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ASSISTED REPRODUCTION TECHNOLOGIES

Optimal embryo management strategies for patients undergoing antagonist protocols in IVF treatment

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Abstract

Purpose Selection of optimal embryo transfer strategies for IVF patients treated with antagonist protocols.

Methods A retrospective study was conducted to assess whether whole embryo culture to the blastocyst stage could enhance the cumulative live birth rate (CLBR). The study included data from the first oocyte retrieval cycle of 4131 patients who underwent IVF treatment between January 2018 and June 2022. Patients were categorized into two strategies based on whether they underwent partial blastocyst culture (PBC) or whole embryo culture (WEC), and were further subdivided into three subgroups according to the number of high-quality embryos on Day 3: 0–2 embryos (subgroup 1), 3–7 embryos (subgroup 2), and 8 or more embryos (subgroup 3). Propensity score matching was used to perform a 1:1 match for patients across the three subgroups. The primary outcome measure was the CLBR per oocyte retrieval cycle.

Results After propensity score matching, there were no statistically significant differences in the baseline data among patients across the three corresponding subgroups for the PBC and WEC strategies. The analysis of CLBR in single oocyte retrieval cycles revealed that subgroup 1 had a significantly higher rate with the PBC strategy compared to the WEC strategy (33.0% vs. 25.7%, $P=0.018$). In subgroups 2 and 3, there were no statistically significant differences in the CLBR between patients using the two embryo management strategies.

Conclusions When patients have 0–2 high-quality embryos on Day 3, opting for Day 3 embryo transfer rather than blastocyst culture can increase the chances of embryo transfer and improve the CLBR.

Keywords IVF/ICSI · Cumulative live birth rate · Antagonist protocol · Blastocyst · Cleavage stage

Introduction

In the clinical practice of in vitro fertilization-embryo transfer (IVF-ET), the role of blastocyst transfer in enhancing both clinical pregnancy rates and live birth rates for infertile patients has been confirmed [1, 2]. For patients with good ovarian reserve function, culturing to the blastocyst stage facilitates the selection of embryos that exhibit greater developmental potential. This approach can improve the implantation rate and live birth per embryo transfer and simultaneously lower the risks associated with multiple gestations and ectopic pregnancies [3, 4]. However, the Blastocyst development rate of blastocyst culture is only 40–60%, which means that there is a certain risk in blastocyst culture, ultimately leading to the cancellation of the transfer cycle [5]. Therefore, the effectiveness of blastocyst culture strategies in patients with a limited number of cleavage-stage embryos remains controversial. Kovacic et al.'s retrospective study suggests that when there are fewer than three embryos on Day 2, blastocyst culture does not improve or reduce the clinical live birth rate [6]. However, these conclusions are confined to the outcomes of fresh embryo transfer, and there is an insufficient amount of long-term data regarding frozen embryos and CLBR. Consequently, the effect of blastocyst culture on the CLBR in IVF patients still warrants further investigation and confirmation.

Therefore, we conducted a retrospective study on the embryo management strategy for patients undergoing antagonist protocol ovarian stimulation, aiming to assess the impact of two different embryo management strategies on the cumulative live birth rate of patients. To ensure

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comparability between groups, we introduced propensity score matching to align the baseline characteristics of the two patient populations and compared and analyzed the pregnancy outcomes of the two groups.

Materials and methods

Study design and population

This study is a single-center, observational, retrospective cohort study, with research subjects primarily from the southwestern region of China. The study covered oocyte retrieval cycles for both IVF and intracytoplasmic sperm injection (ICSI), with sperm sources including fresh or frozen partner sperm. The study period was from January 2018 to June 2022, encompassing 4131 oocyte retrieval cycles. The partial blastocyst culture (PBC) strategy group comprised 2503 patients. In this group, D3 embryos were preferentially selected for transfer or cryopreservation, after ensuring the utilization of embryo transfer on Day 3, the remaining embryos were then subjected to extended blastocyst culture. The whole embryo culture (WEC) strategy group comprised 1628 patients, in which all cleavage-stage embryos underwent blastocyst culture. The study was approved by the institutional ethics committee and, as a retrospective study, did not require patient-informed consent. "Data related to patient treatments were extracted from the electronic medical record system and recorded in a database.

The inclusion criteria for the study were as follows: (1) first-time recipients of IVF treatment; (2) use of gonadotropin-releasing hormone (GnRH) antagonist protocol for controlled ovarian hyperstimulation (COH); and (3) at least one Day 3 embryo met the transfer criteria (grade III or higher). The exclusion criteria included: (1) indications for preimplantation genetic testing (PGT); (2) severe uterine anomalies; (3) moderate to severe intrauterine adhesions; and (4) adenomyosis and endometriosis.

This retrospective study involved clinical treatments conducted according to the clinical guidelines in effect at the time, with all patients receiving personalized antagonist protocols. Ovarian stimulation was initiated between the 2nd and 5th days of the menstrual cycle, with the specific dosage of gonadotropins determined based on each patient's age, antral follicle count (AFC), body mass index (BMI), and follicular growth response. GnRH antagonists were administered daily via subcutaneous injection, following either a fixed or flexible treatment plan, until the trigger day. When a follicle measures 18 mm in diameter or two follicles each measure 17 mm in diameter, the maturation of the oocyte is induced using human chorionic gonadotropin (hCG) and/or gonadotropin-releasing hormone agonist (GnRH-a). On the trigger day, progesterone levels are measured to evaluate the suitability for fresh embryo transfer. Within 36 to 38 h post-trigger, transvaginal ultrasound-guided follicular aspiration is conducted to retrieve oocytes.

Laboratory procedures

Following oocyte retrieval, fertilization was routinely carried out using IVF or ICSI, depending on the sperm quality assessment. After successful fertilization, embryos were cultured continuously in Cook's series of culture mediums. They were cultured individually in an incubator with a covered layer of oil, containing 6% carbon dioxide (CO_2), 5% oxygen (O_2), and 89% nitrogen (N_2), under a constant temperature condition at 37 °C. Fertilization status was checked 16 to 18 h after IVF or ICSI, and embryonic development was assessed daily until the transfer day or freezing. In this retrospective study, we transitioned from Day 3 embryo transfer to blastocyst transfer to enhance implantation rates and reduce the incidence of ectopic pregnancy. Throughout this transition, our laboratory practices remained consistent, with all embryo cultures being conducted using traditional incubators.

