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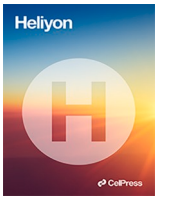

Research article

Outcomes of different transfer strategies for in vitro fertilization/intracytoplasmic sperm injection with poor-quality embryos—Analysis of embryonic development, perinatal period, and neonatal outcomes

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ABSTRACT

During the in vitro fertilization and embryo transfer process, some expectant mothers may not have good embryos to choose from before the embryo transfer. Recommendations for this condition are currently unclear, and relevant clinical and neonatal outcomes are still lacking. This study analyzed the outcomes of poor-quality embryo transfers, including fetal outcomes, in the fresh cycle and frozen–thawed embryo transfer cycle. Embryos were also analyzed for abnormalities during the cleavage stage. The results indicate that in the absence of good embryos, clinicians and embryologists could advise expectant mothers to continue culturing the embryos to the blastocyst stage and undergo transfer if blastocysts are formed. This finding can also be used as a reference for many expectant mothers with frozen embryos that have not yet been thawed.

1. Introduction

The selection of high-quality embryos in the in vitro fertilization (IVF) cycle has always been a topic of great concern for clinicians and embryologists. Recently, there have been many reports on methods of high-quality embryo selection in addition to the traditional morphological observation, such as assessing the metabolic status of embryos [1–3], artificial intelligence technology selection [4–6], evaluating developmental dynamics [7–9], assessing the genetics status [10,11], and prolonging the culture time [12,13]. These methods have greatly helped embryologists select high-quality embryos for transfer and have resulted in successful live births. However, a precondition for being hopeful is that the patient has good embryos.

In an IVF cycle, the number of oocytes obtained from each patient is limited based on the patient's ovarian reserve and absence of ovarian hyperstimulation syndrome. The average number of oocytes retrieved is approximately 10 and may even be less than 3 in some countries and regions [14]. Since the number of embryos obtained may be limited, it further reduces the number of high-quality embryos available. During embryonic development, the cleavage pattern of the embryo is essential to assess the quality and clinical outcome of the embryo [15]. Generally, cleavage embryos undergo their first mitotic cycle approximately 25 h after fertilization, and the second mitosis occurs approximately 10 h later. After another 12 h, the embryo undergoes the third mitosis, which is

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completed in approximately 5 h and forms an 8-cell embryo [16,17]. These embryos are desirable to every embryologist. However, in a single IVF cycle, the proportion of Day 3 high-quality embryos is only 15–30 % [18]. Therefore, at some point, we may encounter patients with no available normal cleavage embryos, especially on Day 3 where only 6-cell, 5-cell, or even 4-cell embryos can be selected. These embryos may be stunted, the cleavage pattern may be incorrect, or part of the blastomere may be broken into small fragments [19]. Hence, we need to determine the approach for patients with only ≤6-cell embryos. In such circumstances, we select the best embryos from these abnormal cleavage embryos for transfer or continue to culture and transfer if the blastocysts are formed. Alternatively, we advise the patient to discontinue this cycle and start a new IVF cycle. Chromosomal analysis can be performed on 4-cell, 5-cell, and 6-cell embryos on Day 3, and we can transfer them if they are euploid. However, for the most part, a regular IVF or intracytoplasmic sperm injection (ICSI) cycle does not involve genetic screening. Therefore, determining the outcomes of such embryo transfers is essential.

This study retrospectively analyzed the outcomes of 4-cell, 5-cell, and 6-cell cleavage-stage embryo transfers, including neonatal outcomes, in the fresh cycle and frozen–thawed embryo transfer (FET) cycle. The embryos were also analyzed for abnormalities during the cleavage stage. We hope that these results will guide embryologists and clinicians on management strategies in the absence of normal cleavage embryos in patients.

2. Materials and methods

2.1. Study design and patients

This single-center cohort study included 27,129 fresh and FET cycles from January 2017 to July 2021. All cycles were either single cleavage embryo transfer (SET) or single blastocyst transfer (SBT) performed at the Reproductive Medicine Center, Tongji Hospital. All patients provided written informed consent and underwent routine clinical treatment at our center. No additional interventions were performed. This study conformed to the Declaration of Helsinki for Medical Research involving humans. It was approved by the Ethical Committee of the Reproductive Medicine Center, Tongji Hospital.

The main population for this experiment comprised patients with only 4-cell, 5-cell, or 6-cell embryos to choose from on Day 3 of the fresh cycle. In this situation, some patients may insist on choosing Day 3 transfer, regardless of the number of cells in the embryo. Other patients may choose to continue the culture and transfer when the blastocysts are available. If the fresh cycle is not suitable for transfer (trigger day progesterone >1.5 ng/mL, estrogen >5000 pg/mL, or number of oocyte >20), some patients may insist on choosing Day 3 to freeze directly and then proceed to the FET cycle. Other patients may choose to continue the culture. If the

