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ORIGINAL ARTICLE ***Fertility control***

Obstetric and perinatal risks in 4601 singletons and 884 twins conceived after fresh blastocyst transfers: a Nordic study from the CoNARTaS group

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STUDY QUESTION: Are obstetric and perinatal outcomes in pregnancies after fresh blastocyst transfer (BT) comparable with those born after fresh cleavage stage transfer (CT) and spontaneous conception (SC)?

SUMMARY ANSWER Fresh BT is associated with a higher risk of placental and perinatal complications.

WHAT IS KNOWN ALREADY: BT optimizes the selection of top-quality embryos and increases pregnancy and live birth rates per transfer compared to CT. However, concerns have been raised as extended culture duration may increase obstetric complications and impair perinatal outcomes. Previous studies have shown a higher risk of preterm birth (PTB) among infants born after BT compared with CT. Pregnancies after BT are also prone to a higher risk of same-sex twins after single embryo transfer (SET).

STUDY DESIGN, SIZE, DURATION: A retrospective register-based cohort study used data from Denmark, Norway and Sweden including three cohorts: 56 557 singletons and 16 315 twins born after fresh IVF/ICSI cycles and 2 808 323 SC singletons in Denmark (birth years 1997–2014), Norway (2010–2015) and Sweden (2002–2015). Of the fresh IVF/ICSI singletons, 4601 were born after BT and 51 956 after CT. The twin cohort consisted of 884 fresh IVF/ICSI children born after BT and 15 431 fresh IVF/ICSI children born after CT.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Data were obtained from a large Nordic cohort of children born after ART and SC initiated by the Committee of Nordic ART and Safety (CoNARTaS). The CoNARTaS cohort was established by cross-linking National ART-, Medical Birth-, and National Patients Registers using the unique personal identification number, allocated to every citizen in the Nordic countries. Obstetric and perinatal outcomes after BT, CT and SC were compared using logistic regression analysis. For perinatal outcomes, we calculated gestational age based on the date of oocyte pick-up (OPU) and in sensitivity analyses on data from Denmark and Norway, we also calculated gestational age based on the second-trimester ultrasonography (US) scan. Risk of pregnancies with same-sex twins after SET was used as a proxy for risk of monozygotic twins. Adjustments were made for child's sex, birth year, parity (0 or >1), maternal age, body mass index, smoking, educational level, fertilization method (IVF/ICSI), the number of aspirated oocytes, SET and country. Information on educational level and the number of aspirated oocytes was not available for Norway. Children born after frozen embryo transfer were not included. The birth cohorts were restricted according to the year in which BT was introduced in the different countries.

MAIN RESULTS AND THE ROLE OF CHANCE: A hi gher risk of placenta previa was found in singleton pregnancies after BT compared with CT (adjusted odds ratio [aOR] 2.11 [95% CI 1.76; 2.52]). Singletons born after BT had a higher risk of PTB (aOR 1.14 [95% CI 1.01;

1.29]) compared with CT singletons, when estimated based on OPU. Furthermore, an altered male/female ratio (aOR 1.13 [95% CI 1.06; 1.21]) with more males following BT compared with CT was seen. Risk of same-sex twins after SET was higher after single BT compared with single CT (aOR 1.94 [95% CI 1.42; 2.60]).

LIMITATIONS, REASONS FOR CAUTION: Residual confounding cannot be excluded, in particular related to duration and cause of infertility that we could not adjust for due to lack of reliable data.

WIDER IMPLICATIONS OF THE FINDINGS: Extended embryo culture to the blastocyst stage has the potential to compromise obstetric and perinatal outcomes in fresh cycles. These results are important since an increasing number of IVF/ICSI treatments are performed as BT.

STUDY FUNDING/COMPETING INTEREST(S): NORDFORSK (project no: 71450). The Research Fund of Rigshospitalet, Copenhagen University Hospital. ReproUnion Collaborative study, co-financed by the European Union, Interreg V ÖKS. Grants from Swedish state under the agreement between the Swedish government and the county councils, the ALF-agreement (LUA/ALF 70940), Hjalmar Svensson Research Foundation. The Research Council of Norway through its Centres of Excellence funding scheme, project number 262700. None of the authors has any conflicts of interests to declare regarding this study.

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Key words: blastocyst transfer / perinatal outcome / obstetric outcome / monozygotic twins / sex ratio

Introduction

Cleavage stage embryo transfer (CT) at Days 2–3 after fertilization has been practised since the early days of ART treatment, but extended culturing of embryos to the blastocyst stage with embryo transfer at Days 5–6 (BT) has gradually been replacing CT.

BT is thought to improve the selection of top-quality embryos and to increase pregnancy rates and live birth rates per transfer (Elgindy *et al*., 2011; De Vos *et al*., 2016; Glujovsky *et al*., 2016). Despite these advantages, a systematic review suggests that there is no high-quality evidence to support the use of either BT or CT when taking into consideration the cumulative live birth rate per started cycle of a fresh embryo transfer followed by a frozen embryo transfer, as extended embryo culture might increase failure to transfer any embryos and reduce the embryo freezing rate (Glujovsky *et al*., 2016).

Furthermore, concerns about BT have been raised regarding impaired maternal-obstetric health and infant health. One study has indicated a higher risk of placental complications after BT compared with CT (Ginström Ernstad *et al*., 2016). In addition, some studies have shown higher rates of preterm (PTB) and very preterm birth (VPTB) (Kalra *et al*., 2012; Dar *et al*., 2013) among infants conceived after fresh BT compared with fresh CT. Some studies also suggest an altered male/female ratio (Chang *et al*., 2009; Hattori *et al*., 2019) and a higher risk of monozygotic twins (MZT) following fresh BT compared with CT (Ikemoto *et al*., 2018; Hattori *et al*., 2019).

This Nordic registry study explores whether obstetric and perinatal outcomes in pregnancies after BT are comparable with those conceived following CT and following spontaneous conception (SC).

