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ORIGINAL ARTICLE *Embryology*

Fresh transfer of Day 5 slow-growing embryos versus deferred transfer of vitrified, fully expanded Day 6 blastocysts: which is the optimal approach?

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STUDY QUESTION: In IVF cycles in which the entire embryo cohort is slow growing, is it optimal to perform fresh transfer in Day 5 or to extend the culture and transfer in subsequent vitrified-warmed cycles?

SUMMARY ANSWER: The outcomes depend on the degree of embryo development on Day 5.

WHAT IS KNOWN ALREADY: Slow-growing blastocysts have lower implantation potential when transferred in fresh cycles. It has been suggested that embryo-endometrial asynchrony could explain this finding. However, studies that compared Days 5 and 6 embryos in frozen embryo transfer (FET) cycles showed contradictory results. There is still a lack of evidence regarding the best approach, performing fresh transfer or deferring transfer and continuing culture until fully developed blastocysts are achieved, when the entire cohort of embryos is slow growing.

STUDY DESIGN SIZE, DURATION: This was a retrospective study that included 477 women aged <40 years who underwent fresh Day 5 single embryo transfer of slow-growing embryos and subsequent FET cycles of fully expanded blastocysts (FEB) originating from the same IVF cycle between 2012 and 2016.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The study included cycles in which the embryos either began blastulation by Day 5 of culture but did not reach the fully expanded stage (Gardner Stage III) or had delayed blastulation with only morula embryos present by Day 5 of culture. All of the subjects in the study underwent elective, single embryo transfer (slow or delayed blastocysts) on Day 5 and had at least one embryo that developed into a FEB on extended culture Day 6 that was suitable for vitrification. All subjects, regardless of the outcome of the fresh transfer, returned for at least one subsequent FET cycle of Day 6 embryos.

MAIN RESULTS AND ROLE OF CHANCE: A total of 1070 embryo transfer cycles (fresh + FET) were included. Of them, 365 women had elective, fresh, single transfer of slow-growing blastocysts (Group I) and 112 had elective, fresh, single morula transfer (Group II). Groups I and II underwent a subsequent 457 and 136 FET cycles, respectively. The mean age of Group I was 33.8 ± 2.9 years, the proportion of Day 5 embryos that developed to FEB by Day 6 was 92%, and the number of blastocysts vitrified was 627 (average of 1.71 blastocysts per cycle). The outcomes of fresh and FET cycles were comparable regarding clinical pregnancy rate (CPR) (31.0 vs. 30.4%, $P = 0.86$) and live birth rate (LBR) (23.3 vs. 20.3%, $P = 0.15$). In Group II, the mean age was 35.8 ± 3.4 years and the proportion of morula embryos that developed to FEB by Day 6 was 72%. The number of blastocysts vitrified on Day 6 was 155 (1.38 per cycle). The transfer of fresh embryos in Group II

resulted in significantly lower clinical pregnancy (5.3 vs. 30.1%, $P < 0.001$) and LBRs (1.8 vs. 20.5%, $P < 0.001$). The results did not change after controlling for possible confounding factors

LIMITATIONS AND REASONS FOR CAUTION: The retrospective design of the study is a major limitation. Although we compared the outcomes of embryos that originated from the same cohort, the FET cycles could have been overrepresented by older patients and those with poorer prognoses. Furthermore, the study included only cycles in which there were blastocysts available for cryopreservation on Day 6; therefore, the results were not be applicable for those who had mandatory Day 5 transfer with no embryos available for vitrification.

WIDER IMPLICATIONS OF THE FINDINGS: Fresh transfer of embryos that begin blastulation on Day 5 results in similar outcomes to the transfer of FEB originating from the same cohort. However, in cases where only morula/compacting embryos are available by Day 5, extending culture until FEB are achieved and then performing subsequent FET will result in significantly higher LBRs.

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Key words: slow-growing blastocyst / morula / compacted embryo / fresh transfer / frozen embryo transfer / Day 6 / Day 5 / fully expanded blastocyst

Introduction

The development of extended embryo culture and vitrification techniques for embryo cryopreservation have resulted in the wide utilization of blastocyst culture and increased the practice of single embryo transfer (Cutting, 2018; Sekhon *et al.*, 2018). Most of the favorable data regarding the success of blastocyst transfer stems from studies that evaluated fresh, fast-growing Day 5 blastocyst transfers. However, as in any biologic system, not all embryos will demonstrate the same pace of development, and some will either fail to begin blastulation by Day 5 or will begin blastulation but will not reach the fully expanded stage.

