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Impact of the outcome of fresh blastocyst transfer on the subsequent frozen-thawed blastocyst transfer cycle

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KEY MESSAGE

This large observational study showed that achieving a positive pregnancy test after fresh blastocyst transfer is an independent factor influencing the outcome of the subsequent sibling frozen-thawed blastocyst transfer, leading to significantly higher clinical pregnancy and live birth rates compared with frozen-thawed blastocyst transfer cycles following unsuccessful fresh blastocyst transfer.

A B S T R A C T

The objective of this observational study was to assess the influence of the outcome of fresh blastocyst transfer on the success rate of the subsequent sibling frozen-thawed blastocyst transfer (FBT) cycle. In total, 1639 FBT cycles were divided into two groups: Group A (*n* = 698) cycles in which a positive pregnancy test result was achieved and Group B (*n* = 941) cycles in which no pregnancy was achieved in the preceding fresh IVF cycle. Mean age at cryopreservation, basal FSH level, number of oocytes retrieved, number of embryos transferred in the fresh cycle and survival rate of the thawed blastocysts in the FBT cycle were comparable between the two groups. Although significantly more thawed blastocysts were transferred in the FBT cycles in Group B compared with Group A, the live birth rate in Group A was significantly higher compared with Group B. After adjusting for potentially confounding variables, the likelihood of a live birth after FBT was significantly higher when a pregnancy was achieved in the preceding fresh IVF cycle. Achieving a pregnancy after fresh blastocyst transfer is an independent factor influencing the outcome of the subsequent sibling FBT.

Introduction

Embryo cryopreservation is an important contributor to the success of assisted reproductive techniques. The trend to limit the number of embryos transferred per cycle in order to reduce the incidence of multiple pregnancy associated with IVF treatment has increased the availability of surplus embryos for cryopreservation. Comparable implantation and pregnancy rates have been reported after frozen-thawed embryo transfers compared with fresh IVF cycles

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(Aflatoonian et al., 2010; Chen et al., 2016; Edgar et al., 2005; Shapiro et al., 2008; Zhu et al., 2011). Data from a recent systematic review (Maheshwari et al., 2012) have suggested that obstetric and perinatal outcomes could be improved after frozen-thawed embryo transfer compared with those after fresh IVF transfer. Multiple factors could influence the outcome of frozen-thawed embryo transfer cycles (El-Toukhy et al., 2011; Salumets et al., 2006; Veleva et al., 2013). Amongst these, the developmental stage of embryos at cryopreservation has been shown to be an independent factor affecting the clinical outcome of frozen-thawed embryo transfers (Mesut et al., 2011; Noyes et al., 2009). Although previous studies carried out using cleavage-stage embryos (Ashrafi et al., 2011; El-Toukhy et al., 2003a, 2003b; Lin et al., 1995; Wang et al., 2001) have suggested that the outcome of the fresh embryo transfer cycle could predict that of the subsequent sibling frozen-thawed embryo transfer cycle, the more recent studies in which frozen-thawed blastocysts had been utilized did not confirm this relationship (Berin et al., 2011; Doherty et al., 2014).

Due to the inconsistency in the results of published literature, the aim of our study was to examine the influence of the outcome of the fresh blastocyst transfer cycle on that of the subsequent frozen-thawed blastocyst transfer (FBT) cycle using a sibling embryo cohort, after accounting for important confounding variables.

Materials and methods

A consecutive series of 1639 FBT cycles performed at Guy's and St Thomas' Hospital Assisted Conception Unit between January 2006 and January 2014 were studied. These cycles were carried out for patients who had previously undergone a fresh IVF, with or without intracytoplasmic sperm injection (ICSI) cycle and blastocyst transfer together with freezing of surplus blastocysts on day 5 or 6 after fertilization. Embryo transfer, freezing and thawing methodology was consistent during the study period with no significant laboratory or clinical protocol changes.

FBT cycles in which the embryos were created from donated oocytes or for the purpose of fertility preservation before cancer therapy, cryopreserved at the pronuclear stage or biopsied for preimplantation genetic diagnosis, were excluded. In addition, frozen cycles that took place in a natural cycle without artificial endometrial preparation were also excluded. Only the first FBT cycle performed after embryo freezing was included.Because the present work did not involve either therapeutic interventions or change to our routine IVF protocols, we did not require additional approval from our institutional ethics committee. However, each couple gave written informed consent upon entering our IVF programme and before starting an FBT cycle.

