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ASSISTED REPRODUCTION TECHNOLOGIES

Increased live births after day 5 versus day 6 transfers of vitrified-warmed blastocysts

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Abstract

Purpose An investigation into the clinical implications of delayed blastulation (day 5 versus day 6) was carried out for cryo cycles, as heterogeneous results persist in the current literature.

Methods We performed a retrospective study comparing clinical pregnancies and live births between 178 blastocysts vitrified and warmed on day 5 versus 149 on day 6. The stage of blastocyst development was taken into account and adjustment for confounding factors was performed.

Results Our results demonstrate a significant difference in clinical pregnancy (43 versus 23% p value < 0.001) and live birth rates (34 versus 16% p value < 0.001) regarding the day of vitrification, in favour of day 5. This difference persisted after adjustment for confounding factors. The adjusted odds ratio for clinical pregnancies and deliveries for the day 5 group compared to that of the day 6 group was 2.83 (95%CI, 1.48 to 5.41) and 2.94 (95%CI, 1.39 to 6.22), respectively. When the stage of development of the blastocyst was taken into consideration, we still observed a significant advantage of day 5 versus day 6 vitrification.

Conclusions Day of vitrification (day 5 versus day 6) appears to be an independent predictor of clinical outcomes. Stratification of our cohort was carried out according to the developmental stage, and significant differences persisted. Although the transfer of day 6 cryopreserved embryos remains a viable option, giving priority to a day 5 embryo would reduce the time to pregnancy.

Keywords Blastocyst · Vitrification · Day 5 · Day 6 · Live births · Assisted reproduction

Introduction

Embryo cryopreservation plays a central role in medically assisted procreation, allowing patients a greater chance of conception per IVF cycle, while limiting the risks of multiple pregnancies, ovarian hyperstimulation syndrome and repeated oocyte retrieval. There currently exist two techniques: the original slow-freezing and the more recent vitrification technique [1]. Vitrification is an ultrafast cryopreservation method that transforms a substance to a glass-like state, while using high-concentration cryoprotectants. Compared to slow-freezing, this technique results in lower levels of intracellular and extracellular ice-crystal formation, and hence minimises mechanical embryo injury [2]. Studies on vitrification have indicated higher clinical pregnancy rates per embryo transfer, as well as improved embryo cryosurvival rates [3, 4]. Over the past decade, vitrification has gained ground, due to its biological and practical advantages and has become the favoured option for embryo cryopreservation [5].

In parallel, the development of efficient culture systems and culture media has guided certain IVF centres to opt for transfers at the blastocyst stage. This has observable advantages; firstly, the synchronisation between embryo and endometrium proceeds more harmoniously [6]; secondly, since expression of the embryonic genome is not achieved until the four- to eight-cell stage [7], examination at the blastocyst stage allows the biologist to identify potentially viable embryos. Due to complications associated with multiple pregnancies, the option of a single embryo transfer for fresh as well as cryo cycles is considered [8]. However, such a policy requires careful identification of embryos with high implantation potential.

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A literature review shows heterogeneous results regarding the clinical implications of a delay in development. Results from a meta-analysis seem to indicate more favourable outcomes for day 5 versus slower developing day 6 vitrified/warmed blastocysts [9]. However, when embryos of the same stage are compared, there would appear to be no significant difference [9]. A few recent studies nevertheless contradict these findings with significantly improved clinical results for day 5 versus day 6 blastocysts at the same developmental stage [10–12].

Within the paradigm of blastocyst vitrification, the current study addresses the implications of a delay in development. A detailed comparison in clinical outcomes was made between blastocysts vitrified on day 5 and those on day 6, in function of their developmental stage, to assess the clinical implications of a delay in blastulation. The establishment of clear clinical guidelines at the CHU St-Pierre, concerning vitrification, embryo-warming and transfer policy was the main goal of this study.