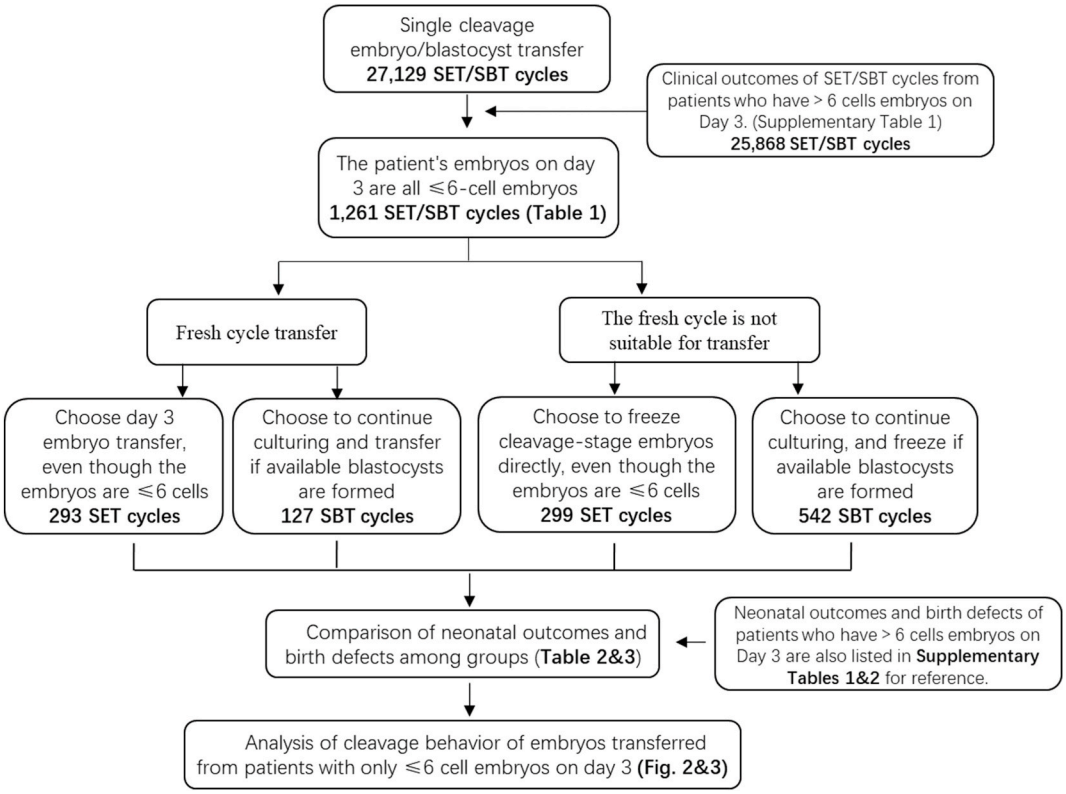

Fig. 1. Flow chart of the study design.

blastocysts are available, they can be frozen, and then the FET cycle can be performed. The flow chart of the experimental design is shown in Fig. 1.

2.2. Embryo incubation

Gamete preparation, insemination, and embryo incubation were performed as described in our previous paper [17]. Briefly, density gradient centrifugation was used to process the semen samples. The morphology, motility, and concentration of the sperms were evaluated according to the fifth edition of the World Health Organization guidelines. In the IVF cycles, cumulus-oocyte complexes were inseminated with 10,000 motile spermatozoa 3–4 h after retrieval. In the ICSI cycles, the cumulus cells and corona radiata of the oocytes were removed by brief exposure to hyaluronidase 2–3 h after retrieval. ICSI was performed on metaphase II oocytes observed under an inverted microscope. All fertilized oocytes were then continuously cultured in the Time-lapse (TL) system (Vitrolife, Denmark). Embryo evaluation was conducted at a relatively fixed time (8:00am to 9:00am) on Day 3. An optimal cleavage-stage embryo was routinely selected for transfer, and the remaining embryos were frozen or cultured until the blastocyst stage and then frozen for subsequent FET cycles.

2.3. Time-lapse monitoring and morphokinetic parameters

All fertilized oocytes were cultured at 37 °C until Day 5 or 6 under 6 % CO_2, 5 % O_2, and 89 % N_2 conditions in the TL system, with each embryo's image captured every 10 min. Morphokinetic parameters and cleavage pattern analysis of the embryos were performed using Embryoviewer software. All embryos were grouped according to the first, second, and third cleavage stages. The first cleavage was divided into normal cleavage (1–2 cells) and abnormal cleavage (direct cleavage, 1 to ≥3 cells). Embryos with a first normal cleavage underwent a second cleavage, which was divided into normal cleavage (2–4 cells) and abnormal cleavage (direct cleavage, 1 to ≥3 cells; or one blastomere that stops developing). The third cleavage was divided into direct cleavage (1–3 cells) to form 6-cell embryos; some blastomeres stop developing to form 5-cell or 6-cell embryos, or all blastomeres stop developing and remain in the 4-cell stage.

2.4. FET protocols

The FET protocols were based on a previous study [20]. Briefly, oral estradiol 2 mg/day was administered from Day 1 to Day 4, 4 mg/day from Day 5 to Day 8, and 6 mg/day from Day 9 to Day 12. On Day 13, when the endometrial thickness reached 8.0 mm or the maximum, 40 mg of progesterone was administered. The cleavage embryo or blastocyst was thawed and transferred on the day after 3/5 days of progesterone administration.

2.5. Outcome measures

Biochemical pregnancy was determined by measuring the serum human chorionic gonadotropin levels 2 weeks after the embryo transfer. Clinical pregnancy was defined as the presence of a gestational sac with fetal cardiac activity on ultrasonography 5 weeks

Table 1
Clinical outcomes of single cleavage embryo/blastocyst transfer cycles from patients with all embryos ≤6 cells on Day 3.

