chromosomal abnormalities was significantly higher in slow-cleaving embryos, too fast-cleaving embryos and uneven-size blastomeres or scattered fragments compared to embryos with eight cells at 62 hours after insemination (8). And it was reported that aneuploidy negatively affects blastocyst developmental speed, inner cell mass (ICM) morphology, and trophectoderm (TE) morphology (9). However, after blastocyst culture, 21% of the poor-quality cleavage embryos (PQEs) reached the blastocyst stage (10–11).

At present, only a few groups reported that low score cleavage embryos could result in successful blastulation and clinical pregnancies (12–13), and there were limited reports on the outcome of blastocyst transfer in patients with only PQEs at cleavage stage, these patients might face the same problem of no available cleavage embryos (AEs) in the next cycles. Whether the clinical outcomes of these blastocysts formed by PQE without AEs and those formed by PQE with AEs were different from those formed by AEs. A recent study showed that when day 5 good-quality blastocysts were transferred, blastocysts formed by good or poor quality embryos on day 2 or 3 did not affect clinical outcomes (14). This results were obtained from the fast-growing day 5 good-quality blastocyst (blastocoel Grade 3 or higher, ICM A/B, TE A/B), and only included people with good prognosis who could obtain multiple good-quality embryos at cleavage stage and exculed people without AEs. However, not all embryos showed the same pace of development, and some would reach the fully expanded stage on day 6, especially for the PQEs. So, it was necessary to determine whether patients witout AEs have different clinical outcomes compared with people who could obtain multiple AEs at cleavage stage after fully expanded blastulation on day 5 or 6.

The aim of this study was to evaluate whether blastocysts cultured from people who faced with PQEs (with or without AEs) could produce similar clinical outcomes compared with those from people who had cleavage embryos suitable for cryopreservation in the vitrified-thawed single blastocyst trasfer cycles.

**Materials And Methods**

**Study population**

All single frozen blastocyst transfer cycles during the period from July 2014 to May 2017 at a single reproductive center were retrospectively analyzed. Inclusion criteria were the following: ≤38 years, single blastocyst transfer (SBTs) in frozen embryo transfer (FET) cycles. The numbers of FET cycles in one in vitro fertilization (IVF) treatment were ≤ 3. Extending embryo culture to the blastocyst stage was done according to the number of AEs or no AEs for transplantation or cryopreservation. According to the patient's condition and informed consent, after 0–4 embryos were frozen, the remaining AEs or PQEs (with and without AEs) could be extended for culture. Exclusion criteria were the following: >38 years, AEs and PQEs to extended culture together, pre-implantation genetic test (PGT), fresh blastocyst transfer, blastocyst from re-vitrification. This study was approved by the Ethics Review Board.
Fertilization methods and embryo culture and evaluation

Conventional IVF or intracytoplasmatic sperm injection (ICSI) was performed as appropriate based on the presence or absence of male factor infertility. Embryos were cultured individually in 25-µl droplet of G1 Plus™ medium (Vitrolife) under Ovoil (Vitrolife) and incubated at 37°C with 6% CO₂ in air. Embryos on day 3 were subsequently cultured in G2 Plus™ medium (Vitrolife) until day 5/6 using tri-gas incubators (5% O₂/6% CO₂/89% N₂). Fertilization was assessed 16–18 h post conventional insemination / injection and embryo quality assessment was performed on day 3 as well as on day 5 and day 6.

AEs scoring criteria on day 3 was: 5–12 cells, ≤ 20% fragmentation, equal sized or incompletely equal sized blastomeres, < 50% vacuoles, < 50% multinucleation. Embryos that did not meet the criteria mentioned above were defined as PQEs.

At the blastocyst stage, embryo quality was assessed on day 5 and day 6 according to the Gardner classification, which considered the expansion grade as well as the development of ICM and TE (15). The blastocysts were frozen when the expansion of Grade 3–6 and the development of the ICM and the TE were at least BC/CB or better.

Blastocyst vitrification and thawing procedures

Prior to vitrification, artificial blastocyst collapse was performed by using the laser system ZILOS-tkTM (Hamilton Thorn Bioscience Inc., Beverly, MA, USA). The blastocysts were then suspended in equilibration solution (containing 7.5%, v/v, dimethyl sulfoxide (DMSO) and 7.5%, v/v, ethylene glycol (EG) for 2 min at 37°C and then were transferred into three 25-mL drops of vitrification solution (containing 15% DMSO, 15% EG and 0.5 mol/l trehalose), holding for 20 s in each drop. The blastocysts were then loaded onto a CryoTop (Kitazato Biopharma) with a minimal volume of vitrification solution and immediately plunged into liquid nitrogen at − 196°C.

For warming, the CryoTop contents were dispensed in warmed thawing solution (TS: 1.0 mol/l sucros) at 37°C for 1 min. Then, the blastocysts were transferred to dilution solution 1 (DS1: 0.5 mol/l sucros) at room temperature for 3 min and DS2 (0.25 mol/l sucros) for 3 min, and finally, blastocysts were washed in washing solution (WS) twice for 3 min each. After warming, the blastocyst was then transferred into G2 Plus™ medium (Vitrolife) and cultured for 2–5 h, and blastocyst survival was defined as the ability of the blastocyst to re-expand during this time.

When blastocyst warmed did not re-expand after 2 h and cells showed signs of degeneration or lyses, the blastocyst was discarded and another one was warmed. After survival assessment, the blastocyst was maintained in an incubator before intrauterine transfer under ultrasound guidance.

Endometrial preparation and embryo transfer

According to menstruation and ovulation, the patients who prepared for frozen embryo transfer adopted natural cycle or artificial cycle. In the artificial cycle, endometrial was priming with daily oral estradiol
valerate (6 mg) beginning on cycle day 2 and 3 for 11 days. When conditions were deemed to be appropriate (e.g. endometrium thickness ≥ 8 mm and progesterone < 4.5 nmol/L), embryo transfer was performed under transabdominal ultrasound guidance on day 6 of progesterone treatment. Vaginal progesterone treatment was initiated at a dose of 200 mg three times per day. In case of pregnancy, luteal support was continued until 8–10 weeks of gestation. In case of inadequate endometrial thickness or progesterone levels higher than 4.5 nmol/L, the embryo transfer was canceled and postponed to another cycle.

**Clinical outcomes**

The primary outcome was the live birth rate (LBR), which was defined as delivery of a viable infant at ≥ 28 weeks of gestation after embryo transfer. Secondary outcomes included clinical pregnancy rate (CPR), early miscarriage rate (EMR), ongoing pregnancy rate (OPR) and neonatal outcomes. CPR was determined by ultrasonographic documentation of at least one fetus with a heartbeat at 6–7 weeks of gestation. EMR was defined as an intrauterine pregnancy loss before 12 weeks of gestation. OPR was defined as pregnancy after 12 weeks. The neonatal outcomes were: gestational age at delivery and preterm birth (< 37 weeks, PTB), low birth weight (< 2500 g, LBW), high birth weight (≥ 4000 g, LGA), and congenital defects (CD).