Materials and Methods

The Committee of Nordic ART and Safety (CoNARTaS) cohort consists of all deliveries in Denmark, Finland, Norway and Sweden. Data were obtained from the national Medical Birth Registries (MBR), where detailed information on maternal, fetal and neonatal health is recorded. Data from the MBR's were cross-linked to National ART- and Patients Registers using the unique personal identification number, allocated to every citizen in the Nordic countries. The study population has been described in more detail in our cohort profile paper (Opdahl *et al*., 2019).

Information on culture duration was not available from Finland, and therefore this study was restricted to data from Denmark, Norway and Sweden including 56 557 singletons and 16 315 twins conceived after fresh embryo transfer and 2 808 323 singletons conceived spontaneously. Of the singletons born after fresh IVF and ICSI, 4601 were conceived after BT and 51 956 after CT. The number of singletons from the three countries was as follows: Denmark (1997–2014) $n = 1\,094\,259$ (BT, $n = 1052$; CT, $n = 22\,089$; SC, $n = 1\,071\,118$); Norway (2010–2015) $n = 344\,027$ (BT, n = 220; CT, $n = 5260$; SC, $n = 338\,547$); Sweden (2002–2015) $n = 1\,426\,594$ (BT, $n = 3329$; CT, n = 24 607; SC, $n = 1\,398\,658$) (Table I). The twin cohort consisted of 884 children conceived after fresh BT and 15 431 after fresh CT. The birth cohorts were restricted according to the year in which BT was introduced in the different countries. Only fresh IVF/ICSI cycles were included in this study as the comparison of obstetric and perinatal outcomes after frozen-thawed embryo BT and CT has been described in a previous study (Ginström Ernstad *et al*., 2019). Furthermore, children born after oocyte donation and preimplantation genetic testing were not included.

A part of the Swedish cohort (children born from 2002 to 2013) was included in a previous study comparing neonatal and maternal outcomes after BT, CT and SC (Ginström Ernstad *et al*., 2016).

Outcomes

Obstetric outcomes (International Statistical Classification of Diseases and Related Health Problems—Tenth Revision [ICD-10 code]) were as follows: hypertensive disorders in pregnancy (HDP: pregnancy-induced hypertension [O13], preeclampsia [O14], eclampsia [O15]), placental abruption (O45), placenta previa (O44), preterm prelabour rupture of membranes (PPROM, O420), induction of labour (O61), caesarean section (O82) and postpartum haemorrhage (PPH, O72: >500 ml in Denmark and Norway, >1000 ml in Sweden).

Perinatal outcomes were child's sex, gestational age (PTB [<37 weeks], VPTB, [<32 weeks]) birth weight (low birth weight [LBW, <2500 g], very low birth weight [VLBW, <1500 g], macrosomia [>4000 and >4500 g]), small for gestational age (SGA), large for gestational age

Table I Maternal and treatment characteristics of singleton pregnancies after fresh blastocyst transfer, fresh cleavage stage transfer and spontaneous conception in the Denmark, Norway and Sweden from 1997 to 2015.

Singletons	Blastocyst transfer *n* = 4601	Cleavage stage transfer *n* = 51 956	Spontaneous conception *n* = 2 808 323
Age mother years, mean (SD)	34.0 (4.3)	34.0 (4.3)	30.5 (5.1)
Age mother years, *n* (%)			
<25	60 (1.3)	641 (1.2)	345 496 (12.3)
25–29	620 (13.5)	7338 (14.1)	845 859 (30.1)
30–34	1802 (39.2)	20 142 (38.8)	1 006 720 (35.8)
35–39	1618 (35.2)	18 487 (35.6)	500 174 (17.8)
>40	501 (10.9)	5348 (10.3)	110 072 (3.9)
Maternal BMI (kg/m^2), median (IQR)	23 (21–26)	23 (21–26)	23.5 (21–27)
Maternal BMI (kg/m^2), *n* (%)			
<18.5	104 (2.5)	961 (2.4)	64 716 (3.1)
18.5–24.9	2534 (61.8)	23 696 (58.6)	1 268 595 (61.2)
25–29.9	1092 (26.6)	11 194 (27.7)	486 432 (23.5)
>30	370 (9.0)	4591 (11.4)	253 287 (12.2)
Unknown	501/4601 (10.9)	11 514/51 956 (22.1)	735 293/2 808 323 (26.2)
Maternal smoking, *n* (%)			
No smoking	4275 (97.7)	46 116 (94.9)	2 320 858 (90.0)
Smoking	99 (2.3)	2481 (5.1)	258 745 (10.0)
Unknown	227/4601 (5.9)	3359/51 956 (6.5)	228 720/2 808 323 (8.1)
Maternal educational level years, *n* (%)[a]			
Low	1656 (39.5)	21 881 (48.9)	1 357 014 (59.6)
Middle	1507 (36.0)	14 457 (32.3)	618 133 (27.2)
High	1027 (24.5)	8441 (18.9)	301 197 (13.2)
Unknown	411/4601 (8.9)	7177/51 956 (13.8)	531 979/2 808 323 (18.9)
Residence, *n* (%)			
Denmark	1052 (22.9)	22 089 (42.5)	1 071 118 (38.1)
Norway	220 (4.8)	5260 (10.1)	338 547 (12.1)
Sweden	3329 (72.4)	24 607 (47.4)	1 398 658 (49.8)
Year of delivery, *n* (%)			
1997–2000	108 (2.3)	3640 (7.0)	253 609 (9.0)
2001–2005	207 (4.5)	12 136 (23.4)	672 728 (24.0)
2006–2010	1360 (29.6)	15 981 (30.8)	864 172 (30.8)
2011–2015	2926 (63.6)	20 199 (38.9)	1 017 814 (36.2)
Parity ≥1, *n* (%)	1587 (34.6)	15 037 (29.1)	1 589 262 (56.9)
Unknown	13/4601 (0.3)	270/51 956 (0.5)	17 280/2 808 323 (6.1)
Years of infertility, *n* (%)			[b]
1–2	1472 (51.2)	9194 (43.2)	
3–4	988 (34.4)	8257 (38.8)	
≥5	414 (14.4)	3851 (18.1)	
Unknown	1727/4601 (37.5)	30 654/51 956 (59.0)	
Cause of infertility, *n* (%)			[b]
Male factor	1158 (32.4)	17 501 (41.6)	
Tubal factor	383 (10.7)	7179 (17.1)	
Endometriosis	244 (6.8)	2926 (6.9)	
Anovulation	668 (18.7)	6622 (15.7)	
Unknown	1025/4601 (22.3)	9854/51 956 (19.0)	

Continued

Table I Continued.