	Fresh transfer cycles		FET cycles	
	SET (≤6 cells on Day3)	SBT (≤6 cells on Day 3)	SET (≤6 cells on Day3)	SBT (≤6 cells on Day 3)
No. of transferred cycles	293	127	299	542
Age (y)	32.0 ± 5.0	32.1 ± 4.7	32.0 ± 4.9	31.9 ± 4.8
FSH	8.20 ± 2.8	7.85 ± 2.4	8.15 ± 2.9	7.90 ± 2.5
AFC	9.6 ± 5.7	10.3 ± 6.1	9.3 ± 5.4	10.0 ± 5.7
AMH	3.38 ± 3.4	3.52 ± 3.0	3.36 ± 3.7	3.44 ± 3.1
Primary infertility (%)	183 (62.8)	80 (63.0)	194 (64.9)	325 (60.0)
Duration of infertility (y)	3.56 ± 3.1	3.86 ± 3.0	3.51 ± 3.3	3.66 ± 3.1
BMI	22.2 ± 2.9	22.2 ± 3.1	22.2 ± 3.0	22.1 ± 2.9
Mean cells	5.86 ± 0.4	–	5.85 ± 0.4	–
IVF cycle (% per ET)	159 (54.3)	77 (60.6)	165 (55.2)	324 (59.8)
ICSI cycle (% per ET)	134 (45.7)	50 (39.4)	134 (44.8)	218 (40.2)
Clinical pregnancy rate (% per ET)	60 (20.5) a	32 (25.2) a	27 (9.0) b	197 (36.3) c
No. of miscarriages (% per clinical pregnancy)	14 (23.3)	5 (15.6)	5 (18.5)	48 (24.4)
No. of ectopic pregnancies (% per clinical pregnancy)	0	0	0	1 (0.5)
No. of induced abortions (% per clinical pregnancy)	0	0	0	1(1.4)
No. of fetal deaths (% per clinical pregnancy)	1 (1.7)	0	0	0
No. of live deliveries (% per ET)	45 (15.4) a	27 (21.2) a	22 (7.4) b	148 (27.3) c
Singletons (% per live delivery)	42 (93.3)	27 (100)	22 (100)	147 (99.3)
Twins (% per live delivery)	3 (6.7) a	0	0	1 (0.7) b

Each group with different superscripts differs significantly ($P < 0.05$). FET, frozen-thaw embryo transfer SET, single cleavage embryo transfer. SBT, single blastocyst transfer.

after the embryo transfer. Neonatal outcome data were obtained via telephone interviews with the parents. The questionnaire collected information regarding the gestational age, sex, birth weight, and congenital birth defects.

2.6. Statistical analysis

All data analyses were performed using the Statistical Package for Social Sciences, version 13.0. Between-group data were analyzed using the non-parametric Mann–Whitney *U* test and chi-square test. Clinical and neonatal outcomes of the SET and SBT cycles were compared using the one-way analysis of variance (ANOVA). The cleavage times for the first, second, and third cleavage stages between the cleavage-stage embryos (≤6 cells) and blastocysts developed from ≤6-cell embryos were compared using one-way ANOVA. The differences in the cleavage process between the cleavage-stage embryos (≤6 cells) and blastocysts developed from ≤6-cell embryos were compared using the chi-square test. The odds ratios and 95 % confidence intervals of the cleavage-stage embryos compared with the blastocysts were calculated. Statistical significance was set at $P < 0.05$.

3. Results

This study included 27,129 single embryo transfer cycles, comprising 1261 SET and SBT cycles of patients with only 4-cell, 5-cell, or 6-cell embryos on Day 3. The clinical outcomes of these patients are shown in Table 1. The basic data were similar between the groups, including age, follicular stimulating hormone level, antral follicle count, anti-mullerian hormone level, primary infertility ratio, duration of infertility, and body mass index. The clinical pregnancy rate in the fresh cycle was slightly lower in the SET group than in the SBT group (20.5 % vs. 25.2 %), without a significant difference ($P > 0.05$). In the FET cycle, the proportion of patients who came for FET for the first time in the SET and SBT groups was 75.3 % (225/299) and 69.2 % (375/542), respectively, with no significant difference. The clinical pregnancy rate was significantly lower in the SET group than in the SBT group (9.0 % vs. 36.3 %) and significantly lower than that of the SET and SBT groups in the fresh cycle. Live birth rates also showed similar results as the clinical pregnancy rates. The results of monozygotic twins showed that the live birth rate was significantly higher in the SET group with ≤6-cell embryos (6.7 %) in the fresh cycle than in the SBT group (0.7 %) in the FET cycle ($P < 0.05$).

Neonatal outcomes of singleton live birth cycles in patients with all embryos ≤6 cells on Day 3 are shown in Table 2. The results were similar among the groups, except for birth weight. The birth weight in the SBT group in the FET cycle (3333 ± 533 g) was significantly higher than that in the SET group in the fresh cycle (3230 ± 589 g), SBT group in the fresh cycle (3301 ± 488 g), and SET group in the FET cycle (3077 ± 431 g) ($P < 0.05$).

The proportions and types of birth defects are shown in Table 3 and Supplementary Table 4). In both fresh and FET cycles, the SET group had higher birth defect rates than the SBT group. In the fresh cycle, the birth defect rate in the SET group was 9.52 %, and no cases of birth defects were observed in the SBT group. Similarly, in the FET cycle, the birth defect rate in the SET group was 4.54 %, and no cases of birth defects were observed in the SBT group. Notably, the SET data of patients with only ≤6-cell embryos on Day 3 was much higher than that of patients with >6-cell embryos available on Day 3 (Supplementary Table 3).

In terms of cleavage patterns, all embryos with ≤6 cells were analyzed using the TL video data. The results are shown in Fig. 2. During the first cleavage, the proportion of direct cleavage in the SET group was significantly higher than that in the SBT group (23 % vs. 16 %; $P < 0.05$). No significant differences were observed between direct cleavage and partial developmental arrest during the second and third cleavage stages.

The TL parameter results corresponding to each cleavage stage are shown in Fig. 3. The results of the first cleavage showed that in both the SET and SBT groups, the cleavage time of the direct cleavage group was significantly later than that of the normal cleavage group by 3.7 h ($P < 0.05$). The results of the second cleavage of embryos with normal first cleavage showed that the cleavage time of

Table 2
Neonatal outcomes of singleton live birth cycles from patients with all embryos ≤6 cells on Day 3.