**Statistical analysis**

Statistical comparisons between groups were performed using the independent sample Student’s t-test or Mann-Whitney’s U test for quantitative variables, as appropriate. We used the chi-square or Fisher’s exact test, where appropriate, for categorical variables. To identify potential confounding variables that could be independently associated with CPR and LBR, we performed univariate analysis and logistic regression analysis. Logistic regression was performed to control for confounders. The confounding factors included the following: AMH, endometrial thickness, ICM grade, TE grade, the day of blastocyst expansion, day 3 embryo source (AEs or PQEs). Odds ratios and 95% CI were calculated. All statistical analyses were performed with the use of SPSS 19.0 for Windows (SPSS Inc., Chicago, IL). A two-tailed P-values < 0.05 were considered statistically significant.

**Results**

Three groups were retrospectively created depending on whether the transferred blastocysts originated from available cleavage embryos (AEs) at day 3 (n = 382), poor-quality cleavage embryo with sibling available embryos (PQEs with AEs) (n = 99), or only poor-quality cleavage embryos (PQEs without AEs) (n = 101). Table 1 showed the progress of day 3 cleaved embryos in all patients during the study period. The patients who had PQEs (with and / or without AEs) were 33.77 ± 4.89 and 34.44 ± 5.26 years old, respectively, 42.31% and 31.51% of them could obtain expanded blastocysts (≥ 3BC/3CB). In PQEs (with and / or without AEs), 21.88% and 16.49% developed into expanded blastocysts, 30.77% and 28.00% of which expanded on day 5. In control group, 95.23% of patients with AEs (31.75 ± 4.46 years old) could
obtain expanded blastocysts, 64.84% of AEs developed into expanded blastocysts, 57.24% of which expanded on day 5.

Table 1
Progression of cleavage embryos on day 3 in all patients during the study period

| Variables                              | People with AEs | People without AEs |
|----------------------------------------|-----------------|--------------------|
|                                        | AEs (control)   | PQEs               | PQEs               |
| Age(y)                                 | 31.75 ± 4.46    | 33.77 ± 4.89       | 34.44 ± 5.26       |
| No. of cycles                          | 1131            | 1782               | 987                |
| Total number of embryos                | 7358            | 5408               | 2577               |
| No. of embryos                         | 6(4,8)          | 2(1,4)             | 2(1,3)             |
| Progression to blastocysts (≥ 3BC/3CB) | 4771(64.84)     | 1183(21.88)        | 425(16.49)         |
| Day 5                                  | 2731(57.24)     | 364(30.77)         | 119(28.00)         |
| Day 6                                  | 2040(42.76)     | 819(69.23)         | 306(72.00)         |
| No. of cycles with Blastocysts (≥ 3BC/3CB) | 1077(95.23)     | 754(42.31)         | 311(31.51)         |

**Baseline characteristics**

Table 2 showed Baseline characteristics. Compared with 382 cycles of AEs group. There were no differences in oocyte age, body mass index (BMI), duration of infertility, pelvic infection, history of preterm birth, history of spontaneous abortion, type of infertility, indication for treatment, blastocyst expansion grade, type of FET cycle, the number of thawed transplants cycles in the same ovarian stimulation cycles, endometrial thickness and progesterone level on the first day of the progesterone administration and assisted hatching (AH). In PQEs with and / or without AEs groups, AMH, good-quality blastocyst, ICM grade, TE grade, insemination method, and the day of blastocyst expansion were significantly different (P < 0.05), compared with AEs group.
Table 2
General characteristics.

| Variables                                | People with AEs | People without AEs | P value a | P value b |
|------------------------------------------|-----------------|--------------------|-----------|-----------|
|                                          | AEs (control, n = 382) | PQEs (n = 99) | PQEs (n = 101) |
| Oocyte age (y)                           | 32.02 ± 3.41    | 32.75 ± 3.04      | 32.74 ± 3.18 | 0.054     | 0.056     |
| BMI (kg/m2)                              | 21.58 ± 3.06    | 21.41 ± 2.8       | 21.39 ± 2.82 | 0.622     | 0.588     |
| Duration of infertility (y)              | 4.48 ± 2.91     | 4.67 ± 3.09       | 4.78 ± 2.89  | 0.584     | 0.368     |
| AMH (ng/mL)                              | 6.45 ± 4.20     | 4.58 ± 3.8        | 4.25 ± 4.30  | 0.000     | 0.000     |
| Pelvic infection                         | 14(3.70)        | 5(5.10)           | 5(5.00)     | 0.737     | 0.765     |
| History of preterm birth                 | 4(1.00)         | 2(2.0)            | 0(0.00)     | 0.788     | 0.584     |
| History of spontaneous abortion          | 54(14.10)       | 10(10.10)         | 13(12.90)   | 0.292     | 0.744     |
| Type of infertility                      |                 |                   | 0.152       | 0.864     |
| Primary                                 | 193(50.50)      | 58(58.6)          | 52(51.50)   |           |           |
| Secondary                               | 189(49.50)      | 41(41.4)          | 49(48.50)   |           |           |
| Indication for treatment                 |                 |                   | 0.847       | 0.797     |
| Tubal factor                             | 186(48.70)      | 46(46.50)         | 50(49.50)   |           |           |
| PCOS and/or anovulation                  | 26(6.80)        | 5(5.10)           | 9(8.90)     |           |           |
| Endometriosis                            | 13(3.40)        | 3(3.00)           | 3(3.00)     |           |           |
| Male factor                              | 63(16.50)       | 21(21.20)         | 19(18.80)   |           |           |
| Combined                                 | 94(24.60)       | 24(24.20)         | 20(19.80)   |           |           |
| Good-quality blastocyst                  | 154(40.31)      | 67(67.68)         | 84(83.17)   | 0.000     | 0.000     |

a poor-quality embryo with available embryos vs. available embryo.

b poor-quality embryo without available embryos vs. available embryo.

c on the first day of the progesterone administration.

