High birth weight and large-for-gestational-age in singletons born after frozen compared to fresh embryo transfer, by gestational week: a Nordic register study from the CoNARTaS group

Terho *et al.*

Large-for-gestational-age after frozen embryo transfer

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ABSTRACT

When do the differences in birth weights become apparent between singletons born after frozen embryo transfer (FET) and fresh embryo transfer (fresh ET)?

Mean birth weights after FET become significantly higher starting from gestational week (GW) 33 among boys and from GW 34 among girls.

In recent years, there has been a steep rise in recorded FET treatments, enabling widespread use of elective single embryo transfer, thus reducing the risks associated with multiple gestations. However, singletons born after FET are heavier and there is a higher risk of large-for-gestational-age (LGA) (birth weight > 90 percentiles) compared to fresh ET. In contrast, risk of small-for-gestational-age (SGA, birth weight < 10 percentiles) is lower in singletons born after FET compared to fresh ET. The reasons, timing and consequences of these differences remain largely unclear. There is limited evidence about whether this difference in growth develops before the last trimester of pregnancy.

This retrospective Nordic register-based cohort study compared singletons born after FET (n = 17 500) to singletons born after fresh ET (n = 69 510) and natural conception (NC, n = 3 311 588). All live born singletons born between the years 2000 and 2015 in Denmark, Norway and Sweden at gestational age ≥22 weeks were included from the population-based Committee of Nordic ART and Safety (CoNARTaS) study population.

Children born after FET were compared to those born after fresh ET and NC for mean birth weight and proportion of LGA and SGA for each GW at birth. Chi-square test and tests for relative proportions were used to compare categorical variables and Student's *t*-test was used to compare continuous variables. Adjusted odds ratios (aORs) for LGA and SGA were calculated using logistic regressions, adjusting for year of birth, maternal age, parity, BMI, chronic hypertension, diabetes, smoking and offspring sex.

Mean birth weights were significantly higher after FET compared to fresh ET starting from GW 33 (range from 75 g to 228 g by week) for boys and starting from GW 34 (range from 90 g to 236 g by week) for girls. Boys born after FET had a significantly higher proportion of LGA (11.0–15.1%) at birth between GW 36 and 42, compared to those born after fresh ET (7.1–9.4%) (range from $P < 0.001$ to $P = 0.048$ by week). For girls born after FET, the difference was seen between GW 37 and 42 (10.6–13.4%) compared to those born after fresh ET (6.6–8.0%) (range from $P < 0.001$ to $P = 0.009$ by week). The proportion of SGA was significantly lower among boys born after FET (7.6–8.7%) compared to fresh ET (11.9–13.6%) between GW 36 and 42 (range from $P < 0.001$ to $P = 0.016$ by week). For girls born after FET, the difference was seen between GW 38 and 42 (7.0–9.3%) compared to those born after fresh ET (13.0–14.6%) ($P < 0.001$). The proportion of LGA (12.3–15.1%) was significantly higher for boys born after FET between GW 38 and 41 ($P < 0.001$) and for girls born after FET (12.6–13.4%) between GW 37 and 40 (range from $P < 0.001$ to $P = 0.018$ by week), compared to naturally conceived boys (9.7–9.9%) and girls (9.0–10.0%). All singletons born after FET had a higher risk of LGA compared to singletons born after fresh ET (aOR 1.87, 95% CI 1.76–1.98) and singletons born after NC (aOR 1.28, 95% CI 1.22–1.35).

There may be residual confounding factors that we were not able to control for, most importantly the causes of preterm birth, which may also influence foetal growth. A further limitation is that we have no knowledge on growth patterns between implantation and GW 22. Finally, the number of children born extremely preterm or post-term was limited even in this large study population.

This is, to date, the largest study on birth weights among preterm and term ART singletons with a population-based design and NC control group. The results suggest that the freeze–thaw process is associated with higher birthweights and greater risk of LGA at least in the last trimester of pregnancy. This is an important aspect of the safety profile of ART. More research is needed on the long-term outcome of these children.

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Introduction

Freezing of human embryos with thawing and transfer in a subsequent cycle (frozen embryo transfer, FET) is a well-established part of ART. In recent years, there has been a steep rise in recorded FET treatments, and the relative proportion of FET to fresh embryo transfer (fresh ET) was 40.3% in Europe in 2015 (De Geyter *et al.*, 2020). FET practice enables the widespread use of elective single embryo transfer (eSET), thus reducing the risks associated with multiple gestations (Thurin *et al.*, 2004; Tiitinen and Gissler, 2004; Henningsen *et al.*, 2015). Still, safety monitoring of different ART modalities is of utmost importance, since already one in 50 children born in Europe is the result of ART treatment (De Geyter *et al.*, 2020).

