Journal of Assisted Reproduction and Genetics (2022) 39:379–388
https://doi.org/10.1007/s10815-021-02380-1

ASSISTED REPRODUCTION TECHNOLOGIES

Day 5 vs day 6 single euploid blastocyst frozen embryo transfers: which variables do have an impact on the clinical pregnancy rates?

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Received: 22 September 2021 / Accepted: 13 December 2021 / Published online: 22 January 2022

Abstract

Objective To determine which variables affect most the clinical pregnancy rate with positive fetal heartbeat (CPR FHB+) when frozen embryo transfer (FET) cycles are performed with day 5 (D5) or day 6 (D6) euploid blastocysts.

Design and method

A single center retrospective study was performed from March 2017 till February 2021 including all single FET cycles with euploid D5 or D6 blastocysts and transferred in natural cycles (NC) or hormone replacement therapy (HRT) cycles. Trophectoderm (TE) and inner cell mass (ICM) qualities were recorded before biopsy.

Results A total of 1102 FET cycles were included, 678 with D5 and 424 with D6 blastocysts. Pregnancy rate (PR), clinical PR (CPR), and CPR FHB+ were significantly higher with D5 blastocysts (*PR*: 70.7% vs 62.0%, *OR* = 0.68 [0.53–0.89], $p = 0.004$; *CPR*: 63.7% vs 54.2%, *OR* = 0.68 [0.52–0.96], $p = 0.002$ and CPR FHB+: 57.8% vs 49.8%, *OR* = 0.72 [0.53–0.96], $p = 0.011$). However, miscarriage rate (12.5% vs 11.4%, *OR* = 0.78 [0.48–1.26], $p = 0.311$) did not differ. From a multivariate logistic regression model, endometrial thickness (*OR* = 1.11 [1.01–1.22], $p = 0.028$), patient's age (*OR* = 1.03 [1.00–1.05], $p = 0.021$), BMI (*OR* = 0.97 [0.94–0.99], $p = 0.023$), and ICM grade C (*OR* = 0.23 [0.13–0.43], $p < 0.001$) were significant in predicting CPR FHB+.

Conclusion Although clinical outcomes are higher with D5 blastocysts, CPR FHB+ is more affected by endometrial thickness, patient age, BMI, and ICM grade C rather than biopsy day or endometrial preparation protocol.

Keywords Frozen-embryo transfer · Day 5 · Day 6 · PGT-A · Embryo quality · Endometrial preparation

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Introduction

Preimplantation genetic testing for aneuploidy (PGT-A) and morphological grading of embryos are still the two main strategies to select a blastocyst from a pool of embryos with the highest implantation potential [1]. Ideally, embryos reach the blastocyst stage on the fifth day of embryo development (116 ± 2 h after insemination) [2]; however, not all embryos have the same developmental rate, leading to a cohort of blastocysts on day 5 (D5), day 6 (D6), or even day 7 (D7) available for embryo transfer (ET) [3].

When selecting blastocysts in fresh ETs, top quality D5 blastocysts are preferentially chosen, as controlled ovarian stimulation does have an impact on the window of implantation due to an endometrial advancement [4, 5]. Not only the asynchrony between endometrial and embryo developmental stage in fresh ET negatively affect pregnancy and implantation rates, other metabolic or intrinsic factors affecting embryo viability might contribute to the higher implantation potential of D5 blastocysts compared to slow developing D6 blastocysts [6]. Subsequently, the quality and day of blastocyst development do play a crucial role in embryo selection in fresh ET when non-genetically tested blastocysts are transferred [7].

Compared to fresh ET cycles, there is no doubt that frozen embryo transfers (FET) have similar, if not higher pregnancy and implantation rates [8–10]. In FET, the importance of the day of blastocyst development seems to persist as slower blastocyst development on D6 results in a significantly lower pregnancy and implantation potential compared to D5 blastocysts with similar quality [11–13]. On the contrary, it has also been demonstrated that clinical outcomes are equivalent when transferring blastocysts on D5 or D6 in FET, generating some controversy on the reduced pregnancy potential of D6 vitrified blastocysts [14, 15]. Therefore, day of blastocyst development is an important determinant in the final decision of which blastocyst to choose for transfer.

The implementation of PGT-A and blastocyst vitrification allows embryologists to choose the best quality euploid blastocyst in FET cycles. While mostly euploid embryos do reach the blastocyst stage, it has also been demonstrated that delayed blastulation is not necessarily associated with increased aneuploidy rates [16]. Therefore, D5, D6, and D7 euploid blastocysts with high clinical potential obtained from a single stimulation cycle can be available when selecting a blastocyst in FET cycles [3].

Analysis of the clinical outcomes of vitrified D5 and D6 euploid blastocysts has been described; however, conflicting results have been published due to the heterogeneity of the study designs [17–20]. Moreover, little is described if different endometrial preparation (EP) protocols affect pregnancy rates when transferring euploid blastocysts biopsied on D5 or D6 in FET cycles [21]. Protocols for EP can widely vary but mainly, they are grouped as natural cycle (NC) or hormone replacement therapy (HRT) [22, 23] and yet, no consensus has been reached if any EP protocol available is superior to another [24].

Consequently, the question remains unanswered of which factor determines the difference in clinical success between D5 and D6 euploid blastocysts in FET cycles: is it the day of blastocyst biopsy, the blastocyst quality, or the type of EP protocol? To address this question, a retrospective analysis was performed to assess the clinical outcomes of single euploid vitrified blastocysts on D5 or D6, transferred in either a NC or HRT cycle.

Material and methods

Study design and study endpoint

All patients that went through a FET cycle between March 2017 and February 2021 with an autologous single euploid blastocyst were included. Only blastocysts that underwent trophectoderm (TE) biopsy on D5 or D6 and were analyzed by next generation sequencing (NGS) for PGT-A were considered for the study. The use of fresh or frozen autologous sperm and fresh or vitrified autologous oocytes did not serve as exclusion criteria. Blastocysts that did not re-expand within 3 h post warming procedure were excluded from the data analysis in this study.

The primary endpoint was to determine which most relevant factor affects clinical pregnancy rate with positive fetal heartbeat (CPR FHB+) when single euploid FET cycles are performed with D5 or D6 blastocysts. As secondary objectives, clinical outcomes such as pregnancy rate (PR), biochemical pregnancy rate (BPR), clinical pregnancy rate (CPR), and miscarriage rate (MR) were analyzed when single euploid D5 or D6 blastocysts were transferred. This retrospective study was approved by the local Ethical Committee (REFA041 and REFA041b) of ART Fertility Clinic, Abu Dhabi, United Arab Emirates.