Singletons	Blastocyst transfer *n* = 4601	Cleavage stage transfer *n* = 51 956	Spontaneous conception *n* = 2 808 323
ART method, *n* (%)			[b]
IVF	2724 (57.2)	29 693 (59.2)	
ICSI	1877 (42.8)	22 263 (40.8)	
Number of aspirated oocytes, *n* (%)[a]			[b]
1–3	71 (1.7)	3691 (9.0)	
4–9	1270 (29.6)	18 962 (46.1)	
10–15	1949 (45.4)	13 371 (32.5)	
≥16	1003 (23.4)	5141 (12.5)	
Unknown	308/4601 (6.7)	10 791/51 956 (20.8)	
Source of semen, *n* (%)			[b]
Homologous	3039 (98.8)	15 755 (94.4)	
Donated	38 (1.2)	930 (5.6)	
Unknown	1524/4601 (33.1)	35 271/51 956 (67.9)	
SET, *n* (%)	3740 (81.3)	28 448 (55.7)	[b]
Unknown	0/4601 (0.0)	850/51956 (1.6)	

IQR, interquartile range; *SET*, single embryo transfer;
[a]no data available from Norway;
[b]not relevant.

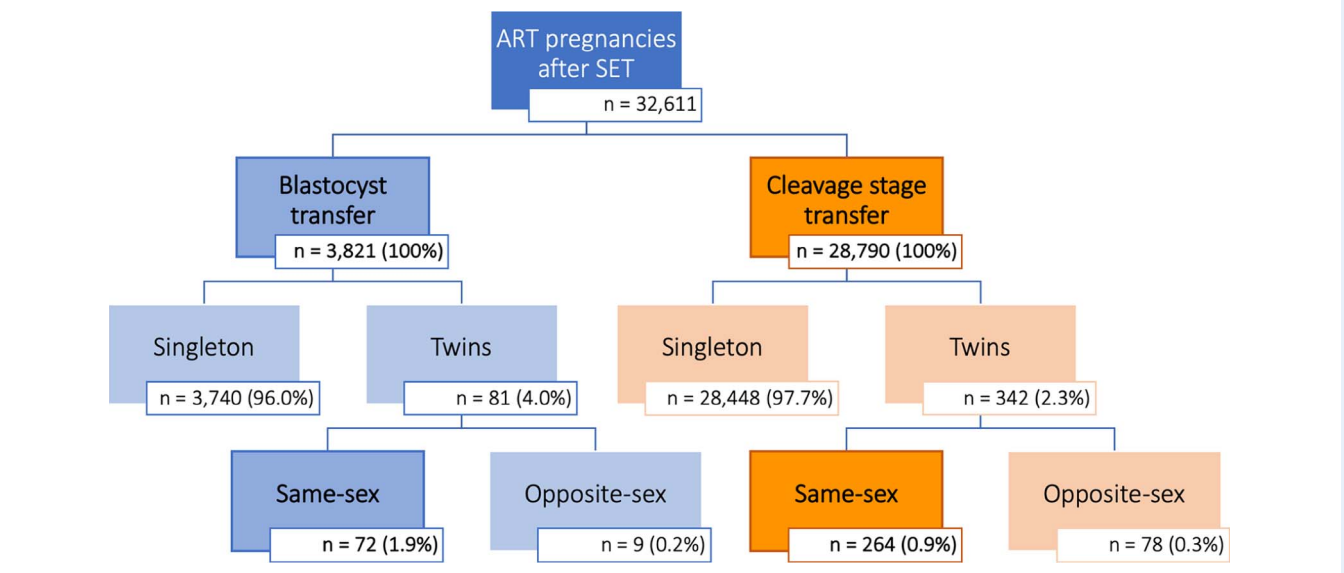

Figure 1 Pregnancies with same-sex and opposite-sex twins after ART and single embryo transfer (SET)—blastocyst and cleavage stage transfer during the period from 1997a to 2015 in Denmark, Norway and Sweden.

(LGA), Apgar (Appearance, Pulse, Grimace, Activity and Respiration) score at 5 min <7, any birth defects (ICD-10 codes Q00–Q99), stillbirth, perinatal and neonatal death and same-sex twins after transfer of a single embryo (SET).

For the IVF/ICSI population, gestational age was calculated based on the date of oocyte pick-up (OPU). For Denmark and Norway, gestational age in the IVF/ICSI population was also calculated based on second-trimester ultrasonography (US) scan. For all countries, the US scan was used to calculate gestational age in the SC population. SGA and LGA were defined as <−2SD and >+2SD from the expected sex-specific birth weight for the given gestational age (Marsal *et al.*, 1996). In the analyses on birth weight and gestational age, we excluded all stillbirths.