It is well known that singletons born after FET have a higher risk of being born large-for-gestational-age (LGA) than singletons born after fresh ET. This has been shown in a number of large register-based cohort studies (Pelkonen *et al.*, 2010; Sazonova *et al.*, 2012; Wennerholm *et al.*, 2013; Pinborg *et al.*, 2014) and meta-analyses (Maheshwari *et*

al., 2018; *Sha et al.*, 2018). In contrast, singletons born after fresh ET have been shown to have a higher risk of small-for-gestational-age (SGA) compared to FET (*Pelkonen et al.*, 2010; *Wennerholm et al.*, 2013; *Pinborg et al.*, 2014; *Maheshwari et al.*, 2018). Both small and large birth weights may be predictors of obesity and cardiometabolic risk factors and disease in adulthood (*Barker*, 2007; *Taal et al.*, 2013; *Kapral et al.*, 2018).

There is limited knowledge on the foetal growth patterns during pregnancies following different ART modalities. There is some evidence that growth differences develop before the last trimester of pregnancy (*Li et al.*, 2014; *Ginod et al.*, 2018). In this study, we aimed to investigate when this difference in birth weights occurs between singletons born after FET compared to singletons born after fresh ET and natural conception (NC). Thus, we estimated mean birth weights and proportions of SGA, appropriate-for-gestational-age (AGA) and LGA according to gestational age and sex.

Materials and methods

Study population

The CoNARTaS study population consists of all ART and NC pregnancies from Denmark, Finland, Norway and Sweden. Data were obtained from national ART registers and Medical Birth Registers and linked at the individual level using the national identity number assigned to all residents in each Nordic country at birth or immigration (*Opdahl et al.*, 2019). For this study, data from Denmark, Norway and Sweden were used, but data on different ART modalities were not available from Finland. Live-born singletons (gestational age ≥ 22 weeks at birth) born between the years 2000 and 2015 were included, $n = 17\,500$ for FET, $n = 69\,510$ for fresh ET and $n = 3\,311\,588$ for NC. Children conceived after ovulation induction and intrauterine insemination (IUI) are included in the NC group in this study. In both FET and fresh ET groups, some oocyte donation pregnancies were included (1.8–2.4%).

Gestational age for ART pregnancies was based on the number of culture days and the date of embryo transfer, and that for NC pregnancies was based on ultrasound (US) examination or last menstrual period if information on US was missing. Gestational weeks (GWs) used in this study are completed weeks. All analyses were performed using <10 percentiles and >90 percentiles according to the CoNARTaS data, as cut-off levels for SGA and LGA, respectively. We excluded cases with birthweight <300 g or ≥ 6000 g and birthweight for gestational age ≤ -6 and $\geq +6$ SDs (*Marsal et al.*, 1996).

As regards to background factors, we had access to data on country, ART characteristics, embryo culture duration, number of embryos transferred, year of birth, maternal age, parity, prepregnancy BMI, smoking in pregnancy, level of education, diabetes, chronic hypertension, hypertensive disorders of pregnancy, induction of labour and caesarean section, as well as paternal age (Table I).

Table I Characteristics of pregnancies originated after frozen embryo transfer (FET), fresh embryo transfer (fresh ET) and natural conception (NC) categorized by birth weight into small-for-gestational-age (SGA, <10 percentiles), appropriate-for-gestational-age (AGA) and large-for-gestational-age (LGA, >90 percentiles). [AQ6]

	FET (n = 17 500)			Fresh ET (n = 69 510)			NC (n = 3 311 588)			P-val- ue, ^a FET vs fresh ET
	SGA (n = 1445)	AGA (n = 13 835)	LGA (n = 2220)	SGA (n = 9135)	AGA (n = 55 251)	LGA (n = 5124)	SGA (n = 326 911)	AGA (n = 2 660 928)	LGA (n = 323 749)	
Country, n (%)										
Denmark	287 (9.0)	2530 (78.9)	388 (12.1)	3165 (13.8)	18 151 (78.9)	1692 (7.4)	93 175 (10.8)	686 965 (79.8)	80 241 (9.3)	
Norway	240 (7.1)	2664 (79.4)	453 (13.5)	1944 (12.9)	12 049 (79.9)	1091 (7.2)	85 610 (9.6)	713 903 (80.5)	87 796 (9.9)	
Sweden	918 (8.4)	8641 (79.0)	1379 (12.6)	4026 (12.8)	25 051 (79.7)	2341 (7.5)	148 126 (9.5)	1 260 060 (80.6)	155 712 (10.0)	

