Birthweight of singletons born after blastocyst-stage or cleavage-stage transfer: analysis of a data set from three randomized controlled trials

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Abstract

Purpose The present post hoc analysis aims to study the neonatal data of singletons born from three randomized controlled trials (RCTs) which compared the outcome of day 3 and day 5 transfers.

Methods Our analysis included 208 liveborn singletons from three existing RCTs (publication dates 2004, 2005, and 2006), 93 children from cleavage-stage transfers and 115 from blastocyst-stage transfers. Vanishing twins were excluded from the analysis. Singleton birthweight was the primary outcome measure. Gestational age and gender of the newborn were accounted for in the multiple regression analysis, along with other confounding factors, such as maternal age, BMI, parity, and smoking behavior.

Results There was no significant difference in gestational age (median, interquartile range) between cleavage-stage transfer (275 days; 267–281) and blastocyst-stage transfer (277 days; 270–281; $p = 0.22$). Singleton birthweight (median, interquartile range) was not significantly different between cleavage-stage transfer (3330 g; 3020–3610) and blastocyst-stage transfer (3236 g; 2930–3630; $p = 0.40$), even following multivariable regression analysis to control for potential maternal and newborn confounders.

Conclusion The gestational age and birthweight were not significantly different after cleavage-stage and blastocyst-stage transfers. One limitation to be recognized is the age of the data, with original data collection dates from 2001 to 2004. Additionally, the RCTs used for the present analysis have a fairly young age restriction.

Keywords Cleavage-stage · Blastocyst-stage · Birthweight · Perinatal outcome

Introduction

Since the start in 1978, the use of assisted reproduction technology (ART) has continuously increased worldwide [1]. Over the last years, a heavier focus towards cryopreservation and extended culture to the blastocyst stage has also occurred in an attempt to further enhance the effectiveness of ART. However, concerns about the potential health implications of these strategies remain [2].

Extended culture to the blastocyst stage, allowing a better selection of embryos with the highest implantation potential, in combination with single-embryo transfer, has proven to be a gold standard for obtaining high live birth rates while reducing multiple pregnancies [3, 4]. Cumulative pregnancy data, including the outcome of frozen embryo transfer cycles, are important when comparing blastocyst-stage with cleavage-stage transfers [5–7]. Whereas blastocyst transfer has been shown to increase delivery rates in the fresh cycle, there is no current evidence for its superiority regarding cumulative live birth rate compared with cleavage-stage transfer [5]. However, with blastocyst transfer, the number of transfer cycles (fresh and vitrified) was significantly lower than with cleavage-stage transfer, thus shortening the time to pregnancy [5]. Although much valued from a patient viewpoint, this apparently modest added value should of course be weighed up against costs and, more importantly, safety considerations.

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Recently, blastocyst-stage transfer has been associated with a higher relative risk of preterm delivery in different meta-analyses [8–10]. An increased risk for being large for gestational age (LGA) has also been reported in small series on blastocyst transfers [11, 12], although these results were not confirmed in other studies [13–15] including a recent meta-analysis [10]. Unfortunately, the limitation of all available studies is their observational design, and confounding by indication cannot fully be excluded, leaving the question whether blastocyst-stage transfer has an influence on neonatal outcome among singleton births yet unresolved. As causality can better be assessed by adequately designed randomized controlled trials (RCTs), we decided to perform a post hoc analysis of the neonatal outcome of singletons born from the largest existing trial comparing single blastocyst-stage and cleavage-stage transfer [16]. This study, however, would only provide data on 36 infants resulting from day 3 transfer and 50 infants from day 5 transfer. In order to increase the power, we pooled the data from two other RCTs performed earlier in our IVF center [17, 18].

Materials and methods

Study design

We analyzed retrospectively the neonatal outcome of all singleton live births obtained from three existing RCTs that were published in 2004, 2005, and 2006 by our center [16–18]. Original data collection periods were respectively January 2001–December 2003, January 2001–November 2003, and July 2003–November 2004. Despite overlapping time periods, there is no overlap between the studies due to differences in inclusion criteria. All three RCTs only involved fresh embryo transfer cycles. They were all designed in order to compare ongoing pregnancy or live birth rates between patients randomized to have (single) cleavage-stage transfer or (single) blastocyst-stage transfer. A total of 208 liveborn singletons resulting from singleton pregnancies were included, 93 liveborn singletons from cleavage-stage transfer and 115 liveborn singletons from blastocyst-stage transfer. Multiple deliveries, vanishing twins, and stillborn deliveries were excluded from the analysis.

The following data have recently been collected for the analysis: singleton birthweights, maternal age, maternal body mass index (BMI), parity of the mother, maternal smoking, and number of embryos transferred. Other variables analyzed included gestational age and gender of the newborn.

