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ASSISTED REPRODUCTION TECHNOLOGIES

Clinical pregnancy rate following frozen embryo transfer is higher with blastocysts vitrified on day 5 than on day 6

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Abstract
Purpose The aim of this study was to compare the pregnancy rates between good quality blastocysts vitrified on day 6 versus blastocysts vitrified on day 5 after fertilization.
Methods This is a retrospective cohort study of 791 freeze-thaw cycles of blastocysts vitrified either on day 5 or on day 6 and transferred between January 2012 and October 2015. Five hundred and thirty-seven cycles included blastocysts vitrified on day 5, and 254 cycles included blastocysts vitrified on day 6.
Results The age of the patients and the proportion of embryos that survived the thawing process were comparable between the two groups. More good quality embryos were transferred in the group in which blastocysts were vitrified on day 6 (1.2 vs. 1.3, $p = 0.005$), but the clinical pregnancy rate (44 vs. 33 %, $p = 0.002$) and the ongoing pregnancy rate (41 vs. 28 %, $p < 0.001$) were higher in the group in which blastocysts were vitrified on day 5. Multivariate regression analysis adjusting for patient's age, number of good quality embryos transferred (≥3BB), and treatment protocol demonstrated that the day 6 vitrified group had a significantly lower clinical pregnancy rate compared to the day 5 vitrified group (OR 0.54, 95 % CI 0.38–0.76).
Conclusions The clinical pregnancy rate following frozen embryo transfer is significantly lower with blastocysts vitrified on day 6 compared to blastocysts vitrified on day 5.

Keywords Vitrification · Blastocyst day 6 · Frozen embryo transfer

Capsule The clinical pregnancy rate following frozen embryo transfer is significantly lower with blastocysts vitrified on day 6 compared to blastocysts vitrified on day 5.

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Introduction

Recently, several studies that compared fresh and frozen-thawed embryo transfer (FET) cycles in normal responders demonstrated a significantly higher clinical pregnancy rate per transfer in the FET cycles versus the fresh cycles [1–4]. This improvement in pregnancy rate was thought to be due to impaired endometrial receptivity in the fresh cycles as a result of stimulation [1–4], and therefore, there has been a trend toward FET cycles in our clinic during the last 3 years.

There is also a recent trend toward blastocyst culture and single embryo transfer (ET) in an attempt to reduce the risk of multiple pregnancy [5, 6]. However, there are many factors that may have an impact on the pregnancy rate that need to be considered before deciding how many embryos to transfer. These factors include the age of the patient, blastocyst quality, and the number of failed IVF cycles in the past [7–10]. Whether the day of the blastocyst formation and vitrification has an influence on the pregnancy rate is still not clear.

There are a few studies showing a lower pregnancy rate after transferring fresh slower developing blastocysts on day 6 [11], but whether a vitrified good quality day 6 embryo has a decreased pregnancy rate compared to a vitrified blastocyst on day 5 is important to determine.

The aim of this study was to compare the pregnancy rates between good quality blastocysts vitrified on day 6 versus blastocysts vitrified on day 5 after fertilization.

Materials and methods

This was a single-center, retrospective cohort study of 791 freeze-thaw cycles of blastocysts vitrified either on day 5 or on day 6 and transferred between January 2012 and October 2015. The study was approved by the Research Ethics Board at Mount Sinai Hospital in Toronto. Embryos that developed to blastocysts were transferred or vitrified on day 5, and non-expanded embryos (morula or cavitating morula (CAVM)) were cultured until day 6. On day 6, only fully expanded blastocysts were transferred or vitrified and the rest were discarded. All the embryos were cultured under the same condition—continuous media despite the day of vitrification.

Five hundred and thirty-seven cycles included blastocysts vitrified on day 5, and 254 cycles included blastocysts vitrified on day 6. All the embryos were thawed and transferred on day 6 of progesterone in hormonally prepared cycles.

