Human Reproduction, Vol.33, No.11 pp. 2018–2022, 2018
Advanced Access publication on September 14, 2018 doi:10.1093/humrep/dey284

human reproduction

SHORT COMMUNICATION *Infertility*

Perinatal outcomes of singleton live births with and without vanishing twin following transfer of multiple embryos: analysis of 113 784 singleton live births

Mohan S. Kamath[1,*], Belavendra Antonisamy[2], Hepsy Y. Selliah[2], and Sesh Kamal Sunkara[3]

[1]Department of Reproductive Medicine, Christian Medical College, Vellore, India [2]Department of Biostatistics, Christian Medical College, Vellore, India [3]Queen's Hospital, Barking Havering Redbridge University Hospitals NHS Trust, Essex, UK

*Correspondence address. E-mail: dockamz@gmail.com

Submitted on June 14, 2018; resubmitted on July 20, 2018; accepted on August 24, 2018

STUDY QUESTION: Does transfer of multiple embryos affect perinatal outcomes of resulting singleton live births following ART?

SUMMARY ANSWER: There is a higher risk of preterm birth (PTB) and low birthweight (LBW) in singleton live births associated with spontaneous reduction of an initial multiple to singleton gestation following transfer of multiple embryos.

WHAT IS KNOWN ALREADY: Singleton pregnancies following ART are at a higher risk of adverse perinatal outcomes compared to spontaneous conceptions. Earlier studies have found an increased risk of PTB and LBW in singletons following transfer of multiple embryos versus single embryo transfer (SET). However, these studies did not address the specific role of vanishing twin, i.e. spontaneous reduction of an initial multiple to singleton gestation.

STUDY DESIGN, SIZE, DURATION: Anonymised data on all ART cycles performed in the UK were obtained from the Human Fertilization and Embryology Authority. Data from 1991 to 2011 involving 508 410 fresh and 131 157 frozen autologous ART cycles resulting in 95 779 and 18 005 singleton live births, respectively, were analyzed.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Fresh and frozen ART cycles were analyzed separately to compare perinatal outcomes of PTB and LBW of singleton live births resulting from transfer of multiple (≥2) embryos versus SET. Logistic regression analysis was performed adjusting for confounders. Subgroup analyses were carried out for perinatal outcomes of singleton live births with initial multiple or initial single gestational sacs following transfer of multiple embryos versus singleton live births following SET.

MAIN RESULTS AND THE ROLE OF CHANCE: In fresh cycles, there was a significantly higher risk of PTB (adjusted odds ratio (aOR) 2.70, CI 2.37–3.05) and LBW (aOR 2.76, CI 2.44–3.13) in singleton live births with initial multiple gestational sacs but there was no significant difference in the risk of PTB (aOR 1.08, CI 1.00–1.16) or LBW (aOR 1.08, CI 1.00–1.16) in singleton live births with an initial single gestational sac following transfer of ≥2 embryos compared to those following SET. In frozen cycles, there was a significantly higher risk of PTB (aOR 2.13, CI: 1.55–2.93) and LBW (aOR 2.61, CI: 1.87–3.64) in singleton live births with initial multiple gestational sacs but there was no significant difference in the risk of PTB (aOR 1.02, CI: 0.88–1.18) or LBW (aOR 0.91, CI: 0.77–1.07) in the singleton live births with an initial single gestational sac following transfer of ≥2 embryos compared to those following SET.

LIMITATIONS, REASONS FOR CAUTION: While the analysis was adjusted for a number of known confounders, the dataset had no information for confounders such as smoking, BMI, previous obstetric history and comorbid medical conditions during pregnancy. The lack of information about the timing of occurrence of the vanishing phenomenon is another limitation because poorer perinatal outcomes of a surviving twin have been reported following second trimester fetal demise compared to the first trimester.

WIDER IMPLICATIONS OF THE FINDINGS: The study results suggest that the vanishing twin phenomenon is associated with increased risk of PTB and LBW in the resulting singleton live births and there was no increased risk when there was a single gestational sac from the outset following transfer of multiple embryos.