The assessment of embryo quality was based on the scoring system, which evaluated the morphological appearance of embryos [7]. For D3 embryos, the evaluation of embryo quality integrated considerations of cell number, blastomere size, and the degree of fragmentation. On Day 3, grade I embryos were defined as having 8 cells with uniform blastomere size and a fragmentation rate not exceeding 10%. Grade II embryos were characterized by a cell count of ≥ 6, with slightly uneven blastomere size or moderate fragmentation (not exceeding 25%). Grade III embryos were characterized by a cell count of ≥ 4, uneven blastomere size, and a fragmentation rate exceeding 25%. Grade IV embryos were those with fewer than 4 cells or a fragmentation rate over 50% [8]. We classified grades I and II embryos as high-quality D3 embryos. If a patient did not have high-quality embryos on Day 3, grade III embryos may have been considered for transfer, but grade IV embryos were not candidates for transfer. Blastocyst assessment also followed the Gardner grading system [9], with AA, AB, and BA grades considered high quality, BB grade as average quality, and BC, CB, AC, and CA as lower quality. Blastocysts graded CC were not used for transfer.

In reproductive medicine, if a patient had multiple embryos, the order of embryo transfer was determined based on the morphological scoring of the embryos. For Day 3 embryos with an equivalent number of cells, uniform size, and similar fragmentation rates, embryos with a pronuclei score of Z1 or Z2 were selected preferentially. If the pronuclei scores were identical, priority was given to embryos that had achieved a higher cell count with uniformly sized blastomeres on Day 2. Regarding blastocysts, the grading

of the inner cell mass (ICM) was considered more critical than that of the trophectoderm (TE). A full blastocyst was deemed superior to an expanded blastocyst. When the expansion stage and grading of the blastocysts were identical, the embryo score on Day 3 was taken into consideration.

Patients on the PBC strategy underwent Day 3 embryo transfer after oocyte retrieval. If fresh embryo transfer was not suitable for the patient, 1–2 D3 embryos were cryopreserved first. Excess embryos were cultured to the blastocyst stage and then cryopreserved using vitrification technology. In the WEC strategy, all embryos were cultured to the blastocyst stage. Only blastocysts that had reached the expanded stage by the morning of Day 5 were considered for fresh transfer. Delayed blastocysts were preserved for extended culture and underwent additional morphological assessment on Day 6 or D7. When delayed blastocysts reached the criteria for transplantability, they would undergo vitrification and cryopreservation for future transplantation. If a fresh blastocyst transfer was not suitable on Day 5, the blastocysts were cryopreserved using vitrification technology.

All embryo cryopreservations were conducted using a vitrification protocol. The cryoprotectant solution consisted of 15% dimethyl sulfoxide, 15% ethylene glycol, and 0.6 M sucrose. The primary reasons for canceling fresh embryo transfer in this study included delayed blastocyst development, an increased risk of ovarian hyperstimulation syndrome (OHSS), prematurely elevated progesterone levels, and the presence of conditions such as endometrial polyps. Additionally, frozen Day 3 embryos were not advanced to blastocyst culture in this research.

Cryopreserved embryo transfer

For patients with regular ovulatory cycles, a natural cycle protocol was commonly selected for frozen embryo transfer (FET). In the natural cycle, ovulation timing was tracked by transvaginal ultrasound monitoring of the follicle, with the day of ovulation set as Day 0. For patients with irregular ovulatory cycles, a hormone replacement cycle was used to prepare the endometrial lining. Patients took 2–6 mg of estradiol valerate orally daily, and the endometrial thickness was monitored using transvaginal ultrasound until the thickness exceeded 8 mm and/or the duration of estradiol valerate use reached 14 days. Subsequently, progesterone injections were used for endometrial maturation. Progesterone treatment was initiated on Day 0. Embryo transfer was planned for either Day 3 or 5, with the embryo thawing and revival process taking place on the morning of the respective transfer day.

Embryo transfer was performed under the guidance of abdominal ultrasound using a Cook catheter. During the study period, embryos did not undergo assisted hatching. The decision to transfer was based on a comprehensive consideration of the woman's age and embryo quality. Typically, only one high-quality blastocyst was transferred, or two Day 3 embryos; if the patient did not have high-quality blastocysts, double blastocyst transfer may have been considered, with a maximum of two embryos transferred per cycle.

Luteal support

The luteal support protocols for patients primarily included the following two types: (1) intravaginal administration, which involved the daily use of 90 mg of micronized progesterone in the form of Crinone® 8% pessaries (Merck Serono); (2) intramuscular injections of progesterone, at a dosage ranging from 40 to 60 mg daily. For fresh embryo transfer, the calculation started from the day of oocyte retrieval, and for FET cycles, luteal support was used from the day of endometrial maturation. On the 14th day following embryo transfer, an hCG test was conducted; if the hCG test result was positive, luteal support continued until the eighth week of pregnancy.

Outcomes

The principal outcome of this study was the cumulative live birth rate following the first oocyte retrieval cycle, which included the results of both fresh and frozen embryo transfer procedures. Secondary outcomes included the implantation rate, clinical pregnancy rate, multiple pregnancy rate, ectopic pregnancy rate, and pregnancy loss rate per oocyte retrieval cycle. Clinical pregnancy was defined as the detection of a gestational sac in an ultrasound examination conducted 28 days after embryo transfer. Pregnancy loss was defined as the failure of pregnancy due to various maternal and fetal factors, excluding ectopic pregnancy. A live birth was defined as an infant born with a heartbeat, respiration, and muscle movement, including both term and preterm infants. CLBR was defined as the sum of live birth events in both fresh and FET cycles, and deliveries of multiple pregnancies were counted as one live birth only. The follow-up endpoints for patients in this study were set at two scenarios: the first was when a patient achieved a live birth, the follow-up was terminated; the second was that the follow-up would be terminated by 31 May 2024. To ensure a comprehensive assessment of patients who had not achieved a live birth, our follow-up study covered at least 24 months starting from the date of oocyte retrieval.

Statistical analysis

Due to the large variation in the number of good-quality embryos among different patients, we plotted a line graph with the number of good-quality embryos on the *x*-axis and

cumulative live birth rates on the *y*-axis. Thereafter, subgroups were delineated based on the turning points of the line chart for statistical analysis, guaranteeing an adequately high sample size in each subgroup to augment the efficacy of the statistical analysis.

The "MatchIt" package in R software was used to perform 1:1 propensity score matching. The PSM model incorporated the following covariates: age, primary or secondary infertility, basal follicle-stimulating hormone (bFSH), AFC, total gonadotropin dosage (TGndose), endometrial thickness on the day of HCG triggering, number of retrieved oocytes, number of MII oocytes, fertilization method, and number of high-quality embryos on Day 3. Propensity scores were estimated using Generalized Linear Models (GLM). The nearest neighbor matching method was employed to ensure that each subject in the treatment group was matched with a control subject with the nearest propensity score. The process of performing the match combined the use of strata with a caliper. With strata based on the high-quality embryos variable, a caliper was applied to restrict the maximum distance between matching pairs to 0.005, ensuring that the covariates within specific subgroups were balanced within each stratum by limiting the maximum propensity score difference between matched pairs. Due to the limited number of cases in subgroup 3, the matching method has been switched from the nearest neighbor matching method to the optimal matching method, while other matching parameters in the model are kept completely consistent with the other two subgroups.