Stillbirth was defined as follows due to differences in registration between the countries during the study period: Denmark, pregnancies >28 completed gestational weeks before 1 January 2004 and >22

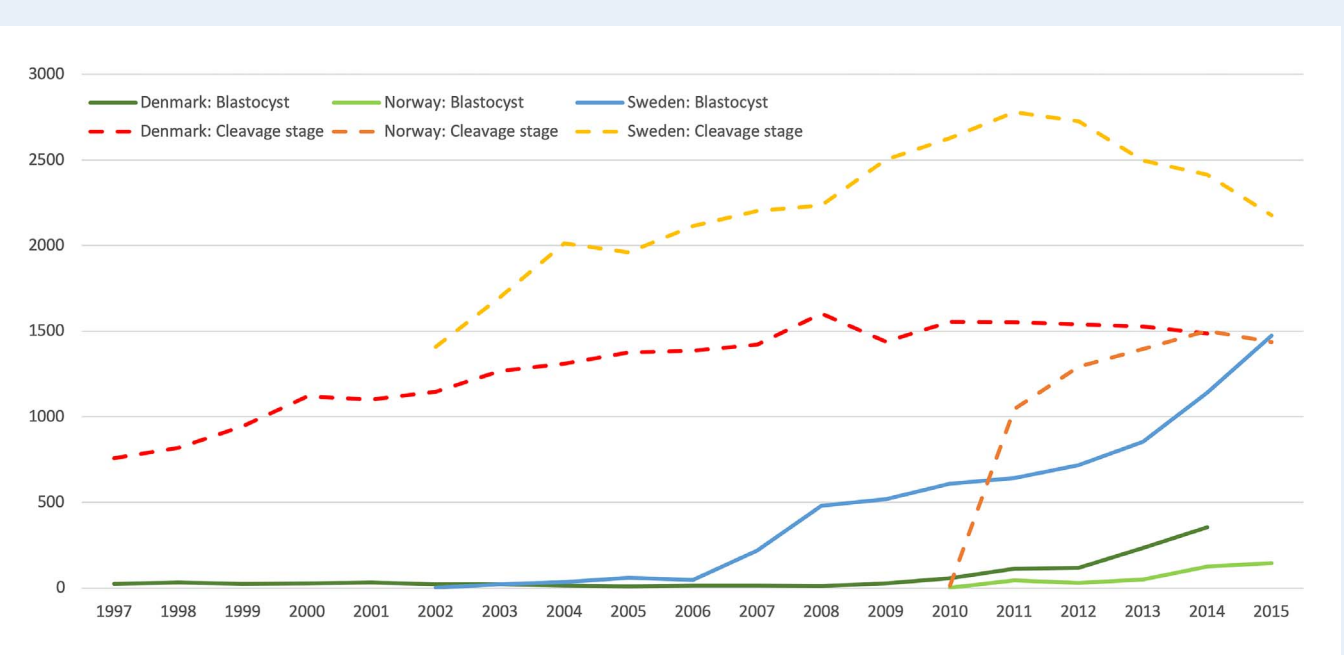

Figure 2 Number of children conceived per year after fresh blastocyst and cleavage stage transfer during the period from 1997b to 2015 in Denmark, Norway and Sweden.

completed gestational weeks after 1 January 2004; Norway, pregnancies >22 completed gestational weeks throughout the study period; and Sweden: pregnancies >28 completed gestational weeks before 1 July 2008 and >22 completed gestational weeks from 1 July 2008. Perinatal death was defined as stillbirth or live birth with death from Day 0 to 6 and neonatal death was defined as live birth with death from Day 0 to 27. Analyses on PPH were restricted to the birth years 2010–2015 for Denmark and Norway due to differences in registration practice throughout the study period. Furthermore, we analysed the risk of pregnancies with same-sex twins after transfer of a SET as a proxy for the rate of MZT, Figure 1.

Statistical analyses and covariates

Descriptive statistics of maternal and treatment characteristics were made for singletons, BT versus CT and BT versus SC using Student's *t*-test for normally distributed continuous data, the Mann–Whitney U test for non-normally distributed continuous data and the Chi-square test for categorical data.

Descriptive statistics are presented as numbers and percentages, means and SDs or medians and interquartile ranges.

Obstetric and perinatal outcomes were compared for singletons. In the twin cohort, we analysed the risk of pregnancies with same-sex twins after SET. All comparisons were made using multiple logistic regression analyses. An adjusted *p*-value <0.05 was considered statistically significant. All analyses were conducted using RStudio, version 3.6.1 (R Core Team, 2018) and the MASS package was used for multiple logistic regression analyses (Venables and Ripley, 2002).

Multiple regression models

In the main model, we adjusted for child's sex (categorical: male/female), birth year (categorical: 1997–2000; 2001–2005; 2006–2010; 2011–2015), parity (categorical: 0 or >1), maternal age (categorical: <25; 25–29; 30–34; 35–39; >40 years), body mass index (categorical: <18.5; 18.5–24.9; 25–29.9; >30 kg/m^2), smoking (categorical: yes/no) and country (categorical). For the comparisons of BT and CT we additionally adjusted for fertilization method (categorical: IVF/ICSI) and SET (categorical: yes/no). Pregnancies with missing values (BT versus CT, $n = 13\,566$ [24.0%]; BT versus SC, $n = 775\,296$ [27.6%]) were excluded from the analyses (Table I).

Due to missing data in some covariates in the main model, we made sensitivity analyses only including child's sex, birth year, parity, maternal age and country. For the comparisons of BT versus CT, we additionally adjusted for fertilization method. Pregnancies with missing values (BT versus CT, $n = 283$ [0.5%]; BT versus SC, ($n = 17\,295$ [0.4%]) were excluded from the analyses (Table I).

Sensitivity analyses on children born in Denmark and Sweden enabled further adjustments for maternal educational level (categorical: low, middle, high) and for comparisons of BT and CT also the number of aspirated oocytes (categorical: 1–3, 4–9, 10–15, >16). This information was not available from Norway.

Ethics approval

In Sweden, permission for data retrieval and linkage was obtained from the scientific Ethics Committee in Gothenburg (Dnr 304/06, T109-08, T087-12 and Dnr 214-12, T422-12, T516-15, T233-16, T300-17, T1144-17, T121-18), the National Board of Health and Welfare and Statistics Sweden. According to Danish legislation, studies based solely on register data, and with no personal involvement of the participants, do not require approval from a scientific ethics committee. For Denmark, data retrieval was approved by the Data Protection Agency (DT-journal number, 2012-58-0004; local journal number, AHH-2016-033;

Table II Logistic regression analyses—obstetric and perinatal outcomes in singleton deliveries from 1997 to 2015 conceived after: blastocyst transfer ($n = 4601$) vs cleavage stage transfer ($n = 51\,956$).