Patients started on days 2–3 of the cycle with an oral administration of 2 mg of estradiol (Estrace, Shire, Canada) twice daily for endometrial preparation, which was increased by a step-up protocol to 8 mg/day. An ultrasound endometrial assessment performed about 10 days later assessed the lining as ready for the ET procedure when the endometrial thickness was ≥7 mm. If not adequate, endometrial estrogen priming continued and ultrasound assessment was undertaken to confirm further endometrial thickening. Participants commenced luteal support via vaginal administration of progesterone suppositories 200 mg three times daily according to the proposed day of embryo thawing and transfer. Embryos vitrified on day 5 were thawed on day 5 of progesterone and transferred after 20–24 h. Embryos vitrified on day 6 were thawed on day 6 of progesterone and transferred after 2–4 h. In both groups, the embryos were transferred on day 6 of progesterone.

Embryos vitrified on day 5 or 6 after PGS were excluded from the study as were cycles with combined transferred embryos from days 5 to 6.

A good quality embryo was defined as an embryo ≥3BB according to the grading scale proposed by Gardner [12] and expanded after warming.

The vitrification method used was the Irvine Scientific Freeze Kit (Cat. no. 90133-SO; Irvine Scientific, Santa Ana, CA, USA) with HSV straws.

The outcomes of the cycles with blastocysts vitrified on day 5 were compared with cycles with blastocysts vitrified on day 6. Clinical pregnancy was defined as visualization of a gestational sac, while ongoing pregnancy necessitated the visualization of fetal cardiac activity on transvaginal ultrasound.

Comparison of continuous variables between the two groups was conducted using Student's *t* test and Mann-Whitney test. Chi-square test was used for comparison of categorical variables. Logistic regression analysis was employed for multivariate analysis. Variables used in the regression model included vitrification day, maternal age at the time of oocyte retrieval, number of transferred completely hatched embryos, and number of top quality embryos transferred. Significance was accepted at $p < 0.05$. Statistical analyses were conducted using the IBM Statistical Package for the Social Sciences (IBM SPSS v. 20; IBM Corporation, Inc., Armonk, NY, USA).

Results

The age of the patients (34.9 vs. 35.3, $p = 0.2$) and the proportion of embryos that survived the warming process (96.4 vs. 95.7, $p = 0.6$) were comparable between the two groups.

More embryos were transferred in the day 5 group per cycle (1.53 vs. 1.39, $p = 0.001$), but more good quality embryos were transferred in the group in which blastocysts were vitrified on day 6 (1.2 vs. 1.3, $p = 0.005$). In the day 6 group, there were significantly more cycles with transfers of vitrified top quality embryos (85 vs. 95 %, $p < 0.001$) and a higher proportion of the vitrified embryos were of good quality in the day 6 group (76 vs. 95 %, $p < 0.001$) (Table 1).

There were no differences between the two groups in the number of completely hatched embryos transferred (1.05 vs. 1.00, $p = 0.8$).

The clinical pregnancy rate (45 vs. 33 %, $p = 0.002$) and the ongoing pregnancy rate (41 vs. 28 %, $p < 0.001$) were higher in the group in which blastocysts were vitrified on day 5 (Table 1). Multivariate regression analysis adjusting for patient's age, number of completely hatched embryos transferred, and number of good top quality embryos transferred (≥3BB) demonstrated that the day 6 vitrified group had a significantly lower clinical pregnancy rate compared to the day 5 vitrified group (OR 0.54, 95 % CI 0.38–0.76) and that age and number of top quality embryos transferred had a significant impact on the pregnancy rate (Table 2).