Materials and methods

Population studied

Single embryo transfers of vitrified-warmed blastocysts that took place between January 2012 and December 2015 were evaluated retrospectively. A total of 327 single blastocyst transfers were analysed. We excluded HCV-/HIV-positive women, donor cycles as well as blastocysts vitrified before May 2011 (protocol change).

Ovarian stimulation and oocyte retrieval

Patients were monitored and managed according to standardised clinical protocols as previously reported [13]. Briefly, ovarian stimulation was performed with hMG (Menopur®; Ferring, Denmark), recombinant FSH (Puregon®; NV Organon, the Netherlands or Gonal-F®; Merck-Serono, Switzerland) (Bemfola®; Finox, Switzerland) or corifollitropine alfa (Elonva®; NV Organon, The Netherlands). The dose of gonadotropins was determined on an individual basis according to the woman's age, day 3 serum FSH value and antral follicle count. Pituitary inhibition was obtained by GnRH analogue (long or short protocol) (Suprefact®; Senofi-Avantis, Germany or Decapeptyl®; Ipsen) or GnRH antagonist (Orgalutran®; NV Organon, the Netherlands or Cetrotide®; Merck-Serono, Switzerland). When three or more leading follicles reached 17 to 18 mm, ovulation was triggered with 5000–10,000 IU of hCG (Pregnyl®; NV Organon, The Netherlands). Oocyte retrieval was performed transvaginally and ultrasound-guided 34–36 h after hCG injection. Apart from Suprefact® which was administered intranasally, all medication was administered by the intradermal route.

Endometrial preparation

A natural cycle was the preferred means of endometrial preparation (88%). Blood/urine LH (luteinizing hormone) levels were measured, and intravaginal ultrasounds performed in order to follow the development of a dominant follicle and identify the moment of the LH surge or to decide to trigger the ovulation with hCG (5000 IU Pregnyl®, NV Organon, The Netherlands). Blastocyst transfer was performed 6 days after LH surge or 7 days after hCG triggering. Vaginal progesterone supplementation was administrated for 15 days. If a pregnancy was achieved, progesterone supplementation was continued until the first ultrasound confirming the clinical pregnancy.

Induction of an artificial cycle was the other means of endometrial preparation. Oestrogen (4 to 10 mg, Progynova®; Bayer Healthcare, Germany) supplementation was administered orally in order to allow for endometrial development. Once the endometrium reached a thickness >8 mm, vaginal progesterone (600 mg utrogestan®; Besins, Thailand) supplementation began in order to achieve endometrial differentiation. Blastocyst (day 5/6) transfer was performed on the sixth day of progesterone administration. Progesterone and oestrogen supplementations were continued for 15 days. If a pregnancy was achieved, oestrogen and progesterone supplementations were continued for a further 3 months.

Embryo culture and selection

After 17–20 h of ICSI/IVF, fertilisation was monitored and zygotes were cultured individually, mainly in two different culture media; G1 (Vitrolife, Sweden) or Global (Life Global, USA) media under 6% CO_2, 37 °C until day 3. Embryos with extended culture were transferred to fresh G2 or Global under the same conditions until days 5/6. Embryo transfers took place on days 2, 3 or 5, though our policy has evolved these last years towards a majority of day 5 transfers [14]. Blastocysts were observed and graded following Gardner and Schoolkraft [15], on the mornings of days 5/6 under an inverted microscope at ×400 magnification. The vitrification policy of supernumerary blastocysts at our centre states that cryopreservation is preferentially carried out on day 5 if the ICM and TE can be clearly distinguished and graded (stages 3 to 5). If not, culture is extended until day 6 and cryopreservation takes place if the embryo achieves sufficient development (stages 3 to 5) and quality. A majority of embryos of A/B quality (95%) were vitrified.

Vitrification cooling protocol

As recommended by the vitrification protocol of Irvine, grade 4 blastocysts (not grades 3 and 5) were submitted to laser shrinking prior to vitrification. A single laser pulse of 500 μs (Hammilton and Thorne, USA) was applied between two TE cells as far away possible from the ICM. The embryo was then replaced in the incubator for approximately 10 min to allow shrinking to occur.