Parameter	Fresh transfer cycles		FET cycles	
	SET (≤6 cells on Day3)	SBT (≤6 cells on Day 3)	SET (≤6 cells on Day3)	SBT (≤6 cells on Day 3)
No. of vaginal deliveries (%)	7 (16.7)	4 (14.8)	2 (9.1)	21 (14.3)
No. of cesarean sections (%)	35 (83.3)	23 (85.2)	20 (90.9)	126 (85.7)
Mean gestational age, wk	38.2 ± 2.0	38.7 ± 1.1	38.1 ± 1.8	38.3 ± 1.6
No. of preterm deliveries (<37 wk) (%)	3 (7.1)	0	0	15 (10.2)
No. of very preterm deliveries (<32 wk) (%)	1 (2.4)	0	0	1 (0.7)
Mean birth weight, g	3230 ± 589 a	3301 ± 488 a	3077 ± 431 a	3333 ± 533 b
Live birth	42	27	22	147
Birth weight <1500 g (%)	0	0	0	2 (1.4)
Birth weight 1500–2499 g (%)	2 (4.8)	1 (3.7)	3 (13.6)	5 (3.4)
Birth weight 2500–3999 g (%)	35 (83.3)	25 (92.6)	19 (86.4)	123 (83.7)
Birth weight ≥4000 g (%)	5 (10.4)	1 (3.7)	0	17 (11.6)
Male	24	9	8	76
Female	18	18	14	71
Sex ratio, male/female	1.33	0.5	0.57	1.07

Each group with different superscripts differs significantly ($P < 0.05$). FET, frozen-thaw embryo transfer SET, single cleavage embryo transfer. SBT, single blastocyst transfer.

Table 3
Types of malformations among babies delivered from singleton live birth cycles.

Malformation type	Fresh transfer cycle		FET cycles	
	SET (≤6 cells on Day3)	SBT (≤6 cells on Day 3)	SET (≤6 cells on Day3)	SBT (≤6 cells on Day 3)
Cardiovascular	2: Congenital heart disease		1: Congenital heart disease	
Digestive system	1: Cleft lip and Palate			
Urogenital	1: Cryptorchidism			
Total birth defects/Total live birth (%)[a]	4 (9.52)	0	1 (4.54)	0

FET, frozen-thaw embryo transfer SET, single cleavage embryo transfer. SBT, single blastocyst transfer.

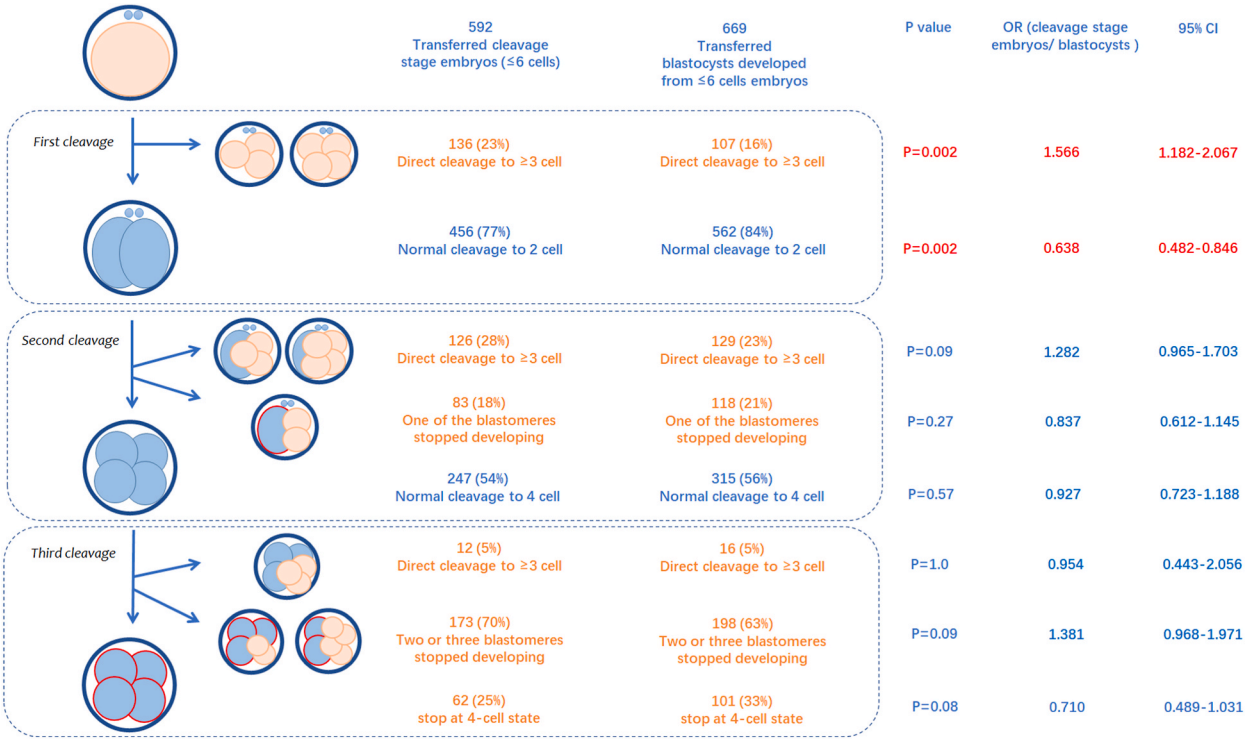

Fig. 2. Distribution of cleavage process of transferred cleavage-stage embryos (≤6 cells) and blastocysts developed from ≤6-cell embryos.

the direct cleavage group was later than that of the normal cleavage group in both SET and SBT groups. However, the cleavage time in blastomeres that stopped developing was similar to that in the normal group ($P > 0.05$). During the third cleavage, no significant difference was observed between the immediate cleavage group and 2- or 3-celled blastomeres that stopped developing, regardless of whether it was the SET or SBT group ($P > 0.05$).