© The Author(s) 2018. Published by Oxford University Press on behalf of the European Society of Human Reproduction and Embryology. All rights reserved.
For Permissions, please e-mail: journals.permissions@oup.com

Downloaded from https://academic.oup.com/humrep/article/33/11/2018/5096856 by guest on 23 April 2024

STUDY FUNDING/COMPETING INTERESTS: Nil.

Key words: ART / single embryo transfer / multiple embryo transfer / preterm birth / low birthweight / vanishing twin

Introduction

Singleton pregnancies following ART are associated with a higher risk of adverse obstetric and perinatal outcomes compared to spontaneous conceptions (Pandey *et al.*, 2012). Underlying infertility is considered as an independent risk factor (Messerlian *et al.*, 2013), and other suggested reasons for the adverse outcomes include ART procedures such as ovarian stimulation, *in vitro* gamete manipulation and embryo culture (Pinborg *et al.*, 2013).

Earlier studies have found an increased risk of preterm birth (PTB) and low birthweight (LBW) in singletons following transfer of multiple embryos compared to singletons from single embryo transfer (SET) (De Sutter *et al.*, 2006; Wang *et al.*, 2009; Luke *et al.*, 2010). These studies did not address the contributory role of the vanishing twin phenomenon to the observed poor perinatal outcomes. Vanishing twin rates have been found to vary between 5% and 30% following ART (Shebl *et al.*, 2008; Gjerris *et al.*, 2012). Other studies have found an increased risk of LBW in singleton births following a vanishing twin compared to those following an initial single gestation (Pinborg *et al.*, 2005; Shebl *et al.*, 2008). These studies did not delineate whether the initial single gestation resulted following transfer of single or multiple embryos. It is not clear whether the finding of an increased risk of poor perinatal outcomes in singletons following transfer of multiple embryos is mainly due to the vanishing twin phenomenon or occurs even with an initial single gestation. We therefore compared perinatal outcomes in singleton live births with and without a vanishing twin following transfer of multiple embryos with those following SET.

Materials and Methods

Anonymised data were obtained from the Human Fertilization and Embryology Authority (HFEA) on all ART treatment cycles in the UK between 1991 and 2011. ART cycles with transfer of a single and ≥2 embryos resulting in singleton live births were analyzed for outcomes of PTB and LBW. Autologous fresh and frozen ART cycles were included and analyzed separately. Multiple births were excluded from the analysis. Data were obtained for the following: female age group (≤34, 35–37, 38–39, 40–42, 43–44, ≥45 years), treatment period (1991–1995, 1996–2000, 2001–2005, 2006–2011), type of infertility (primary or secondary), cause of infertility (tubal disease, ovulatory disorder, male factor, unexplained, endometriosis, cervical factors), previous IVF cycles, previous live birth, number of oocytes retrieved, number of embryos transferred, day of embryo transfer, live birth occurrence, initial number of intrauterine gestational sacs on ultrasound, gestational age at delivery and birthweight.

Live birth was defined as delivery of at least one live newborn at ≥24 weeks gestation. An occurrence of a live birth at <37 weeks gestation was defined as a PTB, and birthweight <2500 g was defined as LBW.

Statistical analysis

The characteristics of the cohorts were described using relative frequencies for categorical variables and means and SD for continuous variables. Adjusted logistic regression was performed for confounding factors: female age category, treatment period, previous live birth occurrence, cause of infertility, number of oocytes retrieved, and day of embryo transfer. Since women could have more than one cause of infertility, therefore the five causes of infertility were included in the regression analysis as dummy variables individually. The intercorrelation of these exposure variables were below 0.38, indicating little or no multicollinearity problem.

Subgroup analyses were carried out for perinatal outcomes of singleton live births following transfer of ≥2 embryos with initial multiple or initial single gestational sacs versus singleton live births following SET. A *P* value less than 0.05 was considered statistically significant. Data were analyzed using the statistical package Stata, version 13 (StataCorp, College Station, TZ, USA).