BMI = body mass index; FSH = follicle-stimulating hormone; AMH = anti-müllerian hormone; ICM = inner cell mass; TE = trophoderm; AH = assisted hatching.
| Variables                        | People with AEs | People without AEs | \( P \) value \( a \) | \( P \) value \( b \) |
|---------------------------------|-----------------|--------------------|-------------------------|--------------------------|
|                                 | AEs (control, n = 382) | PQEs (n = 99) | PQEs (n = 101) |                        |
| Blast expansion grade           |                 |                    |                        |                          |
| 3                               | 20(5.20)        | 2(2.00)            | 6(5.90)                | 0.074                    |
| 4                               | 331(86.60)      | 82(82.80)          | 85(84.20)              | 0.81                     |
| 5                               | 26(6.80)        | 11(11.10)          | 8(7.90)                |                          |
| 6                               | 5(1.30)         | 4(4.00)            | 2(2.00)                |                          |
| ICM grade                       |                 |                    |                        |                          |
| A                               | 211(55.20)      | 25(25.30)          | 11(10.90)              | 0.000                    |
| B                               | 164(42.90)      | 73(73.70)          | 83(82.20)              | 0.000                    |
| C                               | 7(1.80)         | 1(1.00)            | 7(6.90)                |                          |
| TE grade                        |                 |                    |                        |                          |
| A                               | 114(29.80)      | 12(12.10)          | 6(5.90)                | 0.000                    |
| B                               | 244(63.90)      | 63(63.60)          | 69(68.30)              | 0.000                    |
| C                               | 24(6.30)        | 24(24.20)          | 26(25.70)              |                          |
| Previous IVF/ICSI attempts      |                 |                    |                        |                          |
| 0                               | 310(81.20)      | 81(81.80)          | 58(57.40)              | 0.906                    |
| 1                               | 62(16.20)       | 15(15.20)          | 25(24.80)              | 0.000                    |
| >1                              | 10(2.60)        | 3(3.00)            | 18(17.80)              |                          |
| Insemination method             |                 |                    |                        |                          |
| Conventional                    | 298(78.01)      | 79(79.80)          | 79(78.22)              | 0.700                    |

\( a \) poor-quality embryo with available embryos vs. available embryo.

\( b \) poor-quality embryo without available embryos vs. available embryo.

\( c \) on the first day of the progesterone administration.

BMI = body mass index; FSH = follicle-stimulating hormone; AMH = anti-müllerian hormone; ICM = inner cell mass; TE = trophectoderm; AH = assisted hatching.
| Variables                                      | People with AEs | People without AEs | P value a | P value b |
|-----------------------------------------------|-----------------|--------------------|-----------|-----------|
|                                               | AEs (control,  n = 382) | PQEs (n = 99) | PQEs (n = 101) |           |
| ICSI                                          | 84(21.98)       | 20(20.20)         | 22(21.78)  |           |
| Type of FET cycle                             |                 |                   |           | 0.745     | 0.18      |
| Spontaneous                                   | 117(30.60)      | 32(32.30)         | 38(37.60)  |           |
| Hormonal substitution                         | 265(69.40)      | 67(67.70)         | 63(62.40)  |           |
| No. thawed transplants Cycles in one IVF cycles | 0.114           | 0.945             | 0.114     | 0.945     |
| 1                                             | 334(87.40)      | 79(79.80)         | 90(89.10)  |           |
| 2                                             | 43(11.30)       | 18(18.20)         | 10(9.90)   |           |
| 3                                             | 5(1.30)         | 2(2.00)           | 1(1.00)    |           |
| Endometrial thickness(mm) c                   | 8.72 ± 1.53     | 8.78 ± 1.79       | 8.99 ± 1.69| 0.742     | 0.116     |
| Progesterone level(nmol/L) c                  | 2.99 ± 4.18     | 3.69 ± 4.61       | 3.79 ± 4.71| 0.167     | 0.116     |
| the day of blastocyst expansion               |                 |                   |           | 0.000     | 0.000     |
| Day 5                                         | 295(77.20)      | 33(33.30)         | 29(28.70)  |           |
| Day 6                                         | 87(22.80)       | 66(66.70)         | 72(71.30)  |           |
| AH (Yes)                                      | 24(6.30)        | 6(6.10)           | 8(7.90)    | 0.935     | 0.556     |

a poor-quality embryo with available embryos vs. available embryo.

b poor-quality embryo without available embryos vs. available embryo.

c on the first day of the progesterone administration.

BMI = body mass index; FSH = follicle-stimulating hormone; AMH = anti-müllerian hormone; ICM = inner cell mass; TE = trophectoderm; AH = assisted hatching.

**Clinical and neonatal outcomes**

The LBR, CPR and OPR was significantly lower for the PQEs with and / or without AEs groups compared to the AEs group (28.28%, 29.70% versus 44.50% for LBR, P < 0.01; 38.38%, 36.63% versus 57.07% for CPR, P < 0.01; 29.69%, 30.69% versus 46.0% for OPR, P < 0.01) (Table 3). However, no difference was observed in terms of the EMR (23.68%, 13.51% versus 18.81%, P > 0.05). Regarding the neonatal outcomes, infants...
of the two groups had similar mean gestational weeks and birth weights. In addition, preterm birth rate (0.00%, 10.00% versus 8.82%, P > 0.05), male offspring sex rate (50.00%, 53.33% versus 57.06%, P > 0.05), vaginal delivery (35.71%, 46.67% versus 35.88%, P > 0.05), LBW (3.57%, 10.00% versus 5.88%, P > 0.05), LGA (7.14%, 6.67% versus 5.29%, P > 0.05) and congenital defects rate (0.00%, 0.00% versus 1.18%) were comparable.

Table 3
Embryo transfer outcomes.

| Variables                        | People with AEs | People without AEs | P value a | P value b |
|----------------------------------|-----------------|--------------------|-----------|-----------|
|                                  | AEs (control, n = 382) | PQEs (n = 99) | PQEs (n = 101) |          |          |
| Clinical pregnancy               | 218(57.07)      | 38(38.38)          | 37(36.63) | 0.001     | 0.000     |
| Early miscarriage                | 41(18.81)       | 9(23.68)           | 5(13.51)  | 0.484     | 0.439     |
| Ongoing pregnancy                | 176(46.0)       | 29(29.29)          | 31(30.69) | 0.003     | 0.005     |
| Live birth                       | 170(44.50)      | 28(28.28)          | 30(29.70) | 0.003     | 0.007     |
| Gestational weeks at delivery (weeks) | 38.58 ± 2.04    | 38.87 ± 1.02       | 38.38 ± 1.91 | 0.473     | 0.609     |
| Preterm birth                    | 15(8.82)        | 0(0.00)            | 3(10.00)  | 0.135     | 1.000     |
| Offspring sex (male)             | 97(57.06)       | 14(50.00)          | 16(53.33) | 0.486     | 0.704     |
| Vaginal delivery                 | 61(35.88)       | 10(35.71)          | 14(46.67) | 0.986     | 0.261     |
| Mean birth weight, kg            | 3.21 ± 0.54     | 3.27 ± 0.44        | 3.25 ± 0.66 | 0.574     | 0.684     |
| Birth weight < 2500 g            | 10(5.88)        | 1(3.57)            | 3(10.00)  | 0.961     | 0.659     |
| Birth weight ≥ 4000 g            | 9(5.29)         | 2(7.14)            | 2(6.67)   | 1.000     | 1.000     |
| Congenital defects               | 2(1.18)         | 0(0.00)            | 0(0.00)   | 1.000     | 1.000     |

a poor-quality embryo with available embryos vs. available embryo.

b poor-quality embryo without available embryos vs. available embryo.