The Irvine Scientific Freeze Kit (Irvine, USA) combined with CBS-VIT High Security straws from CryoBioSystem was used for vitrification. All basic solutions contained HEPES-buffered Medium-199, gentamicin sulphate 35 μg/mL and 20% *v*/*v* Dextran Serum Supplement (DSS). Embryos were progressively brought to room temperature and then incubated 10 min (blastocysts) in a 30 μL ES drop (equilibration solution 7.5% *v*/*v* of each DMSO and ethylene glycol) followed by two times 5 s and one time 10 s in 20 μL VS drops (vitrification solution 15% *v*/*v* of each DMSO and ethylene glycol, 0.5 M sucrose). The smallest possible volume of VS containing the embryo(s) was loaded into the gutter of the straw, which in turn was inserted into an external sheath; heat-sealed and plunged horizontally into liquid nitrogen (LN_2). The embryos were in contact with the VS between 60 and 90 s. The whole procedure was carried out at room temperature.

Vitrification warming protocol

The Irvine Scientific Thaw Kit (Irvine, USA) was used for warming. Again, all basic solutions contained HEPES-buffered Medium-199, gentamicin sulphate 35 μg/mL and 20% *v*/*v* DSS. Straws to be warmed were transferred into a small recipient containing LN_2. The external sheath was cut; the inner straw removed from LN_2 and plunged rapidly in a large droplet (300 μL) of TS media (thawing solution 1 M sucrose) preheated to 37 °C. The embryo(s) was left in this media for 1 min on a non-heated stage and then transferred into 20 μL of DS media (dilution solution 0.5 M sucrose) for 2 min. This step was repeated a second time. The embryo(s) was then incubated for 3 min in 20 μL WS media (washing solution HEPES-buffered solution of Medium-199 containing gentamicin sulphate 35 μg/mL HEPES and 20%DSS). This step was performed three times. During the last incubation step, embryos were brought progressively back to 37 °C, cultured in media containing 20% HSA for 1 h and then in media with 10% HSA until transfer. Blastocyst compaction state upon warming, survival and re-expansion were evaluated directly after warming and 60 min later. Embryo transfers took place 1 to 3 h post-warming and blastocysts with partial or no damage were considered to have survived and were transferred.

Outcome parameters

Warmed blastocysts were allocated into one of two groups (day 5 or day 6) depending on the day they were vitrified. The clinical outcomes compared between the two groups were clinical pregnancy rates (CPR), live birth rates (LBR) and miscarriage rates (MR). A clinical pregnancy was defined as a pregnancy with a gestational sac. The general characteristics of the fresh and cryopreservation cycles were compared between the two populations.

Statistical analysis

To assess the association between day of vitrification (day 5 versus day 6) and outcomes (CPR, MR, LBR), we used generalised estimating equations (GEE) model, as patients can have multiple cycles. For each outcome (CPR, MR, LBR), we had one model. The GEE model takes into account the dependency between cycles of the same patient by a compound symmetry correlation matrix. We also performed multivariate analysis in order to assess whether the day of vitrification was independently related to the outcome (clinical pregnancies, live births), independently of possible confounding factors. As the number of confounding factors one might consider in a multivariate model depends on the number of cases with the outcome (e.g. number of cases with a clinical pregnancy), not all multivariate models included the same possible confounding variables.

For clinical pregnancies involving all stages of blastocysts combined (stages 3 to 5), the following confounding factors were considered: maternal age at oocyte retrieval, endometrial thickness, duration of infertility, aetiology of infertility, basal FSH value, number of vitrified embryos, quality of vitrified-warmed embryos and the re-expansion status of the blastocyst post-warming. For live birth rates, we could adjust for seven explanatory variables. The number of embryos obtained being highly correlated with the number of cryopreserved embryos was not taken into account. The final re-expansion state of the blastocyst post-warming did not reach significance in the univariate analysis and was therefore the second factor that we did not consider. For clinical pregnancy and live birth analysis after stratification of blastocysts in function of their development stage, age at retrieval and embryo quality were the variables we could adjust for.

