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Perinatal and maternal outcome after vitrification of blastocysts: a Nordic study in singletons from the CoNARTaS group

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STUDY QUESTION: Is transfer of vitrified blastocysts associated with higher perinatal and maternal risks compared with slow-frozen cleavage stage embryos and fresh blastocysts?

SUMMARY ANSWER: Transfer of vitrified blastocysts is associated with a higher risk of preterm birth (PTB) when compared with slow-frozen cleavage stage embryos and with a higher risk of a large baby, hypertensive disorders in pregnancy (HDPs) and postpartum hemorrhage (PPH) but a lower risk of placenta previa when compared with fresh blastocysts.

WHAT IS KNOWN ALREADY: Transfer of frozen-thawed embryos (FETs) plays a central role in modern fertility treatment, limiting the risk of ovarian hyperstimulation syndrome and multiple pregnancies. Following FET, several studies report a lower risk of PTB, low birth weight (LBW) and small for gestational age (SGA) yet a higher risk of fetal macrosomia and large for gestational age (LGA) compared with fresh embryos. In recent years, the introduction of new freezing techniques has increased treatment success. The slow-freeze technique combined with cleavage stage transfer has been replaced by vitrification and blastocyst transfer. Only few studies have compared perinatal and maternal outcomes after vitrification and slow-freeze and mainly in cleavage stage embryos, with most studies indicating similar outcomes in the two groups. Studies on perinatal and maternal outcomes following vitrified blastocysts are limited.

STUDY DESIGN, SIZE, DURATION: This registry-based cohort study includes singletons born after frozen-thawed and fresh transfers following the introduction of vitrification in Sweden and Denmark, in 2002 and 2009, respectively. The study includes 3650 children born after transfer of vitrified blastocysts, 8123 children born after transfer of slow-frozen cleavage stage embryos and 4469 children born after transfer of fresh blastocysts during 2002–2015. Perinatal and maternal outcomes in singletons born after vitrified blastocyst transfer were compared with singletons born after slow-frozen cleavage stage transfer and singletons born after fresh blastocyst transfer. Main outcomes included PTB, LBW, macrosomia, HDP and placenta previa.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Data were obtained from the CoNARTaS (Committee of Nordic ART and Safety) group. Based on national registries in Sweden, Finland, Denmark and Norway, the CoNARTaS cohort includes all children born after ART treatment in public and private clinics 1984–2015. Outcomes were assessed with logistic multivariable regression analysis, adjusting for the country and year of birth, maternal age, body mass index, parity, smoking, parental educational level, fertilisation method (IVF/ICSI), single embryo transfer, number of gestational sacs and the child's sex.

MAIN RESULTS AND THE ROLE OF CHANCE: A higher risk of PTB (<37 weeks) was noted in the vitrified blastocyst group compared with the slow-frozen cleavage stage group (adjusted odds ratio, aOR [95% CI], 1.33 [1.09–1.62]). No significant differences were observed for

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LBW (<2500 g), SGA, macrosomia (≥4500 g) and LGA when comparing the vitrified blastocyst with the slow-frozen cleavage stage group. For maternal outcomes, no significant difference was seen in the risk of HDP, placenta previa, placental abruption and PPH in the vitrified blastocyst versus the slow frozen cleavage stage group, although the precision was limited.
When comparing vitrified and fresh blastocysts, we found higher risks of macrosomia (≥4500 g) aOR 1.77 [1.35–2.31] and LGA aOR 1.48 [1.18–1.84]. Further, the risks of HDP aOR 1.47 [1.19–1.81] and PPH aOR 1.68 [1.39–2.03] were higher in singletons born after vitrified compared with fresh blastocyst transfer while the risks of SGA aOR 0.58 [0.44–0.78] and placenta previa aOR 0.35 [0.25–0.48] were lower.

LIMITATIONS, REASONS FOR CAUTION: Since vitrification was introduced simultaneously with blastocyst transfer in Sweden and Denmark, it was not possible to explore the effect of vitrification *per se* in this study.