When comparing the vitrified day 5 blastocysts with only good quality embryos vitrified on day 6 (Table 3), we found that the age of the patients (34.7 vs. 35.2, $p = 0.1$) and the proportion of embryos that survived the warming process (96.3 vs. 95.3) were comparable between the two groups. The clinical pregnancy rate (50 vs. 34 %, $p = <0.001$) and the ongoing pregnancy rate (47 vs. 29 %, $p < 0.001$) were still higher in the group in which good quality blastocysts were vitrified on day 5 compared to the good quality embryos vitrified on day 6. Next, we analyzed only cycles with single embryo transfer (203 vs. 157 cycles). We included all the good quality blastocysts vitrified on day 5 and the good quality embryos vitrified on day 6 (Table 4). The clinical pregnancy rate (42 vs. 22 %, $p = 0.04$) and the ongoing pregnancy rate (40 vs. 19 %, $p < 0.001$) were significantly higher in the day 5 group.

Table 1 Comparison of frozen cycles between blastocysts vitrified on day 5 and blastocysts vitrified on day 6

	Blastocysts vitrified on day 5	Blastocysts vitrified on day 6	*p*
Cycles (*n*)	537	254	–
Age at retrieval (years)	34.9 ± 4	35.3 ± 5	0.2
Age at transfer (years)	35.7 ± 4	36.3 ± 4	0.05
Number of thawed embryos	852	369	–
Survival rate of the embryos	821/852 (96.4)	353/369 (95.7)	0.6
Number of embryos transferred per cycle (mean)	1.53 ± 0.5	1.39 ± 0.6	0.001
Cycles with GQE transfer/all cycles	459/537 (85.4.0)	242/254 (95.2)	<0.001
GQE embryos/all embryos	630/821 (76.7)	334/353 (94.6)	<0.001
Number of GQE transferred per cycle (mean)	1.17 ± 0.70	1.31 ± 0.64	0.005
Clinical pregnancy rate	240/537 (44.7)	84/254 (33)	0.002
Ongoing pregnancy	221/537 (41.1)	72/254 (28.3)	<0.001
Implantation rate	252/821 (30)	86/353 (24.3)	0.02

GQE good quality embryo

Discussion

Previous studies have showed decreased pregnancy rates when transferring blastocysts on day 6 compared to blastocysts on day 5 in fresh cycles. Barrenetxea et al. compared the pregnancy rate according to the day of transfer in fresh transfer cycles. They found significantly increased pregnancy rate when transferring embryos on day 5 after retrieval compared to blastocysts on day 6, and the pregnancy rate was extremely low in the transfer day 6 group (11 %) [11]. Elgindy and Elsedeek [13] aimed to study the outcome of blastocysts showing expansion on day 5 and transferred on day 5 or 6 in comparison with those unexpanded and transferred on day 6. They found similar pregnancy rate of expanded blastocysts transferred on day 5 or 6, but significantly lower pregnancy rate in the later expanded blastocyst group transferred on day 6. Hashimoto et al. [14] also demonstrated a lower pregnancy rate of slow-growing embryos compared to normally developing embryos. He also showed that the incidence of abnormal spindles in the growth-retarded embryos was significantly higher than that in the normally developing embryos.

Table 2 Regression analysis of all the frozen cycles including blastocyst vitrified on day 5 or 6

	p	OR	95 % CI for OR	
			Lower	Upper
Day of vitrification	0.01	0.539	0.38	0.76
Number of good quality embryos transferred	<0.001	2.35	1.71	3.25
Number of completely hatched embryos transferred	0.24	1.16	0.90	1.49
Age at retrieval	0.03	0.95	0.91	0.98

However, studies involving vitrified-thawed blastocyst transfers have reported conflicting results regarding whether the rate of blastocyst formation prior to cryopreservation affects treatment outcome [15–19]. A meta-analysis concluded that there is a significant increase in the clinical pregnancy rate with day 5 vitrified-thawed blastocyst transfers compared with day 6 vitrified-thawed blastocyst transfers. However, analysis of those studies where the day 5 and day 6 blastocysts had the same morphological quality at the time of freezing showed no difference in clinical pregnancy and ongoing pregnancy rates [20].