Transfer of fresh, slow-growing blastocysts was shown to result in lower implantation and clinical pregnancy rates (CPRs) compared to fully expanded Day 5 blastocysts (Shapiro *et al.*, 2001; Barrenetxea *et al.*, 2005). It has been suggested that embryo-endometrium asynchrony could explain this difference. To overcome the endometrial factor, several studies compared Day 5 and Day 6 embryos in frozen embryo transfer (FET) cycles; however, the results were inconsistent as some studies reported similar outcomes (Liebermann and Tucker, 2006; El-Toukhy *et al.*, 2011; Kovalevsky *et al.*, 2013; Yang *et al.*, 2016) and some reported worse outcomes for Day 6 compared to Day 5 transfer (Levens *et al.*, 2008; Kang *et al.*, 2013; Desai *et al.*, 2016; Haas *et al.*, 2016). The inability to reach firm conclusions is most likely the result of comparing two groups (Day 5 vs. Day 6) that have different prognostic factors, such as older maternal age and lower overall embryo quality in Day 6 compared to Day 5 blastocysts. Therefore, there is still a lack of evidence regarding the optimal approach, whether to perform fresh transfer or to continue culturing until fully expanded blastocysts (FEB) are achieved and then performing subsequent FET cycles, in cases of slow-growing embryos.

Deferring embryo transfer can potentially improve outcomes by improving embryo-endometrium synchrony. The optimal way to answer this question is to investigate cycles in which all embryos demonstrated slow development and underwent fresh Day 5 transfer and compare it to subsequent frozen transfer cycles of FEB vitrified on Day 6 that originated from the same cohort. This would enable better matching of the baseline characteristics by comparing embryos formed from the same cycle. Hence, the objective of this study is to compare the outcomes of delayed or slow-growing blastocysts transferred on Day 5 with the transfer of Day 6 FEB that were achieved from the same cohort and transferred in subsequent vitrified-warmed cycles.

Materials and Methods

We performed a retrospective cohort analysis of 1070 embryo transfer cycles (fresh + FET) from 477 women between January 2012 and April 2016. The patients' demographics, indications for treatment, ovarian stimulation cycle characteristics, embryo development, morphology and outcome following embryo transfer were all recorded prospectively in computerized medical charts. Inclusion criteria were the following: maternal age <40 years at the beginning of ovarian stimulation, elective fresh Day 5 single embryo transfer of morula or slow-growing blastocyst (Gardner Stages I and II), no embryo in the whole cohort attaining full expansion (Gardner Stage III) by Day 5, the presence of at least FEB at culture Day 6 that was suitable for vitrification and the patient returning for at least one vitrified-warmed cycle regardless of the outcome of the fresh transfer. Exclusion criteria were the following: cycles with freeze-all embryos and cycles with PGS. The study was conducted after approval by the institutional review board (study number 15-249)

Ovarian stimulation and embryo procedures

Ovarian stimulation was performed under pituitary suppression using one of the three following protocols: GnRH agonist micro-dose flare protocol, midluteal GnRH agonist long protocol or GnRH antagonist short protocol. Final oocyte maturation was induced with 10 000 IU of hCG, when at least two follicles were >17 mm in diameter. Oocyte collection was performed 36-38 hours after hCG triggering. Insemination of retrieved oocytes was done by conventional IVF or ICSI. Fertilization was assessed 16–18 h after insemination for the appearance of two distinct pro-nuclei and two polar bodies. The zygotes were cultured in cleavage medium (COOK Medical, Sydney, Australia). Extending embryo culture to the blastocyst stage was done if there were at least three good quality embryos on Day 3 (six to nine cells and <20% fragmentation). Embryo development was checked in the morning of Day 5, and another assessment was performed in the afternoon of the same day. According to the clinic protocol, in cases of

extended embryo culture, fresh transfer was performed in the afternoon of Day 5, regardless of the developmental stage of the embryo. In cases in which there were no FEB in culture, the embryo with the best quality was transferred and the other embryos were cultured to Day 6. The two assessments were performed again in the morning and afternoon of Day 6, and only FEB with a morphological score of ≥3 bb were vitrified. All other embryos were discarded.

Luteal phase support after fresh transfer consisted of estradiol valerate (6 mg/day) and micronized progesterone (300 mg/day) that were started on the day of oocyte retrieval and continued until 10 weeks of gestation.

Morula and blastocysts' assessment

Morula grading was performed according to the score proposed by Tao *et al.*, as following: top quality: all the blastomeres are undergoing compaction with smooth sphere shape of the embryo. Good quality: >75% of the blastomeres are underdoing compaction with shallow indentation or >65% undergoing compaction with smooth surface. Average quality: bilobed morula, or full to 75% compaction, with deep indentation. Poor quality: 30% compaction or lobed to two or more with 30% compaction (Tao *et al.*, 2002).