A p value smaller than 0.05 was considered statistically significant.

All statistical analyses were performed using the SAS System version 9.4 (SAS Institute Inc., Cary, NC, USA).

Ethical statement

All our protocols have been approved by the local Ethics Committee and all our patients have given their informed,

written consent prior to treatment. The current study was approved by the Ethics Committee of the CHU St-Pierre (AK/16-11-139/4734).

Results

During the period considered, 327 single blastocyst transfers took place. Initially, 361 embryos were warmed. Out of these, 327 survived and were transferred with a survival rate of 91% (327/361). Similar survival rates for day 5 and day 6 warmed blastocysts were observed (respectively, 92 (178/194) and 89% (149/167)). The overall clinical pregnancy rate per warmed embryo transfer was 34% (111/327) with two monozygotic twin pregnancies. Among the 83 live births, three malformations occurred after the transfer of a day 5 warmed embryo. Additionally, one intra-uterine death due to a trisomy eight was observed (day 5 embryo).

A comparison between the two groups (day 5 and day 6) in terms of the characteristics of the fresh and of the cryopreservation cycles is shown in Table 1 and significant differences are reported. In Table 2, clinical outcomes are compared between days 5 and 6. Significant differences in clinical pregnancy (43 versus 23% $p < 0.001$) and live birth rates (34 versus 16% $p < 0.001$) were observed between the two groups, in favour of day 5 vitrification.

When the stage of development was taken into consideration, a significant difference was still observed for stages 4 and 5, in favour of day 5 (Table 3). For stage 3 blastocysts, few embryos were cryopreserved on day 6 versus day 5.

A multivariate model was carried out for all stages combined in order to adjust for confounding factors. A significant difference in favour of day 5 versus day 6 for clinical pregnancies and live births persisted after adjustment (CPR OR 2.83, 95% CI, 1.48 to 5.41) (LBR OR 2.94, 95%CI, 1.39 to 6.22). We performed a stepwise variable selection and observed that only day 5/6 and numbers of vitrified embryos reached statistical significance for live birth analysis.

Comparisons were then made between days 5 and 6 for combined stages 4 and 5 (to gain statistical power) as well as between stages 3 and combined stages 4 and 5, respectively. Adjustment, for maternal age at retrieval and for quality of warmed embryos, was performed and significant differences between days 5 and 6 were maintained when comparing stages 4 and 5 combined on days 5 and 6 (Table 4).

Discussion

Our study addressed the clinical implications of vitrification on day 5, as compared to delayed day 6 embryos. Results demonstrate vitrification on day 5 to be an independent predictor of clinical pregnancy and live birth, an observation that remains true after adjusting for confounding factors. Stratification of our cohort was carried out according to the developmental stage, and a significant difference persisted.