Results

A total of 1004 488 ART cycles were recorded in the HFEA database during the study period, of which 508 410 fresh and 131 157 frozen ART cycles were included. A total of 13 177 and 82 602 singleton live births following fresh cycles and a total of 3199 and 14 806 singleton live births following frozen cycles with transfer of single and ≥2 embryos were analyzed (Supplementary Figs 1 and 2).

Baseline characteristics of the two cohorts (SET and transfer of ≥2 embryos) in fresh and frozen cycles are detailed in Table I. Day of embryo transfer for the majority of the cycles was less than Day 5 (cleavage stage) in fresh (78.8% with SET, 93.3% with ≥2 embryos) and frozen cycles (99.4% with SET, 99.7% with ≥2 embryos).

The incidence of PTB was 8.4% (1108/13 177) and 9.5% (7851/82 602), and the incidence of LBW was 8.4% (1112/13 177) and 9.6% (7940/82 602) in singleton live births following SET and transfer of ≥2 embryos, respectively, in fresh ART cycles. The incidence of PTB was 8.3% (265/3199) and 1348/14 806 (9.1%), and the incidence of LBW was 6.4% (203/3199) and 985/14 806 (6.6%) in singleton live births following SET and transfer of ≥2 embryos, respectively, in frozen ART cycles.

Perinatal outcomes of singleton live births following multiple versus single embryo transfer in fresh ART cycles

In the main analysis, there was a significantly higher risk of PTB (adjusted odds ratio (aOR) 1.14, 95% CI: 1.06–1.23) and LBW (aOR 1.14, CI: 1.06–1.23) in singleton live births following transfer of ≥2 embryos compared to SET in fresh cycles. The vanishing twin rate following transfer of ≥2 embryos was 3.5% (2910/82 244) in fresh ART cycles. There was a significantly higher risk of PTB (aOR 2.70, CI 2.37–3.05) and LBW (aOR 2.76, CI 2.44–3.13) in singleton live births with initial multiple gestational sacs, but there was no significant difference in the risk of PTB (aOR1.08, CI 1.00–1.16) and LBW (aOR 1.08, CI 1.00–1.16) in singleton live births when there was an initial single gestational sac following transfer of ≥2 embryos compared to SET in fresh ART cycles (Table II).

Downloaded from https://academic.oup.com/humrep/article/33/11/2018/5096856 by guest on 23 April 2024

Table I Baseline characteristics for single versus multiple embryo transfer in fresh and frozen ART cycles.