Blastocyst grading

Blastocyst's grade of expansion, TE and inner cell mass (ICM), were assessed immediately before biopsy and categorized using a modified Gardner and Schoolcraft criteria [25]. Briefly, the ICM was graded as A, when numerous tightly packed cells were seen; B, when several and loosely packed cells were observed; C, in case of very few cells; and D, if no cells were seen or more than 50% of cells were degenerated. Also, the TE was graded as A, when many cells

were organized in stretch contact forming the TE; B, when several cells were organized in a loose epithelium; C, if few cells with abnormal disposition in the TE were present; or D, if only very few irregular large cells with necrotic aspect constituted the TE. The grade of expansion was classified as BL1, when blastocoel was less than half of the volume of the embryo or BL2, when blastocoel was at least half of the volume of the embryo, and both considered early blastocysts with no cell differentiation between ICM and TE. A full blastocyst was classified as BL3, when the blastocoel filled the embryo completely; BL4, when an expanded blastocyst with a thin zona pellucida (ZP) was seen; and BL5, when cells started to herniate through the ZP or BL6, if the blastocyst had completely escaped from the ZP. Blastocysts were evaluated on D5 of embryo culture and if blastocyst quality was deemed sub-optimal and expansion or number of cells present in the TE were insufficient to perform the biopsy, embryos were left in culture and re-evaluated on D6. Blastocysts that were not biopsied on D5 or D6 were re-evaluated on D7.

Embryo biopsy and tubing procedure

All blastocysts available on D5 or D6 with a quality ≥ BL3CC were subjected to TE biopsy for PGT-A analysis by NGS. Blastocysts with an expansion < BL3 or with a grade D in the ICM or TE were not eligible for biopsy and consequently, discarded on D7 of embryo culture. Blastocysts were placed in a drop of 10 µL of Hepes buffered medium supplemented with 5% of human serum albumin (HSA - SOLUTION™, Vitrolife), covered with 8 mL of paraffin oil (Ovoil, Vitrolife) and pre-warmed to 37 °C. The blastocyst was positioned using the holding pipette to locate the ICM at the 12 o'clock position. For the zona drilling, an opening was made by applying three laser pulses in the ZP and a piece of TE was aspirated with a biopsy pipette (Origio, Coopersurgical Group) with an internal diameter of 30 µm, and dissected with an OCTAX laser (NaviLase, Vitrolife) using an intensity of 2.2 ms. A mechanical "flicking" method was used to cut and obtain the TE cells inside the aspiration pipette. Biopsied TE cells were washed and placed into an Eppendorf tube of 0.2 mL containing 2.5 µL of phosphate buffer saline solution (PBS) and stored at −20 °C till NGS for PGT-A was performed by Igenomix Dubai.

Blastocyst vitrification and warming protocol

Blastocysts were individually vitrified 1 h after TE biopsy using a Kitazato vitrification kit (Kitazato, Biopharma) in combination with open Vitrification Straws (Cryotop, Kitazato, Biopharma). The vitrification procedure was performed according to the manufacturer's instructions as previously described elsewhere [26].

In patients with more than one euploid blastocyst available for FET, blastocyst selection was based on morphological score (grade of expansion, TE, and ICM) and day of blastocyst biopsy (preferably D5 blastocysts over D6 as per routine clinical practice) and were warmed using a Kitazato thawing kit (Kitazato, Biopharma) following the manufacturer's protocol. Warmed blastocysts were cultured for up to 3 h before ET and during this period, blastocysts were evaluated for blastocoel re-expansion which was considered a sign of viability [27].

Preimplantation genetic testing for aneuploidies of TE samples

Whole genome amplification (WGA) protocol was performed on all TE samples using PicoPlex technology (Rubicon Genomics, Inc; USA). After WGA, library preparation consisted of the incorporation of individual barcodes for the amplified DNA of each sample. Following amplification and enrichment of the DNA, sequencing was performed in a 316 or 318 chip using the Personal Genome Machine sequencing (Thermo Fisher Scientific, USA). An ion Reporter™ software was employed for sequencing analysis and data interpretation (Thermo Fisher Scientific, USA).

Endometrial preparation (EP) and blastocyst transfer

The EP protocol was chosen according to physician's discretion. For a spontaneous ovulatory natural cycle (NC), transvaginal ultrasound scans were performed to monitor follicular growth with serial measurements of serum luteinizing hormone (LH), estradiol (E_2), and progesterone (P_4) levels to accurately determine the ovulation time (automated Elecsys® immunoanalyzer, Roche Diagnostics, Mannheim, Germany). Vaginal P_4 suppositories were commenced in the evening of the confirmed ovulation day and from day 1 onwards, three times daily until pregnancy test.

In hormone replacement therapy (HRT) cycles, patients commenced oral E_2 tablets daily from day 2 or 3 of menses for 3 days and increased to 6 mg on the fifth day. When an endometrial thickness of at least 6 mm was achieved with a trilaminar appearance, an initial evening P_4 dose of 100 mg was administered vaginally (day 0). The administration of P_4 was increased on day 1 to three times daily and this regimen was continued until pregnancy test.

For the embryo transfer procedure, blastocysts were loaded in a soft pass catheter (GUARDIA™ AccessET Catheter, Cook Medical, USA) in 25 µL of pre-gassed culture medium with the help of a tuberculin syringe and all FET cycles were performed by a physician under abdominal

ultrasound guidance. All blastocysts' FETs were performed 5 days after ovulation was confirmed or on the fifth full day of P_4 administration with an average of 120 [115–125] h of P_4 exposure between P_4 initiation and ET procedure, regardless of the day on which the blastocyst was biopsied (D5 or D6). In case the endometrial thickness did not achieve a thickness ≥ 6 mm even with different endometrial preparation approaches (NC or HRT), ET was performed only when a triple lining pattern was seen.

Clinical outcomes

A pregnancy was defined 12 days after ET by a serum β-hCG value ≥ 15 mIU/mL. Biochemical pregnancy was described by the detection of β-hCG in serum which did not develop into a clinical pregnancy [28]. Clinical pregnancy was defined with a positive result for β-hCG and the presence of at least 1 gestational sac 4 weeks after the FET while CPR FHB+ was defined as a clinical pregnancy with positive fetal heartbeat diagnosed by ultrasound 6 weeks after the FET [28]. A miscarriage was considered when a spontaneous loss of an intrauterine CP or CPR FHB+ occurred at any gestational age. Ectopic pregnancy was only considered in the calculation of pregnancy.

Data collection and statistical analysis

Categorical variables are presented as numbers and percentages while continuous variables are presented as mean ± SD. Groups (D5 vs D6) were compared using Fisher's exact test for dichotomous variables and Mann Whitney test for continuous variables.

A multivariate logistic regression model via generalized estimating equations (GEE) was performed to identify the effect of D5 or D6 blastocysts on CPR FHB+ outcomes adjusted for potential confounding factors that could be independently associated such as: grade of expansion, TE, and ICM morphological score of blastocysts, patient age, antimüllerian hormone (AMH), body mass index (BMI), EP, and endometrial thickness.