WIDER IMPLICATIONS OF THE FINDINGS: The results from the change of strategy to vitrification of blastocysts are reassuring, indicating that the freezing technique *per se* has no major influence on the perinatal and maternal outcomes. The higher risk of PTB may be related to the extended embryo culture rather than vitrification.

STUDY FUNDING/COMPETING INTEREST(S): The study is part of the ReproUnion Collaborative study, co-financed by the European Union, Interreg V ÖKS. The study was also financed by grants from the Swedish state under the agreement between the Swedish government and the county councils, the ALF agreement (LUA/ALF 70940), Hjalmar Svensson Research Foundation and NordForsk (project 71 450). There are no conflicts of interest to declare.

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Introduction

Cryopreservation has become an important part of ART, and in several European countries frozen embryo transfer (FET) accounted for approximately 40% of all *in vitro* fertilisation (IVF) cycles in 2015 (De Geyter *et al.*, 2018). In the USA, the rate of FET has doubled since 2005, being 30% of the non-donor ART cycles in 2015 (cdc.gov). An important reason for this increase in FET is the change in transfer policy to single embryo transfer (SET), particularly in the Nordic countries (Thurin *et al.*, 2004), leading to more supernumerary embryos available for freezing. After the first successful cryopreservation of human embryos and the first live birth reported (Trounson and Mohr, 1983), various protocols for freezing have been introduced, differing particularly in type and concentration of cryoprotectant, cooling rates and type of device used. For more than 20 years, a slow-freezing technique using propylene glycol and sucrose as a cryoprotectant was widely used with the idea to permit cellular dehydration while minimising intracellular ice formation (Lassalle *et al.*, 1985, Testart *et al.*, 1986). Over the last decade, there has been a shift from conventional slow-freezing towards vitrification of human embryos, a cryopreservation method which turns the embryo into a glass-like state without formation of ice (Mukaida *et al.*, 1998, Kuwayama *et al.*, 2005). Compared with slow-freezing, vitrification has resulted in improved embryo survival rates and improved clinical pregnancy/live birth rates (Balaban *et al.*, 2008, Fasano *et al.*, 2014, Levron *et al.*, 2014, Li *et al.*, 2014, Debrock *et al.*, 2015, Rienzi *et al.*, 2017).

FET has been associated with a lower risk of preterm birth (PTB), low birth weight (LBW) and small for gestational age (SGA) yet a higher risk of large for gestational age (LGA) and macrosomia (≥4500 g) compared with fresh transfer. This has been summarised in a systematic review and meta-analysis by Maheshwari in 2018 (Maheshwari *et al.*, 2018). Regarding maternal outcomes, FET has been associated with a higher risk of hypertensive disorders in pregnancy (HDPs) compared with fresh transfers (Sazonova *et al.*, 2012, Opdahl *et al.*, 2015, Maheshwari *et al.*, 2018). However, the technique for cryopreservation and culture duration varies and most of the 26 included studies in this meta-analysis (Maheshwari *et al.*, 2018) used slow-freezing of cleavage stage embryos or a mix of the different techniques and only few studies used vitrification of either blastocysts (Pereira *et al.*, 2016), cleavage stage embryos (Aflatoonian *et al.*, 2010, Shi *et al.*, 2012, Aflatoonian *et al.*, 2016) or both (Kato *et al.*, 2012). These studies, most of them small (Aflatoonian *et al.*, 2010, Shi *et al.*, 2012, Pereira *et al.*, 2016), did not show any significant differences in the rate of PTB or LBW, except for the larger study by Kato (Kato *et al.*, 2012) finding a lower rate of LBW among children from cryopreserved embryos. Neither were any differences regarding maternal outcomes observed (Shi *et al.*, 2012).

Due to the rapid implementation of vitrification of blastocysts following the higher success rates, studies comparing perinatal and maternal outcomes between vitrified and slow-frozen blastocysts or cleavage stage embryos are rare. In a study from Australia (Li *et al.*, 2014), comparing 4721 singletons from vitrified blastocysts with 1965 singletons from slow-frozen blastocysts, no significant differences in perinatal outcome were found. We identified no studies that compared vitrified and slow-frozen blastocysts concerning maternal outcomes. In a small Swedish single-centre study (Wikland *et al.*, 2010), comparing vitrified blastocysts with slow-frozen cleavage stage embryos, the rate of SGA was lower and the rate of postpartum hemorrhage (PPH) was higher following vitrified blastocyst transfer while no other differences for perinatal or maternal outcomes were seen.