4. Discussion

In IVF-ET cycles worldwide, clinical outcomes have improved significantly compared to those reported 10 years ago or earlier. However, despite satisfying patients, there will still be some patients with poor outcomes. The problems experienced can be of many types, including maturity disorders, fertilization disorders, and developmental delays [21,22]. Among these problems, developmental delays are encountered often, particularly in centers where fresh-cycle cleavage-stage embryo transfers are routinely performed. When the embryo develops to the third day, there are only 4-cell, 5-cell, or 6-cell blastomeres, and there are no better embryos to choose from. How should clinicians and embryologists choose in such situations? The patient may also have the same question.

Currently, there are two views. First, fresh-cycle cleavage-stage transfer should be considered, regardless of whether the embryos meet the criteria for high-quality embryos. Second, if there is no high-quality embryo transfer, the cleavage-stage embryo transfer should be abandoned, the embryo should be continued to culture to the blastocyst stage, and then transferred if a blastocyst is formed.

Proponents of cleavage-stage embryo transfer argue that prolonged incubation times may cause more harm or uncertainty [23,24]. No clear conclusion exists on whether prolonged culture with blastocyst stage transfer can achieve better clinical outcomes. Vos et al. [25] concluded that the live birth rate was significantly lower with the transfer of fresh single-cleavage stage embryos than with the transfer of blastocysts (31.3 % vs. 37.8 %; $P = 0.041$). Furthermore, the number of embryo transfers required until the first live birth

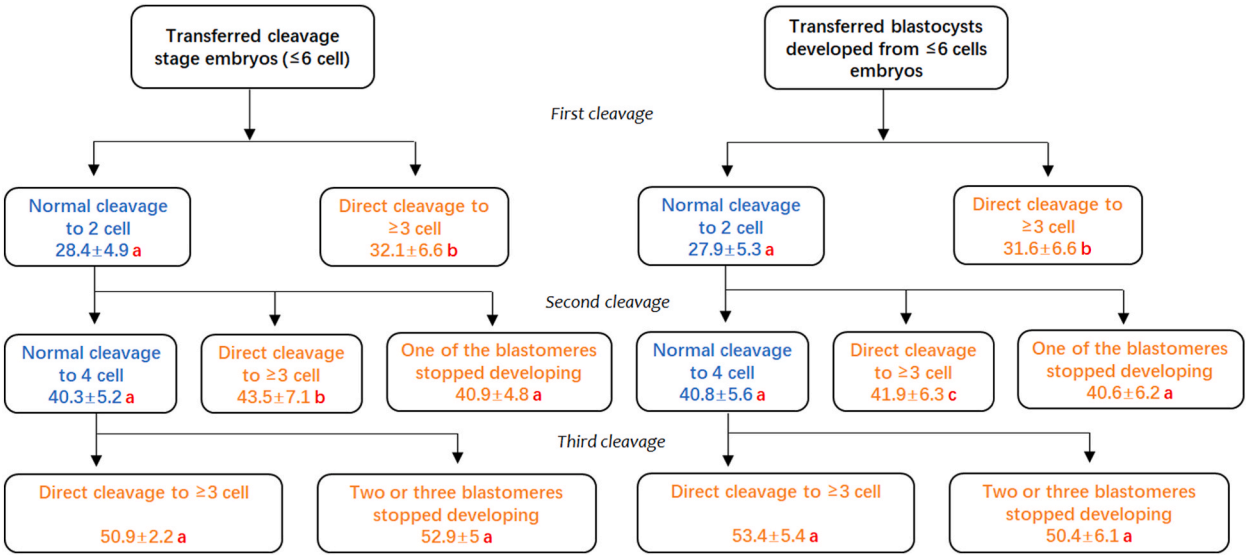

Fig. 3. Comparison results of the cleavage time in each group.

with blastocyst-stage embryos was significantly lower than that with cleavage-stage embryos ($P < 0.001$). However, the cumulative live birth rate was 52.6 % for the cleavage-stage transfer and 52.5 % for the blastocyst-stage transfer ($P = 0.989$). A meta-analysis also found no significant differences in the pregnancy outcomes between the blastocyst-stage and cleavage-stage embryo transfers [26]. In this study, the clinical pregnancy rate was also similar between the fresh cycle cleavage-stage ≤6-cell embryo transfers and the cultured blastocyst group (20.5 % vs. 25.2 %; $P > 0.05$). Third, blastocyst transfer has the potential to alter epigenetic variations and sex ratios in the offspring [27]. In our center, our baseline SBT population with good-quality embryos (>6 cells) had a higher proportion of male fetuses than the SET population, showing a significant difference in the fresh cycle (1.33 vs. 1.11; $P < 0.05$) (Supplementary Table 2). However, in the absence of >6-cell embryos, which was the focus of this study (Table 2), the proportion of male fetuses did not differ significantly ($P > 0.05$).

