A comparison of pregnancy outcomes between day 3 and day 5/6 embryo transfers: does day of embryo transfer really make a difference?

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Abstract

Purpose To determine if day of embryo transfer (ET) affects gestational age (GA) and/or birth weight (BW) at a single university fertility center that primarily performs day 5/6 ET. **Methods** Retrospective cohort study of 2392 singleton live births resulting from IVF/ICSI at a single large university fertility center from 2003 to 2012. Patients were stratified by day 3 or day 5/6 ET. Outcome variables included patient age, gravidity, prior miscarriages, prior assisted reproduction technology cycles, number of embryos transferred, number of single ET, infertility diagnosis, neonatal sex, GA at birth, and BW. Subanalyses were performed on subgroups of preterm infants. A comparison was made between the study data and the Society of Assisted Reproductive Technologies (SART) published data.

Results There was no difference in GA at birth (39 ± 2.1 weeks for day 3 ET, 39 ± 1.9 weeks for day 5/6 ET) or BW between ET groups (3308 ± 568 g for day 3 ET, 3268 ± 543 g for day 5/6 ET). There was also no difference in the number of preterm deliveries (8.5 % for day 3 ET vs. 10.8 % for day 5/6 ET). The day 5/6 ET study data had significantly fewer pre-term deliveries than the SART day 5/6 ET data.

Conclusion In contrast to published SART data, GA and BW were not influenced by day of ET. Data may be more uniform

at a single institution. Day 5/6 ET continues to offer improved pregnancy rates without compromising birth outcomes.

Keywords Embryo transfer · In vitro fertilization · Preterm birth · Birth weight · Blastocyst

Introduction

In assisted reproductive technology (ART) cycles, ET at the blastocyst stage (on day 5/6 of culture) has become increasingly utilized due to several advantages over cleavage stage or day 3 ET. Blastocysts have been found to have higher implantation rates over cleavage stage embryos [1], which may be related to the physiology of the endometrium and the developing embryo. Cleavage stage embryos naturally reside in the oviduct and progress to the blastocyst stage by the time they enter the uterine cavity [2]. The uterine environment, therefore, may be more suited for the blastocyst resulting in improved implantation rates [3, 4]. Higher implantation rates allow for the transfer of fewer embryos with the benefit of decreasing the number of higher order multiple gestations and increasing the success rate of single ET [1, 5, 6]. A recent Cochrane review found that blastocyst ET has a statistically significant higher live birth rate than cleavage stage ET, 32–42 vs 31 % respectively [7]. Furthermore, longer cell culture allows for improved selection of higher grade embryos and decreases the rate of aneuploidy [8–12].

Despite these advantages, a recent analysis of singleton live births from the SART database demonstrated an increased risk of preterm delivery after transfer of blastocyst-stage embryos compared to cleavage-stage embryos (18.6 vs 14.4 % between 32 and 37 weeks gestation and 2.8 vs 2.2 % for less than 32 weeks gestation) [13]. The objective of this study was to determine if the day of ET impacted the gestational age at birth or birth weight of singleton pregnancies at a single large

Capsule This retrospective cohort study of singleton live births resulting from IVF/ICSI at the New York University Fertility Center found no difference in birth outcomes between day 3 and day 5/6 embryo transfers.

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university-based fertility center that primarily performs day 5/6 ETs. We hypothesized that there would be no difference between birth weights and gestational ages at birth between day 3 and day 5/6 ETs given more uniform practices at a large fertility center with experience performing day 5/6 ETs.

Materials and methods

This retrospective cohort study reviewed all singleton live births from 2003 to 2012 resulting from in vitro fertilization (IVF) and/or intracytoplasmic sperm injection (ICSI) at the New York University Fertility Center (NYUFC). The exposure group was defined as a singleton live birth following fresh ET on day 5 or 6 after oocyte retrieval and fertilization. The comparison group was defined as a singleton live birth following fresh ET on day 3 after oocyte retrieval and fertilization. The primary outcome variables were gestational age at birth and birth weight. Secondary variables included patient age, gravidity, parity, prior miscarriages, prior ART cycles, number of embryos transferred, number of single ET, infertility diagnosis, and neonatal sex. Exclusion criteria included donor oocyte cycles, frozen oocyte cycles, cycles undergoing pre-implantation genetic diagnosis (PGD), multiple gestations, still births or pre-viable births, and singleton live births that were initially multiple gestations (vanishing twins).