Data analysis was performed using the statistical software R (version 3.5.0) and a p-value < 0.05 was considered statistically significant. According to the sample size included in this study ($n = 1102$), the margin error of the results was 2.5% with a 90% confidence.

Results

A total of 1102 FET cycles were included in the study of which 678 FETs were performed with blastocysts biopsied on D5 and 424 on D6. For all patients with at least one euploid blastocyst for transfer, the fresh cycle characteristics were the following: the normal fertilization rate (2PN) was 70.9% ± 2.4% with a total blastulation rate on day 5/2PN of 68.8% ± 4.3%. From all blastocysts obtained (D5, D6, and D7/2PN), 54.2% ± 3.5% were biopsied with a total euploidy rate of 44.8% ± 3.5%, according to the PGT-A screening results.

Considering the patient's characteristics, only women age and AMH levels were significantly different between D5 and D6 blastocyst FET cycles (33.4 ± 5.5 years vs 34.4 ± 5.4 years, $p = 0.002$ and 3.5 ± 3.6 ng/mL vs 3.0 ± 2.9 ng/mL, $p = 0.001$, respectively) while BMI, years of infertility, and type of infertility were comparable between groups, as shown in Table 1. Regarding the type of EP protocol, no significant difference was found between the number of FET cycles performed in NC or in HRT when D5 or D6 blastocysts were transferred (38.1% vs 41.0% for NC and 61.9 vs 59.0 for HRT cycles, $p = 0.342$ for D5 or D6, respectively). However, the endometrial thickness differed between D5 and D6 euploid blastocysts FET cycles (7.7 ± 1.3 mm vs 7.9 ± 1.3 mm, $p = 0.034$) (Table 1).

Concerning clinical outcomes, the PR (70.7% vs 62.0%, $OR = 0.68$ [0.53–0.89], $p = 0.004$), CPR (63.7% vs 54.2%, $OR = 0.68$ [0.52–0.87], $p = 0.002$), and CPR FHB+ (57.8% vs 49.8%, $OR = 0.72$ [0.53–0.96], $p = 0.011$) were significantly higher after transferring a D5 euploid blastocyst compared to a D6. In contrast, biochemical pregnancy rate (BPR) (8.3% vs 12.5%, $OR = 1.30$ [0.78–2.17], $p = 0.316$) and MR (12.5% vs 11.4%, $OR = 0.78$ [0.48–1.26], $p = 0.311$) did not differ significantly between FET outcomes with blastocysts biopsied on D5 or D6, regardless the type of EP protocol performed in each FET cycle (Table 2).

Table 1 Patient characteristics stratified according to the transfer of day 5 or day 6 euploid blastocysts

	Day 5	Day 6	*P* value
Number of FET cycles	678	424	
Age (years)	33.4 ± 5.5	34.4 ± 5.4	0.002
BMI (kg/m^2)	26.7 ± 4.9	26.9 ± 5.0	0.463
AMH (ng/mL)	3.5 ± 3.6	3.0 ± 2.9	0.001
Years of infertility	3.2 ± 3.0	3.3 ± 3.0	0.562
Type of infertility:			
Primary (%)	49.0	48.6	0.951
Secondary (%)	51.0	51.4	
Type of endometrial preparation protocol:			
NC (%)	38.1	41.0	0.342
HRT (%)	61.9	59.0	
Endometrial thickness (mm)	7.7 ± 1.3	7.9 ± 1.3	0.034

Values are expressed as mean ± standard deviation or percentage (%); *FET*, frozen embryo transfers; *BMI*, body mass index; *AMH*, antimüllerian hormone; *NC*, natural cycle; *HRT*, hormone replacement therapy

Table 2 Clinical outcomes stratified according to biopsy day (day 5 vs day 6) of euploid blastocysts FET cycles

	Day 5	Day 6	*OR* [95% *CI*]	*P* value
Number of FET cycles	678	424		
Pregnancy rate (%)	70.7	62.0	0.68 [0.53–0.89]	0.004
Biochemical pregnancy rate (%)	8.3	12.5	1.30 [0.78–2.17]	0.316
Clinical pregnancy rate (%)	63.7	54.2	0.68 [0.52–0.87]	0.002
Clinical pregnancy rate fetal heartbeat positive (%)	57.8	49.8	0.72 [0.53–0.96]	0.011
Miscarriage rate (%)	12.5	11.4	0.78 [0.48–1.26]	0.311

Values are expressed as percentage (%); *OR*, odds ratio; 95% *CI*, 95% confidence interval.

A multivariate logistic regression model was performed to evaluate if CPR FHB+ was affected by the day of blastocyst biopsy, blastocyst expansion grade, and ICM and TE quality of the transferred blastocysts (BL3AA blastocyst on day 5 as reference). From this analysis, only ICM grade C was found to have a significant negative influence on CPR FHB+ outcomes (*OR* = 0.27 [0.15–0.46], $p < 0.001$ compared to ICM grade A); no statistically significant impact was seen for the day on which blastocysts were biopsied, nor the different grades of expansion and TE qualities (Table 3).

A second multivariate logistic regression model was performed which also considered the following patient and FET cycle characteristics: age, AMH, BMI, type of EP protocol, and endometrial thickness (Table 4). Only endometrial thickness (*OR* = 1.11 [1.01–1.22], $p = 0.028$), patient age (*OR* = 1.03 [1.00–1.05], $p = 0.021$), BMI (*OR* = 0.97 [0.94–0.99], $p = 0.023$), and specially ICM quality C (*OR* = 0.23 [0.13–0.43], $p < 0.001$) were significantly associated with CPR FHB+ outcomes as it is shown in Fig. 1.

Table 3 Multivariate logistic regression model for clinical pregnancy rate with fetal heartbeat positive (CPR FHB+) considering the day of biopsy, expansion grade, and blastocyst trophectoderm and inner cell mass quality

	OR [95% *CI*]	*P* value
BL3AA day 5 blastocyst *(intercept)*	1.72 [1.12–2.63]	0.013
Day 6 blastocyst	0.91 [0.69–1.19]	0.483
Expansion grade 4 (BL4)	1.14 [0.80–1.62]	0.471
Expansion grade 5 (BL5)	1.24 [0.87–1.76]	0.227
Expansion grade 6 (BL6)	0.71 [0.23–2.15]	0.542
ICM quality B	0.82 [0.60–1.13]	0.223
ICM quality C	0.27 [0.15–0.46]	< 0.001
TE quality B	0.87 [0.63–1.20]	0.403
TE quality C	0.66 [0.42–1.05]	0.080

Multi logistic regression model via generalized estimating equations (GEE) for (CPR FHB+) adjusted by confounding factors of all frozen embryo transfer cycles; *ICM*, inner cell mass; *TE*, trophectoderm; *OR*, odds ratios; 95% *CI*, 95% confidence interval