Univariate logistic regression analysis of the pregnancy rate and live birth rate

Table 4 presented the correlations between individual variables and LBR. In this univariate model, AMH (OR 1.06, 95% CI 1.02–1.10, P < 0.01) and endometrial thickness (OR 1.15, 95% CI 1.03–1.27, P < 0.05) had strong association with the LBR. The odds ratio for LBR was significantly increased with the transfer of a day 5 blastocyst (OR 3.48, 95% CI 2.39–5.07, P < 0.001). ICM score from A to B (OR 0.37, 95% CI
0.27–0.51 for B versus A, \( P < 0.001 \) or from A to C (OR 0.05, 95% CI 0.01–0.36 for C versus A, \( P < 0.01 \)) and TE score from A to B (OR 0.60, 95% CI 0.41–0.90 for B versus A, \( P < 0.05 \)) or from A to C (OR 0.20, 95% CI 0.10–0.40 for C versus A, \( P < 0.001 \)) was indicative of a poorer grade blastocyst, and this significantly reduced LBR. It was important to note that live birth rate was also significantly decreased in PQEs (with and / or without AEs) group compared with the day 3 AEs group (OR 0.49, 95% CI 0.30–0.80, \( P < 0.01 \); OR 0.53, 95% CI 0.33–0.85, \( P < 0.01 \)).
Table 4
Variables affecting clinical pregnancy and live-birth rates in single frozen embryo transfer cycles by univariate logistic regression analysis.

| Variable                                | OR (95% CI)          | P value |
|-----------------------------------------|----------------------|---------|
| **Live-birth rate**                     |                      |         |
| AMH (ng/mL)                             | 1.06 (1.02–1.10)     | 0.004   |
| Endometrial thickness (mm)              | 1.15 (1.03–1.27)     | 0.011   |
| ICM grade                               |                      | 0.000   |
| A                                       | ref.                 |         |
| B                                       | 0.37 (0.27–0.51)     | 0.000   |
| C                                       | 0.05 (0.01–0.36)     | 0.003   |
| TE grade                                |                      | 0.000   |
| A                                       | ref.                 |         |
| B                                       | 0.60 (0.41–0.90)     | 0.013   |
| C                                       | 0.20 (0.10–0.40)     | 0.000   |
| Expansion day of blastocyst (Day 5 VS. Day 6) | 3.48 (2.39–5.07) | 0.000   |
| Day 3 embryo source                     |                      | 0.001   |
| AEs                                     | ref.                 |         |
| PQEs with AEs                           | 0.49 (0.30–0.80)     | 0.004   |
| PQEs without AEs                        | 0.53 (0.33–0.85)     | 0.008   |
| **Clinical pregnancy rate**             |                      |         |
| AMH (ng/mL)                             | 1.05 (1.01–1.09)     | 0.017   |
| Endometrial thickness (mm)              | 1.19 (1.08–1.32)     | 0.001   |
| ICM grade                               |                      | 0.000   |
| A                                       | ref.                 |         |
| B                                       | 0.46 (0.33–0.64)     | 0.000   |
| C                                       | 0.04 (0.01–0.33)     | 0.003   |
| TE grade                                |                      | 0.000   |

^c on the first day of the progesterone administration.

OR = odds ratio; CI = confidence interval;
Variables impacted LBR also had strong association with the CPR, such as AMH (OR 1.05, 95% CI 1.01–1.09, P < 0.05), endometrial thickness (OR 1.19, 95% CI 1.08–1.32, P < 0.05), day 5 blastocyst transfer (OR 3.45, 95% CI 2.43–4.91, P < 0.001), ICM (OR 0.46, 95% CI 0.33–0.64 for B versus A, P < 0.001; OR 0.04, 95% CI 0.01–0.33 for C versus A, P < 0.01), TE (OR 0.62, 95% CI 0.41–0.93 for B versus A, P < 0.05; OR 0.626, 95% CI 0.14–0.48 for C versus A, P < 0.001) and day 3 embryo source (OR 0.47, 95% CI 0.30–0.74, P < 0.01 for PQEs with AEs versus AEs; OR 0.44, 95% CI 0.28–0.68, P < 0.001 for PQEs without AEs versus AEs).

### Multivariate logistic regression analysis of the pregnancy rate and live birth rate

In our logistic regression analysis of the LBR and CPR, the adjusted variables were AMH, endometrial thickness, ICM grade, TE grade, the day of blastocyst expansion, day 3 embryo source, based on the results of unadjusted logistic regression analysis (Table 5). From this multivariate model, we observed that the endometrial thickness continued to predict LBR (OR 1.16, 95% CI 1.03–1.30, P < 0.05). The day of blastocyst expansion was strongly predictive of LBR. LBR of expanded blastocyst on day 5 was 2.28 times as likely as that of expanded blastocyst on day 6 (OR 2.28, 95% CI 1.543–3.65, P < 0.01) in a subsequent frozen transfer cycle, irrespective of patient's age. Furthermore, the ICM score of an expanded blastocyst from A to C decreased the odds of LBR (OR 0.10, 95% CI 0.01–0.76 for C versus A, P < 0.05). There was not clear independent association of blastocyst transfer from PQEs with decreased LBR. Blastocysts formed from PQEs with and / or without AEs showed similar result in a live birth (OR 0.91, 95% CI 0.53–1.59, P > 0.05; OR 0.94, 95% CI 0.50–1.78, P > 0.05) compared with blastocysts formed from AEs.
Table 5
Logistic regression model for clinical pregnancy and live births in frozen-embryo transfer cycles.

| Variable                                      | AOR (95% CI)     | P value |
|-----------------------------------------------|------------------|---------|
| **Live-birth rate**                           |                  |         |
| Endometrial thickness (mm) \(^c\)              | 1.16 (1.03–1.30) | 0.013   |
| ICM grade                                     |                  | 0.018   |
| A                                             | ref.             |         |
| B                                             | 0.68 (0.46–1.01) | 0.058   |
| C                                             | 0.10 (0.01–0.76) | 0.026   |
| Expansion day of blastocyst (Day 5 VS. Day 6) | 2.28 (1.43–3.65) | 0.001   |
| Day 3 embryo source                           |                  | 0.945   |
| AEs                                           | ref.             |         |
| PQEs with AEs                                 | 0.91 (0.53–1.59) | 0.747   |
| PQEs without AEs                              | 0.94 (0.50–1.78) | 0.854   |
| **Clinical pregnancy rate**                   |                  |         |
| Endometrial thickness (mm) \(^c\)              | 1.18 (1.05–1.33) | 0.005   |
| ICM grade                                     |                  | 0.045   |
| A                                             | ref.             |         |
| B                                             | 0.77 (0.50–1.18) | 0.231   |
| C                                             | 0.08 (0.01–0.67) | 0.02    |
| Expansion day of blastocyst (Day 5 VS. Day 6) | 2.52 (1.62–3.94) | 0.000   |
| Day 3 embryo source                           |                  | 0.586   |
| AEs                                           | reference        |         |
| PQEs with AEs                                 | 0.78 (0.46–1.31) | 0.345   |
| PQEs without AEs                              | 0.82 (0.47–1.42) | 0.479   |

\(^c\) on the first day of the progesterone administration.