Patients underwent stimulation using standard gonadotropin protocols with luteinizing hormone suppression using either a gonadotropin-releasing hormone (GnRH) antagonist or agonist. Ovulation was triggered with human chorionic gonadotropin or GnRH agonist, with oocyte retrieval occurring 35 hours later. Oocytes were fertilized using standard insemination in the majority of cases. ICSI was performed only when indicated (e.g. prior fertilization failure, severe oligospermia, sperm acquired via biopsy, etc.). Embryos were cultured until day 3. If a sufficient number of embryos (generally >3 although criteria varied slightly during the study period) were considered viable at that time, then embryo culture was extended until day 5 or 6.

The statistical analysis of continuous outcome variables was conducted using the *t*-test with $p < 0.05$ considered significant. Categorical data were analyzed using Fishers exact test with $p < 0.05$ considered significant. The Kolmogorov-Smirnov Two-sample test was used to compare the distributions of our two data sets in terms of gestational age at birth and birth weight. Multiple linear regression was performed for gestational age and birth weight in order to adjust for possible confounders. In order to normalize the distributions, gestational age was transformed as $-\log(323 - \text{Gestational Age in days})$ and birth weight was transformed as $-\log(7200 - \text{birth weight in grams})$. Multiple linear regression of gestational age was performed considering as independent variables patient age, the diagnosis of diminished ovarian reserve, parity, prior

ART cycles, prior number of spontaneous losses, the year of the procedure, the day of the embryo transfer, and number of embryos transferred. Multiple linear regression of birth weight was performed considering as independent variables patient age, the diagnosis of diminished ovarian reserve, parity, prior ART cycles, prior number of spontaneous losses, the year of the procedure, the day of the embryo transfer, the number of embryos transferred, and the gestational age at birth. Following the multiple regression, gestational age and birth weight could be compared using values adjusted for these possible confounding parameters.

Subanalyses examining groups of preterm infants were performed using Fishers exact test with $p < 0.05$ considered significant. Subgroups of preterm infants were created based on commonly studied perinatology definitions of preterm infants. Very preterm was defined as <32 weeks gestation, moderately preterm as 32–34 weeks gestation, late preterm as 34–37 weeks gestation, and total preterm as <37 weeks gestation. A final comparison was made between day 3 and day 5/6 ET data collected at NYUFC and day 3 and day 5/6 ET SART data collected by Kalra et al. [13] using Chi squared with Yate's correction, $p < 0.05$ considered significant.

A post-hoc multiple regression power analysis was performed to determine if the study sample size was sufficient to detect a difference in gestational age or birth weight between groups. This study was approved by the institutional review board of New York University School of Medicine.

Results

2392 singleton live births resulting from IVF/ICSI at NYUFC were identified from 2003 to 2012. The 78 patients who underwent ET on days 2, 4, or after day 6 were excluded. Of the remaining 2314 births, 421 underwent day 3 ET and 1893 underwent day 5/6 ET. 14 patients were excluded from the day 3 ET group and 57 patients from the day 5/6 ET group for incomplete birth outcome data. 95 patients in the day 5/6 ET group underwent PGD and were thus excluded. 30 cases of vanishing twins were excluded from the day 3 ET group (30/408, 7.4 %) and 257 cases of vanishing twins were excluded from the day 5/6 ET group (257/1741, 14.8 %). 377 day 3 ETs and 1484 day 5/6 ETs were available for analysis. Table 1 outlines the comparison of primary and secondary outcome variables between exposure and comparison groups. Women undergoing day 3 ET were approximately 2 years older than those undergoing day 5/6 ET. There was no difference in the gravidity, parity, or number of prior miscarriages between groups. Women undergoing day 3 ET were more likely to have had prior ART cycles, more embryos transferred, and fewer single ETs than women undergoing day 5/6 ET. They were also more likely to be undergoing ART due to diminished ovarian reserve.

Table 1 Results of primary and secondary outcome variables between Day 3 and Day 5/6 ET