Those who support blastocyst-stage embryo transfer believe that blastocyst transfer has a higher implantation rate and clinical pregnancy rate than cleavage-stage embryo transfer [28,29], and the number of embryos transferred is less [30–32], which greatly reduces multiple pregnancy rates. In our center, our baseline SBT population with good-quality embryos (>6 cells) showed a higher clinical pregnancy rate than the SET population ($P < 0.05$) in both the fresh and FET cycles (Supplementary Table 1). However, as mentioned previously, the clinical outcomes of blastocyst cultures were not significantly altered in the absence of >6-cell embryos [33]. Second, blastocyst transfer and endometrial receptivity may be more synchronized [34]. Additionally, blastocyst-stage embryo transfer is associated with a lower rate of chromosomal abnormalities than cleavage-stage embryo transfer [35]. Although abnormal cleavage conditions, such as direct cleavage, rapid cleavage, and retrograde cleavage, occur in cleavage-stage embryos, these abnormalities may not affect blastocyst euploidy [36,37]. These results suggest that blastocyst culture can help eliminate problematic embryos [38,39]. Moreover, blastocyst transfer avoids premature exposure of embryos to the changing intrauterine environment due to ovulation (excessive estrogen levels reduce endometrial receptivity) [40].

Based on the aforementioned points of view, there are no clear guidelines in cases where >6-cell embryos are not available. In our reproductive center, clinicians and embryologists often encounter this situation, where they inform the patients about their embryonic development and that they can insist on choosing Day 3 transfer or continue to cultivate all of them until Day 5/6 and transfer the available blastocysts if formed. The patients are also informed about the risk of cycle cancellation. Patients are allowed to make their own choices. In this study, we found that there appeared to be no differences in the clinical pregnancy rates, miscarriage rates, and live birth rates between SET and SBT in the fresh cycles when >6-cell embryos were unavailable. This was not known before.

Moreover, this study found that neonatal birth outcomes differed between SET and SBT in the fresh cycle when >6-cell embryos were unavailable. The proportion of birth defects in SET of ≤6-cell embryos was 9.52 % (4/42) in fresh cycles and 0 (0/27) in SBT. Although the sample size of live births in both groups was small owing to the lack of >6-cell embryos, this result deserves our attention. The birth defect rate of SET for ≤6-cell embryos in fresh cycles was quite high (9.52 %) and much higher than that of our baseline SET population with good-quality embryos (>6 cells). Lijuan et al. [41] reported that 246 embryos with only 4–5 cells in a fresh cycle on Day 3 were cultured further to form blastocysts, and 41 patients formed blastocysts for transfer, with 22 live births and no birth defects.

In conclusion, in the absence of available embryos in a fresh cycle, we should directly transfer low-grade cleavage-stage embryos or proceed to blastocyst culture and transfer them after blastocyst formation. By comparing the clinical and neonatal birth outcomes of these two practices, this study shows that the direct transfer of low-grade cleavage embryos may be associated with an increased risk of neonatal birth defects. Therefore, in the absence of >6-cell embryos for fresh-cycle transfer, clinicians and embryologists should recommend that patients continue culturing embryos to the blastocyst stage and transfer them if the blastocysts are formed.

CRediT authorship contribution statement

Lei Jin: Data curation. **Zhou Li:** Methodology. **Keyi Si:** Validation. **Bingxin Ma:** Validation. **Xinling Ren:** Validation. **Bo Huang:** Writing – original draft, Supervision, Conceptualization.

Limitations

The main limitation of this study is that the population with only ≤6-cell embryos available on Day 3 was not very large. Since the sample size was relatively small, it may have been insufficient for observing birth defects.

Ethical approval

It was approved by the Ethical Committee of Reproductive Medicine Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology.

Data availability statement

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

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e40103.

References

[1] G. Schobers, R. Koeck, D. Pellaers, S.J.C. Stevens, M.V.E. Macville, A.D.C. Paulussen, E. Coonen, A. van den Wijngaard, C. de Die-Smulders, G. de Wert, et al., Liquid biopsy: state of reproductive medicine and beyond, Hum. Reprod. 36 (2021) 2824–2839, https://doi.org/10.1093/humrep/deab206.

[2] R. Orvieto, A. Aizer, N. Gleicher, Is there still a rationale for non-invasive PGT-A by analysis of cell-free DNA released by human embryos into culture medium? Hum. Reprod. 36 (2021) 1186–1190, https://doi.org/10.1093/humrep/deab042.

[3] L. Alegre, R. Del Gallego, S. Arrones, P. Hernandez, M. Munoz, M. Meseguer, Novel noninvasive embryo selection algorithm combining time-lapse morphokinetics and oxidative status of the spent embryo culture medium, Fertil. Steril. 111 (2019) 918–927, https://doi.org/10.1016/j.fertnstert.2019.01.022, e913. S0015-0282(19)30060-3 [pii].

[4] M.A. Riegler, M.H. Stensen, O. Witczak, J.M. Andersen, S.A. Hicks, H.L. Hammer, E. Delbarre, P. Halvorsen, A. Yazidi, N. Holst, T.B. Haugen, Artificial intelligence in the fertility clinic: status, pitfalls and possibilities, Hum. Reprod. 36 (2021) 2429–2442, https://doi.org/10.1093/humrep/deab168.

[5] B. Huang, S. Zheng, B. Ma, Y. Yang, S. Zhang, L. Jin, Using deep learning to predict the outcome of live birth from more than 10,000 embryo data, BMC Pregnancy Childbirth 22 (2022) 36, https://doi.org/10.1186/s12884-021-04373-5.

[6] B. Huang, W. Tan, Z. Li, L. Jin, An artificial intelligence model (euploid prediction algorithm) can predict embryo ploidy status based on time-lapse data, Reprod. Biol. Endocrinol. 19 (2021) 185, https://doi.org/10.1186/s12958-021-00864-4, 10.1186/s12958-021-00864-4 [pii].

[7] N. Desai, M. Yao, E.G. Richards, J.M. Goldberg, Randomized study of G-TL and global media for blastocyst culture in the EmbryoScope: morphokinetics, pregnancy, and live births after single-embryo transfer, Fertil. Steril. 114 (2020) 1207–1215, https://doi.org/10.1016/j.fertnstert.2020.06.049. S0015-0282(20)30623-3 [pii].