The aim of this study was to compare perinatal and maternal outcomes for singleton pregnancies following vitrification of blastocysts compared with slow-frozen cleavage stage transfers. A comparison was also made between singleton pregnancies following vitrified and fresh blastocyst transfer.

Materials and Methods

Set-up and study groups

The study population was based on a Nordic population-based cohort (CoNARTaS, Committee of Nordic ART and Safety), which includes all ART pregnancies in Sweden, Denmark, Finland and Norway

Table I Background characteristics in women giving birth to singletons conceived after vitrified blastocyst transfer (BT) compared with slow-frozen cleavage stage transfer (CT) and fresh BT, 2002–2015.

	Treatment		
	Vitrified BT	Slow-frozen CT	Fresh BT
N, total	3650	8123	4469
Maternal age, mean (±SD)	34.7 ± 4.2	34.8 ± 4.1	34.0 ± 4.3
BMI, median (IQR)	23 (21—26)	23 (21—26)	23 (21—26)
BMI missing, *N* (%)	226 (6.2)	583 (7.1)	268 (6.0)
Primiparous, *N* (%)	2196 (60.2)	4422 (54.5)	2874 (64.4)
Smoking, *N* (%)	44 (1.3)	177 (2.3)	76 (1.8)
Smoking missing, *N* (%)	151 (4.1)	364 (4.5)	164 (3.7)
Highest parental educational level			
≤9 years, *N* (%)	61 (1.7)	150 (1.9)	68 (1.5)
10–12 years, *N* (%)	769 (21.3)	2286 (28.4)	1146 (26.0)
Higher education <3 years, *N* (%)	502 (13.9)	1406 (17.5)	740 (16.8)
Higher education ≥3 years, *N* (%)	2281 (63.1)	4194 (52.2)	2460 (55.7)
Duration of infertility, median (years)	2 (2—4)	3 (2—4)	2(2—4)
Duration of infertility missing, *N* (%)	733 (20.1)	2726 (33.6)	1468 (32.8)
Country of birth of child			
Denmark, *N* (%)	63 (1.7)	1225 (15.1)	963 (21.5)
Sweden, *N* (%)	3587 (98.3)	6898 (84.9)	3506 (78.5)
Year of birth of child			
2002–2006, *N* (%)	18 (0.5)	1627 (20.0)	147 (3.3)
2007–2011, *N* (%)	977 (26.8)	3664 (45.1)	1823 (40.8)
2012–2015, *N* (%)	2655 (72.7)	2832 (34.9)	2499 (55.9)
ICSI, *N* (%)	1479 (40.5)	3037 (38.7)	1868 (41.8)
Number of embryos transferred, *N* (%)			
1	3482 (95.5)	5775 (72.4)	3813 (85.3)
2	165 (4.5)	2181 (27.3)	643 (14.4)
3	0 (0.0)	22 (0.3)	12 (0.1)
Number of gestational sacs			
1, *N* (%)	3432 (94.0)	6948 (85.5)	4205 (94.1)
≥2, *N* (%)	68 (1.9)	373 (4.6)	130 (2.9)
Number of gestational sacs missing, *N* (%)	150 (4.1)	802 (9.9)	134 (3.0)
Preimplantation genetic diagnosis	27 (0.7)	33 (0.4)	228 (5.1)

BT: blastocyst transfer, CT: cleavage stage transfer, SD: standard deviation, BMI: body mass index, IQR: interquartile range, ICSI: intracytoplasmic sperm injection.