The distribution characteristics of the data were evaluated using the Kolmogorov–Smirnov test. Those that were normally distributed were compared using the Student's *t* test, while non-normally distributed continuous variables were compared using the Mann–Whitney *U* test. Continuous variables are described with mean ± standard deviation. Categorical data were presented as frequency counts and percentage distributions, and group differences were assessed using the Chi-square statistical test. A *P* value of less than 0.05 was considered to indicate statistical significance. All statistical analyses for this study were performed using the R 4.3.3 software environment.

Results

The process of patient inclusion, exclusion, and subgroup division in this retrospective study cohort is shown in Fig. 1. The study excluded 231 patients with indications for PGT, 219 patients with severe uterine anomalies or moderate to severe intrauterine adhesions, and 276 patients with adenomyosis and endometriosis (Fig. 1). The WEC strategy group included 1628 patients, while the PBC strategy group comprised 2503 patients. A line graph based on unmatched cumulative live birth rate data indicates that when patients have 0 to 2 high-quality embryos on Day 3, the PBC strategy shows a higher cumulative live birth rate compared to the WEC strategy. When the number of high-quality embryos on Day 3 is between 3 and 7, the cumulative live birth rates for

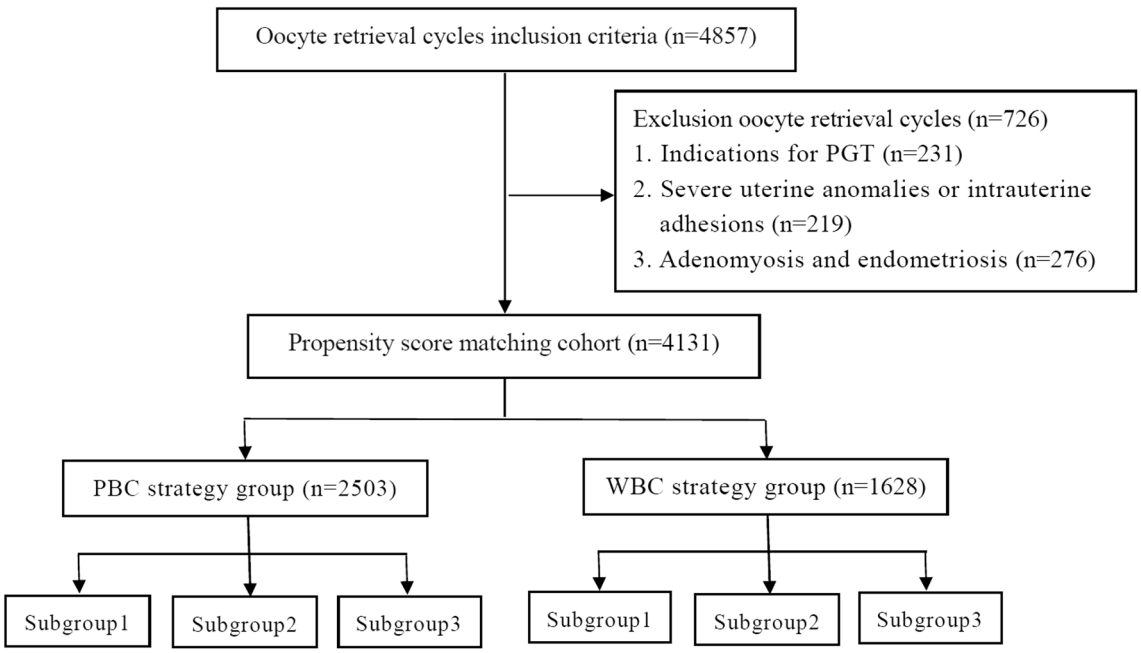

Fig. 1 Flow chart showing the selection of the study cohort. PBC, partial blastocyst culture; WBC, whole blastocyst culture. Subgroup 1: with 0–2 high-quality D3 embryos. Subgroup 2: with 3–7 high-quality D3 embryos. Subgroup 3: high-quality D3 embryos ≥ 8

both the PBC and WEC strategy groups are similar. However, when the number of high-quality D3 embryos ≥ 8, there is greater fluctuation in the cumulative live birth rates between the two groups (Fig. 2). To ensure a sufficiently high sample size across subgroups and enhance statistical power, patients were categorized into three subgroups based on the inflection points of the line chart: subgroup 1 comprises patients with only 0–2 high-quality embryos on D3, subgroup 2 includes patients with 3–7 high-quality embryos on D3, and subgroup 3 consists of patients with 8 or more high-quality embryos on D3. Following PS matching, subgroups 1–3 of the PBC and WEC strategies comprised 442, 387, and 144 female patients, respectively.

Before PS matching, there were no statistically significant differences in baseline characteristics such as age, duration of infertility, BMI, type of infertility, basal FSH and AFC, total dose of gonadotropins, and the number of oocytes retrieved among patients in subgroup 1. In subgroup 2, females in the WEC strategy group were significantly younger, had lower basal FSH levels, and had a higher AFC count ($P < 0.05$). In subgroup 3, couples in the WEC strategy group were significantly younger, with lower basal FSH levels, and had a higher AFC count and oocyte retrieval number ($P < 0.05$), but they had a significantly thinner endometrial ($P < 0.05$) (Table 1).

After PS matching, there were no statistical differences between the subgroups in baseline characteristics such as age, duration of infertility, BMI, type of infertility, basal FSH and AFC, total dose of gonadotropins, number of oocytes retrieved (Table 1), number of mature oocytes, fertilization method, number of 2PN-fertilized oocytes, and number of high-quality D3 embryos. In subgroup 1, the blastocyst formation rate was 30.0% for the PBC group and 36.1% for the WEC group ($P < 0.001$). In subgroup 2, the blastocyst formation rate was 48% for the PBC group and 55.5% for the WEC group ($P < 0.001$). In subgroup 3, the