[8] N. Desai, J.M. Goldberg, C. Austin, T. Falcone, Are cleavage anomalies, multinucleation, or specific cell cycle kinetics observed with time-lapse imaging predictive of embryo developmental capacity or ploidy? Fertil. Steril. 109 (2018) 665–674, https://doi.org/10.1016/j.fertnstert.2017.12.025. S0015-0282(17)32172-6 [pii].

[9] B.X. Ma, L. Jin, B. Huang, Cytoplasmic String between ICM and mTE Is a Positive Predictor of Clinical Pregnancy and Live Birth Outcomes in Elective Frozen-Thawed Single Blastocyst Transfer Cycles: a Time-Lapse Study, 2020.

[10] N. Gleicher, D.H. Barad, P. Patrizio, R. Orvieto, We have reached a dead end for preimplantation genetic testing for aneuploidy, Hum. Reprod. (2022), https://doi.org/10.1093/humrep/deac052.

[11] Y. Yang, W. Tan, C. Chen, L. Jin, B. Huang, Correlation of the position and status of the polar body from the fertilized oocyte to the euploid status of blastocysts, Front. Genet. 13 (2022) 1006870, https://doi.org/10.3389/fgene.2022.1006870, 1006870 1006870 [pii].

[12] E.R. Hammond, L.M. Cree, D.E. Morbeck, Should extended blastocyst culture include Day 7? Hum. Reprod. 33 (2018) 991–997, https://doi.org/10.1093/humrep/dey091.