Blastocysts were graded according to Gardner criteria, and the score was dependent on blastocyst expansion, inner cell mass (ICM) development and trophectoderm (TE) appearance. Degree of expansion included the following six grades: (i) a nonexpanded embryo with the blastocele filling <50%; (ii) the blastocele fills >50% of the embryo; (iii) the blastocele fills the entire blastocyst; (iv) an expanded blastocyst with a thin zona pellucida; (v) a hatching blastocyst and (vi) a hatched blastocyst. The ICM was graded as follows: (A) tightly packed cells; (B) loosely gathered cells and (C) no identifiable cells. The three TE grades were (A) many cells establishing a cohesive epithelial layer; (B) few uneven cells creating a loose epithelium and (C) few large cells (Gardner *et al.*, 2004). In Blastocyst expansion score I and II, it is more challenging to score the ICM and TE compared to expansion score ≥3. However, it was previously shown that the number, shape and cell compaction can be assessed when cavitation starts to develop (Wirleitner *et al.*, 2016). Blastocysts were considered as slow-growing blastocysts (Stages 1 and 2) and FEB (Grade ≥3). Quality of the blastocysts was dependent on ICM and TE scoring: top quality: AA; good quality: AB and BA; average quality: AC, CA and BB and poor quality: BC, CB and CC. Blastocysts were scored before fresh transfer and vitrification and in warming cycle blastocysts were scored again before transfer. In this study, we used embryo score after warming in FET cycles.

Blastocyst vitrification and warming procedures

Prior to vitrification, artificial blastocyst collapse was performed by using the laser system ZILOS-tkTM (Hamilton Thorn Bioscience Inc., Beverly, MA, USA). The blastocysts were then suspended in equilibration solution (containing 7.5%, v/v, dimethylsulfoxide (DMSO) and 7.5%, v/v, ethylene glycol (EG)) for 3 min at 37°C and then were transferred into vitrification solution (containing 15% DMSO, 15% EG and 0.5 mol/l trehalose) for 30 s. The blastocysts were then loaded onto a CryoTop (Kitazato Biopharma) with a minimal volume of vitrification solution and immediately plunged into liquid nitrogen at −196 C.

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

Endometrial preparation for vitrified-warming cycles

Endometrial priming was performed with daily oral estradiol valerate (6 mg) beginning on cycle Days 2 and 3 for 11 days to achieve endometrial thickness of ≥8 mm. If this thickness was not reached, the dose of estradiol valerate was increased by 2 mg/day (until a maximus dose of 12 mg/day) for 3 days and endometrial thickness was checked again. If the 8 mm cutoff was not achieved after the maximal dose and the endometrial thickness was ≥7 mm, embryo transfer was scheduled and micronized vaginal progesterone (300 mg) was started daily and embryo transfer was performed on Day 6 of progesterone treatment. In cases of endometrial thickness <7 mm, the cycle was canceled. In this study all the cycles reached endometrial thickness ≥7 mm. Luteal support was continued until 10 weeks of gestation.

Sample size calculation

Sample size calculation was based on the study of Wirleitner *et al.* (2016). For slow-growing blastocysts cycles and considering a similar live birth rate (LBR) of 25% in fresh and FET cycles, with an alpha error of 5% and power of 90% to detect no difference >10%, a total of 464 cycles (232 in each group) would be needed.

For fresh morula transfer and subsequent vitrified-warmed blastocysts transfer and considering a live birth of 2.8 and 21.7% respectively, with alpha error 5% and power 90%, a total of 118 cycles (59 in each group) would be needed to detect a difference.

Clinical outcomes and statistical analysis

Biochemical pregnancy was considered as positive serum levels of hCG (>10 IU/l) performed 2 weeks after oocyte retrieval or 11 days following FET transfer. Clinical pregnancy was defined as the visualization of gestational sac by vaginal sonography by 6 weeks pregnancy. Live birth was defined as a live birth after 24 weeks of pregnancy. Data was analyzed using JMP®, version (13) (SAS Institute Inc., Cary, NC, USA). Continuous data for patient baseline and cycle characteristics were assessed for normality using the Shapiro–Wilk test and expressed as mean (± SD). Categorical data were presented with their frequency and percentage. Between-group differences were assessed using Chi-square and the *t*-test. A *P* value of <0.05 was considered significant. Logistic regression was performed to control for confounders. The confounding factors included the following: maternal age, BMI, ovarian stimulation protocol, gonadotropin dose, number of FET cycle per patient, number of transferred embryos per cycle and embryo quality.