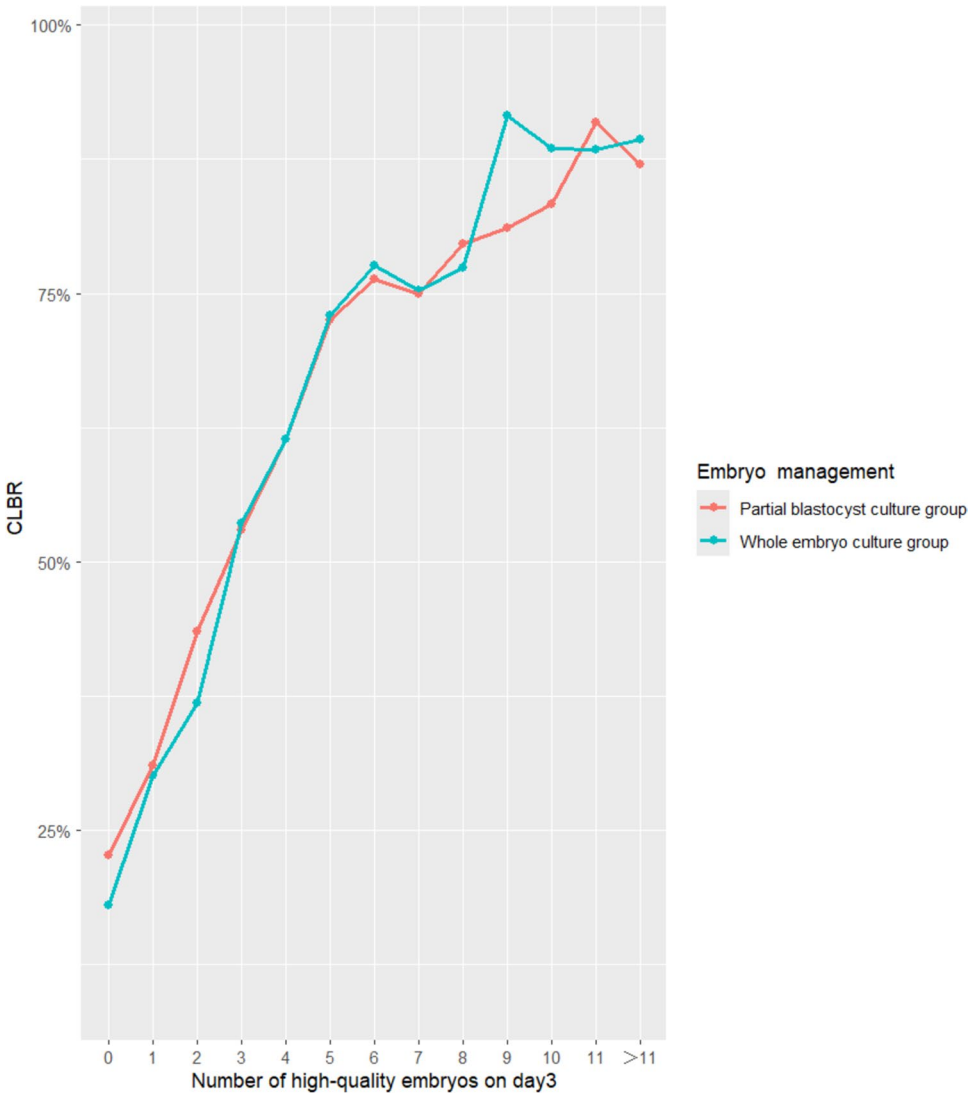

Fig. 2 Comparison of cumulative live birth rates for two embryo management strategies

Table 1 Demographic and baseline IVF characteristics for PS matching

	Before PS matching			After PS matching		
	PBC	WEC	*P* value	PBC	WEC	*P* value
Subgroup 1	**(*N* = 1245)**	**(*N* = 679)**		**(*N* = 442)**	**(*N* = 442)**	
Subgroup 2	**(*N* = 1114)**	**(*N* = 570)**		**(*N* = 387)**	**(*N* = 387)**	
Subgroup 3	**(*N* = 144)**	**(*N* = 409)**		**(*N* = 144)**	**(*N* = 144)**	
Female age (year)						
Subgroup 1 (mean (SD))	34.0 (±4.7)	34.2 (±4.8)	0.537	34.0 (±4.7)	34.4 (±4.7)	0.185
Subgroup 2 (mean (SD))	32.7 (±4.4)	32.2 (±4.6)	0.035	32.7 (±4.6)	32.6 (±4.6)	0.760
Subgroup 3 (mean (SD))	31.9 (±4.4)	30.4 (±4.0)	< 0.001	31.9 (±4.4)	31.7 (±4.3)	0.663
Male age (year)						
Subgroup 1 (mean (SD))	35.6 (±5.5)	35.8 (±5.4)	0.294	35.8 (±5.5)	36.1 (±5.3)	0.437
Subgroup 2 (mean (SD))	34.6 (±5.2)	34.4 (±5.3)	0.448	34.4 (±5.3)	34.7 (±5.2)	0.372
Subgroup 3 (mean (SD))	34.5 (±5.1)	32.6 (±4.4)	< 0.001	34.5 (±5.1)	33.7 (±4.5)	0.166
Infertility duration (year)						
Subgroup 1 (mean (±SD))	4.9 (±3.9)	5.0 (±4.0)	0.638	5.0 (±4.0)	5.2 (±4.2)	0.558
Subgroup 2 (mean (±SD))	4.7 (±3.5)	4.4 (±3.5)	0.146	4.6 (±3.6)	4.4 (±3.6)	0.495
Subgroup 3 (mean (±SD))	4.5 (±3.4)	4.2 (±3.0)	0.621	4.5 (±3.4)	4.5 (±3.2)	0.740
Female BMI (kg/m^2)						
Subgroup 1 (mean (±SD))	21.6 (±2.9)	21.7 (±2.8)	0.624	21.5 (±2.8)	21.8 (±2.8)	0.129
Subgroup 2 (mean (±SD))	21.6 (±3.1)	21.8 (±2.8)	0.289	21.6 (±3.0)	21.9 (±2.7)	0.200
Subgroup 3 (mean (±SD))	21.9 (±2.8)	21.5 (±3.0)	0.233	21.9 (±2.8)	21.4 (±2.9)	0.174
Primary infertility						
Subgroup 1 (*n* (%)	34.5% (430/1245)	38.2% (260/679)	0.102	35.5% (157/442)	32.8% (145/442)	0.396
Subgroup 2 (*n* (%)	37.5% (418/1114)	41.4% (236/570)	0.123	39.5% (153/387)	38.0% (147/387)	0.659
Subgroup 3 (*n* (%)	39.6% (57/144)	46.7% (191/409)	0.141	39.6% (57/144)	41.0% (59/144)	0.812
Basal FSH (IU/L)						
Subgroup 1 (mean (±SD))	6.6 (±2.5)	6.5 (±2.7)	0.554	6.7 (±2.5)	6.7 (±2.6)	0.742
Subgroup 2 (mean (±SD))	5.9 (±1.9)	5.4 (±1.8)	< 0.001	5.6 (±1.7)	5.7 (±1.8)	0.511
Subgroup 3 (mean (±SD))	5.3 (±1.8)	4.8 (±1.5)	0.003	5.3 (±1.8)	5.3 (±1.5)	0.918
AFC						
Subgroup 1 (mean (±SD))	11.1 (±5.9)	11.9 (±7.6)	0.253	10.9 (±5.9)	11.1 (±6.8)	0.652
Subgroup 2 (mean (±SD))	15.1 (±7.6)	18.4 (±9.3)	< 0.001	16.2 (±7.6)	16.3 (±8.4)	0.798
Subgroup 3 (mean (±SD))	19.9 (±9.0)	24.2 (±10.1)	< 0.001	19.9 (±9.0)	20.2 (±9.0)	0.666
Total dose of gonadotropins (IU)						
Subgroup 1 (mean (±SD))	1842.8 (±637.3)	1841.1 (±702.9)	0.958	1842.8 (±658.2)	1914.2 (±755.3)	0.135
Subgroup 2 (mean (±SD))	1814.4 (±513.5)	1825.4 (±528.7)	0.682	1820.4 (±545.1)	1832.9 (±492.2)	0.737
Subgroup 3 (mean (±SD))	1773.4 (±487.9)	1761.0 (±503.8)	0.796	1773.4 (±487.9)	1790.1 (±493.6)	0.772
Endometrial thickness on HCG day (mm)						
Subgroup 1 (mean (±SD))	10.9 (±2.4)	10.9 (±2.8)	0.492	10.9 (±2.4)	10.9 (±2.7)	0.904
Subgroup 2 (mean (±SD))	11.1 (±2.4)	11.0 (±2.6)	0.907	11.0 (±2.4)	11.1 (±2.6)	0.671
Subgroup 3 (mean (±SD))	11.6 (±2.5)	10.8 (±2.3)	0.003	11.6 (±2.5)	11.5 (±2.3)	0.937
Oocytes retrieved						
Subgroup 1 (mean (±SD))	6.6 (±4.2)	7.8 (±6.2)	0.026	6.8 (±4.7)	6.8 (±5.1)	0.518
Subgroup 2 (mean (±SD))	10.6 (±5.0)	14.7 (±6.6)	< 0.001	12.0 (±4.8)	12.0 (±4.8)	0.932
Subgroup 3 (mean (±SD))	16.5 (±5.8)	22.3 (±7.0)	< 0.001	16.5 (±5.8)	17.2 (±4.8)	0.054