(Henningsen *et al.*, 2011). The CoNARTaS cohort is composed of data from the national ART registries and Medical Birth Registries, all with high coverage rates (Henningsen *et al.*, 2011). By using the unique personal identification number assigned to every resident in the Nordic countries, individual follow-up is possible. In Norway, there was no registration on type of freezing procedure during the study period, and for Finland, it was not possible to separate fresh and frozen cycles. Therefore, only pregnancies from Sweden and Denmark were included. All singleton deliveries derived from autologous oocytes following the introduction of vitrification in Sweden in 2002–2015 and in Denmark in 2009–2014 were included. Data on deliveries included all live born children and stillbirths. Stillbirths were recorded at ≥22 completed gestational weeks during the entire study period in Denmark. In Sweden, the definition of stillbirth has changed during the study period. Before 01 July 2008, only stillbirths ≥28 who completed gestational weeks were registered as stillbirths, and from 01 July 2008, stillbirths ≥22 who completed gestational weeks were defined as stillbirths and thus included.

The national ART registries include information about treatment (fresh or frozen-thawed transfer), number of oocytes retrieved, fertilisation method (IVF or ICSI), freezing method (vitrification or slow-freeze), culture duration, date of embryo transfer, number of embryos transferred and number of gestational sacs. The Medical Birth Registries and the National Patient Registries provide data on maternal characteristics (i.e. age, parity, body mass index (BMI), smoking habits, all at first antenatal visit) and data on delivery and neonatal outcomes.

Table II Perinatal and maternal outcome in singleton pregnancies after vitrified blastocyst transfer (BT) compared with slow-frozen cleavage stage transfer (CT) and fresh BT, 2002–2015.

	Treatment		
	Vitrified BT	**Slow-frozen CT**	**Fresh BT**
N, total	3650	8123	4469
Male sex, *N* (%)	1951 (53.5)	4055 (49.9)	2386 (53.4)
Gestational age (days ±SD)	278.0 ± 14.6	278.4 ± 14.6	276.2 ± 15.1
≥42 weeks, *N* (%)	281 (7.7)	620 (7.6)	190 (4.3)
<37 weeks, *N* (%)	271 (7.4)	513 (6.3)	398 (8.9)
<32 weeks, *N* (%)	42 (1.2)	102 (1.3)	66 (1.5)
Mean birth weight (grams ±SD)	3595 ± 593	3581 ± 607	3432 ± 600
<2500 g, *N* (%)	127 (3.5)	304 (3.8)	256 (5.7)
<1500 g, *N* (%)	30 (0.8)	70 (0.9)	43 (1.0)
≥4000 g, *N* (%)	854 (23.4)	1865 (23.0)	645 (14.5)
≥4500 g, *N* (%)	183 (5.0)	410 (5.1)	117 (2.6)
SGA < −2 SD, *N* (%)	92 (2.5)	233 (2.9)	198 (4.4)
LGA > +2 SD, *N* (%)	247 (6.8)	513 (6.3)	181 (4.1)
Apgar score at 5 min			
<7, *N* (%)	76 (2.1)	122 (1.7)	46 (1.2)
<4, *N* (%)	22 (0.6)	24 (0.3)	12 (0.3)
Missing, *N* (%)	35 (1.0)	1066 (13.1)	639 (14.2)
Birth defects, any, *N* (%)	152 (4.2)	338 (4.2)	217 (4.9)
Stillbirth, *N* (%)	0 (0.0)	4 (0.0)	5 (0.1)
Perinatal death, *N* (%)	4 (0.1)	20 (0.2)	11 (0.2)
Neonatal death, *N* (%)	7 (0.2)	21 (0.3)	10 (0.2)
HDP, *N* (%)	258 (7.1)	534 (6.6)	231 (5.2)
Placental abruption, *N* (%)	20 (0.5)	42 (0.5)	49 (1.1)
Placenta previa, *N* (%)	58 (1.6)	82 (1.0)	182 (4.1)
PPH, *N** (%)	362 (10.1)	645 (9.4)	231 (6.6)
Induction of labour, *N* (%)	949 (26.0)	1964 (24.3)	865 (19.4)
Cesarean section, *N* (%)	1078 (29.6)	2205 (27.2)	1205 (27.0)

BT: blastocyst transfer, CT: cleavage stage transfer, SD: standard deviation, SGA/LGA: small/large for gestational age, HDP: hypertensive disorders in pregnancy, PPH: postpartum hemorrhage.
Maternal outcomes included placenta previa (ICD-10 code O44), placental abruption (ICD-10 code O45), hypertensive disorders in pregnancy (ICD-10 codes O13-O15), postpartum hemorrhage (ICD-10 code O72), induction of labour (ICD-10 code O61) and Cesarean section (ICD-10 code O82).
*Only Swedish data.