Results

During the study period, 571 patients had elective transfer of fresh, single slow-growing embryos on Day 5 and fully expanded embryos on Day 6 suitable for vitrification. Of them, 94 patients were excluded because they did not return for subsequent FET cycles. Of the remaining patients, 365 patients had elective single slow-growing blastocyst transfer (Group I) and 112 patients had elective single morula transfer (Group II) (Fig. 1).

Outcomes of fresh and FET cycles in Group I

The mean maternal age was 33.8 years, the median number of retrieved oocytes was 12 and the fertilization rate was 78%. The average number of vitrified blastocysts on Day 6 was 1.74. Overall, 92% of the embryo cohort available on Day 5 continued development and

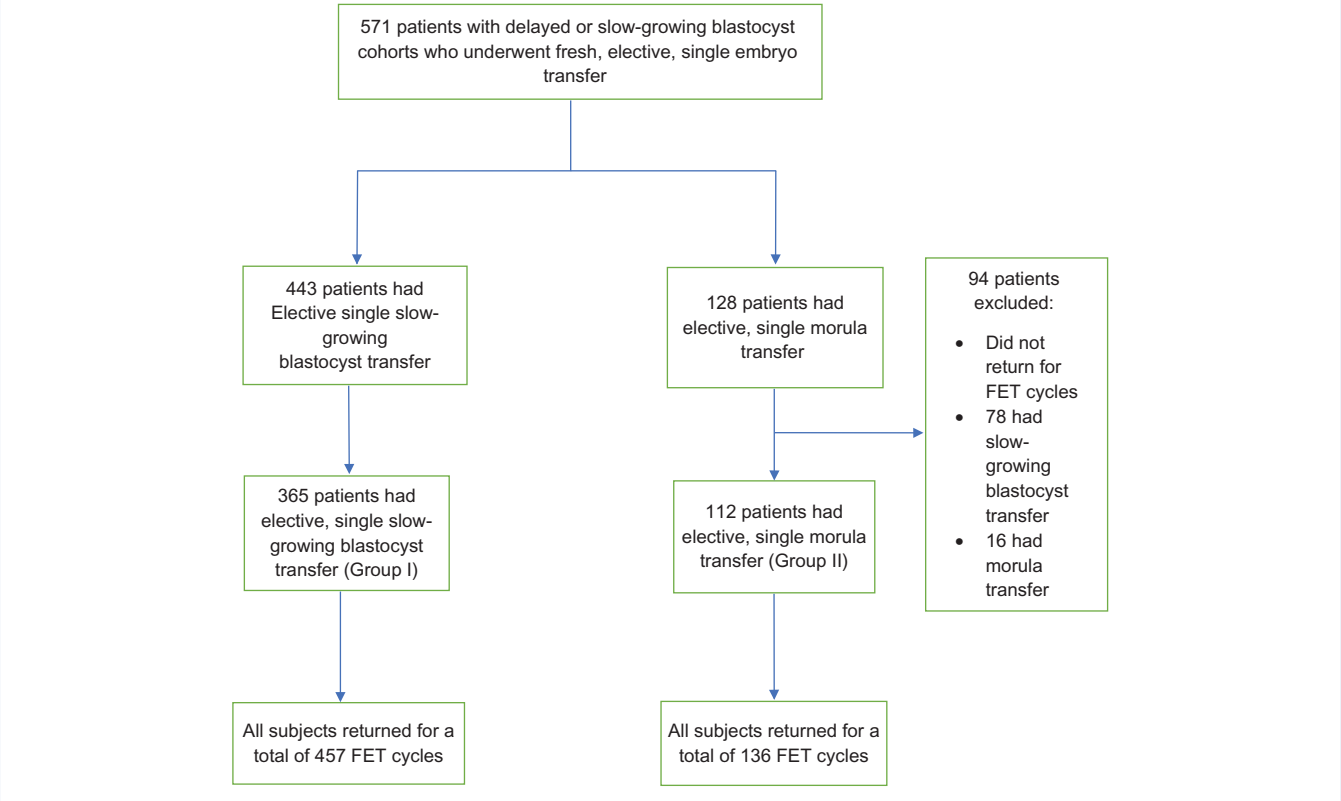

Figure 1 Flow chart of the study groups.

reached full expansion on Day 6. The transfer of fresh single blastocyst resulted in 31 and 23.3% of CPR and LBR, respectively.

Description of the FET cycles is presented in Table I. Overall, 83.2% of the vitrified embryos were utilized, embryo survival rate after thawing was 91.5% and the mean number of embryos transferred per cycle was 1.04. Most of the patients (98%) underwent one or two FET cycles (Table I). This resulted in a CPR and LBR of 30.4 and 20.3%, respectively. When comparing the outcomes of the fresh and FET cycles, the CPR and LBR were similar (31 vs. 30.4% and 23.3 vs. 20.3%). The LBR remained similar after controlling for confounding factors (OR = 1.57, 95% CI = 0.92–2.5, $P = 0.14$) (Table II). Full description of the demographics and cycle parameters are provided in Supplementary Table SI.