Table 4 Multivariate logistic regression model for clinical pregnancy rate with fetal heartbeat positive (CPR FHB+) considering blastocyst, patient, and frozen embryo transfer cycle characteristics

	OR [95% *CI*]	*P* value
BL3AA day 5 blastocyst HRT *(intercept)*	0.61 [0.16–2.34]	0.427
Day 6 blastocyst	0.84 [0.63–1.11]	0.225
Endometrial preparation (NC)	1.14 [0.87–1.48]	0.343
Endometrial thickness (mm)	1.11 [1.01–1.22]	0.028
Patient age (years)	1.03 [1.00–1.05]	0.021
AMH (ng/mL)	1.00 [0.96–1.04]	0.878
BMI (kg/m^2)	0.97 [0.94–0.99]	0.023
Expansion grade 4 (BL4)	1.19 [0.82–1.74]	0.342
Expansion grade 5 (BL5)	1.30 [0.91–1.88]	0.150
Expansion grade 6 (BL6)	1.10 [0.34–3.55]	0.871
ICM quality B	0.81 [0.58–1.13]	0.204
ICM quality C	0.23 [0.13–0.43]	< 0.001
TE quality B	0.90 [0.65–1.24]	0.503
TE quality C	0.72 [0.44–1.16]	0.176

Multi logistic regression model via generalized estimating equations (GEE) for (CPR FHB+) adjusted by confounding factors of all frozen embryo transfer cycles; *HRT*, hormone replacement therapy; *NC*, natural cycle; *AMH*, antimüllerian hormone; *BMI*, body mass index; *ICM*, inner cell mass; *TE*, trophectoderm; *OR*, odds ratio; 95% *CI*, 95% confidence interval

As quality grade C of ICM and TE were related with a reduced OR for CPR FHB+ in the multivariate logistic regression models, clinical outcomes were re-analyzed only considering good quality euploid blastocysts (grade A or B for ICM and TE) transferred on D5 ($N = 596$) or D6 ($N = 281$). From this comparison, clinical outcomes were not statistically significantly different between blastocysts transferred on D5 or D6 in FET cycles (Supplementary Table 1).

A sub-analysis was conducted to evaluate the impact of the EP protocol (NC vs HRT cycles) on the clinical outcomes of all FET cycles. For FET cycles performed in a NC approach, PR (70.9% vs 56.9%, *OR* = 0.54 [0.35–0.83], $p = 0.003$), CPR (65.9% vs 48.8%, *OR* = 0.49 [0.33–0.75], $p < 0.001$), CPR FHB+ (63.6% vs 47.7%, *OR* = 0.52 [0.35–0.79], $p = 0.001$), and MR (8.7% vs 3.0%, *OR* = 0.27 [0.05–0.95], $p = 0.030$) were statistically significantly different comparing D5 and D6 blastocysts, while BPR (6.0% vs 13.1%, *OR* = 1.81 [0.73–4.58], $p = 0.198$) was higher for D6 blastocysts but did not reach a significant difference (Table 5). In contrast to the findings in the NC approach for EP, there was no statistical difference for any of the clinical outcomes between D5 and D6 blastocysts FET in HRT cycles (Table 5).

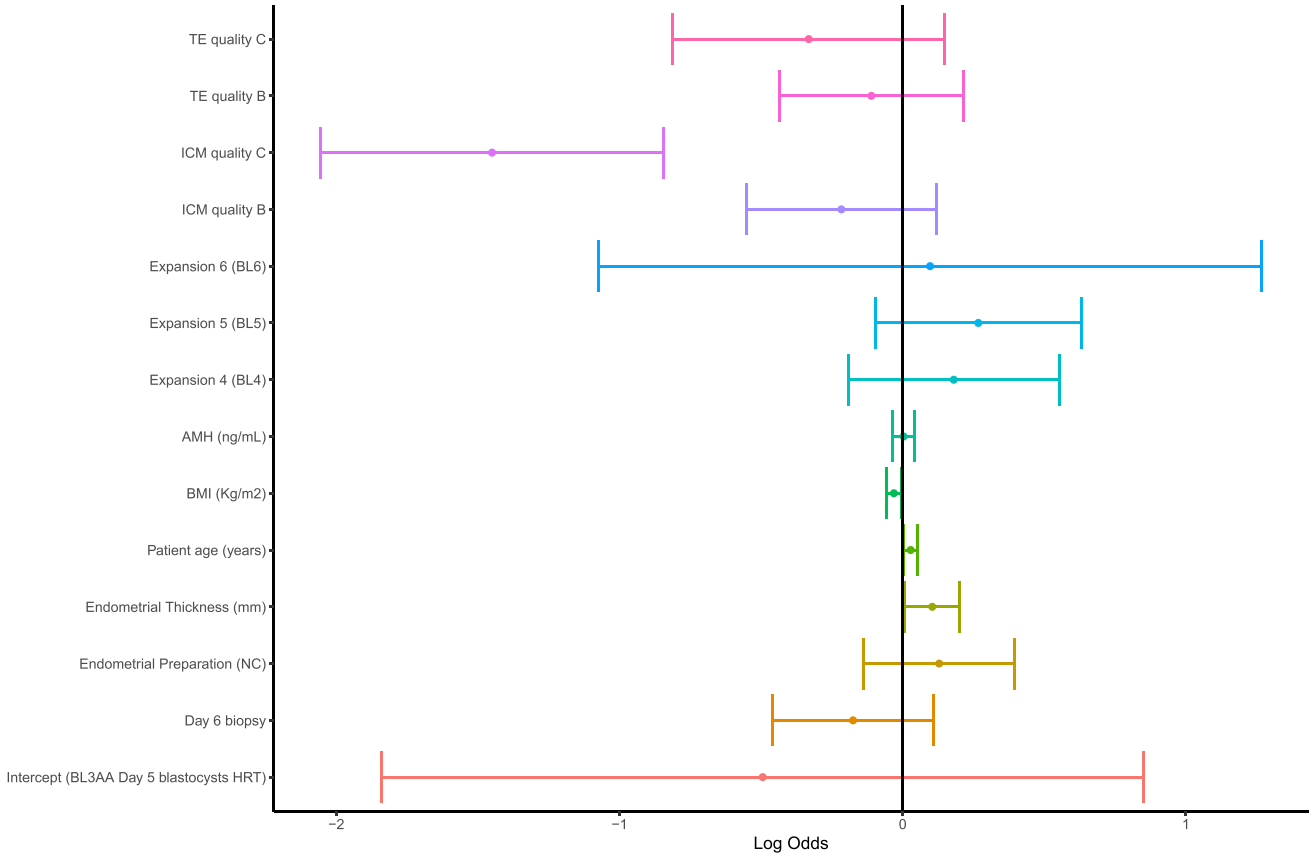

Fig. 1 Multinomial logit model representation including all variables affecting CPR FHB+outcome in FET cycles with euploid blastocyst. Variables affecting CPR FHB+ outcomes are represented in the Y axis and logarithm of the odds ratio are represented in the X axis. Variables not crossing the zero of the X axis are considered statistically significant ($p < 0.05$). As it is shown in the figure, ICM quality C is the most significant variable affecting CPR FHB+ in a negative way (CI on the left side compared to the central zero line). *HRT*, hormone replacement therapy; *NC*, natural cycle; *AMH*, Antimüllerian hormone; *BMI*, body mass index; *ICM*, inner cell mass; *TE*, trophectoderm; *CI*: 95% confidence intervals