Outcome	Singletons		Blastocyst transfer vs Cleavage stage transfer		
	Blastocyst transfer *n* (%)	Cleavage stage transfer *n* (%)	Crude OR (95% CI)	Adjusted OR (95% CI)*	*P*-value*
Obstetric					
HDP	243 (5.3)	2852 (5.5)	0.96 (0.84; 1.10)	1.08 (0.93; 1.25)	0.32
Preeclampsia	166 (3.6)	2070 (4.0)	0.90 (0.77; 1.06)	1.05 (0.88; 1.25)	0.57
Eclampsia	4 (0.1)	42 (0.1)	1.08 (0.32; 2.66)	NA	NA
Placental abruption	51 (1.1)	449 (0.9)	1.29 (0.95; 1.70)	1.37 (0.98; 1.87)	0.06
Placenta previa	188 (4.1)	959 (1.8)	2.27 (1.93; 2.65)	2.11 (1.76; 2.52)	<0.001
PPROM	226 (4.9)	3537 (6.8)	0.71 (0.61; 0.81)	1.03 (0.88; 1.21)	0.69
Induction of labor	866 (18.8)	9995 (19.2)	0.97 (0.90; 1.05)	0.91 (0.83; 0.99)	0.03
C-section	1242 (27.0)	13 409 (25.8)	1.06 (0.99; 1.14)	1.09 (1.01; 1.18)	0.03
PPH	508 (11.0)	5555 (10.7)	1.04 (0.94; 1.14)	1.09 (0.98; 1.22)	0.11
>500 ml[a]	274 (27.5)	3017 (25.5)	1.11 (0.96; 1.28)	1.18 (1.00; 1.38)	0.05
>1000 ml[b]	217 (6.5)	1567 (6.4)	1.03 (0.88; 1.18)	1.06 (0.94; 1.19)	0.34
Perinatal					
Sex (male/female)	2477/2124 (53.8)	26 427/25 529 (51.0)	1.13 (1.06; 1.20)	1.13 (1.06; 1.21)	<0.001
PTB (*OPU*)	337 (8.3)	4054 (7.9)	1.05 (0.94; 1.17)	1.14 (1.01; 1.29)	0.03
VPTB (*OPU*)	68 (1.5)	708 (1.4)	1.08 (0.84; 1.38)	1.32 (0.99; 1.73)	0.05
PTB (*US*)[a]	102 (8.1)	2277 (8.4)	0.96 (0.78; 1.17)	1.08 (0.84; 1.36)	0.54
VPTB (*US*)[a]	24 (1.9)	429 (1.6)	1.21 (0.77; 1.78)	1.39 (0.84; 2.16)	0.17
LBW <2500 g	267 (5.8)	3036 (5.9)	0.99 (0.87; 1.12)	1.10 (0.95; 1.27)	0.18
VLBW <1500 g	50 (1.1)	629 (1.2)	0.89 (0.66; 1.18)	1.03 (0.73; 1.42)	0.86
Macrosomia >4000 g	649 (14.1)	7024 (13.5)	1.05 (0.96; 1.14)	1.03 (0.94; 1.14)	0.49
Macrosomia >4500 g	123 (2.7)	1236 (2.4)	1.13 (0.93; 1.35)	1.11 (0.89; 1.37)	0.34
SGA (*OPU*)	215 (4.7)	3021 (5.9)	0.79 (0.68; 0.91)	0.86 (0.73; 1.00)	0.06
LGA (*OPU*)	222 (4.9)	2137 (4.2)	1.18 (1.02; 1.35)	1.12 (0.95; 1.32)	0.17
SGA (*US*)[a]	69 (5.5)	1403 (5.2)	1.06 (0.82; 1.35)	1.14 (0.85; 1.50)	0.38
LGA (*US*)[a]	51 (4.0)	1006 (3.7)	1.09 (0.81; 1.44)	1.06 (0.70; 1.53)	0.78
Apgar 5 min <7	49 (1.3)	470 (1.5)	0.84 (0.62; 1.13)	0.98 (0.70; 1.34)	0.90
Birth defect, any	232 (5.0)	3183 (6.1)	0.81 (0.71; 0.93)	0.90 (0.77; 1.05)	0.17
Stillbirth	17 (0.4)	207 (0.4)	0.93 (0.54; 1.47)	NA	NA
Perinatal death	27 (0.6)	333 (0.6)	0.92 (0.60; 1.33)	1.46 (0.72; 2.66)	0.25
Neonatal death	12 (0.3)	149 (0.3)	0.91 (0.48; 1.57)	1.63 (0.81; 3.00)	0.14

HDP, hypertensive disorders in pregnancy; *PPROM*, preterm prelabour rupture of membranes; *PPH*, post-partum hemorrhage; *PTB*, preterm birth; *US*, gestational age calculated based on second-trimester ultrasonography scan; *VPTB*, very preterm birth; *OPU*, gestational age calculated based on oocyte pick up; *LBW*, low birthweight; *VLBW*, very low birthweight; *SGA*, small for gestational age; *LGA*, large for gestational age; *Apgar*, appearance, pulse, grimace, activity and respiration; OR, Odds ratio; *NA*, no adjustment made because of few events reported

Data were compared using generalized linear models, including the following covariates:

*Adjusted for the following covariates: child sex, birth year, parity, maternal age, BMI and smoking, fertilization method, SET and country;

[a]no data available from Sweden;

[b]no data available from Denmark and Norway.

I-suite number, 04790). For Norway, ethical approval was given by the Regional Committee for Medical and Health Research Ethics (REK-Nord, 2010/1909).

Results

BT is gradually replacing CT in the Nordic countries (Fig. 2).

Mean maternal age was similar in singleton pregnancies born after BT and CT, but 3.5 years higher when BT was compared with SC. Maternal BMI was similar for singletons in the BT and CT group, but lower in the SC group. Fewer mothers conceiving after BT smoked during pregnancy and their educational level was higher compared with mothers conceiving after CT or SC.