Outcomes of fresh and FET cycles in Group II

The mean age was 35.8 years, the median number of retrieved oocytes was 12 and the fertilization rate was 82%. Of the embryos presented on Day 5, 72% continued development and were vitrified on Day 6 (Table I). The mean number of blastocysts vitrified per cycle was 1.38. Fresh morula transfer resulted in CPR and LBR of 5.3 and 1.8% respectively.

A total of 136 FET cycles were performed in Group II, and vitrified embryo utilization rate was 100% (Table I). The maximum number of FET cycle per patient was 2, and 78.5% of patients ($n = 88$) underwent single FET cycle. When comparing the FET outcomes with the fresh cycles, the CPR (30.1 vs. 5.3%) and the LBR (20.5 vs. 1.8%) were significantly higher in the FET cycles. The results for LBR remained significant after controlling for confounding factors (OR = 9.2, 95% CI = 5.1–14.2, $P < 0.001$) (Table II). Detailed description of cycle characteristics is provided in Supplementary Table SII.

To examine whether there were differences in embryo competence of blastocysts vitrified from Groups I and II, we compared the outcomes of FET cycles that were performed in both groups. In this case, only FET cycles with embryo transfer were included. The results are shown in Table III. The LBR remained similar between the groups after controlling for age, body mass index and embryo quality (OR = 0.69, 95% CI = 0.45–1.83, $P = 0.22$)

Discussion

In this study, we aimed to determine whether deferring the transfer of morula or slow-growing blastocysts by extending embryo culture to Day 6 and transferring FEB in subsequent FET cycles could result in better reproductive outcomes. The improved outcomes could result from better embryo-endometrium synchronization or better embryo selection. The embryo cohorts included had delayed or slow blastulation, and only fresh, single embryo transfers were included to avoid inclusion of cycles in which embryos were at different stages of development. The results demonstrated that the outcome depends on the day the embryos start blastulation. Embryos that started blastulation but did not reach full expansion on Day 5 had similar outcomes to transferring fully expanded Day 6 blastocysts in subsequent vitrified-

Table I Description of vitrified-warmed cycles in Groups I and II.

	Group I	Group II
Proportion of Day 5 embryos vitrified on Day 6	92%	72%
Number of blastocysts vitrified	627	154
Number of blastocysts vitrified per cycle	1.71 ± 0.97	1.38 ± 0.7
Distribution of patients according to the number of FET cycles n (%)		
One cycle	280 (76.7%)	88 (78.5%)
Two cycles	78 (21.3%)	24 (21.5%)
Three cycles	7 (2%)	0
Number of embryos thawed	522	154
Embryo utilization rate	83.2%	100%
Embryo survival rate	91.5%	90.1%
Number of blastocysts transferred	478	140

FET: frozen embryo transfer.

Table II Multivariate logistic regression for the effect on live birth rates when comparing fresh and vitrified-warmed cycles in Groups I and II.

	Group I		Group II	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Live birth rate	1.57 (0.92–2.5)	0.14	9.2 (5.1–14.2)	< 0.001
Maternal age (per year)*	1.04 (0.98–1.1)	0.12	1.56 (1.21–2.01)	0.64
BMI (per kg/m^2)	0.82 (0.74–1.1)	0.21	1.23 (0.78–1.55)	0.4
Ovarian stimulation protocol				
GnRH antagonist short protocol	Reference	-	Reference	
GnRH agonist long protocol	1.76 (0.91–2.2)	0.47	0.66 (0.41–1.65)	0.75
GnRH agonist short protocol	0.56 (0.30–1.66)	0.81	0.4 (0.35–1.38)	0.21
Gonadotropin dose	0.99 (0.99–1.01)	0.36	0.90 (0.88–1.05)	0.82
Number of embryo transfer cycles	1.2 (0.82–1.33)	0.44	1.24 (0.81–1.66)	0.37
Number of embryo transferred per cycle	0.97 (0.92–1.03)	0.76	1.09 (0.91–1.21)	0.15
Embryo quality (top and good vs. average and poor)	1.75 (1.29–2.40)	0.003	1.9 (1.32–2.66)	<0.01
Embryo expansion score (Gardner I and I vs. ≥III)	1.42 (0.89–1.78)	0.15	na	
Embryo expansion (Gardner ≥III vs. morula)	na		13.1 (8.6–23.1)	<0.01

OR: odds ratio.
* Maternal age at oocyte retrieval.

warmed cycles. However, cycles in which the embryos did not start blastulation by Day 5 resulted in significantly lower LBRs compared to embryos from the same cohort that reached full expansion on Day 6 and were then transferred in subsequent vitrified-warmed cycles. It should be noted that single embryo transfer is the standard practice in our clinic, and although the number of embryos transferred in FET cycles was higher than fresh transfers, this difference was negligible and the results did not change after controlling for confounding factors.