	FET (n = 17 500)			Fresh ET (n = 69 510)			NC (n = 3 311 588)			P-value, ^a FET vs fresh ET
	SGA (n = 1445)	AGA (n = 13 835)	LGA (n = 2220)	SGA (n = 9135)	AGA (n = 55 251)	LGA (n = 5124)	SGA (n = 326 911)	AGA (n = 2 660 928)	LGA (n = 323 749)	
Maternal age, mean (SD)	34.7 (4.3)	34.9 (4.2)	35.1 (4.2)	34.3 (4.3)	34.4 (4.3)	34.7 (4.3)	30.4 (5.3)	30.9 (5.1)	31.7 (4.9)	<0.001
Maternal BMI, mean (SD)	23.6 (3.9)	24.1 (4)	25.7 (4.4)	23.7 (4.0)	24.2 (4)	26.1 (4.6)	23.7 (4.6)	24.4 (4.6)	26.5 (5.4)	0.717
Missing, n (%)	205 (14.2)	2 439 (17.6)	437 (19.7)	2283 (25.0)	14 455 (26.2)	1463 (28.6)	103 964 (31.8)	837 681 (31.5)	107 770 (33.3)	
Primiparous, n (%)	1023 (70.8)	7673 (55.4)	922 (41.5)	7314 (80.0)	38 346 (69.4)	2902 (56.6)	189 755 (58.0)	1 144 545 (43)	87 069 (26.9)	<0.001
Missing	5 (0.3)	25 (0.2)	<5 (0.1)	60 (0.7)	326 (0.6)	32 (0.6)	1599 (0.5)	8865 (0.3)	784 (0.2)	
Maternal smoking, n (%)	100 (6.9)	617 (4.5)	81 (3.6)	993 (10.9)	3890 (7.0)	309 (6.0)	70 413 (21.5)	361 940 (13.6)	32 378 (10.0)	<0.001
Missing	48 (3.3)	467 (3.4)	81 (3.6)	288 (3.2)	1805 (3.3)	162 (3.2)	9825 (3)	82 606 (3)	10 964 (3.4)	
Maternal education, ^b n (%)										
Basic/upper secondary	333 (23)	3106 (22.4)	527 (23.7)	1924 (21.1)	11 715 (21.2)	1171 (22.8)	95 871 (29.3)	749 394 (28.2)	95 486 (29.5)	<0.001
Vocational	222 (15.4)	1975 (14.3)	272 (12.3)	1711 (18.7)	9860 (17.8)	955 (18.6)	46 014 (14.1)	376 890 (14.2)	47 955 (14.8)	<0.001
University	564 (39)	5521 (40)	898 (40.5)	3159 (34.6)	19 814 (35.9)	1780 (34.7)	70 445 (21.5)	661 819 (24.9)	79 473 (24.5)	<0.001
Missing	326 (22.6)	3233 (23.4)	523 (23.6)	2341 (25.6)	13 862 (25.1)	1218 (23.8)	114 581 (35)	872 825 (32.8)	100 835 (31.1)	
Paternal age, ^c mean (SD)	37.2 (5.6)	37.2 (5.7)	37.3 (5.6)	36.6 (5.7)	36.5 (5.7)	36.7 (5.8)	33 (6.5)	33.1 (6.2)	33.7 (6)	<0.001
Missing, n (%)	300 (20.8)	2711 (19.6)	421 (19)	3228 (35.3)	18 461 (33.4)	1715 (33.5)	97 658 (29.9)	707 784 (26.6)	81 928 (25.3)	
ICSI, n (%)	534 (37)	4963 (35.9)	805 (36.3)	3669 (40.2)	23 186 (42)	2223 (43.4)	NA	NA	NA	<0.001
Missing	113 (7.8)	1003 (7.2)	184 (8.3)	16 (0.2)	128 (0.2)	15 (0.3)	NA	NA	NA	
Culture duration, days, n (%)										
2–3	976 (67.5)	8840 (63.9)	1437 (64.8)	7383 (80.9)	43 695 (79.1)	3220 (77.1)	NA	NA	NA	<0.001
≥5	280 (19.4)	3083 (22.2)	489 (21.9)	522 (5.7)	3684 (6.6)	362 (7)	NA	NA	NA	<0.001

