Outcomes of singleton births after blastocyst versus nonblastocyst transfer in assisted reproductive technology

Dhanushi Fernando, B.Med.Sci.,[a] Jane L. Halliday, Ph.D.,[b] Susan Breheny, B.S.,[c] and David Lindsay Healy, B.Med.Sci., M.B.B.S., Ph.D.[a]

[a] Monash University, Clayton; [b] Murdoch Children's Research Institute, Parkville; and [c] Monash IVF, Hawthorn, Victoria, Australia

Objective: To compare obstetric and perinatal outcomes of singleton births after assisted reproductive technology (ART) with blastocyst transfer (days 5 to 6) versus nonblastocyst transfer (days 2 to 4).
Design: Retrospective cohort study.
Setting: Monash IVF.
Patient(s): 4,202 women who conceived using in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) between 2004 and 2009.
Intervention(s): Records analysis of fresh and frozen-thawed embryo transfers resulting in singleton births of at least 20 weeks' gestation.
Main Outcome Measure(s): Perinatal outcomes: preterm birth, low birthweight, very low birthweight, small for gestational age, large for gestational age, preeclampsia, antepartum hemorrhage, placental abruption, placenta previa, and postpartum hemorrhage; and covariates: maternal age, year of birth of the baby, private health insurance status, maternal body mass index, smoking status, parity, gender of baby, and variations in treatment procedures.
Result(s): Multivariate analysis found no statistically significant difference between transfers on days 5 and 6 and days 2 and 4 for all maternal and perinatal outcomes. There were modest increases in the adjusted odds ratios for preeclampsia (adjusted odds ratio 1.72, 99% confidence interval 0.93–3.20) and placenta previa (1.65, 0.92–2.98).
Conclusion(s): Obstetric and perinatal outcomes after blastocyst transfer on days 5 to 6 are similar when compared with embryo cleavage-stage transfers on days 2 to 4. (Fertil Steril® 2012;97:579–84. ©2012 by American Society for Reproductive Medicine.)
Key Words: Blastocyst, cleavage stage embryo, frozen-thawed embryo transfer, ICSI, IVF, morula

Blastocyst culture is a relatively new technique and its long-term health implications have not yet been extensively researched. In a natural pregnancy, the cleavage-stage embryo remains in the ampulla of the uterine tube for 3 days, descending into the uterine cavity after compaction, which typically occurs after day 4 (1, 2). The nutritional environment and metabolite composition of the tubal and the uterine fluids are different (3), and the recognition of this, with the creation of stage-specific media by Gardner, has enabled improved blastocyst culture with better survival and implantation rates (4). Schoolcraft et al. (5) reported blastocyst formation rates of up to 48.8% using stage-specific G1/G2 media.

Implantation of the human embryo, which involves the attachment and penetration of the endometrium by the blastocyst, occurs over an "implantation window" (6), a period of time beginning at approximately 5 to 7 days after fertilization. Studies have shown that, although successful implantation can occur with up to 3 days asynchrony, the optimal time for initiation of implantation is within a day of embryo-endometrial asynchrony (6, 7). This suggests that cleavage-stage embryos may still achieve successful implantation, but blastocysts are more synchronized to the uterine environment, giving them greater implantation potential (4, 8).

Embryos make the transition from the maternal to the embryonic genome only after the eight-cell stage (9). In vitro culture of the embryo to the blastocyst stage, where the embryonic genome has experienced complete activation, allows selection based on more objective criteria than the limited and inconsistent assessment of morphologic criteria at the cleavage stage (10, 11). Furthermore, day-3 embryos with particular types of aneuploidy are associated with slow rates of cleavage and developmental arrest (12). The occurrence of chromosomal abnormalities in the embryos of women over

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Reprint requests: David Lindsay Healy, B.Med.Sci., M.B.B.S., Ph.D., Monash University, Department of Obstetrics and Gynaecology, Level 5, Monash Medical Centre, 246 Clayton Road, Clayton, Victoria 3168, Australia (E-mail: david.healy@monash.edu).