Patients	Day 3 ET (n=377)	Day 5/6 ET (n=1484)	P value ^a
Age (y)	37.9±3.9	35.5±4.2	<0.0001
Gravidity	1.1±1.2	1.0±1.2	0.32
Parity	0.37±0.6	0.34±0.6	0.38
Prior Miscarriages	0.7±1.0	0.7±1.0	0.54
Prior ART cycles	1.9±2.0	1.0±1.4	<0.0001
Number Embryos Transferred	2.9±1.3	2.2±0.8	<0.0001
Single Embryo Transfer	31 (8.2 %)	184 (12.4 %)	0.024
Uterine/Tubal Infertility	82 (21.8 %)	308 (20.8 %)	0.67
Polycystic Ovarian Syndrome	25 (6.6 %)	209 (14.1 %)	<0.0001
Endometriosis	28 (7.4 %)	104 (7 %)	0.7375
Diminished Ovarian Reserve	124 (32.9 %)	174 (11.7 %)	<0.0001
Male Factor Infertility	55 (14.6 %)	269 (18.1 %)	0.11
Unexplained Infertility	62 (16.4 %)	416 (28 %)	<0.0001
Male Sex of Neonate	188 (49.9 %)	780 (52.6 %)	0.3562
Female Sex of Neonate	189 (50.1 %)	704 (47.4 %)	0.3562
Gest Age (wks)	39.0±2.1	39.0±1.9	0.93
Gest Age (wks) Adjusted (95%CI) ^{b,c}	39.3 (39.1–39.5)	39.26 (39.22–39.3)	N/A
Birth Weight (gms)	3308±568	3268±543	0.22
Birth Weight (gms) Adjusted (95 % CI) ^{b,d}	3324 (3273–3375)	3311 (3298–3324)	N/A

^a T-test^b Adjusted for patient age, infertility diagnosis of diminished ovarian reserve, year of cycle, parity, prior spontaneous abortions, prior ART cycles, number of embryos transferred^c Linear regression of gestational age transformed as $-\log(323 - \text{Gestational Age in days})$ ^d Linear regression of birth weight transformed as $-\log(7200 - \text{birth weight in grams})$

The distributions of the day 3 ET and day 5/6 ET data sets were plotted as cumulative distributions (Figs. 1 and 2) using the Kolmogorov-Smirnov two-sample test. There was no difference between the distributions of gestational age at birth (Fig. 1, $p>0.999$) or the distributions of birth weights for the two study groups (Fig. 2, $p>0.2$).

Multiple linear regression of gestational age and birth weight provided adjustments for the possible confounders of patient age, the diagnosis with diminished ovarian reserve, parity, prior ART cycles, prior number of spontaneous losses, the year of the procedure, and the number of embryos transferred. There was no significant association between gestational age and day of ET. Adjusted mean values (95 %

confidence limits) of gestational age were 39.3 weeks for day 3 ET (39.1–39.5) and 39.26 weeks for day 5/6 ET (39.22–39.3) (Table 1). Post hoc power was estimated to be >0.99 . There was also no significant association between birth weight and day of ET. Adjusted mean values (95 % confidence limits) of birth weight were 3324 g for day 3 ET (3273–3375) and 3311 g for day 5/6 ET (3298–3324) (Table 1). Post hoc power was estimated to be 0.984.

Subanalyses of preterm groups of infants between day 3 and day 5/6 ET were performed (Table 2). The results revealed no statistical difference between ET groups in the number of very preterm, moderate preterm, late preterm, or total preterm births.

Fig. 1 Cumulative histograms of gestational age at delivery for Day 3 and Day 5/6 ET (Kolmogorov-Smirnov two-sample test, $p>0.999$)

Fig. 2 Cumulative histograms of birth mass at delivery for Day 3 and Day 5/6 ET (Kolmogorov-Smirnov two-sample test, $p>0.2$)

There was an overall difference in the incidence of preterm deliveries between the NYUFC day 3 ET and day 5/6 ET data compared to the SART data published by Kalra et al. [13] (Table 2). There was a significant difference between the NYUFC day 3 ET data and the SART day 3 ET data in the preterm group between 32 and 37 weeks gestation and for the <37 weeks gestation group. The incidence of premature deliveries in the NYUFC day 5/6 ET data was significantly less than the incidence in the SART data for all preterm groups.

Discussion

In contrast to the SART data published by Kalra et al. [13], we found no overall difference in birth weight or gestational age between day 3 and day 5/6 ET for patients at NYUFC. Comparing day 3 and day 5/6 ET we found no difference in the subgroups of very preterm, moderately preterm, late preterm, and total preterm deliveries. When comparing the distributions of data between day 3 and day 5/6 ET in terms of birth weight or gestational age at birth we also did not find a

difference. Furthermore, the incidence of premature delivery among NYUFC patients was significantly less than that observed for the SART data [13] for both day 3 and day 5/6 ET groups.