[13] D. Feil, R.C. Henshaw, M. Lane, Day 4 embryo selection is equal to Day 5 using a new embryo scoring system validated in single embryo transfers, Hum. Reprod. 23 (2008) 1505–1510, https://doi.org/10.1093/humrep/dem419.
[14] O. Ishihara, J.C. Arce, Individualized follitropin delta dosing reduces OHSS risk in Japanese IVF/ICSI patients: a randomized controlled trial, Reprod. Biomed. Online 42 (2021) 909–918, https://doi.org/10.1016/j.rbmo.2021.01.023. S1472-6483(21)00056-0 [pii].
[15] R.J. Paulson, D.E. Reichman, N. Zaninovic, L.R. Goodman, C. Racowsky, Time-lapse imaging: clearly useful to both laboratory personnel and patient outcomes versus just because we can doesn't mean we should, Fertil. Steril. 109 (2018) 584–591, https://doi.org/10.1016/j.fertnstert.2018.01.042. S0015-0282(18)30068-2 [pii].
[16] K. Kirkegaard, A. Ahlstrom, H.J. Ingerslev, T. Hardarson, Choosing the best embryo by time lapse versus standard morphology, Fertil. Steril. 103 (2015) 323–332, https://doi.org/10.1016/j.fertnstert.2014.11.003. S0015-0282(14)02349-8 [pii].
[17] B. Huang, X. Ren, L. Zhu, L. Wu, H. Tan, N. Guo, Y. Wei, J. Hu, Q. Liu, W. Chen, et al., Is differences in embryo morphokinetic development significantly associated with human embryo sex?dagger, Biol. Reprod. 100 (2019) 618–623, https://doi.org/10.1093/biolre/ioy229, 5145577 [pii].
[18] B. Huang, X. Ren, W. Li, L. Zhu, B. Xu, Y. Li, J. Ai, L. Jin, Q.Y. Sun, Elevated progesterone levels on the day of oocyte maturation may affect top quality embryo IVF cycles, PLoS One 11 (2016) e0145895.
[19] J. Bellver, A. Mifsud, N. Grau, L. Privitera, M. Meseguer, Similar morphokinetic patterns in embryos derived from obese and normoweight infertile women: a time-lapse study, Hum. Reprod. 28 (2013) 794–800, https://doi.org/10.1093/humrep/des438, des438 [pii].
[20] B. Huang, D. Hu, K. Qian, J.H. Ai, Y.F. Li, L. Jin, G.J. Zhu, H.W. Zhang, Is frozen embryo transfer cycle associated with a significantly lower incidence of ectopic pregnancy? An analysis of more than 30,000 cycles, Fertil. Steril. 102 (2014), https://doi.org/10.1016/j.fertnstert.2014.07.1245.
[21] Y. Ouyang, Y. Peng, S. Zhang, F. Gong, X. Li, A simple scoring system for the prediction of early pregnancy loss developed by following 13,977 infertile patients after in vitro fertilization, Eur. J. Med. Res. 28 (2023) 237, https://doi.org/10.1186/s40001-023-01218-z.
[22] Y. Fang, Y. He, W. Wang, Z. Zhang, P. Zhou, Y. Cao, X. He, Y. Xu, Z. Wei, Influencing factors and predictive model of live birth involving low-grade blastocyst frozen–thawed transfer: a retrospective study, Eur. J. Med. Res. 28 (2023) 117, https://doi.org/10.1186/s40001-023-01045-2.
[23] P.G. McGovern, A.J. Llorens, J.H. Skurnick, G. Weiss, L.T. Goldsmith, Increased risk of preterm birth in singleton pregnancies resulting from in vitro fertilization-embryo transfer or gamete intrafallopian transfer: a meta-analysis, Fertil. Steril. 82 (2004) 1514–1520, https://doi.org/10.1016/j.fertnstert.2004.06.038. S0015-0282(04)02326-X [pii].
[24] M. Berkkanoglu, K. Coetzee, H. Bulut, K. Ozgur, Optimal embryo transfer strategy in poor response may include freeze-all, J. Assist. Reprod. Genet. 34 (2017) 79–87, https://doi.org/10.1007/s10815-016-0825-y.
[25] A. De Vos, L. Van Landuyt, S. Santos-Ribeiro, M. Camus, H. Van de Velde, H. Tournaye, G. Verheyen, Cumulative live birth rates after fresh and vitrified cleavage-stage versus blastocyst-stage embryo transfer in the first treatment cycle, Hum. Reprod. 31 (2016) 2442–2449, https://doi.org/10.1093/humrep/dew219, dew219 [pii].
[26] D. Glujovsky, A.M. Quinteiro Retamar, C.R. Alvarez Sedo, A. Ciapponi, S. Cornelisse, D. Blake, Cleavage-stage versus blastocyst-stage embryo transfer in assisted reproductive technology, Cochrane Database Syst. Rev. 5 (2022) CD002118, https://doi.org/10.1002/14651858.CD002118.pub6.
[27] E.L. Niemitz, A.P. Feinberg, Epigenetics and assisted reproductive technology: a call for investigation, Am. J. Hum. Genet. 74 (2004) 599–609, https://doi.org/10.1086/382897. S0002-9297(07)61887-4 [pii].
[28] M. Kamath, A. Mangalaraj, K. Muthukumar, T. Aleyamma, A. Chandy, K. George, Comparison of clinical outcomes following vitrified warmed day 5/6 blastocyst transfers using solid surface methodology with fresh blastocyst transfers, J. Hum. Reprod. Sci. 6 (2013) 59–64.
[29] D. Zhu, J. Zhang, S. Cao, B.C. Heng, M. Huang, X. Ling, T. Duan, Q.T. Guo, Vitrified-warmed blastocyst transfer cycles yield higher pregnancy and implantation rates compared with fresh blastocyst transfer cycles–time for a new embryo transfer strategy? Fertil. Steril. 95 (2011) 1691–1695.
[30] E. Kadour-Peero, I. Feferkorn, V. Bellemare, S. Arab, W. Buckett, A comparison of frozen-thawed embryo transfer protocols in 2920 single-blastocyst transfers, Arch. Gynecol. Obstet. (2022), https://doi.org/10.1007/s00404-022-06588-z, 10.1007/s00404-022-06588-z [pii].
[31] H.J. Kim, J.K. Park, J.H. Eum, H. Song, W.S. Lee, S.W. Lyu, Embryo selection based on morphological parameters in a single vitrified-warmed blastocyst transfer cycle, Reprod. Sci. 28 (2021) 1060–1068, https://doi.org/10.1007/s43032-020-00349-6, 10.1007/s43032-020-00349-6 [pii].
[32] H. Ji, Y. Zhou, S. Cao, J. Zhang, X. Ling, C. Zhao, R. Shen, Effect of embryo developmental stage, morphological grading, and ploidy status on live birth rate in frozen cycles of single blastocyst transfer, Reprod. Sci. 28 (2021) 1079–1091, https://doi.org/10.1007/s43032-020-00381-6, 10.1007/s43032-020-00381-6 [pii].
[33] I. De Croo, R. Colman, P. De Sutter, D. Stoop, K. Tilleman, No Difference in Cumulative Live Birth Rates between Cleavage versus Blastocyst Transfer in Patients with Four or Fewer Zygotes: Results from a Retrospective Study, vol. 2022, Human Reproduction Open, 2022, https://doi.org/10.1093/hropen/hoac031.
[34] N. Doyle, J.C. Combs, S. Jahandideh, V. Wilkinson, K. Devine, J.E. O'Brien, Live birth after transfer of a single euploid vitrified-warmed blastocyst according to standard timing vs. timing as recommended by endometrial receptivity analysis, Fertil. Steril. 118 (2022) 314–321, https://doi.org/10.1016/j.fertnstert.2022.05.013. S0015-0282(22)00316-8 [pii].
[35] N. Gleicher, J. Metzger, G. Croft, V.A. Kushnir, D.F. Albertini, D.H. Barad, A single trophectoderm biopsy at blastocyst stage is mathematically unable to determine embryo ploidy accurately enough for clinical use, Reprod. Biol. Endocrinol. 15 (2017) 33, https://doi.org/10.1186/s12958-017-0251-8, 10.1186/s12958-017-0251-8 [pii].
[36] L. Jin, X. Dong, W. Tan, B. Huang, Incidence, dynamics and recurrences of reverse cleavage in aneuploid, mosaic and euploid blastocysts, and its relationship with embryo quality, J. Ovarian Res. 15 (2022) 91, https://doi.org/10.1186/s13048-022-01026-9, 10.1186/s13048-022-01026-9 [pii].
[37] B. Huang, W. Tan, Z. Li, L. Jin, An artificial intelligence model (euploid prediction algorithm) can predict embryo ploidy status based on time-lapse data, Reprod Biol Endocrin 19 (2021) 1–10.
[38] B.-X. Ma, L. Yang, Y. Tian, L. Jin, B. Huang, Cytoplasmic strings between ICM and mTE are a positive predictor of clinical pregnancy and live birth outcomes: a time-lapse study, Front. Med. 9 (2022), https://doi.org/10.3389/fmed.2022.934327.
[39] Y. Yang, X. Dong, J. Bai, L. Jin, B. Huang, Faster fertilization and cleavage kinetics reflect competence to achieve a live birth: data from single-embryo transfer cycles, BioMed Res. Int. 2022 (2022) 8501362, https://doi.org/10.1155/2022/8501362.
[40] H.M.F. Popovic-Todorovic, Implantation in assisted reproduction: a look at endometrial receptivity, Reprod. Biomed. Online 27 (2013) 530–538.
[41] L. Fan, W. Shi, W. Wen, S. Liang, Y. Chen, W. Zhao, B. Meng, J. Zhao, H. Zhou, Pregnancy outcomes of blastocysts from discarded embryos, J. Reprod. Med. 31 (2022) 166–170.