Table 5 Clinical outcomes of day 5 or day 6 euploid blastocysts in frozen embryo transfer cycles split by the type of endometrial preparation protocol (natural cycle or hormone replacement therapy cycles)

	NC				HRT			
	Day 5	Day 6	*OR* [95% *CI*]	*P* value	Day 5	Day 6	*OR* [95% *CI*]	*P* value
Number of FET cycles	258	174			420	250		
Pregnancy rate (%)	70.9	56.9	0.54 [0.35–0.83]	0.003	70.5	65.6	0.80 [0.56–1.13]	0.197
Biochemical pregnancy rate (%)	6.0	13.1	1.81 [0.73–4.58]	0.198	9.8	11.6	1.11 [0.57–2.10]	0.758
Clinical pregnancy rate (%)	65.9	48.8	0.49 [0.33–0.75]	< 0.001	62.4	58.0	0.83 [0.60–1.16]	0.288
Clinical pregnancy rate fetal heartbeat positive (%)	63.6	47.7	0.52 [0.35–0.79]	0.001	54.3	51.2	0.88 [0.64–1.22]	0.471
Miscarriage rate (%)	8.7	3.0	0.27 [0.05–0.95]	0.030	14.9	16.5	1.03 [0.60–1.76]	0.897

Values are expressed as percentage (%); *OR*, odds ratio; 95% CI, 95% confidence interval

Finally, from the results obtained, a priority was assigned to each blastocyst based on the day of biopsy, grade of expansion, and quality of ICM and TE which were recorded before TE biopsy in order to select the best embryo at the time of warming (Supplementary Table 2).

Discussion

The selection of the embryo with the highest implantation potential is an ongoing challenge for the embryologist [29–31]. The combination of PGT-A with morphological grading presents the most important criteria for the selection process, especially as nowadays biopsy on D5, D6, or even D7 of embryo development is becoming a routine practice in ART cycles [3]. The aim of this study was therefore, to evaluate the various factors influencing the CPR FHB+ outcomes. The results of this study demonstrated that the day at which blastocysts are biopsied does not seem to be as critical as other factors to achieve a successful CPR FHB+ result in FET cycles with D5 and D6 euploid blastocysts. The increased clinical potential of D5 blastocysts reported in literature cannot just be simplified to the day at which the blastocysts are biopsied; endometrial thickness, patient age, BMI, and mainly ICM grade C do have a relevant importance on achieving a viable pregnancy.

Despite all published studies focusing on the clinical potential of D5 and D6 vitrified blastocysts, the evidence of the superiority of D5 blastocysts over D6 is still not conclusive [32–34]. Our herein presented data add to the current knowledge and suggest that, when a FET is performed with a single euploid blastocyst, PR, CPR, and CPR FHB+ are higher with D5 blastocysts compared to D6 (70.7% vs 62.0%, $p = 0.004$; 63.7% vs 54.2%, $p = 0.002$ and 57.8% vs 49.8%, $p = 0.011$, respectively), coinciding with the existing published literature [34]. On the contrary, BPR and MR are not significantly different in FET cycles with D5 and D6 euploid blastocysts (8.3% vs 12.5%, $p = 0.316$ and 12.5% vs 11.4%, $p = 0.311$, respectively). It can be questioned if the difference in BPR between groups should be considered as clinically relevant, as it is detrimental to both CPR and CPR FHB+. In general, the etiology of a biochemical pregnancy can be explained by poor endometrial receptivity, defects in the gametes, or genetically abnormal embryos [35]. Since in our study only euploid embryos were transferred, and since BPR was also not different between D5 and D6 blastocysts in the different EP protocols (NC vs HRT cycles), the origin of these differences in biochemical pregnancy outcomes remains to be elucidated.

As embryo quality does have an impact on clinical results, a multivariate logistic regression model was performed to explore if the day of TE biopsy and the blastocyst quality at the time of embryo selection were associated with the CPR FHB+ outcomes. This analysis showed that ICM quality C was an independent factor which negatively affected the CPR FHB+ outcomes, regardless of blastocysts expansion, TE quality, and day of biopsy, suggesting that CPR FHB+ is reduced as the ICM quality decreases from grade A to C (modified Gardner and Schoolcraft scoring), thereby confirming the results of other publications [36, 37]. Although TE quality was not statistically significant for predicting CPR FHB+, ORs were reduced for grade C compared to grade B or A indicating that TE has a relevant clinical impact on CPR FHB+ despite of the statistical result, as published before [38, 39]. Moreover, not only the ICM quality C was statistically significant in predicting a CPR FHB+, but also endometrial thickness, patient age, and BMI were associated with the clinical outcome analyzed in a second multivariate analysis, indicating that these variables have a clinically relevant effect besides the quality of the ICM. The results of this analysis do match with previous studies which reported a negative correlation between live birth and endometrial thickness, maternal age, BMI, and ICM type C in FET cycles with single euploid blastocysts [40]. The multivariate analysis performed in this study clearly showed that other variables (endometrial thickness, maternal age, BMI, and lower ICM quality) do have a stronger impact on the CPR FHB+ outcome rather than day of biopsy (Table 4), which can be directly associated with poorer prognosis patients. Although the day of biopsy showed no significant association with CPR FHB+ in the multivariate analysis, the significant difference observed in the univariate analysis probably indicates a clinical relevance (Table 2). Beside this, the ICM quality C constitutes the strongest predictive factor related to clinical success in ART as it can be observed in Fig. 1, matching with the outcomes when non-genetically tested blastocysts are transferred [37].

The fact that delayed blastocyst formation is a sign of suboptimal embryo development does not necessarily mean that they are adversely chromosomally affected [3, 41], therefore euploid D6 embryos are an option when selecting blastocysts for FET cycles. From the results of this study, clinical outcomes are more affected by lower quality embryos rather than the day of blastocysts biopsy. Additionally, from the re-analysis performed considering only good quality blastocysts (grade A and/or B for ICM and TE), clinical outcomes were not statistically significantly different between blastocysts biopsied on D5 or D6 (Supplementary Table 1), as reported previously [42]; however, there was a trend in favor of D5 euploid blastocysts over D6. Clearly, higher blastocyst quality increases pregnancy outcomes; therefore, blastocyst morphology still plays an important role in embryo selection, even when considering euploid blastocysts. The adverse effect of decreasing ICM and TE quality from A or B to C on clinical outcomes may be explained by a reduction in cell number or altered homeostasis of the blastocyst that ultimately affects the develop into a viable pregnancy [43].