Fewer children born after BT were the first-born child compared with CT but more often the first-born child compared with SC children. Children born after BT were more often born after ICSI and SET and their mothers were more likely to have more than 10 oocytes aspirated compared with mothers of CT singletons (Table I).

Table III Logistic regression analyses—obstetric and perinatal outcomes in singleton deliveries from 1997 to 2015 conceived after: blastocyst transfer ($n = 4601$) vs spontaneous conception ($n = 2\,808\,323$).

Outcome	Singletons		Blastocyst transfer vs Spontaneous conception		
	Blastocyst transfer *n* (%)	Spontaneous conception *n* (%)	Crude OR (95% CI)	Adjusted OR (95% CI)*	*P*-value
Obstetric					
HDP	243 (5.3)	115 299 (4.1)	1.30 (1.14; 1.48)	1.03 (0.90; 1.18)	0.64
Preeclampsia	166 (3.6)	81 763 (2.9)	1.25 (1.06; 1.45)	1.06 (0.90; 1.24)	0.49
Eclampsia	4 (0.1)	1469 (0.1)	1.66 (0.52; 3.87)	1.43 (0.44; 3.34)	0.48
Placental abruption	51 (1.1)	12 632 (0.4)	2.48 (1.86; 3.23)	2.59 (1.90; 3.44)	<0.001
Placenta previa	188 (4.1)	10 203 (0.4)	11.68 (10.05; 13.49)	9.52 (8.10; 11.12)	<0.001
PPROM	226 (4.9)	140 330 (5.0)	0.98 (0.86; 1.12)	1.08 (0.92; 1.25)	0.33
Induction of labor	866 (18.8)	405 587 (14.4)	1.37 (1.27; 1.48)	1.05 (0.97; 1.14)	0.22
C-section	1242 (27.0)	473 712 (16.9)	1.82 (1.71; 1.94)	1.36 (1.27; 1.46)	<0.001
PPH	508 (11.0)	229 174 (8.2)	1.40 (1.27; 1.53)	1.21 (1.09; 1.33)	<0.001
>500 ml[a]	274 (27.5)	118 189 (19.3)	1.58 (1.37; 1.81)	1.27 (1.09; 1.47)	0.002
>1000 ml[b]	217 (6.5)	70 560 (5.0)	1.31 (1.14; 1.50)	1.10 (0.95; 1.26)	0.20
Perinatal					
Sex (male/female)	2477/2124 (53.8)	1 443 596/1 364 727 (51.4)	1.10 (1.04; 1.17)	1.10 (1.03; 1.17)	0.004
PTB	337 (8.3)	67 883 (4.9)	1.70 (1.38; 2.07)	1.65 (1.47; 1.85)	<0.001
VPTB	68 (1.5)	9872 (0.7)	2.69 (1.74; 3.93)	2.17 (1.65; 2.78)	<0.001
LBW <2500 g	267 (5.8)	89 941 (3.2)	1.85 (1.63; 2.09)	1.71 (1.50; 1.95)	<0.001
VLBW <1500 g	50 (1.1)	15 589 (0.6)	1.96 (1.46; 2.55)	1.81 (1.31; 2.44)	<0.001
Macrosomia >4000 g	649 (14.1)	507 800 (18.1)	0.74 (0.68; 0.81)	0.83 (0.76; 0.91)	<0.001
Macrosomia >4500 g	123 (2.7)	95 440 (3.4)	0.78 (0.65; 0.93)	0.86 (0.70; 1.04)	0.13
SGA	215 (4.7)	95 421 (3.4)	1.32 (1.14; 1.51)	1.16 (1.00; 1.34)	0.05
LGA	222 (4.9)	127 961 (4.6)	0.93 (0.81; 1.07)	1.14 (0.97; 1.34)	0.10
Apgar 5 min <7	49 (1.3)	20 496 (1.2)	1.11 (0.83; 1.46)	0.91 (0.66; 1.22)	0.56
Birth defect, any	232 (5.0)	126 428 (4.5)	1.13 (0.98; 1.28)	1.10 (0.95; 1.27)	0.18
Stillbirth	17 (0.4)	9827 (0.3)	1.06 (0.63; 1.64)	1.09 (0.18; 3.39)	0.90
Perinatal death	27 (0.6)	13 687 (0.5)	1.21 (0.80; 1.72)	1.84 (0.95; 3.17)	0.04
Neonatal death	12 (0.3)	4915 (0.2)	1.49 (0.80; 2.51)	2.10 (1.08; 3.61)	0.01

UL, gestational age calculated based on second-trimester ultrasonography scan; *NA*, no adjustment made because of few events reported;
Data were compared using generalized linear models, including the following covariates:
*Adjusted for the following covariates: child sex, birth year, parity, maternal age, BMI and smoking and country.
[a]No data available from Sweden;
[b]no data available from Denmark and Norway.

Singletons born after blastocyst transfer compared with singletons born after cleavage stage transfer

In singleton pregnancies, the risk of placenta previa was higher for BT versus CT (adjusted odds ratio [aOR] 2.11 [95% CI 1.76; 2.52]). Likewise, the risk of caesarean section (aOR 1.09 [95% CI 1.01; 1.18]) was higher, whereas a lower risk of induction of labour (aOR 0.91 [95% CI 0.83; 0.99]) was observed after BT compared with CT (Table II).

When gestational age was calculated based on OPU, singletons conceived after BT had a higher risk of PTB (aOR 1.14 [95% CI 1.01; 1.29]). The odds of male infants was higher after BT (aOR 1.13 [95% CI 1.06; 1.21]) compared with CT singletons (Table II). We found no increased risks of perinatal or neonatal deaths after BT (Table II).

When gestational age was calculated based on OPU, the risk of PTB was not significant in analyses including restricted covariates (Supplementary Table SI). Sensitivity analyses restricted to Swedish and Danish data including adjustment for maternal educational level and the number of aspirated oocytes did not change the results.