Information on birth defects is retrieved from the Registry of Birth Defects and the National Patient Registry for Sweden and from the National Patient Registry for Denmark. Information about parental educational level is obtained from Statistics Sweden and Statistics Denmark.

During recent years, there has been a gradual shift from cleavage stage transfer and slow-freezing to vitrification of blastocysts in the Nordic countries. In the Nordic countries, cleavage stage transfer is mainly performed on Day 2. Today, the majority of embryos transferred in FET are vitrified blastocysts with some inter-clinical differences. If few embryos are available on Day 2, most clinics transfer a fresh embryo on Day 2 and culture surplus embryos to the blastocyst stage for vitrification. If many embryos are available on Day 2, most clinics culture all embryos to the blastocyst stage for transfer and for vitrification. Freezing of Day 2 embryos is usually performed as slow-freezing.

Singleton deliveries following transfer of vitrified blastocysts and singleton deliveries following transfer of slow-frozen cleavage stage embryos were compared. Comparisons were also made between singleton deliveries following transfer of vitrified blastocysts and singleton deliveries following transfer of fresh blastocysts.

Outcomes

The main outcomes were PTB (<37 weeks), LBW (<2500 g), fetal macrosomia (≥4500 g), HDP (pregnancy-induced hypertension (International Statistical Classification of Diseases and Related Health Problems *(ICD)-10 code O13)* and preeclampsia *(ICD-10 code O14-O15)*)

Table III Crude and adjusted odds ratios of perinatal and maternal outcome in singleton deliveries after vitrified blastocyst transfer (BT) compared with slow-frozen cleavage stage transfer (CT) and fresh BT, 2002–2015.

	Vitrified BT vs. slow-frozen CT		Vitrified BT vs. fresh BT	
	Crude OR (95% CI)	aOR * (95% CI)	Crude OR (95% CI)	aOR * (95% CI)
Male sex	1.15 [1.07—1.25]	**1.16 [1.05—1.27]**	1.00 [0.92—1.09]	**1.03 [0.93—1.13]**
<37 weeks	1.20 [1.03—1.40]	**1.33 [1.09—1.62]**	0.82 [0.70—0.97]	0.86 [0.71—1.04]
<32 weeks	0.93 [0.64—1.35]	0.98 [0.60—1.62]	0.78 [0.53—1.16]	0.84 [0.81—1.39]
≥42 weeks	1.01 [0.87—1.17]	1.01 [0.85—1.20]	1.87 [1.54—2.26]	**1.61 [1.31—1.98]**
<1500 g	0.92 [0.74—1.13]	1.17 [0.65—2.11]	0.82 [0.51—1.32]	0.92 [0.50—1.70]
<2500 g	0.92 [0.60—1.42]	0.91 [0.70—1.19]	0.59 [0.47—0.73]	**0.57 [0.44—0.74]**
≥4000 g	1.02 [0.93—1.12]	1.06 [0.95—1.18]	1.81 [1.62—2.03]	**1.68 [1.48—1.92]**
≥4500 g	0.99 [0.83—1.18]	0.93 [0.76—1.15]	1.96 [1.55—2.48]	**1.77 [1.35—2.31]**
SGA < −2 SD	0.87 [0.68—1.12]	0.85 [0.63—1.13]	0.56 [0.43—0.72]	**0.58 [0.44—0.78]**
LGA > +2 SD	1.07 [0.92—1.26]	1.10 [0.91—1.32]	1.72 [1.41—2.10]	**1.48 [1.18—1.84]**
Apgar <7 at 5 min	1.20 [0.90—1.61]	1.19 [0.84—1.68]	1.74 [1.20—2.52]	**1.79 [1.19—2.70]**
Apgar <4 at 5 min	1.70 [0.93—3.10]	**2.42 [1.07—5.48]**	1.77 [0.86—3.62]	1.56 [0.72—3.38]
Birth defects, any	0.99 [0.82—1.21]	1.16 [0.91—1.47]	0.85 [0.69—1.05]	0.99 [0.77—1.27]
Perinatal death	0.52 [0.18—1.50]	0.97 [0.17—5.73]	0.70 [0.20—2.39]	0.40 [0.07—2.16]
Neonatal death	0.58 [0.22—1.56]	0.93 [0.26—3.38]	0.68 [0.23—2.03]	0.67 [0.18—2.44]
HDP	1.08 [0.92—1.26]	0.97 [0.81—1.17]	1.39 [1.16—1.67]	**1.47 [1.19—1.81]**
Placental abruption	1.06 [0.62—1.80]	1.78 [0.93—3.40]	0.50 [0.29—0.84]	0.65 [0.37—1.16]
Placenta previa	1.58 [1.13—2.22]	1.48 [0.98—2.24]	0.38 [0.28—0.51]	**0.35 [0.25—0.48]**
PPH**	1.09 [0.95—1.25]	1.03 [0.88—1.25]	1.59 [1.34—1.89]	**1.68 [1.39—2.03]**
Induction of labour	1.10 [1.01—1.20]	**1.14 [1.03—1.27]**	1.46 [1.31—1.62]	**1.67 [1.48—1.88]**
Cesarean section	1.12[1.03—1.22]	**1.17 [1.05–1.30]**	1.13 [1.03—1.25]	**1.15 [1.03–1.29]**