Besides the retrospective nature of the study design, differences in age and AMH between D5 and D6 groups suggest that better prognosis patients (lower age and higher AMH) are more likely to transfer a D5 euploid blastocyst rather than a D6, which has already been published [3]. Even

though a significant difference in endometrial thickness was found between groups (7.7 ± 1.3 vs 7.9 ± 1.3, $p = 0.034$), clinically these values can be considered equal since only 0.2 mm differed between D5 and D6 FET cycles. Additionally, different EP (NC versus HRT) regimen may pose a limitation for this study; however, the proportion of FET cycles performed in NC vs HRT was similar in both groups (D5 or D6). Although clinical outcomes differed when D5 or D6 blastocysts were transferred in different EP protocols, the type of EP was chosen according to the clinician's discretion as, based on the current published literature, there is no recommendation to choose one EP method over another in FET [22, 44–47]. As clinical outcomes seem to differ when D5 or D6 blastocysts are transferred in NC and not in HRT cycles, this suggests that the type of EP protocol might explain the difference in clinical outcomes when comparing D5 vs D6 euploid blastocysts in the univariate analysis (Table 2). More observational studies are warranted to evaluate live birth outcomes when transferring D5 or D6 euploid blastocysts in NC or HRT cycles to evaluate if FET cycles could be personalized according to the day of blastocyst development to increase outcomes.

To conclude, integrating morphological grading with ploidy assessment is a strategy to shorten the time to pregnancy by enhancing embryo selection and thereby decreasing the number of transfer cycles required to achieve a clinical pregnancy [48, 49]. This study confirms that the clinical outcome of a single euploid blastocyst is independent of the day of blastocyst biopsy when good quality blastocysts are transferred; however, there is an increased positive tendency in favor of D5 euploid blastocysts over D6. Therefore, since not all euploid blastocyst are equivalent, a proposal of embryo priority selection was suggested based on the results obtained in this study (Supplementary Table 2). Nevertheless, caution should be given as not all IVF laboratories apply the same grading system as reported in this study and not all perform a "freeze all strategy". The clinical potential of a D5 or D6 euploid blastocyst cannot be reduced only to the day at which TE biopsy is performed, there are many variables that can have a profound effect on the clinical outcomes, principally ICM grade C. From the embryologist's perspective, the morphological grading of the blastocysts at the time of biopsy is the strongest predictive factor when selecting an euploid blastocyst with the greatest clinical potential. In future, studies should focus on evaluating the influence of TE biopsy day in single euploid D5 or D6 blastocysts and how to personalize FET cycles according to patient characteristics, blastocyst quality, EP protocol, and endometrial thickness. Meanwhile, internal research should be performed in each fertility clinic to elucidate a pathway for selecting the blastocyst with the highest clinical potential to optimize FET outcomes based on their own clinical routine practice.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10815-021-02380-1.

Acknowledgements We thank our biostatistician Ms. Gioia Altobelli for the statistical analysis and critical review of the manuscript.

Author contribution AAb, ND, HF: conception and design of the study; AAb, ND, IE, AAr, AD, AB, LM, BL, HF: oocyte retrieval; ND, IE, AAr, AD, AB, AAb: IVF and ICSI procedure and embryo evaluations; ND, IE, AAr, AD, AB, AAb: blastocyst biopsy, tubing and freezing; ND, IE, AB, AAr, LM, BL, HF: embryo transfers; AAb, GA: construction of the database and data interpretation; AAb: drafting the manuscript; ND, IE, AAr, AD, AB, LM, BL, HF: critical review and final approval of the manuscript.

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Capalbo A, Rienzi L, Cimadomo D, Maggiulli R, Elliott T, Wright G, Nagy ZP, Ubaldi FM. Correlation between standard blastocyst morphology, euploidy and implantation: an observational study in two centers involving 956 screened blastocysts. Hum Reprod. 2014;29(6):1173–81. https://doi.org/10.1093/humrep/deu033.
2. Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod. 2011;26(6):1270–83. https://doi.org/10.1093/humrep/der037.
3. Tiegs AW, Sun L, Patounakis G, Scott RT. Worth the wait? Day 7 blastocysts have lower euploidy rates but similar sustained implantation rates as day 5 and day 6 blastocysts. Hum Reprod. 2019;34(9):1632–9. https://doi.org/10.1093/humrep/dez138 Erratum in: Hum Reprod. 2019;34(12):2559-2560.
4. Tannus S, Cohen Y, Henderson S, Al Ma'mari N, Shavit T, Son WY, Dahan MH. Fresh transfer of day 5 slow-growing embryos versus deferred transfer of vitrified, fully expanded day 6 blastocysts: which is the optimal approach? Hum Reprod. 2019;34(1):44–51. https://doi.org/10.1093/humrep/dey351.
5. Shapiro BS, Daneshmand ST, Restrepo H, Garner FC, Aguirre M, Hudson C. Matched-cohort comparison of single-embryo transfers in fresh and frozen-thawed embryo transfer cycles. Fertil Steril. 2013;99(2):389–92. https://doi.org/10.1016/j.fertnstert.2012.09.044.
6. Bourdon M, Pocate-Cheriet K, Finet de Bantel A, Grzegorczyk-Martin V, Amar Hoffet A, Arbo E, Poulain M, Santulli P. day 5 versus day 6 blastocyst transfers: a systematic review and meta-analysis of clinical outcomes. Hum Reprod. 2019;34(10):1948–64. https://doi.org/10.1093/humrep/dez163.
7. Bakkensen JB, Brady P, Carusi D, Romanski P, Thomas AM, Racowsky C. Association between blastocyst morphology and pregnancy and perinatal outcomes following fresh and cryopreserved embryo transfer. J Assist Reprod Genet. 2019;36(11):2315–24. https://doi.org/10.1007/s10815-019-01580-0.
8. Roque M, Bedoschi G, Cecchino GN, Esteves SC. Fresh versus frozen blastocyst transfer. Lancet. 2019;394(10205):1227–8. https://doi.org/10.1016/S0140-6736(19)31393-5.
9. Shi Y, Sun Y, Hao C, Zhang H, Wei D, Zhang Y, Zhu Y, Deng X, Qi X, Li H, Ma X, Ren H, Wang Y, Zhang D, Wang B, Liu F, Wu