Fresh IVF cycle and blastocyst cryopreservation

Our protocol for ovarian stimulation, IVF/ICSI and embryo culture has been described in detail elsewhere (El-Toukhy et al., 2002; Khalaf et al., 2008). Extended embryo culture to day 5 of *in vitro* culture and blastocyst transfer was performed in the fresh cycle. The quality of the fresh blastocysts transferred was scored using the Gardner and Schoolcraft blastocyst grading system (Gardner and Schoolcraft, 1999).

Blastocysts were selected for cryopreservation if they were of grade 3CC or better without signs of degeneration on day 5 or day 6 of *in vitro* culture. A standard slow freezing protocol, employing 1,2-propanediol and sucrose as cryoprotectants, was used throughout the study period (El-Toukhy et al., 2004; Kaufman et al., 1995; Lassalle et al., 1985). Women undergoing fresh blastocyst transfer received micronized progesterone pessaries (Cyclogest; Shire Pharmaceuticals Ltd, Hants, UK) 400 mg twice a day, from the day of oocyte retrieval up to 8 weeks of gestation if pregnancy had occurred.

FBT cycles and endometrial hormonal preparation

Oestradiol valerate 6 mg daily (Climaval; Novartis Pharmaceuticals, Surrey, UK, or Progynova; Bayer plc, Newbury, Berkshire, UK) was commenced orally on day 1 or 2 of menstruation after pituitary suppression, and continued for 13–15 days, after which endometrial thickness was evaluated. If endometrial thickness was <7 mm, the dose of oestradiol valerate was increased to 8 mg daily for a further 7–12 days. If endometrial thickness had failed to reach 7 mm after this period, the cycle was usually cancelled. Progesterone supplementation in the form of micronized progesterone pessaries (Cyclogest) 400 mg twice daily was commenced 5 days before the day of transfer.

Embryos were thawed rapidly by removal from liquid nitrogen and exposure to air for 45 s followed by immersion in a water bath at 30°C for 30 s. Propanediol was then removed by a three-step process in the presence of 0.2 M sucrose at room temperature for 5 min per each step until final rehydration in a HEPES-buffered salt solution. Thawed blastocysts were then assessed for cell survival using an inverted microscope (Nikon UK, Kingston, Surrey, UK) at a magnification of ×200 before being transferred into culture medium at 37°C. Blastocyst re-expansion was assessed 1–2 h post-thawing. Blastocysts were considered not suitable for transfer if over 50% cell degeneration and no re-expansion was observed post-thaw.

Between one and three thawed blastocysts were transferred to the uterus using an Edwards-Wallace (Sims Portex, Hythe, Kent UK) or Cook embryo transfer catheter (Cook Medical, Limerick, Ireland). After embryo transfer, hormonal supplementation was continued for 11 days until a urine pregnancy test was performed using commercially available kits. Patients who had a positive test continued with hormonal supplementation until they were 12 weeks pregnant.

Cycle outcome

Pregnancy was diagnosed by a positive urine test for human chorionic gonadotrophin (HCG) 11 days after FBT. A clinical pregnancy was defined as the observation of a gestational sac with fetal cardiac pulsations on ultrasound scanning 3–4 weeks after the positive pregnancy test. Implantation rate was defined as the number of gestational sacs observed on ultrasound scanning compared with the number of blastocysts transferred. All pregnancies were followed until delivery.

Data collection and statistical analysis

Data were collected prospectively for patient demographics and fresh IVF/ICSI and FBT cycle characteristics and outcomes. Univariate analysis of the study outcome measures and associated clinical variables was performed using a two-sample t-test, chi-squared test or Fisher's exact test, as appropriate.

A step-wise multiple logistical regression analysis was used to assess the FBT cycle outcome using important confounding variables including patient age, infertility cause, history of previous pregnancy, basal FSH level, outcome of the fresh IVF/ICSI cycle,

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number of surplus blastocysts frozen in the fresh IVF/ICSI cycle, number of blastocysts thawed and survived in the FBT cycle, blastocyst re-expansion and number of thawed blastocysts transferred in the FBT cycle (Veleva et al., 2013). Odds ratios (OR) and their 95% confidence intervals (CI) were calculated using the exact method. The Statview software package for Macintosh (Statview 4.1, Abacus Concepts Ltd., Berkeley, CA, USA) was used for statistical analysis. A *P*-value of <0.05 was considered statistically significant.