blastocyst formation rate was 64.7% for the PBC group and 67.9% for the WEC group ($P > 0.05$) (Table 2).

It is noteworthy that in the WEC strategy, subgroup 1 had 153 cycles (36.8%) with no transferable blastocysts formed, and another 75 cycles (17.0%) experienced delayed blastocyst development; subgroup2 had 8 cycles (2.1%) with no transferable blastocysts, and another 50 cycles (12.9%) had delayed blastocyst development. In

Table 2 The laboratory parameters and clinic outcomes in fresh transfer cycles

	Before PS matching			After PS matching		
	PBC	WEC	*P* value	PBC	WEC	*P* value
Subgroup 1	**(*N* = 1245)**	**(*N* = 679)**		**(*N* = 442)**	**(*N* = 442)**	
Subgroup 2	**(*N* = 1114)**	**(*N* = 570)**		**(*N* = 387)**	**(*N* = 387)**	
Subgroup 3	**(*N* = 144)**	**(*N* = 409)**		**(*N* = 144)**	**(*N* = 144)**	
No. of MII oocytes						
Subgroup 1 (mean (±SD))	6.0 (±3.8)	7.0 (±5.5)	0.124	6.1 (±4.2)	6.2 (±4.7)	0.769
Subgroup 2 (mean (±SD))	9.8 (±4.5)	13.5 (±5.9)	< 0.001	11.1 (±4.2)	11.2 (±4.2)	0.921
Subgroup 3 (mean (±SD))	15.6 (±5.1)	20.9 (±6.6)	< 0.001	15.6 (±5.1)	16.2 (±4.5)	0.060
ICSI cycles						
Subgroup 1 (%)	19.4% (241/1245)	24.9% (169/679)	0.005	20.8% (92/442)	23.5% (104/442)	0.332
Subgroup 2 (%)	16.2% (181/1114)	17.8% (101/570)	0.444	17.6% (68/387)	16.0% (62/387)	0.566
Subgroup 3 (%)	12.5% (18/144)	13.0% (53/409)	0.902	12.5% (18/144)	13.2% (19/144)	0.863
No. of 2PN fertilization						
Subgroup 1 (mean (±SD))	3.6 (±2.6)	4.2 (±3.8)	0.662	3.6 (±2.7)	4.0 (±3.5)	0.404
Subgroup 2 (mean (±SD))	6.2 (±3.1)	9.2 (±4.4)	< 0.001	7.5 (±2.8)	7.7 (±3.2)	0.717
Subgroup 3 (mean (±SD))	11.1 (±3.7)	14.8 (±5.4)	< 0.001	11.1 (±3.7)	12.0 (±3.9)	0.046
No. of high-quality embryos on day 3						
Subgroup 1 (mean (±SD))	1.3 (±0.7)	0.68 (±0.8)	< 0.001	0.94 (±0.8)	0.96 (±0.8)	0.781
Subgroup 2 (mean (±SD))	3.3 (±1.3)	5.0 (±1.4)	< 0.001	4.6 (±1.4)	4.7 (±1.3)	0.807
Subgroup 3 (mean (±SD))	9.6 (±1.8)	11.5 (±3.9)	< 0.001	9.6 (±1.8)	9.7 (±2.1)	0.672
Blastocyst rate						
Subgroup 1 (%)	30.5% (887/2907)	32.1% (1046/3252)	0.172	30.0% (305/1015)	36.1% (719/1989)	< 0.001
Subgroup 2 (%)	47.9% (2887/6023)	52.5% (3052/5811)	< 0.001	48.0% (1193/2484)	55.5% (1838/3313)	< 0.001
Subgroup 3 (%)	64.7% (979/1512)	65.0% (4433/6824)	< 0.001	64.7% (979/1512)	67.9% (1316/1939)	0.059
Fresh transfer cycles rate						
Subgroup 1 (%)	94.8% (1180/1245)	25.0% (170/679)	< 0.001	94.1% (416/442)	26.7% (118/442)	< 0.001
Subgroup 2 (%)	89.9% (1002/1114)	35.3% (201/570)	< 0.001	88.1% (341/387)	42.6% (165/387)	< 0.001
Subgroup 3 (%)	70.8% (102/144)	15.6% (64/409)	< 0.001	70.8% (102/144)	31.9% (46/144)	< 0.001
No. of transferred embryos						
Subgroup 1 (mean (±SD))	1.9 (±0.4)	1.5 (±0.5)	< 0.001	1.8 (±0.4)	1.5 (±0.5)	< 0.001
Subgroup 2 (mean (±SD))	1.9 (±0.2)	1.5 (±0.5)	< 0.001	1.9 (±0.2)	1.5 (±0.5)	< 0.001
Subgroup 3 (mean (±SD))	1.9 (±0.2)	1.6 (±0.5)	< 0.001	1.9 (±0.2)	1.6 (±0.5)	< 0.001
Implantation rate per fresh embryo						
Subgroup 1 (%)	25.3% (552/2178)	27.3% (71/260)	0.541	22.7% (170/749)	27.1% (49/181)	0.251
Subgroup 2 (%)	35.0% (685/1959)	39.3% (119/303)	0.164	37.2% (246/662)	40.2% (99/246)	0.439
Subgroup 3 (%)	41.7% (83/199)	45.7% (48/105)	0.583	41.7% (83/197)	46.7% (35/75)	0.547
Clinical pregnancy per fresh transfer						
Subgroup 1 (%)	38.9% (458/1180)	37.1% (63/170)	0.664	34.6% (144/416)	37.3% (44/118)	0.591
Subgroup 2 (%)	54.2% (543/1002)	51.7% (104/201)	0.526	58.1% (198/341)	52.7% (87/165)	0.259
Subgroup 3 (%)	61.8% (63/102)	62.5% (40/64)	0.928	61.8% (63/102)	65.2% (30/46)	0.696
Multiple pregnancies per fresh transfer						
Subgroup 1 (%)	7.8% (92/1180)	5.3% (9/170)	0.246	6.3% (26/416)	5.1% (6/118)	0.666
Subgroup 2 (%)	13.7% (137/1002)	7.5% (15/201)	0.012	13.2% (45/341)	7.3% (12/165)	0.045
Subgroup 3 (%)	19.6% (20/102)	10.9% (7/64)	0.146	19.6% (20/102)	6.5% (3/46)	0.039
Pregnancy loss per fresh pregnancy						
Subgroup 1 (%)	18.8% (86/458)	17.5% (11/63)	0.824	17.4% (25/144)	15.9% (7/44)	0.846
Subgroup 2 (%)	15.7% (85/543)	14.4% (15/104)	0.769	22.7% (45/198)	16.1% (14/87)	0.266
Subgroup 3 (%)	7.9% (5/63)	22.5% (9/40)	0.046	7.9% (5/63)	23.3% (7/30)	0.054