Singletons born after blastocyst transfer compared with spontaneously conceived singletons

Singleton pregnancies after BT had a higher risk of placental abruption (aOR 2.59 [95% CI 1.90; 3.44]), placenta previa (aOR 9.52 [95% CI 8.10; 11.12]), caesarean section (aOR 1.36 [95% CI 1.27; 1.46]) and PPH (aOR 1.21 [95% CI 1.09; 1.33]) compared with SC singletons (Table III).

Singletons conceived after BT had a higher risk of PTB (aOR 1.65 [95% CI 1.47; 1.85]) VPTB (aOR 2.17 [95% CI 1.65; 2.78]), LBW (aOR 1.71 [95% CI 1.50; 1.95]) and VLBW (aOR 1.81 [95% CI 1.31; 2.44]) but a lower risk of macrosomia >4000 g (aOR 0.83 [95% CI 0.76; 0.91]). The odds of male infants was higher (aOR 1.10 [95% CI 1.03; 1.17]) compared with SC singletons (Table III). We found a higher risk of neonatal death (aOR 2.10 [95% CI 1.08; 3.61]) comparing singletons conceived after BT versus SC (Table III).

Analyses including restricted covariates did not change the results except for the risk of neonatal death, which was no longer significant (Supplementary Table SII). In sensitivity analyses, restricted to Swedish and Danish data including adjustment for maternal educational, the risk of SGA (aOR 1.22 [95% CI 1.04; 1.42]) was increased and the risk of macrosomia >4500 g (aOR 0.81 [95% CI 0.66; 0.99]) was decreased after BT compared with SC (Supplementary Table SII).

Monozygotic twinning

Transferring a single embryo resulted in 1.9% and 0.9% same-sex twin pregnancies in the BT and CT group, respectively (Fig. 1). The risk of having same-sex twins after SET was significantly increased after BT (aOR 1.94 [95% CI 1.42; 2.60]) compared with CT. Sensitivity analyses including restricted covariates or analyses on data from Denmark and Sweden did not change the results. However, in sensitivity analyses on data from Denmark and Sweden we were not able to adjust for the maternal educational level due to few events (data not shown). Risk of pregnancies with opposite-sex twins after SET was similar for the two groups (0.2% versus 0.3% for BT and CT, respectively).

Discussion

In this Nordic registry-based cohort study including data on all IVF/ICSI children conceived after fresh BT and CT in Denmark, Norway and Sweden during 1997–2015, we found a higher risk of placenta previa and PTB when gestational age was calculated based on OPU comparing BT to CT. Additionally, we found a higher male/female ratio and an increased risk of same-sex twins after single embryo fresh BT compared with single embryo fresh CT.

Strengths and limitations

A major strength of this study is the inclusion of the complete birth cohorts of singletons and twins conceived after fresh BT and CT in Denmark, Norway and Sweden. The Nordic countries are comparable in terms of demography, health care and social security systems including reimbursement of IVF treatment, which justifies analyses on pooled data. The Nordic registries include information on important factors known to have an impact on obstetric and perinatal outcomes, allowing us to adjust for potential confounders. Moreover, the risk of selection bias is minimized in nationwide studies.

In the main analysis, we were able to adjust for a wide range of covariates; however, 24% of pregnancies were excluded from the analyses due to missing values in the comparisons of BT and CT singletons. Sensitivity analyses including only variables with very few missing data (<0.5%) and sensitivity analyses restricted to data from Denmark and Sweden including further adjustments for maternal educational level and the number of aspirated oocytes resulted in the same conclusions as the main analyses (Supplementary Table SI–SII). Hence, we consider that our analyses are robust regarding missing values. Furthermore, we have no reason to assume an unequal distribution of missing data between the BT and the CT population. However, we cannot exclude that residual confounding may be present, as we were not able to adjust for the duration of infertility or cause of infertility due to missing data. If an adjustment for duration of infertility had been possible, one may have expected an increased difference between the two groups since the women in the BT cohort had a shorter history of infertility compared with women in the CT cohort.

We could not confirm MZT by genetic testing but used same-sex twins after SET as a proxy for MZ twinning. Although we cannot exclude same-sex dizygotic twins (DZT) as a potential source of misclassification in these analyses, any DZT following IVF/ICSI treatment with SET must result from a simultaneous spontaneous conception or from a transfer of more than one embryo (despite registered as a SET), regardless of whether BT or CT is performed. The fact that the risk of opposite-sex twins after SET (obligate DZT) was similar for BT and CT suggests that same-sex DZT does not explain the higher risk of same-sex twins following single blastocyst transfer.

Meaning of the findings in relation to other studies—obstetric and perinatal outcomes

Obstetric outcomes in pregnancies after BT have been investigated in previous cohort studies (Sazonova *et al*., 2011; Fernando *et al*., 2012; Ishihara *et al*., 2014; Oron *et al*., 2014; Ginström Ernstad *et al*., 2016). Placental complications (placenta previa and/or placental abruption) were not associated with BT in three studies from Australia (fresh BT, $n = 1279$), Japan (fresh BT, $n = 5981$) and Canada (fresh BT, $n = 279$) (Fernando *et al*., 2012; Ishihara *et al*., 2014; Oron *et al*., 2014). The inconsistency in the risk of placenta previa between these studies and ours may be due to limited sample sizes in the previous studies. The aetiology of placenta previa remains unclear, but advanced maternal age, smoking, multiparity, male offspring, prior caesarean section or abortion and IVF/ICSI treatment are found to be associated with placenta previa (Faiz and Ananth 2003; Karami *et al*., 2018). The three previous studies were all able to control for the known important factors related to placenta previa (Fernando *et al*., 2012; Ishihara *et al*., 2014; Oron *et al*., 2014). In the Japanese study, the risk of placenta previa was low (0.80% and 0.88% after BT and CT, respectively) compared with our study (4.1% and 1.8% after BT and CT, respectively) (Ishihara *et al*., 2014). Hence, the different findings might be explained by varying diagnostic criteria for placenta previa or different prenatal surveillance between the countries. In line with our study, two studies showed no association between BT and preeclampsia (Fernando *et al*., 2012; Oron *et al*., 2014). Moreover, Fernando *et al*., (2012) investigated the risk of postpartum haemorrhage, and Ishihara *et al*., (2014) investigated the risk of pregnancy-induced hypertension and found no significant associations (Fernando *et al*., 2012; Ishihara *et al*., 2014).