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36 years decreases from 59% at day 3 to 35% by day 5 (13). Extending in vitro culture to day 5 thus allows identification of the embryos that are fated to arrest and selects in favor of more developmentally competent embryos with a greater likelihood of implantation (12, 14).

The timing of embryo transfers to align with periods of less frequent uterine contractions is also an important consideration in embryo transfer. Uterine contractions at transfer are of concern due to the risk of expulsion of the replaced embryo. A significant decrease in the level of uterine contractions coinciding with the development of the embryo to the blastocyst stage in fresh transfers therefore favors blastocysts over cleavage-stage embryos from being expelled (15).

Blastocyst embryo transfer has a significantly higher rate of pregnancy and live births than cleavage-stage transfer when equal numbers of embryos are transferred (8, 14, 16). In a Cochrane review, Blake described a significantly higher live-birth rate with blastocyst transfer than with cleavage-stage transfer (odds ratio [OR] 1.35; 95% confidence interval [CI], 1.05–1.74) (16). Eighteen studies were accepted into this review, totalling 2,616 women and 395 blastocyst implantations. The total number of births in this study was unclear, and there were variations in the trials reviewed, so generalizing these results to the entire population seeking assisted reproductive technology (ART) is difficult (17). Nonetheless, a meta-analysis similarly demonstrated that live-birth rates were higher after blastocyst transfer (OR 1.39; 95% CI, 1.10–1.76, $P=.005$) when compared with cleavage-stage transfer (8). We can find no detailed analysis of obstetric outcomes after blastocyst transfer and birth. We compared obstetric and perinatal outcomes of singleton births after ART and embryo transfer on culture days 5 to 6 versus transfer on days 2 to 4.

MATERIALS AND METHODS

Study Design

The design of this analysis was a retrospective cohort study of 4,202 women who conceived with intracytoplasmic sperm injection (ICSI) or IVF between 2004 and 2009, and delivered a singleton baby of at least 20 weeks' gestation. Human research ethics approval and permission to access patient histories for the purpose of this study was obtained from the Monash University Human Research Ethics Committee, Monash Surgical Private Human Research Ethics Committee, and Epworth Healthcare Human Research Ethics Committee.

All data were obtained from the Monash IVF patient database. It is a legal requirement in Australia that detailed data are recorded for mandated reporting to the Australian and New Zealand Assisted Reproduction Database. Where information was incomplete, patient history files were manually accessed to complete the record. Patient records were entered into an Excel spreadsheet, and the identifying information was removed.

Study Population

Typically, cleavage-stage transfers are performed on days 2 to 3 and blastocyst-stage transfers on days 5 to 6. There were 2,486 singleton births after embryo transfer on days 2 to 4. Of these transfer procedures on days 2 to 4, 70.8% were cleavage-stage embryos, 27.6% were morula-stage embryos, and 2.6% were blastocysts. There were 1,716 transfers on days 5 to 6, of which 95.3% were blastocysts. Cook Series Media was used in all our patients for embryo culture during the timeframe of this study.

Exclusions

Pregnancies after embryo transfers where the patients had undergone assisted reproductive procedures other than IVF or ICSI were excluded. These procedures were not performed frequently enough to be analyzed as a separate entity. Pregnancies resulting from donor oocytes or embryos were also excluded from this study. Assisted hatching was not used at Monash IVF and was not used in this study. Uncommon stimulation protocols, multiple births, or pregnancies that did not reach 20 weeks' gestation were excluded. Furthermore, where a woman had more than one singleton birth in the study period, only the first listed birth on the database was selected for analysis.

Data Collection

The Monash IVF database was used to obtain information on maternal age at embryo transfer, year of birth of baby, private health insurance status (PHI), body mass index (BMI), smoking status, parity, gender of baby, and number of embryos transferred. Information regarding the etiology of infertility, type of assisted conception treatment undertaken, stimulation of cycle, cryopreservation of embryos, whether ICSI was used, and method of delivery were also obtained.

Outcomes of Interest

The primary obstetric outcomes of interest were placenta previa (PP), placental abruption (PA), preeclampsia, antepartum hemorrhage (APH), and postpartum hemorrhage (PPH). The primary perinatal outcomes were very preterm birth (<32 weeks' gestation), preterm birth (<37 weeks' gestation), very low birth weight (VLBW; <1,500 g at birth), low birth weight (LBW; <2,500 g at birth), small for gestational age (SGA; <10th percentile on intrauterine growth chart), and large for gestational age (LGA; >90th percentile).