Confounding variables include AMH, endometrial thickness, ICM grade, TE grade, transfer day, day 3 embryo source.

The variables impacted LBR also were found to be independently associated with the CPR: endometrial thickness was independently associated with a significant increase in the CPR (OR = 1.18, 95% CI 1.05–
1.33, $P < 0.01$), blastocyst expansion at day 5 was independently associated with a significant increase in the CPR compared to day 6 blastocyst expansion (OR = 2.52, 95% CI 1.62–3.94, $P < 0.01$), furthermore, the ICM score of an expanded blastocyst from A to C decreased the odds of CPR (OR 0.08, 95% CI 0.01–0.67 for C versus A, $P < 0.05$). Blastocysts transfer formed from AEs did not have a significant positive impact on the CPR (OR 0.78, 95% CI 0.46–1.31, $P > 0.05$ for PQEs with AEs versus AEs; OR 0.82, 95% CI 0.47–1.42, $P > 0.05$ for PQEs without AEs versus AEs).

**Pre-freeze expansion day, quality of blastocyst and outcomes**

We stratified and compared the clinical outcomes of blastocysts on different expansion days and found that blastocysts on the same expansion day could obtain similar CPR (62.67%, 51.72%, vs. 62.37% on day 5 expansion; 24.24%, 30.56%, vs. 39.08% on day 6 expansion) and LBR (57.58%, 37.93%, vs. 50.17% on day 5 expansion; 13.64%, 26.39%, vs. 25.29% on day 6 expansion) regardless of the quality of cleavage embryos. We further stratified and compared the clinical outcomes of high-quality blastocysts on different expansion days, and found that no matter what the quality of cleavage embryos, the same expansion day of high-quality blastocysts could obtain similar CPR (66.67%, 52.17%, vs. 53.93% on day 5 expansion and high quality; 25%, 27.87%, vs. 38.46% on day 6 expansion and high quality) and LBR (53.33%, 39.13%, vs. 40.45% on day 5 expansion and high quality; 13.46%, 22.95%, vs. 21.54% on day 6 expansion and high quality) (Table 6).
Table 6
Pre-freeze expansion day, quality of blastocyst and outcomes

|                    | People with AEs | People without AEs |   |   |
|--------------------|----------------|-------------------|---|---|
|                    | AEs(control) | PQEs | PQEs | P value | P value |
| **Day 5 expansion** | n = 295  | n = 33  | n = 29  | 0.628 | 0.261 |
| Clinical pregnancy | 184(62.37)  | 22(66.67)  | 15(51.72) | 0.053 | 0.263 |
| Live birth        | 148(50.17)  | 19(57.58)  | 11(37.93) | 0.076 | 0.874 |
| **Day 6 expansion** | n = 87  | n = 66  | n = 72  | 0.358 | 0.88  |
| Clinical pregnancy | 34(39.08)  | 16(24.24)  | 22(30.56) | 0.35  | 0.908 |
| Live birth        | 22(25.29)  | 9(13.64)  | 19(26.39) | 0.258 | 0.849 |
| **Day 5 expansion and high quality** | n = 89  | n = 15  | n = 23  | 0.122 | 0.207 |
| Clinical pregnancy | 48(53.93)  | 10(66.67)  | 12(52.17) | 0.122 | 0.207 |
| Live birth        | 36(40.45)  | 8(53.33)  | 9(39.13)  | 0.258 | 0.849 |
| **Day 6 expansion and high quality** | n = 65  | n = 52  | n = 61  | 0.258 | 0.849 |
| Clinical pregnancy | 25(38.46)  | 13(25.00)  | 17(27.87) | 0.122 | 0.207 |
| Live birth        | 14(21.54)  | 7(13.46)  | 14(22.95) | 0.258 | 0.849 |

a poor-quality embryo with available embryos vs. available embryo.
b poor-quality embryo without available embryos vs. available embryo.

Discussion

Because transfer of embryos at the blastocyst stage would enable better embryo selection and better embryo–endometrium synchronization, pregnancy rate of blastocyst transfer was higher than that of cleavage embryo transfer. In addition, the high pregnancy rate obtained by blastocyst transfer made single embryo transfer possible, thus generating a reduced incidence of multiple pregnancies (15–17). The emergence of vitrification technology also provided a possibility for the cryopreservation of blastocysts with high pregnancy and implantation rates (18–19).

Most studies had shown that blastocyst transfer of PQEs could increase the pregnancy rate and cumulative delivery rate (12, 20), but few studies had evaluated the impact of PQEs at cleavage stage on the outcome of blastocyst transfers compared with AEs (14), especially for people without AEs.
Our study found that the overall outcomes of SBTs from AEs, including CPR, and LBR, were better than those from PQEs (with and/or without AEs), but there was no statistical difference in clinical outcomes after controlling for confounding variables such as AMH, endometrial thickness, ICM grade, TE grade, the day of blastocyst expansion. The results showed that endometrial thickness, ICM grade, and the day of blastocyst expansion were the factors affecting the outcome, and as long as blastocysts were developed, similar outcomes could be obtained regardless of the quality of cleavage embryos, once the blastocyst was available, the quality of cleavage embryos had no effect on subsequent clinical outcomes.

This was consistent with previous reports. Guerif et al. (7) reported that the blastocyst rate of top-quality embryos at day 2 (four even cells, <20% fragmentation rate) was higher than that of no top-quality embryos, and that the LBR of each blastocyst transfer was similar. However, this study only analyzed AEs (high or non-high quality) and excluded low-quality embryos. Other studies emphasized that poor or good embryo quality on day 2 and 3 in a good prognosis population did not predict the implantation of day 5 high-quality blastocysts (14, 21). However, those studies were limited to patients with good prognosis and did not compare implantation rates on day 6 blastocyst and average quality blastocyst. In fact, PQEs were more likely to result in expanded blastocysts on day 6 than on day 5, and they were more likely to form non-high quality blastocysts, compared with available day 3 embryos. In our study, 66.7% – 71.3% of SBTs formed from PQEs (with and/or without AEs) expanded on day 6, compared with 22.8% of SBTs formed from AEs. We stratified and compared the clinical outcomes of SBTs from different expansion days, and found that blastocysts from the same expansion days could obtain similar clinical outcomes regardless of the quality of cleavage embryos. The results showed that the day of blastocyst expansion rather than the quality of cleavage embryos was the factor affecting the pregnancy outcome of SBTs.