In fresh cycles, superior clinical results for day 5 versus day 6 transfers have been demonstrated [16]. Delayed embryo growth, as well as a displaced window of implantation, might explain these observations. A recent study, where elective day 5 and day 6 transfers were compared, therefore eliminating the contribution of delayed embryo growth, confirmed significantly increased implantation odds for day 5 (OR 0.34;95% CI 0.22–0.52) [17]. For cryo cycles, a meta-analysis was performed in 2010 by Sunkara et al. [9], including 15 studies, four of which used the vitrification technique. Significantly higher clinical pregnancy rates and ongoing pregnancy rates were observed for day 5 versus day 6 transfers. When the stage of embryo development was taken into account, this advantage disappeared (four studies). The authors concluded that there is clearly a lack of well-designed studies before conclusions can be made. Since then, several additional studies on the subject have been published. A large study, including 764 embryo transfers, was carried out by Kovalevsky and colleagues [18]. Implantation, clinical and ongoing clinical pregnancy rates were significantly increased for day 5 versus day 6 transfers. However, this study included slow-freezing and vitrification methods of cryopreservation and encompassed a 12-year study period. Additionally, no comparisons were made between embryo quality and stages of development for the different days of cryopreservation. Hashimoto and colleagues [11] reached the same conclusions in an equally large study for vitrified-warmed single blastocyst transfers of equivalent quality between days 5 and 6. However, no adjustment for confounding factors was performed. In a recent study including 1629 vitrified-warmed blastocysts, the authors observed a significant drop in implantation and clinical pregnancy rates when poor-quality blastocysts were compared between days 5 and 6 [19]. This was not the case when high-quality blastocysts of grades 3BB or above were analysed. A multivariate regression analysis was performed by Haas and coworkers [10] and showed significantly lower clinical pregnancy rates for the day 6 vitrified group compared to that of the day 5 group (OR 0.54, 95% CI 0.38–0.76). One should keep in mind, however, that selection of embryos to be transferred and grading post-warming were not performed at the same level for both groups. Indeed, although in both cases, transfers occurred on day 6 of progesterone, day 5 embryos were transferred 20–24 h post-warming, whereas day 6 embryos after 2–4 h of development. A last study to demonstrate an advantage of day 5 versus day 6 transfers for cryo cycles was performed by Desai et al. [12]. Their multivariate regression analysis equally demonstrated clinical pregnancy and live birth rates to be three times higher after the transfer of a day 5 vitrified-warmed blastocyst compared with those vitrified on day 6 (CPR OR 3.08, 95% CI

Table 1 Univariate analysis of the variables of the fresh and cryopreservation cycles in relation to the day (5 versus 6) of blastocyst vitrification

	Day 5 ($N = 178$)		Day 6 ($N = 149$)		p value
Maternal age at retrieval					
Mean ± std	32.3 ± 4.8		34.0 ± 4.8		0.005
Median (min–max)	32.2 (20.2 to 42.6)		34.7 (20.2 to 44.3)		
Maternal BMI					
Mean ± std	24.2 ± 4.1		23.2 ± 3.9		0.03
Median (min–max)	23.0 (16.6 to 36.8)		22.5 (16.7 to 33.5)		
Endometrial thickness					
Mean ± std	9.4 ± 1.8		9.6 ± 2.1		0.24
Median (min–max)	9.3 (5.0 to 15.5)		9.3 (5.8 to 18)		
Rank of trial					
Mean ± std	1.6 ± 0.9		1.8 ± 1.4		0.02
Median (min–max)	1 (1 to 6)		2 (1 to 11)		
Aetiology of infertility					
Male infertility	78	44%	47	32%	0.03
Female infertility	39	22%	24	16%	
Mixed infertility	50	28%	65	44%	
Unknown infertility	11	6%	13	9%	
Duration of infertility					
Primary	115	65%	79	53%	0.046
Secondary	63	35%	69	47%	
Fertilisation method					
IVF	34	19%	33	22%	0.34
ICSI	144	81%	116	78%	
Type of fresh cycle					
Antagonist	146	82%	106	71%	0.03
Agonist	32	18%	43	29%	
Day 3 basal FSH					
Mean ± std	6.7 ± 3.5		7.1 ± 3.3		0.46
Median (min–max)	6.5 (1 to 25.7)		6.9 (1 to 20)		
Total FSH administered					
Mean ± std	1461 ± 872		1714 ± 942		0.02
Median (min–max)	1363 (150 to 4500)		1800 (100 to 4800)		
Culture media					
Global	53	30%	36	24%	0.009
G1/2	87	49%	94	63%	< 0.001 (G1/2 vs. rest)
CLM/BLM	27	15%	11	7%	
Other	11	6%	8	5%	
N oocytes obtained at retrieval					
Mean ± std	11.0 ± 4.6		9.8 ± 4.5		0.049
Median (min–max)	10 (2 to 26)		9 (2 to 28)		
N day 3 embryos obtained					
Mean ± std	7.6 ± 3.6		6.5 ± 3.0		0.001
Median (min–max)	7 (2 to 18)		6 (2 to 16)		
N embryos vitrified on day 5/6					
Mean ± std	3.2 ± 2.0		2.1 ± 1.5		< 0.001
Median (min–max)	3 (1 to 14)		1 (1 to 8)		
N excellent day 3 embryos					
Mean ± std	2.1 ± 2.2		1.7 ± 1.5		0.02
Median (min–max)	2 (0 to 13)		1 (0 to 7)		