Results

During the study period, all FBT cycles that satisfied the study inclusion criteria were included. In total, 1639 FBT cycles were included in our study and analysed. In these cycles, 2778 frozen blastocysts were thawed (mean ± SD of 1.7 ± 0.7 blastocysts per thaw), 2432 blastocysts (88%) survived the process of thawing and 2371 blastocysts (85%) were transferred (mean of 1.5 ± 0.5 blastocysts/transfer).

In 11 cycles (0.7%), no blastocysts survived thawing. In the remaining 1628 FBT cycles, the transfer involved either single (*n* = 1057, 65%) or double (*n* = 570, 35%) frozen-thawed blastocysts, except one FBT cycle in which three frozen-thawed blastocysts were transferred. The treatment resulted in 775 pregnancies, 556 clinical pregnancies and 484 deliveries, giving an overall pregnancy, clinical pregnancy and delivery rate per FBT cycle of 47%, 34% and 30%, respectively. The implantation rate in the study was 32% (750/2371). The multiple birth rate was 14%. All multiple deliveries were twins.

The FBT cycles were subdivided according to whether a positive pregnancy test result was achieved in the related fresh cycle. Group A (*n* = 698) were cycles in which the preceding fresh IVF/ICSI cycle achieved a positive pregnancy test result and Group B (*n* = 941) were cycles in which no pregnancy had occurred in the preceding fresh IVF/ICSI cycle.

Table 1 compares the two study groups with respect to their demographic and fresh cycle characteristics. Age at fresh transfer cycle, cause of infertility, history of previous pregnancy and basal FSH level were not significantly different between the two study groups. The mean number of oocytes retrieved and normally fertilized and number of embryos transferred and frozen in the fresh transfer cycles were also comparable between the two groups (**Table 1**).

Table 2 depicts the FBT cycle characteristics in both groups. Similar proportions of FBT cycles in each group were performed throughout the study period. Pituitary suppression was achieved in 99% of FBT cycles in both groups prior to starting oestradiol therapy. There was no significant difference in the mean duration of oestradiol therapy and endometrial thickness achieved prior to starting progesterone supplementation between the two groups. The survival rate of the thawed blastocysts and proportion of transfers in which all the frozen-thawed blastocysts had shown evidence of post-thaw re-expansion were not statistically different between the two groups. Significantly more frozen blastocysts were thawed (1.8 ± 0.7 versus 1.6 ± 0.7, *P* < 0.0001), survived (1.6 ± 0.6 versus 1.3 ± 0.5, *P* < 0.0001) and transferred (1.6 ± 0.5 versus 1.3 ± 0.5, *P* < 0.0001) in Group B compared with Group A, respectively. The percentage of FBT cycles involving double blastocyst transfer was also significantly higher in Group B compared with Group A (42% versus 25%, respectively, *P* < 0.001). However, the implantation, clinical pregnancy and live birth rates in the FBT cycle in Group A were significantly higher compared with Group B (36% versus 29%; *P* = 0.003), (38% versus 31%; *P* = 0.01) and (32% versus 27%; *P* = 0.03), respectively (**Table 2**). The live birth rate was higher in Group A compared with Group B across all patient age groups (**Table 2**).

Table 1 – Patient demographics and fresh cycle characteristics stratified according to whether (Group A) or not (Group B) a pregnancy[a] was achieved in the fresh cycle.

	Group A (*n* = 698)	Group B (*n* = 941)
Age at fresh cycle, years	33.4 ± 3.8	33.6 ± 4.0
≤35 years, %	70 (487/698)	69 (648/941)
36–39 years, %	26 (181/698)	26 (242/941)
≥40 years, %	4 (30/698)	5 (51/941)
Fresh IVF cycle order	1.4 ± 0.8	1.4 ± 0.9
Cause of infertility		
tubal, %	16 (113/698)	19 (175/941)
non-tubal, %	84 (585/698)	81 (766/941)
male	*38*	*35*
anovulation	*14*	*11*
endometriosis	*5*	*6*
unexplained	*27*	*29*
Previous pregnancy, %	24 (168/698)	28 (261/941)
Basal FSH level, IU/l	6.5 ± 2.0	6.7 ± 3.1
No. of oocytes retrieved	15.8 ± 6.8	15.6 ± 6.7
ICSI used for fertilization, %	70 (491/698)	70 (658/941)
No. of normally fertilized oocytes	10.1 ± 4.5	10.0 ± 4.5
No. of fresh blastocysts transferred	1.2 ± 0.4	1.2 ± 0.4
No. of blastocysts frozen	4.0 ± 2.5	3.8 ± 2.5
day 5 freezing, %	22 (151/698)	19 (176/941)
day 6 freezing, %	39 (272/698)	43 (407/941)
combined day 5 and 6 freezing, %	39 (275/698)	38 (358/941)

[a] At least a positive pregnancy test result. Values are provided as mean ± SD, or as otherwise stated. There were no statistically significant differences between the two groups.
ICSI = intra-cytoplasmic sperm injection.