Perinatal outcomes in children conceived after BT have been reviewed in a recent meta-analysis by Alviggi *et al*., (2018) including 14 cohort studies with 90 105 and 103 677 singleton pregnancies after BT and CT, respectively (Alviggi *et al*., 2018). In the pooled analyses children conceived after fresh BT had a significantly higher risk of PTB (Risk ratio (RR) 1.15 [95% CI 1.05; 1.25]) and VPTB (RR 1.16 [95% CI 1.02; 1.31]) compared with those conceived after fresh CT (Alviggi

et al., 2018). These findings are in line with our results when gestational age was calculated based on OPU but not when based on the US scan.

However, Alviggi et al., (2018) also reported that children conceived after fresh BT had a lower risk of SGA, which is in contrast to our study, where the risk of SGA was similar after fresh BT and CT. In line with our study Alviggi et al., (2018) found a similar risk of LGA after fresh BT compared with fresh CT. Although 14 cohort studies were eligible for the meta-analyses by Alviggi et al., (2018), the authors concluded that the findings should be interpreted with caution, due to a low level of evidence and a wide study heterogeneity.

Two Swedish registry-based studies, which included two sub-cohorts of our study (birth cohorts 2002–2006 [fresh BT, $n = 1026$] and 2002–2013 [fresh BT, $n = 3026$], respectively), found, in line with our study, an increased risk of placenta previa in singletons conceived after BT compared with CT when adjusting for transfer of a fresh/frozen embryo (Sazonova et al., 2011; Ginström Ernstad et al., 2016). The most recent Swedish study by Ginström Ernstad et al., (2016) also showed an increased risk of placental abruption after BT, which could not be confirmed in our study. The differences in placental abruption may be due to differences in registration practices between countries. Similarly, to our study, Ginström Ernstad et al., (2016) found no association between BT and preeclampsia. Although gestational age was calculated according to OPU, they found no increased risk of PTB (aOR 0.97 [0.72; 1.31]), in contrast to our study (Ginström Ernstad et al., 2016).

The higher risk of placenta previa and PTB after BT indicates that extended culture time may interfere with implantation and placentation. Extended embryo culture increases the exposure to the potential external stressors such as temperature, pH and oxygen concentration. Moreover, culture media may influence the expression of specific genes expressions (Kleijkers et al., 2015) and impact perinatal outcomes (Dumoulin et al., 2010; Kleijkers et al., 2014).

Blastocyst transfer reduces the time for embryo and endometrium communication prior to implantation, which may compromise their synchronisation. Furthermore, a study of bovine showed that transfer of a blastocyst embryo triggered genetic and epigenetic changes in trophectoderm cells (Rizos et al., 2002).

In line with most previous studies, male/female-ratio was higher following BT compared to CT (Luna et al., 2007; Dean et al., 2010; Tarin et al., 2014; Sotiroska et al., 2015; Hattori et al., 2019) while smaller studies have found an unchanged male/female ratio after BT and CT (Richter et al., 2006; Csokmay et al., 2009; Weston et al., 2009). Potential faster growth of male embryos may lead to over-representation of blastocysts of male origin; supporting this theory is one study in which male blastocysts reached higher embryo morphologic scorings and were prioritized higher than female embryos (Alfarawati et al., 2011). Tarín et al., (2014) have suggested that post-implantation foetal demise is higher in female pregnancies from BT, possibly due to abnormal inactivation of the X-chromosomes in blastocysts. Thus, the skewed male/female ratio is not yet fully explained.

The association between BT and risk of MZT has been confirmed in a recent meta-analysis by Hviid et al., (2018), including 21 original articles, which revealed a 2-fold increase in the risk of MZT after BT. The majority of previous studies used the number of fetal heartbeats seen at ultrasound scans in relation to the number of embryos transferred as a proxy for MZT (Luke et al., 2014; Nakasuji et al., 2014; Mateizel et al., 2016; Hviid et al., 2018). In two recent studies, MZT was diagnosed as pregnancies in which the number of foetuses exceeded the number of gestational sacs (Ikemoto et al., 2018; Hattori et al., 2019). Ikemoto et al., 2018 found an adjusted OR of 2.20 (95% CI 1.83; 2.66) for same-sex twins after blastocyst SET, which is comparable to our findings.

Conclusion

In conclusion, we found that fresh BT is associated with a higher risk of placenta previa, PTB, higher male/female-ratio and higher same-sex twin rate after SET compared with CT.

Wider implications of the findings

Safety aspects are of utmost interest to both the infertile couples and to society as well as to health care professionals. Different IVF/ICSI techniques, such as BT, need surveillance to ensure implementation and safety.

Blastocyst culture plays a crucial role in IVF/ICSI treatment. In combination with vitrification, BT has increased survival rates of frozen-thawed embryos, hence facilitates the use of single BT and reduces the overall occurrence of twin births. However, our results taken together with findings from other large cohort studies on adverse perinatal outcomes after BT should be taken into consideration in line with pregnancy and live birth rates when deciding on clinical embryo transfer strategies. With these finding it is difficult to justify a BT policy for all IVF/ICSI patients, rather BT should be considered when there is a need for embryo selection to lower the time to live birth.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

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Authors' roles

All authors participated in the study design and interpretation of the data. ALS analysed the data and drafted the first manuscript. JF contributed to statistical analyses. The manuscript was finalized and approved by all co-authors.

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Conflict of interest

None of the authors has any conflicts of interests to declare regarding this study.

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