Statistical Analysis

We used SPSS software (Statistical Package for the Social Sciences) version 17.0 to compare the obstetric and perinatal outcomes of the singleton IVF and ICSI pregnancies. For univariate analysis of categorical variables, chi-square tests were used. Where the analysis involved a 2×2 table, the Yates' correction for continuity was performed to compensate for the overestimation of the chi-square value when used with a 2×2 table. Where the chi-square analysis of a 2×2 table did not meet the assumption that less than 20% of expected values were less than 5, the Fisher's exact test was used. Independent *t*-tests were used for continuous variables.

Covariates were regarded as known risk factors for the adverse birth outcomes, based on published findings.

A covariate was adjusted for in the multivariate analysis if the distribution between the exposure groups were unequal at a chi-square value of <0.20. These items were used in multivariable models as possible confounders:

1. Maternal age (continuous variable)
2. BMI (categorical: <18.5 kg/m^2, 18.5–24.99, ≥25.00, unknown)
3. Private health insurance status (categorical, nominal: private, public, unknown)
4. Year of birth (categorical, nominal: 2004, 2005, 2006, 2007, 2008, 2009)
5. Smoking status (categorical, nominal: yes, no)
6. Parity (categorical, nominal: 0, 1, ≥2)
7. Gender of baby (categorical, nominal: male, female)
8. Stimulation of cycle (categorical, nominal: fresh embryo in a stimulated cycle, frozen-thawed embryo in an artificial cycle, frozen-thawed embryo in a natural cycle)
9. Number of embryos transferred (categorical, nominal: single-embryo transfer [SET], double-embryo transfer [DET])
10. Type of treatment (categorical, nominal: IVF, ICSI)
11. Mode of delivery (categorical, nominal: normal vaginal delivery, cesarean section)
12. Vanishing twins (categorical, nominal: yes, no, unknown)

Simple binary logistic regression was used to generate the OR, 95% confidence interval, and *P* value for the final univariate analysis. Multivariable binary logistic regression was used to generate the adjusted odds ratio (AOR) with 99% CI, accounting for potential confounders through simultaneous input of covariates. $P<.05$ was considered statistically significant for univariate analyses, and $P<.01$ was considered statistically significant for multivariate analyses (due to the effects of multiple comparisons).

RESULTS

The frequencies of mother and baby characteristics for singleton births involving embryo transfers on days 5 to 6 when compared with transfers on days 2 to 4 showed that maternal age, year of birth of baby, PHI, BMI, smoking status, parity, gender of the baby, stimulation type, number of embryos transferred, and whether ICSI or IVF was performed were unevenly distributed between the two populations (Table 1). We adjusted for these in the multivariate analysis. The difference between the exposure groups for mode of delivery of the baby was statistically insignificant ($P>.20$, chi-square).

The frequencies of adverse outcomes are presented in Table 2 to demonstrate absolute risk. Univariate and multivariate analysis of birth outcomes after embryo transfer on days 5 to 6 versus days 2 to 4 are presented in Table 3.

There were no differences in any of the perinatal outcomes when births resulting from transfer on days 5 to 6 were compared with transfers on days 2 to 4. Preeclampsia and PP were marginally increased (preeclampsia: AOR 1.72; 99% CI, 0.93–3.20; and PP: AOR 1.65; 95% CI, 0.92–2.98), but this did not reach significant statistical difference at $P<.01$. Multivariate analysis did not demonstrate strong evidence for differences between the two exposure groups for any other obstetric outcomes. Multivariate analysis, including adjustment for the possible effects of APH in the analysis of very preterm birth, preterm birth, and tubal factor infertility on the analysis of LGA, also made no statistically significant difference to the AOR.