Previous studies had also shown that LBR of frozen-thawed blastocyst transfer with day 6 was significantly lower than that with day 5 regardless of embryo quality (22). And studies had shown that chromosomal abnormalities were common during cleavage, even in embryos with the best morphological scores, on the contrary, blastocysts with good morphological scores and faster development progression had a greater possibility of euploidy (23–24). Campbell A found that embryos with delayed initiation of blastulation were at a higher risk of aneuploidy (25). Yang et al. (26) pointed to a non-statistically significant trend toward increased pregnancy and implantation rates when euploid blastocysts with early initiation of blastulation were compared with euploid blastocysts with delayed initiation times. However, others believed that the euploidy rate was similar between day 5 and slow-growing day 6 blastocysts (27). In our study, blastocysts of grades 3 to 6 formed by day 5 were two times as likely to result in a live birth as those of the same graded slow-growing day 6 blastocyst. Blastocyst morphology based on blastocoel expansion, ICM and TE must also be considered in the evaluation of FET outcomes. Compared with ICM of Grade A, CPR and LBR of blastocyst transferred with ICM of grade C decreased by 90%, 92%, respectively (28–29).

Although blastocyst morphology was associated with aneuploidy (9), it was important to underline that the trying to select an euploid blastocyst only on the basis of its morphological aspect could be extremely hazardous, because many aneuploid blastocysts were able to reach top quality scores. Whether
blastocysts needed to be detected by PGT-aneuploidy (PGT-A), especially blastocysts from PQEs, we did not recommend PGT-A for these patients who faced with only poor-quality day 3 embryos, because we believed that under the same morphology and blastocyst expansion days, blastocysts from people without cleavage AEs could obtain similar outcomes compared with those from people with AEs. And some studies suggested that the PGT-A did not improve general outcomes in the overall patient population (24, 30). In addition, there was still considerable controversy about the clinical and economic effectiveness of the PGT-A approach (31).

There were obviously some limitations of our study. First, the study was retrospective and not randomized; therefore, several biases could been introduced. Second, different definitions of cleavage PQEs could affect the results. Herbemont C defined it as a PQE when one of these conditions was met on day 3 (< 6 cells, > 10 cells, > 20% fragmentation, multinucleation), which was different from our definition(< 5 cells, > 12 cells, > 20% fragmentation, seriously unequal sized blastomeres, ≥ 50% vacuoles, ≥ 50% multinucleation). Third, 70% of the patients in our center underwent double blastocyst transplantation. The standard of SBTs was medical indication or only one blastocyst remains. the sample size was limited and further stratified research according to the difference characters of PQES was needed.

**Conclusion**

We would suggest that patients who canceled the fresh transfer due to PQEs should choose to continue to culture and cryopreservation of blastocysts for future use, especially for the patients with recurrent PQEs. We concluded that after successful blastulation, low scoring cleavage embryos that were not considered to be transferable could obtain pregnancy rates and live birth rates, which was similar to blastocyst transplantation from available day 3 embryos under the same morphology and blastocyst expansion days.

**Declarations**

*Ethics approval and consent to participate*

This study was approved by the ethics committee of the Third Affiliated Hospital of Guangzhou Medical University. Each patient had signed an informed consent on obtaining and analyzing their clinical data before the initiation of IVF/ICSI-ET treatment.

*Consent for publication*

Not applicable.

*Availability of supporting data*
The datasets used in the current study are available from the corresponding author on reasonable request.

**Competing interests**

All of the authors declared that there were no conflicts of interests.

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**Author contributions**

Hanyan Liu and Shaoquan Zhan analyzed the data and drafted the article. Xiangjin Kang and Jianqiao Liu designed the study, Xiangjin Kang revised the article. and Xiaolin Long, Lei Li, and Hongzi Du gave some useful suggestions regarding data screening and analysis. All authors approved the definitive version of the article to be published.

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**Abbreviations**

ICM, inner cell mass; TE: trophectoderm; AEs, available cleavage embryos; PQEs, poor-quality cleavage embryos; SBTs, single blastocyst transfers; FET: frozen embryo transfer; IVF, in vitro fertilization; PGT, pre-implantation genetic test; ICSI, intracytoplasmatic sperm injection; DMSO, dimethyl sulfoxide; EG, ethylene glycol; LBR, live birth rate; CPR, clinical pregnancy rate; EMR, early miscarriage rate; OPR, ongoing pregnancy rate; PTB, preterm birth; LBW, low birth weight; LGA, high birth weight; CD, congenital defects; BMI, body mass index; FSH, follicle-stimulating hormone; AMH, anti-müllerian hormone; AH, assisted hatching; OR, odds ratio; CI, confidence interval.

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Tables

Table 1. Progression of cleavage embryos on day 3 in all patients during the study period

| Variables                        | People with AEs (AEs) | People without AEs (PQEs) |
|----------------------------------|-----------------------|----------------------------|
| Age (y)                          | 31.75±4.46            | 33.77±4.89                 | 34.44±5.26                 |
| No. of cycles                    | 1131                  | 1782                       | 987                        |
| Total number of embryos          | 7358                  | 5408                       | 2577                       |
| No. of embryos                   | 6(4,8)                | 2(1,4)                     | 2(1,3)                     |
| Progression to blastocysts (≥3BC/3CB) | 4771(64.84)           | 1183(21.88)                | 425(16.49)                 |
| Day 5                            | 2731(57.24)           | 364(30.77)                 | 119(28.00)                 |
| Day 6                            | 2040(42.76)           | 819(69.23)                 | 306(72.00)                 |
| No. of cycles with Blastocysts (≥3BC/3CB) | 1077(95.23)           | 754(42.31)                 | 311(31.51)                 |