Q, Wang Z, Bai H, et al. Transfer of fresh versus frozen embryos in ovulatory women. N Engl J Med. 2018;378(2):126–36. https://doi.org/10.1056/NEJMoa1705334.
10. Stormlund S, Sopa N, Zedeler A, Bogstad J, Prætorius L, Nielsen HS, Kitlinski ML, Skouby SO, Mikkelsen AL, Spangmose AL, Jeppesen JV, Khatibi A, la Cour FN, Ziebe S, Polyzos NP, Bergh C, Humaidan P, Andersen AN, Løssl K, Pinborg A. Freeze-all versus fresh blastocyst transfer strategy during in vitro fertilization in women with regular menstrual cycles: multicentre randomised controlled trial. BMJ. 2020;370:m2519. https://doi.org/10.1136/bmj.m2519.
11. Desai N, Ploskonka S, Goodman L, Attaran M, Goldberg JM, Austin C, Falcone T. Delayed blastulation, multinucleation, and expansion grade are independently associated with live-birth rates in frozen blastocyst transfer cycles. Fertil Steril. 2016;106(6):1370–8. https://doi.org/10.1016/j.fertnstert.2016.07.1095.
12. Sciorio R, Thong KJ, Pickering SJ. Increased pregnancy outcome after day 5 versus day 6 transfers of human vitrified-warmed blastocysts. Zygote. 2019;27(5):279–84. https://doi.org/10.1017/S0967199419000273.
13. Ferreux L, Bourdon M, Sallem A, Santulli P, Barraud-Lange V, Le Foll N, Maignien C, Chapron C, de Ziegler D, Wolf JP, Pocate-Cheriet K. Live birth rate following frozen-thawed blastocyst transfer is higher with blastocysts expanded on day 5 than on day 6. Hum Reprod. 2018;33(3):390–8. https://doi.org/10.1093/humrep/dey004.
14. Sunkara SK, Siozos A, Bolton VN, Khalaf Y, Braude PR, El-Toukhy T. The influence of delayed blastocyst formation on the outcome of frozen-thawed blastocyst transfer: a systematic review and meta-analysis. Hum Reprod. 2010;25(8):1906–15. https://doi.org/10.1093/humrep/deq143.
15. El-Toukhy T, Wharf E, Walavalkar R, Singh A, Bolton V, Khalaf Y, Braude P. Delayed blastocyst development does not influence the outcome of frozen-thawed transfer cycles. BJOG. 2011;118(13):1551–6. https://doi.org/10.1111/j.1471-0528.2011.03101.x.
16. Reignier A, Lammers J, Barriere P, Freour T. Can time-lapse parameters predict embryo ploidy? A systematic review. Reprod Biomed Online. 2018;36(4):380–7. https://doi.org/10.1016/j.rbmo.2018.01.001.
17. Barash OO, Ivani KA, Willman SP, Rosenbluth EM, Wachs DS, Hinckley MD, Pittenger Reid S, Weckstein LN. Association between growth dynamics, morphological parameters, the chromosomal status of the blastocysts, and clinical outcomes in IVF PGS cycles with single embryo transfer. J Assist Reprod Genet. 2017;34(8):1007–16. https://doi.org/10.1007/s10815-017-0944-0.
18. Irani M, O'Neill C, Palermo GD, Xu K, Zhang C, Qin X, Zhan Q, Clarke RN, Ye Z, Zaninovic N, Rosenwaks Z. Blastocyst development rate influences implantation and live birth rates of similarly graded euploid blastocysts. Fertil Steril. 2018;110(1):95–102.e1. https://doi.org/10.1016/j.fertnstert.2018.03.032.
19. Kaye L, Will EA, Bartolucci A, Nulsen J, Benadiva C, Engmann L. Pregnancy rates for single embryo transfer (SET) of day 5 and day 6 blastocysts after cryopreservation by vitrification and slow freeze. J Assist Reprod Genet. 2017;34(7):913–9. https://doi.org/10.1007/s10815-017-0940-4.
20. El-Damen A, Elkhatib I, Bayram A, Arnanz A, Abdala A, Samir S, Lawrenz B, De Munck N, Fatemi HM. Does blastocyst mitochondrial DNA content affect miscarriage rate in patients undergoing single euploid frozen embryo transfer? J Assist Reprod Genet. 2021;38(3):595–604. https://doi.org/10.1007/s10815-020-02050-8.
21. Greco E, Litwicka K, Arrivi C, Varricchio MT, Caragia A, Greco A, Minasi MG, Fiorentino F. The endometrial preparation for frozen-thawed euploid blastocyst transfer: a prospective randomized trial comparing clinical results from natural modified cycle and exogenous hormone stimulation with GnRH agonist. J Assist Reprod Genet. 2016;33(7):873–84. https://doi.org/10.1007/s10815-016-0736-y.
22. Ghobara T, Gelbaya TA, Ayeleke RO. Cycle regimens for frozen-thawed embryo transfer. Cochrane Database Syst Rev. 2017;7(7):CD003414. https://doi.org/10.1002/14651858.CD003414.pub3.
23. Glujovsky D, Pesce R, Sueldo C, Quinteiro Retamar AM, Hart RJ, Ciapponi A. Endometrial preparation for women undergoing embryo transfer with frozen embryos or embryos derived from donor oocytes. Cochrane Database Syst Rev. 2020;10:CD006359. https://doi.org/10.1002/14651858.CD006359.pub3 Accessed 19 July 2021.
24. Groenewoud ER, Cantineau AE, Kollen BJ, Macklon NS, Cohlen BJ. What is the optimal means of preparing the endometrium in frozen-thawed embryo transfer cycles? A systematic review and meta-analysis. Hum Reprod Update. 2013;19(5):458–70. https://doi.org/10.1093/humupd/dmt030 Erratum in: Hum Reprod Update. 2017 Mar 1;23(2):255-261.
25. Gardner DK, Schoolcraft WB. In vitro culture of human blastocyst. In: Jansen R, Mortimer D, editors. Towards reproductive certainty: infertility and genetics beyond 1999. Carnforth: Parthenon Press; 1999. p. 378–88.
26. Kuwayama M. Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method. Theriogenology. 2007;67:73–80.
27. Shu Y, Watt J, Gebhardt J, Dasig J, Appling J, Behr B. The value of fast blastocoele re-expansion in the selection of a viable thawed blastocyst for transfer. Fertil Steril. 2009;91(2):401–6. https://doi.org/10.1016/j.fertnstert.2007.11.083.
28. Fatemi HM, Kyrou D, Bourgain C, Van den Abbeel E, Griesinger G, Devroey P. Cryopreserved-thawed human embryo transfer: spontaneous natural cycle is superior to human chorionic gonadotropin-induced natural cycle. Fertil Steril. 2010;94(6):2054–8. https://doi.org/10.1016/j.fertnstert.2009.11.036.
29. Davis O, Zaninovic N, Xu K, Rosenwaks Z. Morphologic grading of euploid blastocysts influences implantation and ongoing pregnancy rates. Fertil Steril. 2017;107(3):664–70. https://doi.org/10.1016/j.fertnstert.2016.11.012.
30. Lee CI, Chen CH, Huang CC, Cheng EH, Chen HH, Ho ST, Lin PY, Lee MS, Lee TH. Embryo morphokinetics is potentially associated with clinical outcomes of single-embryo transfers in preimplantation genetic testing for aneuploidy cycles. Reprod BioMed Online. 2019;39(4):569–79. https://doi.org/10.1016/j.rbmo.2019.05.020.
31. Minasi MG, Colasante A, Riccio T, Ruberti A, Casciani V, Scarselli F, Spinella F, Fiorentino F, Varricchio MT, Greco E. Correlation between aneuploidy, standard morphology evaluation and morphokinetic development in 1730 biopsied blastocysts: a consecutive case series study. Hum Reprod. 2016;31(10):2245–54. https://doi.org/10.1093/humrep/dew183.
32. Park DS, Kim JW, Chang EM, Lee WS, Yoon TK, Lyu SW. Obstetric, neonatal, and clinical outcomes of day 6 vs. day 5 vitrified-warmed blastocyst transfers: retrospective cohort study with propensity score matching. Front Endocrinol (Lausanne). 2020;11:499. https://doi.org/10.3389/fendo.2020.00499.
33. Ji H, Zhou Y, Cao S, Zhang J, Ling X, Zhao C, Shen R. Effect of embryo developmental stage, morphological grading, and ploidy status on live birth rate in frozen cycles of single blastocyst transfer. Reprod Sci. 2021;28(4):1079–91. https://doi.org/10.1007/s43032-020-00381-6.
34. Li YX, Wang J, Sun TZ, Lv MQ, Ge P, Li HN, Zhou DX. Pregnancy outcomes after day 5 versus day 6 blastocyst-stage embryo transfer: a systematic review and meta-analysis. J