Reanalysis of the cohort comparing only cleavage-stage embryos of days 2 to 3 (n = 1,735) and only blastocysts of days 5 to 6 (n = 1,636) showed no statistically significant difference in obstetric and perinatal outcomes in exposure groups. Analysis of the cohort comparing only day-2 (n = 425) or day-3 (n = 1,310) cleavage-stage embryos with only day-5 blastocysts (n = 1,611) also demonstrated no statistically significant difference in outcomes. For example, the AOR for LBW after day-5 transfer of blastocysts only was 1.20 (99% CI, 0.60–2.42; $P=.50$) when compared with day-2 cleavage-stage embryos, and 0.92 (99% CI, 0.58–1.46; $P=.64$) when compared with day-3 cleavage-stage embryos. For APH, the AORs were 0.81 (99% CI, 0.24–2.69; $P=.65$) and 1.02 (99% CI, 0.43–2.42; $P=.94$) for day-5 blastocysts when compared with day-2 and day-3 cleavage-stage embryo transfers, respectively.

DISCUSSION

When singleton births resulting from embryo transfers on days 5 to 6 were compared with transfer from days 2 to 4, no statistically significant differences between the groups were demonstrated for birth outcomes. This is a notable finding. It indicates that birth outcomes may not be adversely affected by extended embryo culture. A strength of our study was that only a single type of embryo culture medium was used. Possible adverse effects of different in vitro culture media affecting birth weight did not complicate our investigation (18).

A Swedish study by Kallen et al. (19) investigating neonatal outcomes after cleavage versus blastocyst transfer indicated a statistically significantly higher likelihood of preterm birth (OR 1.35; 95% CI, 1.07–1.71) after blastocyst transfer. They attributed this to the possibly higher rate of monozygotic twinning in the blastocyst group, resulting in more preterm births. Our larger but single-center study of singleton births only has shown no increase in prematurity. Kallen et al. (19) found no statistically significant differences between blastocysts and cleavage-stage embryo transfers for very preterm birth, VLBW, LBW, or SGA, supporting the findings from our research.

A large study on 5,497 singleton births by Shih et al. (20) found birth weight was not significantly affected by the morphologic quality or number of cells in cleavage-stage embryos transferred. However, an earlier study on 447 singleton births found the average cell number in day-3 transfers did influence birth weight (21). Our research was of different design to this latter study. We found no statistically significant differences in singletons when comparing cleavage-stage to blastocyst-stage transfers.

In our analysis, potential confounders were regarded as covariates that were known risk factors for the adverse birth outcomes and whose distribution between the exposure

TABLE 1

Summary of demographics and treatment measures for embryo transfer, days 5 to 6 versus days 2 to 4.

	Day 2–4 (n = 2,486)		Day 5–6 (n = 1,716)		
	n	%	n	%	*P* value
Age					
Mean	34.0	–	33.6	–	<.001
Standard deviation	4.1	–	4.1	–	
Body mass index					
<18.5	67	2.7%	22	1.3%	<.001
18.5–24.99	79	3.2%	49	2.9%	
≥25.00	1,386	55.8%	1,075	62.6%	
Unknown	954	38.4%	570	33.2%	
Private health insurance status					
Private	1,742	70.1%	1,243	72.4%	<.001
Public	737	29.6%	469	27.3%	
Unknown	7	0.3%	4	0.2%	
Year of birth of baby					
2004	519	20.9%	4	0.2%	<.001
2005	629	25.3%	28	1.6%	
2006	545	21.9%	185	10.8%	
2007	353	14.2%	408	23.8%	
2008	265	10.7%	644	37.5%	
2009	175	7.0%	447	26.0%	
Smoking					
Nonsmoker	2,280	91.7%	1,608	93.7%	.02
Smoker	206	8.3%	108	6.3%	
Parity group					
0	1,835	73.8%	1,441	84.0%	<.001
1	533	21.4%	207	12.1%	
≥2	118	4.7%	68	4.0%	
Gender					
Male	1,205	48.5%	923	53.8%	.001
Female	1,281	51.5%	793	46.2%	
Stimulation					
Fresh embryo, stimulated cycle	1,758	70.7%	1,279	74.5%	.02
FET, artificial cycle	184	7.4%	115	6.7%	
FET, natural cycle	544	21.9%	322	18.8%	
No. of embryos transferred					
1	1,103	44.4%	1,354	78.9%	<.001
2	1,383	55.6%	362	21.1%	
Type of treatment					
IVF	876	35.2%	658	38.3%	.04
ICSI	1,610	64.8%	1,058	61.7%	
Mode of delivery					
Normal vaginal delivery	1,352	54.4%	964	56.2%	.26
Cesarean section	1,134	45.6%	752	43.8%	
Vanishing twin					
No	2,234	89.9%	1,602	93.4%	<.001
Yes	177	7.1%	60	3.5%	
Unknown	75	3.0%	54	3.1%	