Table 2. General characteristics.
| Variables                        | People with AEs (control, n=382) | People without AEs PQEs (n=99) | People without AEs PQEs (n=101) | P | P  |
|---------------------------------|-----------------------------------|-------------------------------|-------------------------------|---|---|
| Oocyte age (y)                  | 32.02±3.41                        | 32.75±3.04                    | 32.74±3.18                    | 0.054 | 0.056 |
| BMI (kg/m2)                     | 21.58±3.06                        | 21.41±2.8                     | 21.39±2.82                    | 0.622 | 0.588 |
| Duration of infertility (y)     | 4.48±2.91                         | 4.67±3.09                     | 4.78±2.89                     | 0.584 | 0.368 |
| AMH (ng/mL)                     | 6.45±4.20                         | 4.58±3.8                      | 4.25±4.30                     | 0.000 | 0.000 |
| Pelvic infection                | 14(3.70)                          | 50.10                         | 5(5.00)                       | 0.737 | 0.765 |
| History of preterm birth        | 4(1.00)                           | 20.00                         | 0(0.00)                       | 0.788 | 0.584 |
| History of spontaneous abortion | 54(14.10)                         | 10±10                         | 13±12.90                      | 0.292 | 0.744 |
| Type of infertility             |                                   |                               |                               | 0.152 | 0.864 |
| Primary                         | 193(50.50)                        | 58±58.6                       | 52(51.50)                     | 0.847 | 0.797 |
| Secondary                       | 189(49.50)                        | 41±41.4                       | 49(48.50)                     | 0.000 | 0.000 |
| Indication for treatment        |                                   |                               |                               | 0.074 | 0.81  |
| Tubal factor                    | 186(48.70)                        | 46(46.50)                     | 50(49.50)                     | 0.000 | 0.000 |
| PCOS and/or anovulation         | 26(6.80)                          | 5(5.10)                       | 9(8.90)                       | 0.847 | 0.797 |
| Endometriosis                   | 13(3.40)                          | 3(3.00)                       | 3(3.00)                       | 0.000 | 0.000 |
| Male factor                     | 63(16.50)                         | 21(21.20)                     | 19(18.80)                     | 0.000 | 0.000 |
| Combined                        | 94(24.60)                         | 24(24.20)                     | 20(19.80)                     | 0.000 | 0.000 |
| Good-quality blastocyst         | 154(40.31)                        | 67(67.68)                     | 84(83.17)                     | 0.000 | 0.000 |
| Blast expansion grade           |                                   |                               |                               | 0.000 | 0.000 |
| 3                               | 20(5.20)                          | 2(2.00)                       | 6(5.90)                       | 0.000 | 0.000 |
| 4                               | 331(86.60)                        | 82(82.80)                     | 85(84.20)                     | 0.000 | 0.000 |
| 5                               | 26(6.80)                          | 11(11.10)                     | 8(7.90)                       | 0.000 | 0.000 |
| 6                               | 5(1.30)                           | 4(4.00)                       | 2(2.00)                       | 0.000 | 0.000 |
| ICM grade                       |                                   |                               |                               | 0.000 | 0.000 |
| A                               | 211(55.20)                        | 25(25.30)                     | 11(10.90)                     | 0.000 | 0.000 |
| B                               | 164(42.90)                        | 73(73.70)                     | 83(82.20)                     | 0.000 | 0.000 |
| C                               | 7(1.80)                           | 1(1.00)                       | 7(6.90)                       | 0.000 | 0.000 |
| TE grade                        |                                   |                               |                               | 0.000 | 0.000 |
| A                               | 114(29.80)                        | 12(12.10)                     | 6(5.90)                       | 0.000 | 0.000 |
| B                               | 244(63.90)                        | 63(63.60)                     | 69(68.30)                     | 0.000 | 0.000 |
| C                               | 24(6.30)                          | 24(24.20)                     | 26(25.70)                     | 0.000 | 0.000 |
| Previous IVF/ICSI attempts      |                                   |                               |                               | 0.906 | 0.000 |
| 0                               | 310(81.20)                        | 81(81.80)                     | 58(57.40)                     | 0.700 | 0.964 |
| 1                               | 62(16.20)                         | 15(15.20)                     | 25(24.80)                     | 0.745 | 0.18  |
| >1                              | 10(2.60)                          | 3(3.00)                       | 18(17.80)                     | 0.014 | 0.945 |
| Insemination method             |                                   |                               |                               | 0.114 | 0.945 |
| Conventional                    |                                   |                               |                               | 0.114 | 0.945 |
| ICSI                            |                                   |                               |                               | 0.114 | 0.945 |
| Type of FET cycle               |                                   |                               |                               | 0.114 | 0.945 |
| Spontaneous                     |                                   |                               |                               | 0.114 | 0.945 |
| Hormonal substitution           |                                   |                               |                               | 0.114 | 0.945 |
| No.thawed transplants Cycles in one IVF cycles |                 |                               |                               | 0.114 | 0.945 |
| 1                                | 334(87.40)                        | 79(79.80)                     | 90(89.10)                     | 0.745 | 0.18  |
| 2                                | 43(11.30)                         | 18(18.20)                     | 10(9.90)                      | 0.014 | 0.945 |
| 3                                | 5(1.30)                           | 2(2.00)                       | 1(1.00)                       | 0.114 | 0.945 |
| Endometrial thickness(mm)       | 8.72±1.53                         | 8.78±1.79                     | 8.99±1.69                     | 0.742 | 0.116 |
Progesterone level (nmol/L) \(^c\) in the day of blastocyst expansion

|        | People with AEs (control, \(n=382\)) | People without AEs (PQEs, \(n=99\)) | People without AEs (PQEs, \(n=101\)) | \(P\) value | \(P\) value |
|--------|--------------------------------------|-------------------------------------|-------------------------------------|-------------|-------------|
| Day 5  | 295(77.20)                           | 33(33.30)                           | 29(28.70)                           |             |             |
| Day 6  | 87(22.80)                            | 66(66.70)                           | 72(71.30)                           |             |             |
| AH (Yes)| 24(6.30)                             | 6(6.10)                             | 8(7.90)                             | 0.935       | 0.556       |

\(^a\) poor-quality embryo with available embryos vs. available embryo.

\(^b\) poor-quality embryo without available embryos vs. available embryo.

\(^c\) on the first day of the progesterone administration.

BMI=body mass index; FSH=follicle-stimulating hormone; AMH=anti-müllerian hormone; ICM=inner cell mass; TE=trophectoderm; AH=assisted hatching.

Table 3. Embryo transfer outcomes.

| Variables                        | People with AEs | People without AEs | \(P\) value | \(P\) value |
|----------------------------------|-----------------|--------------------|-------------|-------------|
|                                 | AEs (control, \(n=382\)) | PQEs (\(n=99\)) |             |             |
| Clinical pregnancy               | 218(57.07)      | 38(38.38)          | 37(36.63)   | 0.000       | 0.000       |
| Early miscarriage                | 41(18.81)       | 9(23.68)           | 5(13.51)    | 0.484       | 0.439       |
| Ongoing pregnancy                | 176(46.0)       | 29(29.29)          | 31(30.69)   | 0.003       | 0.005       |
| Live birth                       | 170(44.50)      | 28(28.28)          | 30(29.70)   | 0.003       | 0.007       |
| Gestational weeks at delivery    | 38.58±2.04      | 38.87±1.02         | 38.38±1.91  | 0.473       | 0.609       |
| (weeks)                          |                 |                    |             |             |
| Preterm birth                    | 15(8.82)        | 0(0.00)            | 3(10.00)    | 0.135       | 1.000       |
| Offspring sex (male)             | 97(57.06)       | 14(50.00)          | 16(53.33)   | 0.486       | 0.704       |
| Vaginal delivery                 | 61(35.88)       | 10(35.71)          | 14(46.67)   | 0.986       | 0.261       |
| Mean birth weight, kg            | 3.21±0.54       | 3.27±0.44          | 3.25±0.66   | 0.574       | 0.684       |
| Birth weight < 2500 g            | 10(5.88)        | 1(3.57)            | 3(10.00)    | 0.961       | 0.659       |
| Birth weight ≥ 4000 g            | 9(5.29)         | 2(7.14)            | 2(6.67)     | 1.000       | 1.000       |
| Congenital defects               | 2(1.18)         | 0(0.00)            | 0(0.00)     | 1.000       | 1.000       |

\(^a\) poor-quality embryo with available embryos vs. available embryo.