Blastocyst transfer has become the standard practice thanks to the development of efficient culture and media systems and the introduction of the vitrification technique for embryo cryopreservation. However, even under the same conditions, embryos in extended culture can display heterogeneity in development rate and can reach the blastocyst stage on Days 5, 6, or 7 (Ivec *et al.*, 2011). Indeed, studies showed that up to 30% of embryos can be slow growing (Shapiro *et al.*, 2008; Capalbo *et al.*, 2014) and that advanced maternal age and aneuploidy are associated with slow-growing embryos (Shapiro *et al.*, 2002; Kort *et al.*, 2015; Minasi *et al.*, 2016).

In fresh transfer, it seems that fast-growing embryos yield higher pregnancy rates. Shapiro *et al.* (2001) analyzed 183 fresh blastocyst transfer cycles and reported higher pregnancy rates in cycles that included Day 5 transfer compared to Day 6 (59.3 vs. 32.3%). In

Table III Comparison of the outcomes of FET cycles between Group I and Group II.

	Group I ($n = 441$)	Group II ($n = 126$)	*P* value
Maternal age (years)	34.40 ± 1.7	36.6 ± 2.2	0.01
BMI (kg/m^2)	23.8 ± 3.2	24.3 ± 2.5	0.1
Number of blastocysts transferred per cycle	1.08 ± 0.24	1.07 ± 0.18	0.19
Morphology score			
Top quality	3.5%	7.8%	0.03
Good quality	52.1%	46.7%	0.23
Average quality	41.4%	39.3%	0.65
Poor quality	3%	6.2%	0.05
Biochemical pregnancy rate	232 (52.6%)	56 (44.4%)	0.61
Clinical pregnancy rate	139 (31.5%)	42 (33.3%)	0.53
Live birth rate	93 (21%)	28 (22.2%)	0.22**

**After adjusting for maternal age, BMI and embryo quality; OR = 0.69, 95% CI = 0.45–1.83.

another study by Barrenetexia *et al.* (2005) that included 136 cycles, Day 6 transfer resulted in significantly lower pregnancy rates compared to Day 5 (11.1 vs. 38.3%). The authors suggested that embryo-endometrium asynchrony can explain these results. However, this assumption should be considered with caution because the Day 5 group had better prognostic features. For example, in Shapiro *et al.* (2001) study, the Day 5 group had higher blastocyst formation rate, more cycles with blastocyst cryopreservation and more embryos transferred per cycle. Similarly, in Barrenetexia *et al.*'s (2005) study, both the number of formed blastocysts and the number of transferred blastocysts per cycle were higher in the Day 5 group.

To overcome the possible effect of embryo-endometrium asynchrony, several studies compared Days 5 and 6 embryos in FET cycles. In a meta-analysis performed in 2010 that included 15 studies, a significantly higher CPR and ongoing pregnancy rates were observed for Day 5 compared to Day 6 mature blastocysts (OR = 1.14, 95% CI = 1.03–1.26, $P = 0.01$). However, no differences were found in outcomes when embryos were matched according to morphology score (Sunkara *et al.*, 2010). Since this meta-analysis, several other retrospective studies tried to explore this issue with conflicting results. Kaye *et al.* (2017) analyzed 468 cycles of Day 5 versus Day 6 mature blastocysts and found no difference in the ongoing pregnancy rates (51.7 vs. 44.9%). Conversely, Ferreux *et al.* (2018) found LBRs to be higher for Day 5 compared to Day 6 mature blastocysts (29.6 vs. 17%) and the difference persisted when the comparison was stratified by embryo morphology (Ferreux *et al.*, 2018).

The contradictory results of these studies can be partially explained by a sample bias in favor of Day 5 blastocysts when comparing FET cycles. For example, in the study by Ferreux *et al.* (2018), the proportion of good quality embryos was higher in Day 5 compared to Day 6 blastocysts (82 vs. 68%), which could mean a better overall competence of the Day 5 group. Similarly, in another study that showed the superiority of Day 5 transfer, subjects in the Day 6 group were older had different infertility etiologies and had a lower number of oocytes retrieved (Tubbing *et al.*, 2018). This selection bias can explain the similar outcomes of Days 5 and 6 embryos when only euploid embryos were transferred (Minasi *et al.*, 2016).