Data from a single fertility center may be more uniform compared to a nationwide database such as SART that represents a wide range of practices with inherent variations. This may explain why there was a statistically significant difference between the NYUFC day 3 and day 5/6 ET data compared to the SART day 3 and day 5/6 ET data. These differences may be secondary to differences in embryo culture techniques, embryo selection criteria, patient demographics, or regional access to specialists during the antenatal period.

Our data excluded pregnancies with documented vanishing twins. Singleton pregnancies that began as multiple gestations are known to be at increased risk of pre-term birth [14, 15]. There were significantly more vanishing twins among day 5/6 ETs than day 3 ETs (14.8 vs 7.4 %, $p<0.0001$). This may be secondary to improved implantation rates with blastocyst transfers [1, 3–5]. The SART data did not exclude vanishing

Table 2 Number of patients delivered preterm in Day 3 and Day 5/6 ET groups

Preterm Groups	Day 3 ET NYUFC (n=377)	Day 3 ET SART ^a (n=32351)	Day 5/6 ET NYUFC (n=1484)	Day 5/6 ET SART ^a (n=14743)	NYUFC Day 3 vs Day 5 ET P value ^b	NYUFC vs SART ^a Day 3 ET P value ^c	NYUFC vs SART ^a Day 5/6 ET P value ^c
<32 weeks	6 (1.6 %)	714 (2.2 %)	12 (0.8 %)	414 (2.8 %)	0.23	0.53	<0.0001
32–34 weeks	1 (0.3 %)	N/A	16 (1.1 %)	N/A	0.22	N/A	N/A
34–37 weeks	25 (6.6 %)	N/A	132 (8.9 %)	N/A	0.18	N/A	N/A
32–37 weeks	26 (6.9 %)	4645 (14.4 %)	148 (10 %)	2743 (18.6 %)	0.07	<0.0001	<0.0001
<37 weeks	32 (8.5 %)	5359 (16.6 %)	160 (10.8 %)	3170 (21.5 %)	0.22	<0.0001	<0.0001

^a Kalra et al., 2012 [13]

^b Fishers Exact Test

^c Chi squared with Yate's Correction

twins; however, the study was able to adjust for reduction in fetal heart tones to diminish this confounder [13]. The possible contribution of vanishing twins to preterm birth with day 5/6 ET should be further investigated. Because day 5/6 ETs may be associated with a higher rate of vanishing twins with a theoretical increased risk of preterm birth, transfer of fewer blastocysts or single ET should be considered with blastocyst transfers.

This study was limited by its retrospective design and possible selection bias. Patients with poor prognostic factors such as age >35yo, fewer embryos available for transfer, or multiple failed IVF cycles were more likely to undergo day 3 ET due to the risk of losing viable embryos in the process of extended embryo culture. These poor prognostic factors in and of themselves may increase the risk of poor birth outcomes such as preterm birth or low birth weight. The study data spans 9 years, and over this time period laboratory techniques, clinical practices, and culture conditions likely changed. Over time, more patients became candidates for day 5/6 ET and a greater proportion of day 5/6 ETs were performed. An attempt to control for these confounders was made by performing a multiple linear regression. When controlling for patient age, diminished ovarian reserve diagnosis, year of cycle, parity, prior spontaneous abortions, prior ART cycles, and number of embryos transferred, transfer day was not significantly associated with gestational age or birth weight. Our study groups were smaller than the published SART data [13]; however, post-hoc power analysis revealed that our study was sufficiently powered to detect a difference in birth weight or gestational age between groups (Gestational Age Power>0.99, Birth Weight $P=0.984$).

Extended embryo culture to the blastocyst stage has emerged as a way to select the single best embryo for transfer. Elective single blastocyst embryo transfer has been shown to have equivalent implantation and pregnancy rates compared to double embryo transfer while significantly reducing the rate of twin gestations [16]. Emerging technology like preimplantation genetic screening (PGS) is used to select a single euploid embryo for transfer, therefore, improving pregnancy rates and decreasing the miscarriage rate due to aneuploidy [17–20]. PGS relies more on blastocyst-stage biopsies which pose less risk of damage to the embryo and allow for the genetic analysis of a greater number of cells reducing the risk of misdiagnosis secondary to mosaicism [21–24]. With the increasing reliance of blastocyst-stage ET in ART cycles, any adverse perinatal outcomes associated with extended embryo culture needs to be identified.

Our results do not support the national SART trends published by Kalra et al. [13]. Prospective studies are needed to investigate the relationship between day of embryo transfer and birth outcomes. Day 5/6 ETs continue to offer the advantages of enhanced pregnancy rates, improved implantation rates, and decreased risk of multiple gestations with single ET.

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

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