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

Tarek El-Toukhy [a,*], *Julia Y Kopeika* [a], *Yusuf Beebeejaun* [a], *Omar El Tokhy* [a], *Jyotsna Pundir* [b], *Yacoub Khalaf* [a]

[a] *Assisted Conception Unit, Guy's and St. Thomas' Hospital NHS Foundation Trust, London, UK*
[b] *Centre for Reproductive Medicine, St Bartholomew's Hospital, London, UK*

Tarek El-Toukhy qualified at Cairo University in 1991. He is a consultant in Reproductive Medicine and Surgery at Guy's and St Thomas' Hospital in London and a scientific editor for the British Journal of Obstetrics and Gynaecology. His special interests are embryo freezing, recurrent implantation failure, hysteroscopic surgery and PGD.

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

* Corresponding author.
E-mail address: tarek.el-toukhy@gstt.nhs.uk (T El-Toukhy).

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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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