Note: FET = frozen-thawed embryo transfer; ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization.

Fernando. Birth outcomes after blastocyst transfer. Fertil Steril 2012.

groups were unequal at a chi-square value of $P<.20$. It was not possible to account for all confounders, and this was a limitation of our study. Information regarding potential confounders such as ethnicity, previous cesarean section, medical history of prior complications, and family history were not available. Previous cesarean section is a important flag for future adverse outcomes, including PP, and forthcoming studies should endeavor to obtain this information. All assessable covariates including SET or DET, IVF or ICSI, cryopreservation of embryos, transfer into a stimulated or natural cycle, and cesarean section were adjusted where appropriate. This reduced the effect of confounding on the results.

Maternal age is an important risk factor for several obstetric and neonatal outcomes, and advanced maternal age is often associated with poorer results. Preeclampsia, PA, and PP have been found to be increased with advanced maternal age (22, 23), and preterm delivery is also higher in women over 40 years when compared with women 20 to 40 years of age (24). The year of birth of the baby was adjusted for in the analysis to account for variation and improvement in procedures and techniques over the study period. Payment in the form of PHI was also accounted for during analysis because it is potentially an indicator of socioeconomic status and is associated with poorer obstetric outcomes and different rates of obstetric intervention (25).

TABLE 2

Absolute risk of adverse outcomes for embryo transfer, days 5 to 6 versus days 2 to 4.

	Day 2–4 transfer (n = 2,486)		Day 5–6 transfer (n = 1,716)	
Adverse outcome	**n**	**%**	**n**	**%**
Very preterm	58	2.33	37	2.16
Preterm	228	9.17	165	9.62
VLBW	47	1.89	31	1.81
LBW	181	7.28	127	7.40
SGA	214	8.61	141	8.22
LGA	268	10.8	184	10.7
Preeclampsia	74	2.98	65	3.79
APH	67	2.70	35	2.04
PA	4	0.16	5	0.29
PP	72	2.90	64	3.73
PPH	13	0.52	33	1.92

Note: APH = antepartum hemorrhage; LBW = low birth weight; LGA = large for gestational age; PA = placental abruption; PP = placenta previa; PPH = postpartum hemorrhage; SGA = small for gestational age; VLBW = very low birth weight.

Fernando. Birth outcomes after blastocyst transfer. Fertil Steril 2012.

We were unable to determine BMI (calculated using preprocedure or registration weight) for some patients because of missing weights or heights on their registration forms. These patients were not excluded from the study. Clinician and staff observations are that the majority of people who do not provide weight details are self-conscious of their (increased) weight. If this were the case, excluding this subset of patients would introduce bias into the study, as these are patients likely to have more adverse outcomes.

The number of cigarettes smoked by a patient was recorded. However, the covariate used in the analysis was binary and only distinguished whether or not a patient was a smoker at the time of embryo transfer. Inaccurate measures of this covariate may resonate from patient underreporting as well as administration errors in collecting and recording the data.

Nulliparous women are known to have a higher risk of preterm birth (26, 27) and preeclampsia (28). Parous women are more at risk of PA and PP (23, 29). Studies have also found increasing mean birth weight with increasing parity (30). Parity was therefore adjusted for in the analysis. Parity was calculated by summation of all births before registration with the IVF clinic and any ART births (postregistration) occurring before the conception of the singleton being analyzed. As the registration form is not renewed, any natural conceptions or ART conceptions with the clinics outside Monash IVF occurring after the initial registration with Monash IVF but before the conception of the singleton in question are not recorded by the system. This is a potential source of inaccuracy in the measure of parity.