Table 2 (continued)

	Before PS matching			After PS matching		
	PBC	WEC	*P* value	PBC	WEC	*P* value
Ectopic pregnancy per fresh transfer						
Subgroup 1 (*n* (%))	1.6% (19/1180)	2.9% (5/170)	0.214	0.5% (2/416)	3.4% (4/118)	0.024
Subgroup 2 (*n* (%))	3.3% (33/1002)	3.0% (6/201)	0.859	2.9% (10/341)	3.0% (5/165)	0.952
Subgroup 3 (*n* (%))	1.4% (2/144)	0	NA	1.4% (2/144)	0	NA
Live birth per fresh transfer						
Subgroup 1 (*n* (%))	30.0% (353/1180)	27.6% (47/170)	0.550	28.1% (117/416)	28.0% (33/118)	0.981
Subgroup 2 (*n* (%))	42.4% (425/1002)	41.3% (83/201)	0.772	41.9% (143/341)	41.2% (68/165)	0.879
Subgroup 3 (*n* (%))	54.9% (56/102)	48.4% (31/64)	0.423	54.9% (56/102)	50.0% (23/46)	0.586

PBC, partial blastocyst culture; *WEC*, whole embryo culture; *MII*, metaphase II; *ICSI*, intracytoplasmic sperm injection; *2PN*, 2pronuclei. $P<0.05$ indicates a statistical difference between the two subgroups. *P* values reflect the comparative outcomes between the PBC and WEC groups for each category

subgroup 3, there were no cycles canceled due to embryonic factors for fresh transfer. In the PBC strategy, no patients had fresh embryo transfer cycles canceled due to embryonic factors.

PBC, partial blastocyst culture; *WEC*, whole embryo culture; *BMI*, body mass index; *FSH*, follicle stimulating hormone; *AFC*, antral follicle count. $P<0.05$ indicates a statistical difference between the two subgroups. *P* values reflect the comparative outcomes between the PBC and WEC groups for each category.

Fresh embryo transfer cycles

In fresh embryo transfer cycles, both the clinical pregnancy rate and the live birth rate increased with the number of high-quality embryos on D3. In subgroup analysis, the PBC strategy had a higher rate of fresh embryo transfer per oocyte retrieval cycle in all subgroups ($P<0.001$), and the average number of embryos transferred was also greater than that in the WEC strategy group ($P<0.001$). The implantation rate of embryos in each PBC subgroup was lower than that in the corresponding WEC strategy ($P>0.05$), but there were no statistically significant differences in clinical pregnancy rates among the subgroups ($P>0.05$). In subgroups 2 and 3, we observed that the multiple pregnancy rate with the PBC strategy was significantly higher than with the WEC strategy ($P<0.05$). In subgroups 1 and 2, the pregnancy loss rate with the PBC strategy was slightly higher than with the WEC strategy, but not statistically significant ($P>0.05$). In subgroup 1, the ectopic pregnancy rate with the WEC strategy was slightly higher than with the PBC strategy ($P<0.05$). Finally, the live birth rate following fresh embryo transfer was higher in all PBC strategy subgroups compared to the WEC strategy, but the difference was not statistically significant ($P>0.05$) (Table 2).

Frozen embryo transfer cycles

In FET cycles, as the number of high-quality D3 embryos increased, the clinical pregnancy rate and live birth rate of both strategies were improved. In subgroup analysis, the WEC strategy group underwent more thawing transfer cycles, and there was no statistically significant difference in the proportion of hormone replacement cycles (HRC) and the average number of transferred embryos among the three subgroups ($P>0.05$). In subgroup 2, the PBC strategy group had a higher average age and a significantly lower mean endometrial thickness on the transplantation day compared to the WEC strategy group ($P<0.05$). Conversely, Subgroups 1 and 3 demonstrated no statistically significant differences in average age or mean endometrial thickness on the transplantation day ($P>0.05$). Among the three subgroups, there were no statistically significant differences in clinical pregnancy rates between the PBC and WEC strategies ($P>0.05$). The miscarriage rates in each PBC subgroup were higher than their corresponding WEC subgroups, but the differences were not statistically significant ($P>0.05$). The ectopic pregnancy rates during the FET cycles ranged from 0 to 3.4%, showing no significant variation between the PBC and WEC strategies ($P>0.05$). Regarding the live birth rate, the WEC strategy had higher rates in subgroups 1 and 2 compared to the PBC strategy, while subgroup 3 had a lower rate than the PBC strategy, yet none of these differences were statistically significant ($P>0.05$).

Cumulative live birth rates

In a comparison of cumulative live birth rates within a single oocyte retrieval cycle, subgroup 1 demonstrated a significantly higher rate with the PBC strategy compared to the WEC strategy (33.0% vs. 25.7%, $P=0.018$). In subgroup 2, the PBC strategy showed a slightly higher cumulative

live birth rate than the WEC strategy (66.4% vs. 65.6%, $P=0.820$). Similarly, subgroup 3 had a marginally higher cumulative live birth rate with the PBC strategy (82.6% vs. 81.3%, $P=0.762$), but these differences were not statistically significant (Table 3).