The odds of clinical pregnancy and live birth after the FBT cycle were significantly higher in Group A compared with Group B (OR = 1.30, 95% CI 1.07–1.62, *P* = 0.01 and OR = 1.27, 95% CI 1.02–1.57, *P* = 0.03, respectively). The live birth rate after single frozen-thawed blastocyst transfer (FBT) in Group A was similar to that after transferring two frozen-thawed blastocysts in Group B (29% versus 29%, OR = 0.99, 95% CI 0.75–1.33). There was no significant difference in the multiple birth rate between the two groups (11% versus 16%).

After adjusting for important confounding variables including patient age, infertility cause (tubal versus non-tubal), basal FSH level, number of surplus blastocysts frozen in the fresh IVF/ICSI cycle, number of blastocysts thawed and survived in the FBT cycle, blastocyst re-expansion and number of blastocysts transferred in the FBT cycle using multivariable logistic regression, the odds of clinical pregnancy and live birth in the FBT cycle remained significantly associated with the outcome of the previous fresh cycle (adjusted OR for clinical pregnancy = 1.50, 95% CI 1.16–1.946; *P* = 0.002, and adjusted OR for live birth = 1.43, 95% CI 1.10–1.87, *P* = 0.008).Of the 1155 patients who did not achieve a live birth after the first FBT cycle in our study, a significantly higher proportion of patients in Group A [60% (281/472)] compared with Group B [53% (364/683), *P* = 0.04] had surplus frozen blastocysts remaining in storage for future use. Group A patients also tended to have a higher mean number of surplus frozen blastocysts remaining in storage after the first FBT cycle compared with Group B (3.2 ± 2.6 versus 2.7 ± 2.1 blastocysts, respectively), although the difference was not statistically significant.

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Table 2 – FBT cycle characteristics and outcomes stratified according to whether (Group A) or not (Group B) a pregnancy was achieved in the fresh cycle.

	Group A (*n* = 698)	Group B (*n* = 941)	*P*-value
Percentage of FBT cycles performed			
2006–2010	46	45	NS
2011–2014	54	55	
Number of cycles reaching transfer (%)	692 (99.1)	936 (99.5)	–
Percentage of FBT cycle regime:			
with pituitary down-regulation, %	99	99	NS
without pituitary down-regulation, %	1	1	
Mean duration of oestrogen supplementation, days	19.2 ± 5.3	19 ± 5.3	NS
Mean endometrial thickness, mm	9.6 ± 2.0	9.4 ± 1.8	NS
Mean no. of blastocysts thawed	1.6 ± 0.7	1.8 ± 0.7	<0.0001
Blastocyst survival rate, %	86 (936/1085)	88 (1496/1693)	NS
No. of thawed blastocysts survived	1.3 ± 0.5	1.6 ± 0.6	<0.0001
No. of thawed blastocysts transferred	1.3 ± 0.5	1.6 ± 0.5	<0.0001
single blastocyst transfer, %	75	58	<0.001
double blastocyst transfer, %	25	42	
Percentage of transfers with all blastocysts re-expanded, %	65 (452/692)	63 (590/936)	NS
Implantation rate, %	36 (332/910)	29 (418/1461)	0.003
Clinical pregnancy rate, %	38 (262/698)	31 (294/941)	0.01
Live birth rate according to age group, %	32 (226/698)	27 (258/941)	0.03
≤35 years, %	35	30	
36–39 years, %	28	24	
≥40 years, %	23	13	
Multiple delivery rate, %	11 (24/226)	16 (42/258)	NS

[a] At least a positive pregnancy test result. Values are provided as mean ± SD, or as otherwise stated. NS = not statistically significant.
FBT = frozen-thawed blastocyst transfer.