The present retrospective study was approved by the local ethical committee of the Universitair Ziekenhuis Brussel (B.U.N. 143201629046).

Data collection and statistical analysis

Vanishing twins were defined as spontaneous disappearance of one or more gestational sacs or embryos in an ongoing pregnancy, documented by ultrasound. Gestational age was calculated from the day of oocyte retrieval, defined as day 14 of the menstrual cycle. Preterm birth was defined as delivery before 37 completed weeks of gestation. Low birthweight was defined as birthweight < 2500 g. Smoking habits, maternal weight, and height were documented at the start of the treatment cycle.

Continuous data are presented as mean ($\pm$ SD) or median (IQR), depending on the normality of the distribution. Categorical data are presented as number of events and percentages. The between-group differences were assessed using either the two-sample *t* test, Wilcoxon rank-sum test, Pearson's chi-squared test, or Fisher's exact test, as appropriate.

Multivariable linear regression analysis was performed to examine the independent effect of transfer day (day 3 cleavage-stage or day 5 blastocyst-stage) on birthweight while controlling for potential maternal and newborn confounders. The confounders included in the model are maternal age, maternal BMI, maternal parity, maternal smoking, gestational age, and gender of the newborn. Despite the fact that the data included in this study were derived from RCTs, we still performed this approach to adjust for potential confounding because, given that we included only a subset of patients in this study, the balance between the study groups in terms of potential confounding can no longer be guaranteed.

A *p* value of < 0.05 was considered statistically significant. For all analyses, we used Stata version 13.1.

We also performed a power analysis to estimate the minimum effect size necessary to have at least 80% to detect a difference in birthweight between both study arms. Assuming the mean ($\pm$ standard deviation) birthweight after a cleavage-stage transfer of 3300 g $\pm$ 540 g, the difference between both study arms would need to be at least 211 g to achieve $\geq$ 80% power, assuming a *p* value of 5%.

Results

The data set analyzed and related to each of the three RCTs is presented in Table 1.

The main maternal and treatment characteristics according to transfer day are shown in Table 2. No differences in maternal age, maternal BMI, maternal parity, or maternal smoking behavior were observed between the

Table 1 Data set of the three RCTs

Study	Day 3				Day 5			
	a	b	c	Total	a	b	c	Total
Clinical pregnancies	75	27	41	143	75	42	58	175
Deliveries	67	23	38	128	72	38	56	166
Multiple delivery	20	7	2	29	15	14	0	29
Vanishing twin	5	1	0	6	4	6	1	11
Stillborn delivery	0	0	0	0	3	0	2	5
Lost to follow-up	0	0	0	0	2	1	3	6
Liveborn singletons	42	15	36	93	48	17	50	115

^a Kolibianakis et al., 2004 [17]; ^b Papanikolaou et al., 2005 [18];
^c Papanikolaou et al., 2006 [16]

Multiple deliveries, vanishing twins, and stillborn deliveries were excluded from the analysis

two groups. The number of embryos transferred was not different according to transfer day.

Table 3 shows the parameters of delivery and children born according to the transfer day. No differences were observed between the two groups regarding gestational age, gender of the newborn, or median singleton birthweight. The median singleton birthweight was 3330 g (IQR 3020–3610) after cleavage-stage transfer and 3236 g (IQR 2930–3630) after blastocyst-stage transfer ($p = 0.40$). The incidence of preterm birth and low birthweight was not different between the two groups.

Table 2 Maternal and treatment characteristics according to transfer day

	Day 3 (<i>n</i> = 93)	Day 5 (<i>n</i> = 115)	<i>p</i> value
Mean maternal age (SD)	30.9 (3.9)	31.0 (3.7)	0.86
Median maternal BMI (IQR)	22.9 (20.0, 25.8)	22.8 (20.3, 26.0)	0.76
Maternal parity			
Nulliparous (%)	75 (80.6)	88 (76.5)	0.47
Multiparous (%)	18 (19.4)	27 (23.5)	
Maternal smoking			
Non-smoker (%)	78 (88.6)	100 (87.7)	0.84
Smoker (%)	10 (11.4)	14 (12.3)	
Number of embryos transferred			
Single (%)	44 (47.3)	60 (52.2)	0.49
Two or more (%)	49 (52.7)	55 (47.8)	

Maternal age was compared by two-sample *t* test; maternal BMI was compared by Wilcoxon rank-sum test; and categorical variables were compared by Pearson's chi-squared test

In Table 4, the beta regression coefficients of multiple models are shown with birthweight as the dependent variable. For each variable, we present their respective regression coefficients, 95% confidence interval, and *p* values from two regression models: the unadjusted regression coefficient on the left (in which only the variable itself is added to the model as a factor) and, on the right, the adjusted regression coefficient, in which all variables (maternal age, maternal BMI, maternal smoking, maternal parity, embryo day of transfer, gestational age, and liveborn gender) presented in the table are added simultaneously in the model. Continuous variables present regression coefficient per unit increase (for example, per year of increase in maternal age), while for dichotomous variables, the regression coefficient is presented as a contrast between an elected base (referent) level, which we have reported by the use of the word “reference”.