Characteristic	Fresh ART cycles		Frozen ART cycles	
	Single embryo transfer *n* = 72 372	Transfer of ≥2 embryos *n* = 436 038	Single embryo transfer *n* = 26 683	Transfer of ≥2 embryos *n* = 104 474
Age at treatment				
18–34 years	34 395 (47.5%)	2 13 043 (48.9%)	11 072 (41.5%)	50 109 (48.0%)
35–37 years	16 871 (23.3%)	10 6951 (24.5%)	6639 (24.9%)	24 689 (23.6%)
38–39 years	9871 (13.6%)	59 430 (13.6%)	4035 (15.1%)	13 304 (12.7%)
40–42 years	8219 (11.4%)	45 430 (10.4%)	3342 (12.5%)	11 131 (10.7%)
43–44 years	2233 (3.1%)	9054 (2.08%)	816 (3.1%)	2878 (2.8%)
≥45 years	783 (1.1%)	2130 (0.5%)	779 (2.9%)	2363 (2.3%)
Type of infertility				
Female primary	22 343 (30.9%)	2 02 249 (46.4%)	10 785 (40.4%)	50 184 (48.0%)
Cause of infertility*				
Tubal disease	15 225 (21.0%)	1 17 739 (27.0%)	6401 (24%)	29 575 (28.36%)
Ovulatory disorder	8879 (12.3%)	48366 (11.1%)	3767 (14.1%)	17 204 (16.5%)
Male factor	35 216 (48.7%)	2 25 966 (51.8%)	11 992 (44.9%)	50 463 (48.3%)
Unexplained	22 577 (31.2%)	1 37 141 (31.5%)	7921 (29.7%)	29 198 (28.0%)
Endometriosis	5378 (7.4%)	31537 (7.2%)	1526 (5.7%)	6706 (6.4%)
Number of previous ART cycles				
0	43 174 (59.7%)	2 21 324 (50.8%)	887 (3.3%)	4408 (4.2%)
1	14 855 (20.5%)	99 428 (22.8%)	10 440 (39.1%)	41 401 (39.6%)
2	7196 (9.9%)	54 459 (12.5%)	6483 (24.3%)	24 572 (23.5%)
3	3501 (4.8%)	28 023 (6.4%)	3695 (13.9%)	14 403 (13.8%)
4+	3646 (5.0%)	32 804 (7.5%)	5178 (19.4%)	19 690 (18.9%)
Year of treatment				
1991–1995	6578 (9.1%)	57 353 (13.2%)	2066 (7.7%)	11 576 (11.1%)
1996–2000	9573 (13.2%)	1 01 064 (23.2%)	3709 (13.9%)	24 692 (23.6%)
2001–2005	10 536 (14.6%)	1 13 768 (26.1%)	5296 (19.9%)	28 902 (27.7%)
2006–2011	45 685 (63.1%)	1 63 853 (37.6%)	15612 (58.5%)	39 304 (37.6%)
Oocytes number: median (IQR)	6 (3, 11)	10 (6, 14)	NA	NA
Embryos created: median (IQR)	2 (1, 6)	6 (4, 9)	NA	NA
**Day of embryo transfer				
<Day 5	56 812 (78.8%)	4 05 316 (93.3%)	26 282 (99.4%)	1 02 915 (99.7%)
≥Day 5	15 319 (21.2%)	29 135 (6.7%)	151 (0.6%)	365 (0.3%)
Previous live birth (yes)	4815 (6.7%)	29 706 (6.8%)	7815 (29.3%)	20 627 (19.7%)

IQR, interquartile range; NA, not applicable.
*Causes of infertility are not mutually exclusive.
**<Day 5 – cleavage stage transfer; ≥Day 5 – blastocyst stage.

Perinatal outcomes of singleton live births following multiple versus single embryo transfer in frozen ART cycles

In the main analysis, there was no significant difference in the risk of PTB (aOR 1.05, CI: 0.91–1.21) and LBW (aOR 0.95, CI: 0.81–1.12) in singleton live births following transfer of ≥2 embryos compared to SET in frozen cycles. The vanishing twin rate in frozen ART cycles following transfer of ≥2 embryos was 2.4% (355/ 14 708). There was a significantly higher risk of PTB (aOR 2.13, CI: 1.55–2.93) and LBW (aOR 2.61, CI: 1.87–3.64) in singleton live births with initial multiple gestational sacs but there was no significant difference in the risk of PTB (aOR 1.02, CI: 0.88–1.18) and LBW (aOR 0.91, CI: 0.77–1.07) in singleton live births when there was an initial single gestational sac following transfer of ≥2 embryos compared to SET in frozen ART cycles (Table II).

Discussion

This study showed higher risk of PTB and LBW in singleton live births in both fresh and frozen ART cycles following multiple embryo transfer

Downloaded from https://academic.oup.com/humrep/article/33/11/2018/5096856 by guest on 23 April 2024

Downloaded from https://academic.oup.com/humrep/article/33/11/2018/5096856 by guest on 23 April 2024

Table II Perinatal outcomes of singleton live births based on initial single and multiple gestational sac after fresh and frozen ART cycles: subgroup analysis.