Table 1 (continued)

	Day 5 ($N = 178$)		Day 6 ($N = 149$)		p value
Quality of warmed embryos					
Top	132	74%	82	55%	0.001
Intermediate	40	22%	56	38%	
Poor	6	3%	11	7%	
Stage of vitrified embryos					
Stage 3	85	48%	15	10%	< 0.001
Stage 4	45	25%	53	36%	
Stage 5	47	27%	81	54%	
Re-expansion at 60 min					
No re-expansion	79	44%	91	61%	0.001
Partial re-expansion	42	24%	35	23%	
Total re-expansion	57	32%	23	15%	

Total FSH administered represents the total dose of gonadotropins administered. Day 3 basal FSH is expressed in UI/L. Culture media: Global (LifeGlobal), G1/G2 (Vitrolife), CLM/BLM (Cook). Excellent day 3 embryos were defined as having 7–10 stage-specific cells with < 20% fragmentation. The quality of the warmed embryos (top, intermediate or poor) was defined as follows: (top: aA, aB, bA; intermediate: bB; poor: bC, cB, aC, cA). Re-expansion at 60 min represents the cavitation status of the blastocyst at 60 min after warming

1.88–5.12 and LBR OR 2.93, 95% CI 1.79–4.85). Several recent publications contradict these studies. No differences with respect to clinical pregnancy rate, ongoing pregnancy rate, live birth rate, miscarriage rate or rate of multiple gestations between day 5 and day 6 vitrified and frozen embryos in a study that examined exclusively single embryo transfers were observed [20]. Similar to our study, El-Toukhy et al. [21] observed that patients receiving a day 5 cryo embryo transfer were of a better prognostic than those receiving a day 6 transfer. Indeed, numbers of retrieved oocytes, fertilised oocytes and cryopreserved blastocysts were significantly increased for the day 5 population. Interestingly, they observed that high-grade blastocysts frozen on days 5 and 6 had equivalent pregnancy potential, which is not in line with our findings. Indeed, we equally observed that day 5 vitrification is associated to a group of patients with a more favourable prognosis; however, this group had significantly increased clinical outcomes even after adjustment for confounding factors. In the cycles analysed in our study, a third involved embryos exclusively cryopreserved on day 5, a third of the embryos was exclusively cryopreserved on day 6 and a third of the embryos was cryopreserved on both days. Among the cycles were a choice between warming a day 5 and a day 6 embryo took place (24% of the cycles), priority was given to a day 5 embryo (77%) (data not shown). For cycles involving a warmed day 6 embryo, 75% exclusively had embryos cryopreserved on day 6. Survival rates were similar for days 5 and 6 embryos, though a higher number of day 5 embryos showed re-expansion an hour after warming.