Multivariable linear regression analysis (controlling for maternal age, maternal BMI, maternal parity, maternal smoking, gestational age, and gender of the newborn) confirmed the non-significant difference in singleton birthweight according to transfer day (Table 4; adjusted regression coefficient = −88.49; 95% confidence interval, −204.29, 27.32). Within the present data set, singleton birthweight was associated with maternal smoking and gestational age.

Discussion

The present post hoc neonatal outcome analysis of three existing RCTs does not confirm a higher relative risk of preterm delivery when transferring blastocysts. Neonatal birthweight was also not significantly different between the two groups. The present data provide important evidence on the safety using extended culture to the blastocyst stage.

Adverse perinatal outcomes with blastocyst transfer have been reported only in large observational studies, lacking proper control for specific confounders, such as age, parity, BMI, number of embryos transferred, and vanishing twins [8, 9, 19]. Recently, it was also shown that cryopreservation of the embryos represents an additional confounder [10, 20]. Whereas controversy persists, most will agree that adequately powered and well-designed RCTs are needed in order to allow a definitive conclusion on this matter. We have raised caution before, regarding the feasibility of such trials, needing large numbers of patients in each group [21]. In the absence of these RCTs, the present post hoc neonatal outcome analysis on three existing RCTs is performed in our center. The largest existing randomized trial comparing single blastocyst-stage and cleavage-stage transfer [16], however, involved too few singletons born (respectively 36 and 50). In order to increase the power, we pooled the data from two other RCTs performed in our center as well [17, 18], transferring one or two embryos, but equal numbers in both the cleavage and the blastocyst

Table 3 Main pregnancy and infant characteristics according to transfer day

	Day 3 (<i>n</i> = 93)	Day 5 (<i>n</i> = 115)	<i>p</i> value
Median gestational age (days) (IQR)	275 (267, 281)	277 (270, 281)	0.22
Preterm birth (< 37 weeks)			
Yes (%)	7 (7.5)	6 (5.2)	0.49
No (%)	86 (92.5)	109 (94.8)	
Liveborn gender			
Female (%)	33 (35.5)	56 (48.7)	0.056
Male (%)	60 (64.5)	59 (51.3)	
Median liveborn birthweight (g) (IQR)	3330 (3020, 3610)	3236 (2930, 3630)	0.40
Low birthweight (< 2500 g)			
Yes (%)	4 (4.3)	7 (6.1)	0.76
No (%)	89 (95.7)	108 (93.9)	

Continuous variables (gestational age and birthweight) were compared by Wilcoxon rank-sum test; preterm birth and liveborn gender were compared by Pearson's chi-squared test; and low birthweight was compared by Fisher's exact test

groups. An additional strength of the analysis is that it is a single-center study only involving fresh embryo transfers.

One limitation to be recognized is the age of the data, with original data collection dates ranging from 2001 to 2004. Unfortunately, over the last 15 years, there has been no further contribution to literature comparing cleavage-stage and blastocyst-stage single-embryo transfer in a randomized way, at least with live birth or neonatal outcome as the primary outcome. The authors are well aware that practice patterns and culture media have shifted in this time, which may impact the outcomes. We have used different sequential media over the years (Vitrolife, 2001–2004; Medicult/Vitrolife, 2004–

2009; Sage, 2010–2013), although always respecting a medium switch on day 3 of embryo culture. Individual embryo culture in droplets under oil has not changed since 2001. Bench-top incubators with separate chambers and better temperature and gas regulations have been introduced in 2012. Adjusted singleton birthweight after extended culture has been fairly stable over the years in our center: 3250 g [22], 3250 g [21], and 3236 g (present data) and the same applies to adjusted singleton birthweights resulting from cleavage-stage transfers. This is in contrast to recently reported changes in birthweight over time [23]. It remains however difficult to pinpoint the underlying cause: changes in the underlying

Table 4 Univariable and multivariable linear regression analyses for neonatal birthweight (g)