Male babies have a tendency to be heavier than female babies, but a meta-analysis demonstrated similarity in gestational age and prematurity (30). Furthermore, a recent study has shown varying sex ratios for different ART procedures (31). They found a higher rate of males after blastocyst SET (54.1%) when compared with cleavage SET (49.9%) (31). Gender was therefore adjusted in our study.

The "vanishing twin" phenomenon occurs in approximately 10% of singleton pregnancies after DET and is the result of a spontaneous reduction of a twin pregnancy to a single, ongoing pregnancy (32). This can adversely affect outcomes for the surviving twin. In our study, 3.5% of births from transfer on days 5 to 6 and 7.1% after transfer on days 2 to 4 involved a vanishing twin (Table 1). This was statistically significantly different, and therefore was accounted for in the multivariate analysis.

Overall, this study has shown that extended embryo culture does not adversely affect obstetric and perinatal outcomes. This may indicate that the benefits conferred by improved assessment of the quality of embryos, better embryo-endometrial synchrony, and fewer uterine contractions with transfer on

TABLE 3

Summary of results for embryo transfer, days 5 to 6 versus days 2 to 4.

Adverse outcome	Odds ratio (95% CI)	*P* value	Adjusted odds ratio (99% CI)	*P* value
Days 2 to 4	1.00		1.00	
Days 5 to 6				
Very preterm	0.92 (0.61–1.40)	.70	0.65 (0.34–1.24)[a]	.08
Preterm	1.05 (0.85–1.30)	.63	0.93 (0.66–1.30)[a]	.57
VLBW	0.95 (0.60–1.51)	.84	0.73 (0.35–1.52)[b]	.26
LBW	1.02 (0.80–1.29)	.88	0.91 (0.62–1.33)[b]	.52
SGA	0.95 (0.76–1.19)	.65	1.05 (0.73–1.52)[a]	.71
LGA	0.99 (0.81–1.21)	.95	1.17 (0.83–1.64)[a]	.23
Preeclampsia	1.28 (0.91–1.80)	.15	1.72 (0.93–3.20)[a]	.02
APH	0.75 (0.50–1.14)	.18	0.75 (0.39–1.44)[a]	.25
PA	1.81 (0.49–6.76)	.38	0.65 (0.11–3.93)[a]	.53
PP	1.30 (0.92–1.83)	.13	1.65 (0.92–2.98)[a]	.03
PPH	3.73 (1.96–7.11)	<.001	0.97 (0.40–2.37)[c]	.94

Note: Odds ratios were calculated using simple binary logistic regression, and adjusted odds ratios were calculated using multivariable binary logistic regression. APH = antepartum hemorrhage; BMI = body mass index; CI = confidence interval; DET = double-embryo transfer; ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization; LBW = low birth weight; LGA = large for gestational age; PA = placental abruption; PHI = private health insurance status; PP = placenta previa; PPH = postpartum hemorrhage; SET = single-embryo transfer; SGA = small for gestational age; VLBW = very low birth weight.

[a] Adjusted for maternal age, year of birth, parity, PHI, BMI, smoking, stimulation of cycle (including cryopreservation of embryo), IVF/ICSI, SET/DET, and vanishing twins.

[b] Adjusted for maternal age, year of birth, parity, PHI, BMI, smoking, gender of baby, stimulation of cycle (including cryopreservation of embryo), IVF/ICSI, SET/DET, and vanishing twins.

[c] Adjusted for maternal age, year of birth, parity, PHI, BMI, smoking, stimulation of cycle (including cryopreservation of embryo), mode of delivery, APH, IVF/ICSI, SET/DET, and vanishing twins.

Fernando. Birth outcomes after blastocyst transfer. Fertil Steril 2012.

days 5 to 6 counterbalance the potentially negative effects of extended in vitro culture on the embryo. Our analysis of obstetric and birth outcomes in women who have conceived through assisted reproduction and delivered singletons provides important information for both ART practitioners and patients. Our results may assist in decision-making in laboratory practice and provide support for offering blastocyst transfer to appropriate patients.

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