Outcomes in fresh or frozen ART cycles	SET	Transfer of ≥2 embryos (with initial single gestational sac)	Transfer of ≥2 embryos (with initial multiple gestational sac)	OR (95% CI) (transfer of ≥2 embryos with initial single gestational sac versus SET)	aOR* (95% CI) (transfer of ≥2 embryos with initial single gestational sac versus SET)	OR (95% CI) (transfer of ≥2 embryos with initial multiple gestational sac versus SET)	aOR* (95% CI) (transfer of ≥2 embryos with initial multiple gestational sac versus SET)
PTB (<37 weeks), (fresh transfer)	1108/13177 (8.4%)	7205/79334 (9.1%)	614/2910 (21.1%)	1.08 (1.01, 1.16)**	1.08 (1.00, 1.16)	2.91 (2.61, 3.24)**	2.70 (2.37, 3.05)**
LBW (<2500 g), (fresh transfer)	1112/13177 (8.4%)	7271/79334 (9.1%)	635/2910 (21.8%)	1.09 (1.02, 1.16)**	1.08 (1.00, 1.16)	3.02 (2.72, 3.37)**	2.76 (2.44, 3.13)**
PTB (<37 weeks), (frozen transfer)	265/3199 (8.3%)	1283/14353 (8.9%)	61/355 (17.1%)	1.08 (0.94,1.24)	1.02 (0.88, 1.18)	2.29 (1.70, 3.11)**	2.13 (1.55, 2.93)**
LBW (<2500 g), (frozen transfer)	203/3199 (6.4%)	922/14353 (6.4%)	59/355 (16.6%)	1.01 (0.86, 1.18)	0.91 (0.77, 1.07)	2.94 (2.15, 4.02)**	2.61 (1.87, 3.64)**

PTB, preterm birth; LBW, low birthweight; OR, odds ratio; aOR, adjusted OR; SET, single embryo transfer.
Logistic regression analyses were used for perinatal outcomes of singleton live births following transfer of ≥2 embryos with initial multiple or initial single gestational sacs versus singleton live births following SET adjusting for the confounding variables. Unadjusted and adjusted odds ratios reported with 95% CIs and *P*-values; **Significant ($P < 0.05$).
*Adjusted for age of women, period of treatment, causes of infertility
(tubal disease, ovulatory disorder, male factor, unexplained, endometriosis, cervical factors), previous live birth, number of oocytes and day of embryo transfer.

and a vanishing twin phenomenon compared to singleton live births following SET. No increased risk of PTB or LBW was observed in singleton live births in both fresh and frozen ART cycles following multiple embryo transfer with an initial single gestation from the outset.

This is the largest study comparing perinatal outcomes in singleton live births following transfer of ≥2 embryos versus SET, analyzing fresh and frozen cycles separately. One of the limitations of the study was the absence of information on potential confounders such as maternal smoking, body mass index, previous obstetric history and comorbid medical conditions during pregnancy. The lack of information about the timing of occurrence of vanishing phenomenon is another limitation due to reported poorer perinatal outcomes of surviving twin following second trimester fetal demise compared to first trimester (Pinborg *et al.*, 2005).

An earlier study reported perinatal outcomes following double embryo transfer (DET) versus SET (Wang *et al.*, 2009). Among singleton births, the risk of PTB (aOR 1.13, CI 1.05–1.22) and LBW (aOR 1.15, CI 1.05–1.26) was significantly higher following DET compared to SET. While their findings are in agreement with our results, the subgroup analysis in the current study found no difference in the risk of PTB and LBW in singleton live births without vanishing twin. This suggests a major contributory role of vanishing twin in observed adverse perinatal outcomes. Another study evaluated the effects of number of transferred embryos on perinatal outcomes of singletons and twin pregnancies (Luke *et al.*, 2010). The study excluded singletons following vanishing twin. The study reported no significant difference in risk of LBW in singletons following transfer of two (9.4%), three (8.7%) and four embryos (8.5%) compared to SET (8.7%). This is in agreement with the current study finding of no significant difference in the risk of LBW in singleton live births without vanishing twin after transfer of multiple versus SET.