Discussion

The present study examined whether achieving a pregnancy after blastocyst transfer in a fresh IVF/ICSI cycle could influence the outcome of the subsequent FBT cycle using a sibling embryos cohort. Our results demonstrated significantly higher clinical pregnancy, live birth and implantation rates in medicated FBT cycles when at least a positive pregnancy test was achieved in the preceding fresh blastocyst transfer cycle.

To date, this study is the largest to evaluate the relationship between the outcomes of the fresh and subsequent frozen-thawed embryo transfer using high-quality blastocysts. The study accounted for potential confounding variables that could affect the outcome of a FBT cycle, including fresh and frozen cycle characteristics (Veleva et al., 2013).

Our findings are consistent with those of previous studies which demonstrated a more favourable outcome after a frozen-thawed embryo transfer when the preceding fresh cycle was successful using sibling cleavage-stage embryos as well as embryos frozen at the two pronuclear stage (Ashrafi et al., 2011; El-Toukhy et al., 2003a, 2003b; Lee et al., 2012; Lin et al., 1995; Toner et al., 1991; Urman et al., 2007; Wang et al., 2001).

The reported increase in the pregnancy rates after frozen embryo transfer when the previous fresh cycle was successful could be explained by the concept of 'cohort homogeneity'. This theory postulates that if pregnancy occurs after transfer of an embryo belonging to a particular cohort of embryos, then the chance of pregnancy with the use of sibling frozen embryos is increased owing to the presence of similar developmental potential amongst sibling embryos (Lightman et al., 1997; Trounson, 1986), particularly in those embryos that had reached the blastocyst stage.

An additional explanation for the relationship between the outcome of the fresh IVF cycle and that of the subsequent frozen embryo transfer is enhanced endometrial receptivity. It is recognized that a history of previous pregnancy and live birth, whether natural or as a result of IVF treatment, significantly improves the success of assisted reproductive techniques in subsequent cycles (Templeton and Morris, 1998; Templeton et al., 1996). This could explain the significant differences in implantation and pregnancy rates in our two study groups. In our study, only high-quality surplus blastocysts were frozen and we used blastocyst survival and re-expansion rates as markers of post-thaw blastocyst quality (Ahlström et al., 2013). The fact that those markers of embryo quality were comparable between the two groups (**Table 2**) is consistent with a relative difference in endometrial receptivity in favour of Group A as demonstrated by the pregnancy achieved in their preceding fresh IVF/ICSI cycle.

Our findings are, however, in disagreement with two previous retrospective studies (Berin et al., 2011; Doherty et al., 2014). Berin et al. (2011) reported on 243 FBT and included more than one cycle per patient, whilst Doherty et al. (2014) included 125 frozen blastocyst transfer cycles. Both studies concluded that the live birth rate was higher in frozen cycles that followed previously unsuccessful fresh IVF cycles. However, the two studies had a relatively small sample size, did not account for some of the important confounding variables related to the fresh or frozen cycle characteristics, and included frozen transfer cycles in which blastocysts had originated from both autologous and donated oocytes, potentially influencing the study results (Shapiro et al., 2008).

A strength of the present study is that analysis was limited to the first FBT cycle to avoid inclusion bias if multiple FBT cycles for the same patient were included. However, our results show that a significantly higher proportion of patients who achieved a live birth in their first FBT cycle (Group A) compared with those who did not

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(Group B) had surplus frozen blastocysts remaining in storage for future use (60% versus 54%, $P = 0.04$). This suggests that it is likely that the cumulative live birth rate in Group A would be even higher compared with Group B when all frozen blastocysts have been used, further underscoring the validity of our results and challenging the concept that only a few embryos within a cohort are competent to result in a live birth. It would also be interesting to observe if similar results could be reproduced in centres using exclusively blastocyst vitrification techniques.

Our study could have practical implications when counselling patients preparing for an FBT cycle. Two-thirds of the FBT cycles in our study involved the transfer of a single frozen-thawed blastocyst and the overall multiple birth rate was 14%. Based on our results, it would be reasonable to advocate single blastocyst transfer in cycles where a pregnancy was achieved in the fresh IVF cycle, in order to further reduce the multiple birth rate (Ferraretti et al., 2013).

Conclusion

This large study demonstrates that the outcome of the fresh IVF cycle in which elective blastocyst transfer has taken place can have a significant impact on the success rate of the subsequent medicated FBT using a sibling embryos cohort. Achieving pregnancy in the fresh cycle significantly improves the probability of pregnancy and live birth after the FBT cycle. This information should be helpful when counselling patients embarking on a frozen embryo transfer cycle.

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Uncited references

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