\(^b\) poor-quality embryo without available embryos vs. available embryo.

Table 4. Variables affecting clinical pregnancy and live-birth rates in single frozen embryo transfer cycles by univariate logistic regression analysis.
| Variable                              | OR (95% CI)       | P value |
|--------------------------------------|-------------------|---------|
| **Live-birth rate**                  |                   |         |
| AMH (ng/mL)                          | 1.06 (1.02-1.10)  | 0.004   |
| Endometrial thickness (mm)c           | 1.15 (1.03-1.27)  | 0.011   |
| ICM grade                            |                   | 0.000   |
|                                      | A ref.            |         |
|                                      | B                 | 0.37 (0.27-0.51) | 0.000 |
|                                      | C                 | 0.05 (0.01-0.36) | 0.003 |
| TE grade                             |                   | 0.000   |
|                                      | A ref.            |         |
|                                      | B                 | 0.60 (0.41-0.90) | 0.013 |
|                                      | C                 | 0.20 (0.10-0.40) | 0.000 |
| Expansion day of blastocyst (Day 5 VS. Day 6) | 3.48 (2.39-5.07) | 0.000   |
| Day 3 embryo source                  |                   | 0.001   |
|                                      | AEs ref.          |         |
|                                      | PQEs with AEs     | 0.49 (0.30-0.80) | 0.004 |
|                                      | PQEs without AEs  | 0.53 (0.33-0.85) | 0.008 |
| **Clinical pregnancy rate**          |                   |         |
| AMH (ng/mL)                          | 1.05 (1.01-1.09)  | 0.017   |
| Endometrial thickness (mm)c           | 1.19 (1.08-1.32)  | 0.001   |
| ICM grade                            |                   | 0.000   |
|                                      | A ref.            |         |
|                                      | B                 | 0.46 (0.33-0.64) | 0.000 |
|                                      | C                 | 0.04 (0.01-0.33) | 0.003 |
| TE grade                             |                   | 0.000   |
|                                      | A ref.            |         |
|                                      | B                 | 0.62 (0.41-0.93) | 0.019 |
|                                      | C                 | 0.26 (0.14-0.48) | 0.000 |
| Expansion day of blastocyst (Day 5 VS. Day 6) | 3.45 (2.43-4.91) | 0.000   |
| Day 3 embryo source                  |                   | 0.000   |
|                                      | AEs ref.          |         |
|                                      | PQEs with AEs     | 0.47 (0.30-0.74) | 0.001 |
|                                      | PQEs without AEs  | 0.44 (0.28-0.68) | 0.000 |

C on the first day of the progesterone administration.

OR=odds ratio; CI=confidence interval;

Table 5. Logistic regression model for clinical pregnancy and live births in frozen-embryo transfer cycles.
| Variable                                      | AOR (95% CI) | P value |
|-----------------------------------------------|--------------|---------|
| **Live-birth rate**                          |              |         |
| Endometrial thickness (mm) c                  | 1.16(1.03-1.30) | 0.013   |
| ICM grade                                     |              | 0.018   |
| A                                             |              |         |
| B                                             | 0.68(0.46-1.01) | 0.058   |
| C                                             | 0.10(0.01-0.76) | 0.026   |
| Expansion day of blastocyst (Day 5 VS. Day 6) | 2.28(1.43-3.65) | 0.001   |
| Day 3 embryo source                           |              | 0.945   |
| AEs                                           |              |         |
| PQEs with AEs                                 | 0.91(0.53-1.59) | 0.747   |
| PQEs without AEs                              | 0.94(0.50-1.78) | 0.854   |
| **Clinical pregnancy rate**                   |              |         |
| Endometrial thickness (mm) c                  | 1.18(1.05-1.33) | 0.005   |
| ICM grade                                     |              | 0.045   |
| A                                             |              |         |
| B                                             | 0.77(0.50-1.18) | 0.231   |
| C                                             | 0.08(0.01-0.67) | 0.02    |
| Expansion day of blastocyst (Day 5 VS. Day 6) | 2.52(1.62-3.94) | 0.000   |
| Day 3 embryo source                           |              | 0.586   |
| AEs                                           |              |         |
| PQEs with AEs                                 | 0.78(0.46-1.31) | 0.345   |
| PQEs without AEs                              | 0.82(0.47-1.42) | 0.479   |

\(^c\) on the first day of the progesterone administration.

Confounding variables include AMH, endometrial thickness, ICM grade, TE grade, transfer day, day 3 embryo source.

Table 6. Pre-freeze expansion day, quality of blastocyst and outcomes
|                          | People with AEs (control) | People without AEs PQEs | P value a | P value b |
|--------------------------|---------------------------|-------------------------|-----------|-----------|
| **Day 5 expansion**      | n=295                     | n=33                    | n=29      |           |
| Clinical pregnancy       | 184 (62.37)               | 22 (66.67)              | 15 (51.72)| 0.628     | 0.261     |
| Live birth               | 148 (50.17)               | 19 (57.58)              | 11 (37.93)| 0.42      | 0.208     |
| **Day 6 expansion**      | n=87                      | n=66                    | n=72      |           |
| Clinical pregnancy       | 34 (39.08)                | 16 (24.24)              | 22 (30.56)| 0.053     | 0.263     |
| Live birth               | 22 (25.29)                | 9 (13.64)               | 19 (26.39)| 0.076     | 0.874     |
| **Day 5 expansion and high quality** | n=89                      | n=15                    | n=23      |           |
| Clinical pregnancy       | 48 (53.93)                | 10 (66.67)              | 12 (52.17)| 0.358     | 0.88      |
| Live birth               | 36 (40.45)                | 8 (53.33)               | 9 (39.13) | 0.35      | 0.908     |
| **Day 6 expansion and high quality** | n=65                      | n=52                    | n=61      |           |
| Clinical pregnancy       | 25 (38.46)                | 13 (25.00)              | 17 (27.87)| 0.122     | 0.207     |
| Live birth               | 14 (21.54)                | 7 (13.46)               | 14 (22.95)| 0.258     | 0.849     |

a poor-quality embryo with available embryos vs. available embryo.

b poor-quality embryo without available embryos vs. available embryo.