The question of higher aneuploidy rates and genetic abnormalities among blastocysts with delayed development has been evoked. Hashimoto et al. [11] demonstrated lower implantation rates for day 6 vitrified embryos, as well as an increased incidence of abnormal spindles. A study by Kroener et al. [22] showed that although aneuploidy is associated with embryos that did not achieve blastulation, there was no higher incidence among embryos that attained the

Table 2 Univariate analysis for clinical outcomes in relation to the day of vitrification

	Day 5 ($N = 178$)		Day 6 ($N = 149$)		p value
Not pregnant	81	46%	101	68%	< 0.001
Biochemical pregnancy	21	12%	13	9%	0.44
Clinical pregnancy	76	43%	35	23%	< 0.001
Extra-uterine pregnancy	1	< 1%	1	< 1%	
Miscarriage	14	8%	9	6%	0.42
Live birth	61	34%	24	16%	< 0.001

Table 3 Clinical pregnancies and live births in relation to the day of vitrification stratified for the stage of the blastocyst at the time of vitrification

	Day 5 ($N = 178$)		Day 6 ($N = 149$)		p value
Clinical pregnancy					
All patients	43%	(76/178)	23%	(35/149)	< 0.001
Stage 3 ($N = 100$)	36%	(31/85)	13%	(2/15)	0.16
Stage 4 ($N = 98$)	49%	(22/45)	28%	(15/53)	0.048
Stage 5 ($N = 128$)	47%	(22/47)	22%	(18/81)	0.004
Live birth					
All patients	34%	(61/178)	16%	(24/149)	< 0.001
Stage 3 ($N = 100$)	26%	(22/85)	13%	(2/15)	0.28
Stage 4 ($N = 98$)	42%	(19/45)	15%	(8/53)	0.007
Stage 5 ($N = 128$)	38%	(18/47)	17%	(14/81)	0.0009

Table 4 Comparisons of clinical outcomes in relation to the stage and the day of vitrification

Day 5		Day 6		
Clinical pregnancy				*p* value
Stage 4 + stage 5		Stage 4 + stage 5		
44/92	48%	33/134	25%	0.003
Stage 3		Stage 4 + stage 5		
31/85	36%	33/134	25%	0.12
Live birth				
Stage 4 + stage 5		Stage 4 + stage 5		
37/92	40%	22/134	16%	< 0.001
Stage 3		Stage 4 + stage 5		
22/85	26%	22/134	16%	0.07

Blastocysts of stages 4 and 5 were combined in order to gain statistical power

blastocyst stage by day 6 post-retrieval. Several additional studies came to the same conclusion, with euploidy rates found to be similar between day 5 and day 6 groups [19, 23, 24].

To date, the literature remains contradictory. The debate may be attributed in a certain extent to the variation in laboratory protocols, endometrial preparation, culture strategies, vitrification and warming policies and exclusions, as well as differences in study design. Indeed, few studies took into account confounding factors of the fresh cycle. In most studies cited above, it was confirmed that transfers occurred on the same day for day 5 and day 6 blastocysts, thus eliminating the possibility of reduced implantation due to an inadequate window of implantation. In terms of embryo survival and re-expansion rates, not all studies provided comparisons between the two groups.

Our study is hampered by several limitations linked to its retrospective design. On the other hand, single embryo transfers were analysed and confounding factors from both cryo and fresh cycles were taken into account. We demonstrate that day 5 blastocyst vitrification is an independent predictor of pregnancy, regardless of the stage of development. However, we do note that day 6 blastocyst transfers have resulted in clinical pregnancies and remain therefore a viable option. These results are in concordance with several recent studies [10–12] and confirm that transfer of a day 5 embryo compared to that of a day 6 embryo of the same developmental stage could reduce the time to pregnancy and, in turn, reduce patient dropout. The contradictory literature underlines the importance of carrying out such studies in one's own laboratory. It would appear that in the field of blastocyst culture, there remain many unknown variables that influence outcomes, whose clinical implications are as of yet, not fully understood. What produces favourable outcomes in one laboratory may not be suited to the workings of another. Each laboratory must investigate their protocols in order to understand what works best for them.

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Compliance with ethical standards All our protocols have been approved by the local Ethics Committee and all our patients have given their informed, written consent prior to treatment. The current study was approved by the Ethics Committee of the CHU St-Pierre (AK/16-11-139/4734).

Conflict of interest The authors declare that they have no conflict of interest.

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