BT: blastocyst transfer, CT: cleavage stage transfer, OR: odds ratio, CI: confidence interval, SGA/LGA: small/large for gestational age, HDP: hypertensive disorders in pregnancy, PPH: postpartum hemorrhage.
*Adjusted for country of birth of child, year of birth of child, maternal age, BMI, parity, smoking, parental educational level, fertilisation method (IVF/ICSI), single embryo transfer, number of gestational sacs and child's sex.
**Only Swedish data.
Bold indicates statistical significance.

and placenta previa (*ICD-10 code O44*). Secondary outcomes were very preterm birth (VPTB, <32 weeks), post-term birth (≥42 weeks), very low birth weight (VLBW, <1500 g), SGA and LGA (<−2 SD below or >+2 SD above the Scandinavian growth standard, adjusted for gestational age and gender, respectively) (Marsal *et al.*, 1996), Apgar score < 7 at 5 min, Apgar score < 4 at 5 min, stillbirth, perinatal mortality (stillbirth and death in the first week of life), neonatal mortality (death <28 days postpartum) any birth defect (*ICD-10 codes beginning with Q*), placental abruption (*ICD-10 code O45*), PPH (*ICD-10 code O72; >1000 mL in Sweden*), induction of labour (*ICD-10 code O61*) and Cesarean section (*ICD-10 code O82*). Due to different definitions of PPH in Sweden and Denmark, we chose to include only Swedish data in the analysis of PPH. In Sweden, PPH is defined as bleeding >1000 mL and in Denmark bleeding >500 mL.

Gestational age was determined according to day of embryo transfer and number of days in culture for Swedish data and by first trimester ultrasound for Danish data.

Statistical methods

Descriptive statistics are given by number (*n*) and percentages for categorical variables and by mean and SD or median and interquartile range (IQR) for continuous variables. Multivariable logistic regression analyses were performed and adjustment made for child's country and year of birth, maternal age, BMI, parity, smoking, parental educational level, fertilisation method (IVF/ICSI), SET, number of gestational sacs and the child's sex. Crude and adjusted odds ratios (aORs) with 95% confidence intervals (CIs) for each outcome were calculated. Missing data was not imputed. Analyses were conducted using statistical software SPSS, version 15.0.

Ethical approval

Permission from the Scientific Ethics Committee was given in Sweden (the Regional Ethical Committee in Sweden, at the University of Gothenburg, Dnr 304/06, T109-08, T087-12 and Dnr 214-12, T422-12, T516-15, T233-16, T300-17, T1144-17, T121-18) but not required in Denmark for register-based research. The study was approved by the National Board of Health and Welfare and Statistics Sweden in Sweden and the Data Protection Agency in Denmark.