In the current study, no increased risk of PTB and LBW was observed in frozen cycles following transfer of ≥2 embryos versus SET except in the vanishing twin subgroup. The likely reason for the noted difference between fresh and frozen results could be due to a lower rate of vanishing twin in frozen compared to fresh cycles (2.4% versus 3.5%, respectively). The lower vanishing twin rate with frozen cycles could be due to the better quality embryos, given that only good quality embryos fulfilling certain criteria are cryopreserved and survival of the embryos after thawing is likely to be a self selecting process.

A systematic review exploring the role of vanishing twin on perinatal outcomes following ART pooled five studies and found no significant difference in PTB (risk ratio (RR) 1.33, CI 0.91–1.94; $n = 1233$) and LBW (RR 1.85, CI 0.88–3.86; $n = 1461$) (Sun *et al.*, 2017). These results are contrary to current study findings and one likely reason could be smaller pooled numbers analyzed in the meta analysis, which could have led to type II error.

The current study finding of significantly higher risk of PTB and LBW in singleton live births following transfer of ≥2 embryos versus SET is in agreement with earlier studies (De Sutter *et al.*, 2006; Wang *et al.*, 2009) and further validates the findings with a large dataset. However, a finding of no increase in adverse perinatal outcomes with transfer of multiple embryos in the absence of vanishing twin is additional information that merits attention. Future studies are needed to validate these findings and should include additional perinatal outcomes such as stillbirth, neonatal deaths and congenital anomalies.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Authors' roles

MSK and SKS conceived the study question and analysis. MSK drafted the manuscript. BA and HS performed statistical analysis. All authors critically appraised manuscript for its intellectual content.

Funding

No external sources of funding.

Conflict of interest

None.

References

De Sutter P, Delbaere I, Gerris J, Verstraelen H, Goetgeluk S, Van der Elst J, Temmerman M, Dhont M. Birthweight of singletons after assisted reproduction is higher after single- than after double-embryo transfer. *Hum Reprod* 2006;**21**:2633–2637.

Gjerris AC, Tabor A, Loft A, Christiansen M, Pinborg A. First trimester prenatal screening among women pregnant after IVF/ICSI. *Hum Reprod Update* 2012;**18**:350–359.

Luke B, Brown MB, Stern JE, Grainger DA, Klein N, Cedars M. Effect of embryo transfer number on singleton and twin implantation pregnancy outcomes after assisted reproductive technology. *J Reprod Med* 2010; **55**:387–394.

Messerlian C, Maclagan L, Basso O. Infertility and the risk of adverse pregnancy outcomes: a systematic review and meta-analysis. *Hum Reprod* 2013;**28**:125–137.

Pandey S, Shetty A, Hamilton M, Bhattacharya S, Maheshwari A. Obstetric and perinatal outcomes in singleton pregnancies resulting from IVF/ICSI: a systematic review and meta-analysis. *Hum Reprod Update* 2012;**18**:485–503.

Pinborg A, Lidegaard O, la Cour Freiesleben N, Andersen AN. Consequences of vanishing twins in IVF/ICSI pregnancies. *Hum Reprod* 2005;**20**:2821–2829.

Pinborg A, Wennerholm UB, Romundstad LB, Loft A, Aittomaki K, Söderström-Anttila V. Why do singletons conceived after assisted reproduction technology have adverse perinatal outcome? Systematic review and meta-analysis. *Hum Reprod Update* 2013;**19**:87–104.

Shebl O, Ebner T, Sommergruber M, Sir A, Tews G. Birth weight is lower for survivors of the vanishing twin syndrome: a case-control study. *Fertil Steril* 2008;**90**:310–314.

Sun L, Jiang LX, Chen HZ. Obstetric outcome of vanishing twins syndrome: a systematic review and meta-analysis. *Arch Gynecol Obstet* 2017; **295**:559–567.

Wang YA, Sullivan EA, Healy DL, Black DA. Perinatal outcomes after assisted reproductive technology treatment in Australia and New Zealand: single versus double embryo transfer. *Med J Aust* 2009;**190**: 234–237.

Downloaded from https://academic.oup.com/humrep/article/33/11/2018/5096856 by guest on 23 April 2024