	RC (95% CI)	<i>p</i> value	aRC (95% CI)	<i>p</i> value
Maternal age (per year)	10.47 (− 8.14, 29.09)	0.270	− 2.11 (− 17.65, 13.42)	0.790
Maternal BMI (per kg/m ²)	14.91 (− 2.06, 31.88)	0.085	11.81 (− 2.17, 25.78)	0.098
Maternal smoking				
No	Reference		Reference	
Yes	− 162.00 (− 382.36, 58.36)	0.150	− 219.03 (− 400.91, − 37.15)	0.018
Maternal parity				
Nulliparous	Reference		Reference	
Multiparous	126.02 (− 44.84, 296.87)	0.148	133.20 (− 10.50, 276.90)	0.069
Embryo transfer day				
Day 3	Reference		Reference	
Day 5	− 41.09 (− 183.19, 101.00)	0.571	− 88.49 (− 204.29, 27.32)	0.134
Gestational age (per day)	27.73 (22.46, 32.99)	0.000	28.05 (22.83, 33.28)	0.000
Liveborn gender				
Female	Reference		Reference	
Male	62.27 (− 80.38, 32.99)	0.392	48.11 (− 68.92, 165.14)	0.420

RC, regression coefficient; aRC, adjusted regression coefficient (with all variables presented included in the model)

patient population or changes in ART practice, such as optimized embryo culture and incubation systems.

Randomization at intake of the three RCTs, allocating patients to cleavage or blastocyst transfer, did not hold any guarantee that they would achieve a successful outcome (i.e., singleton delivery from a singleton pregnancy) and that the confounders that do matter for neonatal outcome would remain balanced. Nonetheless, no statistical differences were observed between cleavage and blastocyst transfer groups concerning maternal age, maternal BMI, maternal parity, maternal smoking, and number of embryos transferred. Our data showed that singleton birthweight after embryo culture was associated with maternal smoking and gestational age. Although an effort was made to control for as many confounders as possible, it must be added that pregnancy-associated factors possibly influencing birthweight (such as diabetes, hypertension, pre-eclampsia) could not be included in the analysis.

It must be recognized that the present study may be underpowered to detect clinically relevant differences. Specifically, for the outcome birthweight, it is possible that the effect sizes below the pre-estimated 211 g may have failed to achieve statistical significance due to the low number of patients included. Despite this limitation, we consider that this study is of relevance for the scientific community given that most of the previous literature is based on retrospectively collected data. Such studies often fail to collect all relevant covariates.

Most of the RCTs used for the present analysis have a fairly young age restriction and thus young mean ages in the two groups (cleavage-stage, 31.3 [17], 29.6 [18], 30.5 [16] and blastocyst-stage, 31.5 [17], 29.9 [18], 30.4 [16]). For this reason, the present data are only valid within younger age populations. Whether the present outcome would be applicable in older age groups remains open. However, extended culture and blastocyst-stage transfer are a clinical practice specifically for younger patients with a good response (number of oocytes, number of good-quality embryos). In a previous retrospective analysis, we showed no significant effect of maternal age on singleton birthweight, which was associated with maternal parity, maternal smoking, gestational age, and newborn gender [22].

The present data, small in sample size and based on relatively old data sets (2001–2004), are in line with more recent, larger sized retrospective data sets published by our group [21, 22] (data collection periods, January 2010–December 2013 and April 2004–December 2009, respectively, 331 and 2098 babies born).

Of reference, the entire embryo culture was performed in a low-oxygen atmosphere of 5%, a physiological condition recently emphasized to possibly prevent potential adverse outcomes [24]. This key variable is most often not reported in registry-based, retrospective observational studies, whereas variability in the use of either low- or high-oxygen tension

has been documented [25]. Our data are in line with the notion that those clinics utilizing physiological (low) concentrations of oxygen do not report adverse effects on children born following extended culture [14, 23, 26].

Our small cohort of singletons born after cleavage or blastocyst transfer did not confirm the previously reported altered sex ratio after blastocyst transfer [27]. A meta-analysis, including 14 retrospective studies, shows an association between blastocyst transfer and a higher male to female (M:F) ratio (OR = 0.89; 95% CI, 0.86 to 0.93, $I^2 = 19.8\%$) [26]. The disproportionate male to female ratio in the present cleavage-stage group (60/33, close to significance 0.056) is hard to explain. Especially because thin finding, true or coincident, was not confirmed in our more recent, larger, retrospective data sets [21, 22].

The present data provide an adequate neonatal follow-up analyzing the impact of fresh blastocyst transfer on neonatal outcome at a time when well-designed properly powered prospective follow-up studies are lacking. Further prospective research should be encouraged to assess more definitively whether blastocyst-stage transfer has a potential adverse impact on neonatal outcome. A next step could be to combine the data of all currently existing clinical trials followed by an individual patient database meta-analysis.

Compliance with ethical standards The present retrospective study was approved by the local ethical committee of the Universitair Ziekenhuis Brussel (B.U.N. 143201629046).

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