Results

In total, 3650 singletons were born following the transfer of vitrified blastocysts, 8123 following slow-frozen cleavage stage transfer and

4469 following fresh blastocyst transfer. Regarding cleavage stage transfer, 91% were transferred on Day 2 and 9% were transferred on Day 3. Demographic background data are presented in Table I. Perinatal and maternal outcomes and statistical analysis are summarised in Tables II and III.

Transfer of vitrified blastocysts versus slow-frozen cleavage stage embryos

Transfer of vitrified blastocysts was associated with a higher risk of PTB (<37 weeks) aOR [95%] 1.33 [1.09–1.62] and Apgar score < 4 at 5 min aOR 2.42 [1.07–5.48] compared with transfer of slow-frozen cleavage stage embryos. No significant differences were found for LBW (<2500 g), macrosomia (≥4500 g) or other perinatal outcomes as listed in Table III. The male/female ratio was significantly higher following vitrified blastocyst transfer compared with slow-frozen cleavage stage transfer.

For maternal outcomes, no significant differences were observed for HDP, placental abruption, placenta previa or PPH. Transfer of vitrified blastocysts was associated with a higher risk of induction of labour aOR 1.14 [1.03–1.27] and Cesarean section aOR 1.17 [1.05–1.30].

Transfer of vitrified blastocysts versus fresh blastocysts

No significant difference could be seen for PTB (<37 weeks) when comparing vitrified blastocysts with fresh blastocysts. Transfer of vitrified blastocysts was associated with a lower risk of LBW (<2500 g) aOR 0.57 [0.44–0.74] and SGA aOR 0.58 [0.44–0.78] yet a higher risk of macrosomia (≥4500 g) aOR 1.77 [1.35–2.31] and LGA aOR 1.48 [1.18–1.84]. Moreover, a higher risk of post-term birth (≥42 weeks) aOR 1.61 [1.31–1.98] and Apgar <7 at 5 min aOR 1.79 [1.19–2.70] was seen.

For maternal outcomes, transfer of vitrified blastocysts was associated with a higher risk of HDP aOR 1.47 [1.19–1.81], PPH aOR 1.68 [1.39–2.03], induction of labour aOR 1.67 [1.48–1.88] and Cesarean section aOR 1.15 [1.03–1.29] but a lower risk of placenta previa aOR 0.35 [0.25–0.48].

Discussion

The present study shows that a shift from slow-frozen cleavage stage transfer to transfer of vitrified blastocysts is associated with a higher risk of PTB while the other main perinatal and maternal outcomes were not different in singletons conceived after FET. Compared to transfer of fresh blastocysts, vitrified blastocysts are associated with lower risks of PTB, LBW and SGA but higher risks for LGA, macrosomia, PPH and HDP.

In the present study, the comparison of vitrified blastocysts and slow-frozen cleavage stage embryos included two treatment characteristics, i.e. culture time and cryopreservation method, while a more adequate comparison would have been vitrified versus slow-frozen blastocysts. However, since the combination of blastocysts and vitrification turned out to be more successful (Stehlik *et al.*, 2005), studies comparing vitrified and slow-frozen blastocysts are rare. We are aware of only one study that compared vitrified and slow-frozen blastocysts (Li *et al.*, 2014), showing no differences in perinatal outcomes. In our study, we found a higher risk of PTB following vitrification of blastocysts compared with slow-frozen cleavage stage transfer. A small Swedish study, including 103 singletons born following vitrified blastocysts, found no differences in the risk of PTB, LBW and LGA but a lower risk of SGA when compared to 194 singletons following transfer of slow-frozen cleavage stage embryos (Wikland *et al.*, 2010). A Finnish study did not show any difference in the risk of PTB following vitrified and slow-frozen cleavage stage embryos in singleton pregnancies, suggesting that the freezing technique *per se* might not influence the rate of PTB (Kaartinen *et al.*, 2016). These results are supported by the study by Li *et al*. on the effect of the two different freezing techniques on blastocysts (Li *et al.*, 2014). Previous systematic reviews and meta-analyses (Dar *et al.*, 2014, Martins *et al.*, 2016, Palomba *et al.*, 2016, Alviggi *et al.*, 2018) have shown a higher risk of PTB following transfer of blastocysts compared to transfer of cleavage stage embryos. In the study by Alviggi *et al*., the increase in risk was, however, only seen when comparing fresh embryos. In addition to PTB, extended culture has been associated with increased birth weight, as shown in some studies with fresh cycles (Makinen *et al.*, 2013, Zhu *et al.*, 2014). Besides cryopreservation and culture length, differences in culture conditions and oxygen concentrations might affect the embryo and hence the outcome (Gardner, 2016, Mani and Mainigi, 2018).