Discussion

In the published literature, most studies select subjects based on factors such as patient age, oocyte count, or zygote numbers to explore the impact of different transfer strategies on CLBR [10–14]. However, using age, oocyte count, or zygote number to determine transfer strategies has limitations. Focusing solely on the number of retrieved oocytes when deciding on the transfer date may overlook the crucial factor of low fertilization rates. Additionally, when analyzing subsequent embryo transfer strategies using zygote metrics, there may be no transferable embryos on Day 3. Therefore, we selected the number of high-quality embryos on D3 as the basis for formulating embryo transfer strategies, consistent with the choices of Yang, De Vos, and Stimpfel et al. [11, 15, 16]. The advantage of this approach is that it enables reproductive physicians to determine, based on the condition of D3 embryos, whether patients require blastocyst culture followed by blastocyst transfer. In this study, the choice between D3 embryo transfer and blastocyst transfer was guided by the evolution of embryo transfer strategies over time, rather than by any single clinical factor. This method ensures a degree of fairness in the objective allocation of patients to various transfer strategies.

In this retrospective cohort study, to eliminate the influence of different ovulation induction protocols, we included only oocyte retrieval cycles that utilized a GnRH antagonist protocol [17]. We also limited our sample to patients undergoing their first IVF treatment to avoid "survivor bias," which could arise from the outcomes of previous oocyte retrieval cycles and potentially affect clinical outcomes. Additionally, our cohort study excluded patients with indications for PGT, as these patients require the culture of D3 embryos to the blastocyst stage, followed by trophectoderm biopsy [18]. The study also excluded patients with moderate to severe intrauterine adhesions, uterine anomalies, and severe uterine septa, as these conditions can compromise the success of embryo transfer [19, 20]. Finally, women with adenomyosis and endometriosis were excluded, as these conditions are known to decrease clinical pregnancy rates and increase the risk of miscarriage [21, 22].

As a retrospective, observational cohort study, the allocation of subjects into groups was non-random. To mitigate this, we employed propensity score (PS) matching to address the non-random assignment of observational data, thereby reducing biases between study groups due to confounding factors such as demographics, clinical medication use, and embryology [23]. In the field of clinical research on assisted reproduction, the effectiveness of PS matching for handling non-randomly assigned data has been validated [24, 25]. After PS matching, subgroups 1–3 consisted of 442, 387, and 144 female patients, respectively. During the oocyte retrieval cycle, there were no statistically significant differences in baseline characteristics—such as age, duration of infertility, BMI, type of infertility, basal FSH and AFC, gonadotropin dosage, number of oocytes retrieved, number of mature oocytes, fertilization method, number of 2PN fertilizations, and number of high-quality D3 embryos—among the subgroups (Table 2). After controlling for confounding factors with PS matching, the cumulative live birth rates between the corresponding subgroups became comparable [26].

In fresh embryo transfer cycles, the implantation rate per transfer increased with the number of high-quality Day 3 embryos. Although the WEC strategy group had higher implantation rates than the PBC strategy group, the difference was not statistically significant ($P>0.05$). The PBC strategy involved a higher average number of embryos transferred compared to the WEC strategy, yet there were no statistically significant differences in clinical pregnancy rates or live birth rates between the two groups ($P>0.05$). These findings are consistent with a randomized controlled study conducted by Levi-Setti in women under 39 years of age [12]. In subgroups 2 and 3, the PBC strategy showed a significantly higher multiple pregnancy rate compared to the WEC strategy ($P<0.05$), likely due to the PBC strategy's usual selection of transferring two Day 3 embryos when a sufficient number of high-quality embryos were available. Miscarriage rates did not differ significantly between the WEC and PBC strategies across all subgroups ($P>0.05$), suggesting that blastocyst transfer does not effectively reduce the risk of pregnancy loss. This is corroborated by a systematic review by Glujovsky et al., which included 18 randomized controlled trials, indicating that blastocyst transfer does not reduce the risk of pregnancy loss [27]. In subgroup 1, the rate of ectopic pregnancy with the WEC strategy was slightly higher than with the PBC strategy ($P<0.05$). However, due to the overall low number of ectopic pregnancy cases (4 vs. 2), we cannot exclude the impact of random factors.

In FET cycles, the clinical pregnancy rate, multiple pregnancy rate, and live birth rate increase with the number of high-quality Day 3 embryos. It is important to note that the PBC strategy also predominantly involves blastocyst transfer. Consequently, improvements in pregnancy outcomes are primarily attributed to patient-specific factors, and there are no statistically significant differences among the subgroups. In comparisons of clinical pregnancy rates, miscarriage rates, and live birth rates, there