The main purpose of studies comparing the outcomes of fast- and slow-growing blastocysts is to develop a strategy regarding which embryos to give priority to when transferring to achieve pregnancy. This question is especially important in the era of single embryo transfer. When fresh transfer is planned, the priority is usually given to fast-growing blastocysts that achieve full expansion on Day 5. In FET cycles, although the data are still contradictory, it seems that the priority should be given to blastocysts vitrified on Day 5.

Nevertheless, there is still a lack of evidence in the literature regarding the optimal approach, whether to perform fresh transfer or to continue culture until expanded blastocysts are achieved and transfer these in a subsequent frozen cycle, in cycles in which the entire embryo cohort is slow growing. The results of this study are in accordance with those reported by Wirleitner *et al.*, who compared fresh and frozen-thawed blastocyst transfer and found very low LBRs when morula transfer was compared to fully developed blastocysts originating from the same cohort (2.8 vs. 21.7%, $P < 0.001$). However, the number of FET cycles in this case was small and included only 23 cycles (Wirleitner *et al.*, 2016). Nevertheless, our results confirm these observations and support a strategy of extending embryo culture in cases of delayed blastocyst development until FEB are achieved and then transferring them in subsequent FET cycles.

The low LBR in cases of morula transfer can be explained by the fact that 28% of these embryos will not continue development to blastocysts. Furthermore, it is possible that in cases of embryos that continue development, embryo-endometrium asynchrony is another limiting factor for successful implantation. Another contributing factor for the low LBR could be the high aneuploidy rate in Day 5 morula, which was reported to be between 79.8 and 92.9% (Kroener *et al.*, 2012).

Recently, it was shown that embryos achieving blastulation on Day 7 that were then transferred in FET cycles had acceptable LBRs compared to Day 5 blastocysts (25.15 vs. 46.5%, $P < 0.01$) and that there was no difference in neonatal weight, the incidence of malformation, or neonatal death (Du *et al.*, 2018). Therefore, it can be an appropriate strategy, especially in cases of delayed blastulation, to extend embryo culture beyond Day 6. This may increase the yield of embryos suitable for cryopreservation and improve outcomes.

The main weakness of the current study is the retrospective design. Furthermore, although we compared outcomes of embryos originating from the same cycle, these embryos were transferred in different cycles with different hormonal milieus (fresh and frozen) and the FET cycles may have been overrepresented by older patients, those with poorer prognoses and other unmeasured biases. Moreover, the laboratory policy is to extend embryo culture only when three good quality cleavage embryos are present and this may underestimate the prevalence of slow-growing embryos. We usually perform an artificial collapse of blastocysts before vitrification, and this technique was shown to be associated with better survival rate post thawing without compromising the implantation rate (Van Landuyt *et al.*, 2015).

In conclusion, our results show that cases in which embryos that begin blastulation but fail to reach full expansion by Day 5 are transferred in fresh cycles are expected to have similar outcomes to those of fully expanded embryos vitrified on Day 6. However, in cases of delayed blastulation (morula embryo transfer), fresh Day 5 transfer resulted in very low LBRs. In this case, deferring fresh embryo transfer in favor of extending embryo culture until fully expanded embryos are achieved for later vitrified-warmed transfer can significantly improve the LBRs.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Authors' roles

S.T. contributed to the study design and writing the manuscript. Y.C. contributed to the data acquisition, statistical analysis and approving the final version. S.H. contributed to the data acquisition and approving the final version. N.A. contributed to the data acquisition and approving the final version. T.S. contributed to the data acquisition and approving the final version. W.S. contributed to the study design, critical discussion and approving the manuscript. M.D. contributed to the study design, critical discussion and approving the manuscript.

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Conflict of interest

None.

References

Barrenetxea G, Lopez de Larruzea A, Ganzabal T, Jimenez R, Carbonero K, Mandiola M. Blastocyst culture after repeated failure of cleavage-stage embryo transfers: a comparison of day 5 and day 6 transfers. *Fertil Steril* 2005;**83**:49–53.

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**:1173–1181.

Cutting R Single embryo transfer for all. *Best Pract Res Clin Obstet Gynaecol* 2018. pii: S1521-6934(18)30152-4.

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**:1370–1378.

Du T, Wang Y, Fan Y, Zhang S, Yan Z, Yu W, Xi Q, Chen Q, Mol BW, Lyu Q *et al.* Fertility and neonatal outcomes of embryos achieving blastulation on Day 7: are they of clinical value? *Hum Reprod* 2018;**33**:1038–1051.

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**:1551–1556.

Ferreux L, Bourdon M, Sallem A, Santulli P, Barraud-Lange V, Le Foll N, Maignien C, Chapron C, de Ziegler D, Wolf JP *et al.* Live birth rate following frozen-thawed blastocyst transfer is higher with blastocysts expanded on Day 5 than on Day 6. *Hum Reprod* 2018;**33**:390–398.