Obstet Gynaecol Res. 2020;46(4):595–605. https://doi.org/10.1111/jog.14188.
35. Annan JJ, Gudi A, Bhide P, Shah A, Homburg R. Biochemical pregnancy during assisted conception: a little bit pregnant. J Clin Med Res. 2013;5(4):269–74. https://doi.org/10.4021/jocmr1008w.
36. Chen L, Diao Z, Wang J, Xu Z, Zhang N, Fang J, Lin F. The effects of the day of trophectoderm biopsy and blastocyst grade on the clinical and neonatal outcomes of preimplantation genetic testing-frozen embryo transfer cycles. Zygote. 2021;28:1–6. https://doi.org/10.1017/S0967199421000435.
37. Ai J, Jin L, Zheng Y, Yang P, Huang B, Dong X. The morphology of inner cell mass is the strongest predictor of live birth after a frozen-thawed single embryo transfer. Front Endocrinol (Lausanne). 2021;12:621221. https://doi.org/10.3389/fendo.2021.621221.
38. Zhao YY, Yu Y, Zhang XW. Overall blastocyst quality, trophectoderm grade, and inner cell mass grade predict pregnancy outcome in euploid blastocyst transfer cycles. Chin Med J. 2018;131(11):1261–7. https://doi.org/10.4103/0366-6999.232808.
39. Lou H, Li N, Guan Y, Zhang Y, Hao D, Cui S. Association between morphologic grading and implantation rate of euploid blastocyst. J Ovarian Res. 2021;14(1):18. https://doi.org/10.1186/s13048-021-00770-8.
40. Boynukalin FK, Gultomruk M, Cavkaytar S, Turgut E, Findikli N, Serdarogullari M, Coban O, Yarkiner Z, Rubio C, Bahceci M. Parameters impacting the live birth rate per transfer after frozen single euploid blastocyst transfer. PLoS One. 2020;15(1):e0227619. https://doi.org/10.1371/journal.pone.0227619.
41. Kimelman D, Confino R, Okeigwe I, Lambe-Steinmiller J, Confino E, Shulman LP, Zhang JX, Pavone ME. Assessing the impact of delayed blastulation using time lapse morphokinetics and preimplantation genetic testing in an IVF patient population. J Assist Reprod Genet. 2019;36(8):1561–9. https://doi.org/10.1007/s10815-019-01501-1.
42. Nazem TG, Sekhon L, Lee JA, Overbey J, Pan S, Duke M, Briton-Jones C, Whitehouse M, Copperman AB, Stein DE. The correlation between morphology and implantation of euploid human blastocysts. Reprod BioMed Online. 2019;38(2):169–76. https://doi.org/10.1016/j.rbmo.2018.10.007.
43. Guzman L, Nuñez D, López R, Inoue N, Portella J, Vizcarra F, Noriega-Portella L, Noriega-Hoces L, Munné S. The number of biopsied trophectoderm cells may affect pregnancy outcomes. J Assist Reprod Genet. 2019;36(1):145–51. https://doi.org/10.1007/s10815-018-1331-1.
44. Casper RF, Yanushpolsky EH. Optimal endometrial preparation for frozen embryo transfer cycles: window of implantation and progesterone support. Fertil Steril. 2016;105(4):867–72. https://doi.org/10.1016/j.fertnstert.2016.01.006.
45. Lawrenz B, Coughlan C, Fatemi HM. Individualized luteal phase support. Curr Opin Obstet Gynecol. 2019;31(3):177–82. https://doi.org/10.1097/GCO.0000000000000530.
46. Lawrenz B, Coughlan C, Melado L, Fatemi HM. The ART of frozen embryo transfer: back to nature! Gynecol Endocrinol. 2020;36(6):479–83. https://doi.org/10.1080/09513590.2020.1740918.
47. Poletto KQ, Lobo MP, Giovanucci M, Approbato MS, Castro EC. Pregnancy rates from natural and artificial cycles of women submitted to frozen embryo transfers: a metanalysis. JBRA Assist Reprod. 2019;23(3):268–72. https://doi.org/10.5935/1518-0557.20190018.
48. Kim MK, Park JK, Jeon Y, et al. Correlation between morphologic grading and euploidy rates of blastocysts, and clinical outcomes in in vitro fertilization preimplantation genetic screening. J Korean Med Sci. 2019;34(4):e27. Published 2019 Jan 10. https://doi.org/10.3346/jkms.2019.34.e27.
49. Maxwell SM, Grifo JA. Should every embryo undergo preimplantation genetic testing for aneuploidy? A review of the modern approach to in vitro fertilization. Best Pract Res Clin Obstet Gynaecol. 2018;53:38–47. https://doi.org/10.1016/j.bpobgyn.2018.07.005.
50. Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, de Mouzon J, Sokol R, Rienzi L, Sunde A, Schmidt L, Cooke ID, Simpson JL, van der Poel S. The International Glossary on Infertility and Fertility Care, 2017. Fertil Steril. 2017;108(3):393–406. https://doi.org/10.1016/j.fertnstert.2017.06.005.