Table 3 The clinical outcomes in FET cycles and cumulative live birth rates

	Before PS matching			After PS matching		
	PBC	WEC	*P* value	PBC	WEC	*P* value
Subgroup 1	**(*N* = 1245)**	**(*N* = 679)**		**(*N* = 442)**	**(*N* = 442)**	
Subgroup 2	**(*N* = 1114)**	**(*N* = 570)**		**(*N* = 387)**	**(*N* = 387)**	
Subgroup 3	**(*N* = 144)**	**(*N* = 409)**		**(*N* = 144)**	**(*N* = 144)**	
FET cycles						
Subgroup 1 (*n*)	266	306		96	217	
Subgroup 2 (*n*)	659	625		259	392	
Subgroup 3 (*n*)	122	560		122	185	
FET female age (year)						
Subgroup 1 (mean (SD))	33.0 (±4.9)	33.1 (±4.1)	0.907	32.6 (±4.9)	33.4 (±4.0)	0.193
Subgroup 2 (mean (SD))	33.4 (±4.5)	32.2 (±4.5)	< 0.001	33.8 (±4.6)	32.7 (±4.5)	0.002
Subgroup 3 (mean (SD))	32.0 (±4.6)	30.6 (±4.0)	0.003	32.0 (±4.6)	31.7 (±4.2)	0.652
Hormone replacement cycles						
Subgroup 1 (*n* (%))	73.3% (195/266)	76.8% (235/306)	0.338	72.9% (70/96)	74.7% (162/217)	0.743
Subgroup 2 (*n* (%))	74.2% (489/659)	79.4% (496/625)	0.029	73.7% (191/259)	74.2% (291/392)	0.887
Subgroup 3 (*n* (%))	82.8% (101/122)	87.0% (487/560)	0.233	82.8% (101/122)	85.9% (159/185)	0.456
Endometrial thickness on ET day (mm)						
Subgroup 1 (mean (SD))	10.1 (±2.2)	10.2 (±2.1)	0.913	10.1 (±1.9)	10.2 (±2.0)	0.750
Subgroup 2 (mean (SD))	9.9 (±2.0)	10.2 (±2.1)	0.004	9.8 (±2.0)	10.3 (±2.2)	0.003
Subgroup 3 (mean (SD))	10.2(2.2)	9.8 (±1.7)	0.049	10.2 (±2.2)	9.9 (±1.8)	0.209
No. of transferred embryos						
Subgroup 1 (median [Q1,Q3])	1.4 (±0.5)	1.4 (±0.5)	0.887	1.4 (±0.5)	1.4 (±0.5)	0.824
Subgroup 2 (median [Q1,Q3])	1.5 (±0.5)	1.6 (±0.5)	0.263	1.6 (±0.5)	1.5 (±0.5)	0.292
Subgroup 3 (median [Q1,Q3])	1.7 (±0.5)	1.6 (±0.5)	0.493	1.7 (±0.5)	1.6 (±0.5)	0.482
Clinical pregnancy per FET						
Subgroup 1 (*n* (%))	40.6% (108/266)	49.3% (151/306)	0.037	38.5% (37/96)	47.8% (104/217)	0.126
Subgroup 2 (*n* (%))	52.7% (347/659)	59.4% (371/625)	0.016	55.9% (145/259)	58.7% (230/392)	0.498
Subgroup 3 (*n* (%))	63.1% (77/122)	70.5% (395/560)	0.112	63.1% (77/122)	62.7% (116/185)	0.944
Multiple pregnancies per FET						
Subgroup 1 (*n* (%))	4.9% (13/266)	8.8% (27/306)	0.067	4.2% (4/96)	7.8% (17/217)	0.241
Subgroup 2 (*n* (%))	9.7% (64/659)	12.8% (80/625)	0.096	9.3% (24/259)	12.2% (48/392)	0.238
Subgroup 3 (*n* (%))	18.9% (23/122)	21.3% (119/560)	0.564	18.9% (23/122)	17.3% (32/185)	0.727
Pregnancy loss per FET pregnancy						
Subgroup 1 (*n* (%))	21.3% (23/108)	17.9% (27/151)	0.496	23.5% (8/37)	22.1% (23/104)	0.967
Subgroup 2 (*n* (%))	19.3% (67/347)	17.3% (64/371)	0.478	21.4% (31/145)	18.3% (42/230)	0.460
Subgroup 3 (*n* (%))	18.2% (14/77)	15.6% (62/396)	0.568	18.2% (14/77)	17.1% (20/116)	0.842
Ectopic pregnancy per FET						
Subgroup 1 (*n* (%))	0	0	NA	0	0	NA
Subgroup 2 (*n* (%))	0.3% (2/659)	0.3% (2/625)	0.960	0	0.5% (2/392)	NA
Subgroup 3 (*n* (%))	0	1.4% (8/560)	NA	0	1.1% (2/185)	NA
Live birth per FET						
Subgroup 1 (*n* (%))	32.0% (85/266)	40.5% (124/306)	0.034	30.2% (29/96)	37.3% (81/217)	0.227
Subgroup 2 (*n* (%))	42.2% (278/659)	48.8% (305/625)	0.017	44.0% (114/259)	47.4% (186/392)	0.391
Subgroup 3 (*n* (%))	51.6% (63/122)	57.9% (324/560)	0.212	51.6% (63/122)	50.8% (94/185)	0.888
CLBR per oocyte retrieval cycle						
Subgroup 1 (*n* (%))	35.2% (438/1245)	25.2% (171/679)	< 0.001	33.0% (146/442)	25.8% (114/442)	0.018
Subgroup 2 (*n* (%))	63.1% (703/1114)	68.1% (388/570)	0.043	66.4% (257/387)	65.6% (254/387)	0.820
Subgroup 3 (*n* (%))	82.6% (119/144)	86.8% (355/409)	0.223	82.6% (119/144)	81.3% (117/144)	0.762

PBC, partial blastocyst culture; *WEC*, whole embryo culture; *FET*, frozen embryo transfer; *CLBR*, cumulative live birth rate. $P < 0.05$ indicates a statistical difference between the two subgroups. *P* values reflect the comparative outcomes between the PBC and WEC groups for each category

are no statistically significant differences between the PBC and WEC strategy groups.

Our retrospective study of 884 patients with 0–2 high-quality embryos on Day 3 reached a similar conclusion, suggesting that prioritizing Day 3 embryo transfer can yield higher cumulative live birth rates. This could be because the endometrium provides a more suitable environment for embryo development than artificial culture media [28]. Furthermore, studies on the transfer outcomes of blastocysts derived from different quality cleavage-stage embryos have shown that blastocysts from poor-quality Day 3 embryos have a significantly higher pregnancy loss rate after transfer compared to those from high-quality Day 3 embryos [29, 30]. This implies that poor-quality Day 3 embryos, even when cultured into blastocysts, do not improve patient live birth outcomes.

In this retrospective study cohort, the overall blastocyst formation rate for 4131 patients was 50.5%, meeting the laboratory quality control management requirements for blastocyst culture formation rates as per the Vienna Consensus [5]. Due to the selection of high-quality embryos for transfer or vitrification freezing on Day 3 in the PBC strategy group, the blastocyst formation rate in each subgroup was lower than that in the corresponding WEC subgroup. This indirectly confirms that the differences in the cumulative live birth rate in subgroup 1 were not due to substandard blastocyst culture techniques. Corresponding to the reduction in the number of cleavage-stage embryos is an increased risk of blastocyst formation failure, with significant variability in the cancellation rate of transfer strategies across different patient populations [24, 31]. In our study, the proportion of patients in Subgroup 1 with no transferable blastocysts under the WEC strategy reached 36.8%. Similarly, in Croo et al.'s study of 571 blastocyst culture cycles, the cycle cancellation rate due to the absence of blastocysts was 35.2% [32]. This indicates that promoting blastocyst transfer with a limited number of Day 3 embryos can increase the cycle cancellation rate for patients [33]. Early research suggests that patients benefit from blastocyst transfer when there are at least four high-quality embryos on Day 3 [34], a threshold that aligns with our findings. These results underscore the importance of considering the number and quality of embryos and specific patient conditions when devising individualized embryo transfer strategies to optimize cumulative live birth rates and minimize pregnancy risks [35–37].

Limitations

Our study had its limitations: the data were derived from a single-center IVF practice, with a limited collection of data on male factors and a higher proportion of patients undergoing double embryo transfer. Additionally, a subset of patients in the PBC strategy group received blastocyst transfer. Although we addressed various potential confounders and inconsistencies in treatment through propensity score matching [24, 25], selection bias in patient grouping may still have impacted the study outcomes and potentially limited the generalizability of our findings to other IVF practices.

Conclusions

Our study results indicate that when patients have 0–2 high-quality embryos on Day 3, opting for D3 embryo transfer rather than blastocyst culture can increase the chances of embryo transfer and improve cumulative live birth rates. However, when patients had three or more high-quality embryos on Day 3, there was no statistically significant difference in the cumulative live birth rate between the two embryo management strategies.

Author contribution Hao Wei: project development, data collection, and manuscript writing. BaoPing Zhu: data collection. LeiYu Deng: data collection. MeiFang Zeng: data collection. JinLiang Duan: project development.

Data Availability Requests for access to the data that support the findings of this study should be directed to the corresponding author at susuqinzenmeifang@126.com.

Declarations

Consent for publication All authors agreed to the publication of this study.

Competing interests The authors declare no competing interests.

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