Gardner DK, Surrey E, Minjarez D, Leitz A, Stevens J, Schoolcraft WB. Single blastocyst transfer: a prospective randomized trial. *Fertil Steril* 2004;**81**:551–555.

Haas J, Meriano J, Laskin C, Bentov Y, Barzilay E, Casper RF, Cadesky K. Clinical pregnancy rate following frozen embryo transfer is higher with blastocysts vitrified on day 5 than on day 6. *J Assist Reprod Genet* 2016;**33**:1553–1557.

Ivec M, Kovacic B, Vlaisavljevic V. Prediction of human blastocyst development from morulas with delayed and/or incomplete compaction. *Fertil Steril* 2011;**96**:1473–1478.e1472.

Kang SM, Lee SW, Yoon SH, Kim JC, Lim JH, Lee SG. Comparison of clinical outcomes between single and double vitrified-warmed blastocyst embryo transfer according to the day of vitrification. *J Assist Reprod Genet* 2013;**30**:779–785.

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**:913–919.

Kort JD, Lathi RB, Brookfield K, Baker VL, Zhao Q, Behr BR. Aneuploidy rates and blastocyst formation after biopsy of morulae and early blastocysts on day 5. *J Assist Reprod Genet* 2015;**32**:925–930.

Kovalevsky G, Carney SM, Morrison LS, Boylan CF, Neithardt AB, Feinberg RF. Should embryos developing to blastocysts on day 7 be cryopreserved and transferred: an analysis of pregnancy and implantation rates. *Fertil Steril* 2013;**100**:1008–1012.

Kroener L, Ambartsumyan G, Briton-Jones C, Dumesic D, Surrey M, Munne S, Hill D. The effect of timing of embryonic progression on chromosomal abnormality. *Fertil Steril* 2012;**98**:876–880.

Levens ED, Whitcomb BW, Hennessy S, James AN, Yauger BJ, Larsen FW. Blastocyst development rate impacts outcome in cryopreserved blastocyst transfer cycles. *Fertil Steril* 2008;**90**:2138–2143.

Liebermann J, Tucker MJ. Comparison of vitrification and conventional cryopreservation of day 5 and day 6 blastocysts during clinical application. *Fertil Steril* 2006;**86**:20–26.

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**:2245–2254.

Sekhon L, Lee JA, Flisser E, Copperman AB, Stein D. Blastocyst vitrification, cryostorage and warming does not affect live birth rate, infant birth weight or timing of delivery. *Reprod Biomed Online* 2018;**37**:33–42.

Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Ross R. Contrasting patterns in in vitro fertilization pregnancy rates among fresh autologous, fresh oocyte donor, and cryopreserved cycles with the use of day 5 or day 6 blastocysts may reflect differences in embryo-endometrium synchrony. *Fertil Steril* 2008;**89**:20–26.

Shapiro BS, Richter KS, Harris DC, Daneshmand ST. A comparison of day 5 and day 6 blastocyst transfers. *Fertil Steril* 2001;**75**:1126–1130.

Shapiro BS, Richter KS, Harris DC, Daneshmand ST. Influence of patient age on the growth and transfer of blastocyst-stage embryos. *Fertil Steril* 2002;**77**:700–705.

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**:1906–1915.

Tao J, Tamis R, Fink K, Williams B, Nelson-White T, Craig R. The neglected morula/compact stage embryo transfer. *Hum Reprod* 2002; **17**:1513–1518.

Tubbing A, Shaw-Jackson C, Ameye L, Colin J, Rozenberg S, Autin C. Increased live births after day 5 versus day 6 transfers of vitrified-warmed blastocysts. *J Assist Reprod Genet* 2018;**35**:417–424.

Van Landuyt L, Polyzos NP, De Munck N, Blockeel C, Van de Velde H, Verheyen G. A prospective randomized controlled trial investigating the effect of artificial shrinkage (collapse) on the implantation potential of vitrified blastocysts. *Hum Reprod* 2015;**30**:2509–2518.

Wirleitner B, Schuff M, Stecher A, Murtinger M, Vanderzwalmen P. Pregnancy and birth outcomes following fresh or vitrified embryo transfer according to blastocyst morphology and expansion stage, and culturing strategy for delayed development. *Hum Reprod* 2016;**31**: 1685–1695.

Yang H, Yang Q, Dai S, Li G, Jin H, Yao G, Sun Y. Comparison of differences in development potentials between frozen-thawed D5 and D6 blastocysts and their relationship with pregnancy outcomes. *J Assist Reprod Genet* 2016;**33**:865–872.