We did not see any significant differences regarding HDP, placenta previa and placental abruption when comparing vitrified blastocysts and slow-frozen cleavage stage embryos; these results are in line with a previous study investigating the effect of the freezing method on maternal outcomes (Wikland *et al.*, 2010).

When comparing transfer of vitrified and fresh blastocysts, our results show a higher risk of macrosomia and LGA and a lower risk of LBW and SGA for vitrified blastocysts, in accordance with several previous studies showing larger babies following FET (Pelkonen *et al.*, 2010; Wennerholm *et al.*, 2013; Luke *et al.*, 2017; Berntsen and Pinborg, 2018; Maheshwari *et al.*, 2018; Sha *et al.*, 2018; Zhang *et al.*, 2018) independently of culture duration.

The reason behind the higher rate of post-term birth following transfer of vitrified blastocysts compared to fresh blastocysts found in our study remains unclear but is in accordance with previous Swedish studies showing higher rate of post-term pregnancies following FET compared to fresh transfers (Wennerholm *et al.*, 2013, Ginstrom Ernstad *et al.*, 2019). Although the higher birth weight in FET may be associated with the higher rate of post-term births, LGA, being independent of gestational age, is increased to a similar extent as macrosomia, suggesting that other mechanisms are responsible for the higher birth weight. Our findings of a higher risk of HDP and PPH as well as a lower risk of placenta previa following transfer of vitrified blastocysts compared with fresh blastocysts are in line with earlier studies (Sazonova *et al.*, 2012, Opdahl *et al.*, 2015, Maheshwari *et al.*, 2018, Sha *et al.*, 2018). The reason behind the differences in perinatal and maternal outcomes between frozen and fresh cycles is probably multifactorial. Different cycle regimens used in FET have been shown to influence maternal outcome, especially the rate of HDP and PPH (Ginstrom Ernstad *et al.*, 2019). Reasons for differences in perinatal outcome are not known but might be due to selection of better-quality embryos surviving freezing and thawing and/or epigenetic changes following the cryopreservation procedures. The higher rate of post-term birth, Cesarean section and larger babies might also contribute

to the higher risk of PPH (Ehrenthal *et al.*, 2012, Buzaglo *et al.*, 2015, Beta *et al.*, 2019) following transfer of vitrified versus fresh blastocysts.

A major strength of the current study is the size containing a complete birth cohort of singletons following vitrification of blastocyst, slow-frozen cleavage stage embryos and fresh blastocysts in Sweden and Denmark. The registry-based study setting, using registries with high coverage rates and high validity, limits the risk of selection bias. Moreover, we were able to adjust for several confounders. Since vitrification was introduced simultaneously with blastocyst transfer in Sweden and Denmark, it was not possible to explore the effect of vitrification *per se* which is the main limitation of the study. Even though some children were born after vitrified cleavage stage embryos, the number was too small for statistical comparison. Another limitation is the possibility of residual confounding caused by known and unmeasured confounders, e.g. years of infertility, as well as unknown confounders.

In conclusion, for main outcomes, transfer of vitrified blastocysts is associated with a higher risk of PTB compared with transfer of slow-frozen cleavage stage embryos, an effect that might be related to culture duration more than the freezing technique. The underlying causes of FET leading to larger babies and higher risks of HDP and PPH compared with fresh embryo transfer need further attention.

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Authors' roles

All authors participated in the study design and interpretation of the data. E.G.E, A-L.S. and S.S.M analysed the data. E.G.E and S.S.M drafted the manuscript, and it was finalised by all co-authors. The final version of the manuscript was approved by all authors.

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Conflict of interest

None reported.

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