Whether slower-growing blastocysts have a higher rate of aneuploidy is still debatable.

Kroener et al. showed that delayed blastulation is not associated with increased aneuploidy rates, but the absence of blastulation is associated with increased aneuploidy [21]. Similarly, Capalbo et al. demonstrated that faster-growing embryos (day 5 blastocysts) showed a similar euploidy rate

Table 3 Comparison between only good quality embryos vitrified on day 5 versus day 6

	Blastocysts vitrified on day 5	Blastocyst vitrified on day 6	*p*
Cycles (*n*)	442	241	–
Age (years)	34.7 ± 4	35.2 ± 5	0.12
Number of thawed embryos	713	347	–
Survival rate of the embryos	687/713 (96.3)	331/347 (95.3)	0.5
Number of embryos transferred per cycle (mean)	1.55 ± 0.5	1.37 ± 0.5	<0.001
Clinical pregnancy rate	223/442 (50.4)	83/241 (34.4)	<0.001
Ongoing pregnancy	208/442 (47)	71/241 (29.3)	<0.001

Table 4 Comparison between single embryo transfers of good quality blastocysts vitrified on day 5 and good quality blastocysts vitrified on day 6

	Vitrified on day 5	GQE vitrified on day 6	*p*
Clinical pregnancies	85/203 (41.9)	35/157 (22.2)	<0.001
Ongoing pregnancies	81/203 (39.9)	30/157 (19.1)	<0.001

compared with slower-growing ones (day 6 blastocysts) [22].

In contrast, Taylor et al. [23] examined the euploidy rates and outcomes between day 5 and day 6 blastocysts and showed that day 5 blastocysts had a higher chance of being euploid than day 6 blastocysts. He also showed that when only euploid day 5 or euploid day 6 blastocysts were transferred during a cryopreserved embryo transfer, the cycle outcomes were similar.

In this study, we demonstrated that the pregnancy rate is significantly lower during FET cycles with day 6 vitrified blastocysts, even if they were morphologically graded as good quality embryos compared to blastocysts vitrified on day 5. We demonstrated that the blastocysts vitrified on day 6 were of higher quality compared to the blastocyst vitrified on day 5 but still resulted with a significantly lower pregnancy rate.

This study is the first to evaluate the pregnancy outcome after transfer of vitrified slow-growing good quality embryos. The embryos in both groups were transferred on day 6 of progesterone due to our method of thawing the vitrified day 5 blastocysts on day 5 of progesterone and transferring them on day 6 of progesterone, and therefore, the different pregnancy rates cannot be explained by the different transfer days. The comparable survival rates (96.4 vs. 95.7, $p = 0.6$) is an important indicator on validating the homogeneity between the two groups.

Our findings of lower clinical pregnancy rate (22 %) and lower ongoing pregnancy rate (19 %) with the single embryo transfer of a good quality embryo, vitrified day 6 blastocyst compared to day 5 blastocysts (41 %) can have an important influence on our decision regarding the number of embryos to transfer, especially in older patients.

There are a few limitations in our study. There are many factors that can influence the pregnancy rate such as the physician or the embryologist performing the transfer, difficulty in inserting the transfer catheter, endometrial thickness and pattern, and subendometrial contractions to name a few. Those factors were not controlled for in the study. Moreover, the embryos were transferred on day 6 of progesterone in both groups, but the vitrified day 5 blastocysts were thawed on day 5 of progesterone and transferred after 20–24 h and the embryos vitrified on day 6 were thawed on day 6 of progesterone and transferred after 2–4 h, and due to the study being a retrospective study, we could not correct this possible confounder.

In conclusion, even when the day 6 vitrified blastocyst morphology is at least as good as that of blastocysts vitrified on day 5, the clinical pregnancy rate following frozen embryo transfer is significantly lower with blastocysts vitrified on day 6 compared to blastocysts vitrified on day 5.

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