	FET (n = 17 500)			Fresh ET (n = 69 510)			NC (n = 3 311 588)			<i>P</i> -value, ^a FET vs fresh ET
	SGA (n = 1445)	AGA (n = 13 835)	LGA (n = 2220)	SGA (n = 9135)	AGA (n = 55 251)	LGA (n = 5124)	SGA (n = 326 911)	AGA (n = 2 660 928)	LGA (n = 323 749)	
Missing	189 (13.1)	1912 (13.8)	294 (13.2)	1230 (13.5)	7872 (14.2)	814 (15.9)	NA	NA	NA	
SET, n (%)	1042 (72.1)	10 023 (72.4)	1619 (73)	5073 (55.5)	30 257 (54.7)	2647 (51.6)	NA	NA	NA	<0.001
Missing	24 (1.7)	308 (2.2)	53 (2.4)	387 (4.2)	2 453 (4.4)	238 (4.6)	NA	NA	NA	
Diabetes, ^d n (%)	<5 (0.2)	39 (0.3)	41 (1.8)	12 (0.1)	135 (0.2)	62 (1.2)	351 (0.1)	5039 (0.2)	3771 (1.2)	<0.001
Chronic hypertension, n (%)	25 (1.7)	115 (0.8)	19 (0.9)	141 (1.5)	490 (0.9)	57 (1.1)	3809 (1.2)	17 501 (0.7)	2354 (0.7)	<0.001
HDP, n (%)	175 (12.1)	984 (7.1)	150 (6.8)	910 (10.0)	2863 (5.2)	330 (6.4)	26 680 (8.2)	104 017 (3.9)	14 082 (4.3)	<0.001
Induction of labour, n (%)	504 (34.9)	3364 (24.3)	578 (26)	2266 (24.8)	10 270 (18.6)	1190 (23.2)	65 035 (19.9)	366 202 (13.8)	55 822 (17.2)	<0.001
Caesarean section, n (%)	423 (29.3)	3731 (27)	862 (38.8)	2425 (26.5)	13 358 (24.2)	1902 (37.1)	61 595 (18.8)	415 118 (15.6)	76 959 (23.8)	<0.001
Preterm births, n (%)	93 (6.4)	909 (6.6)	130 (5.9)	686 (7.5)	4341 (7.9)	428 (8.4)	15 829 (4.8)	129 068 (4.9)	15 978 (4.9)	<0.001
Male	44 (6.0)	465 (6.6)	79 (6.6)	377 (8.3)	2350 (8.3)	258 (9.5)	8594 (5.2)	70 093 (5.1)	8701 (5.2)	
Female	49 (6.9)	444 (6.5)	51 (5.0)	309 (6.7)	1991 (7.3)	170 (7.0)	7235 (4.5)	58 975 (4.6)	7277 (4.7)	
Year of birth, n (%)										
2000–2004	137 (8.1)	1326 (78.6)	224 (13.3)	1984 (12.1)	12 924 (79.1)	1436 (8.8)	96 142 (9.5)	805 423 (79.6)	110 562 (10.9)	
2005–2009	442 (8.8)	3928 (78.4)	640 (12.8)	3002 (13.3)	17 858 (79.2)	1683 (7.5)	104 752 (9.8)	859 543 (80.6)	101 724 (9.5)	
2010–2015	866 (8.0)	8581 (79.4)	1356 (12.6)	4149 (13.5)	24 469 (79.9)	2005 (6.5)	126 017 (10.2)	995 962 (80.7)	111 463 (9.0)	
Offspring male sex, n (%)	732 (50.7)	7051 (51.0)	1190 (53.6)	4537 (49.7)	28 154 (51.0)	2708 (52.8)	166 498 (50.9)	1 368 431 (51.4)	167 559 (51.8)	<0.001

HDP, hypertensive disorders in pregnancy; NA, not applicable; SET, single embryo transfer.

a Chi-square test, Student's *t*-test.

b Data on education are not available for Norway.

c Data on paternal age are not available for Denmark.

d Data on diabetes included any diabetes due to different registration practices in the study countries.

Ethical approval

Approvals for data retrieval and linkage were obtained in each country (Opdahl *et al.*, 2019). In Denmark, ethical approval is not required for scientific projects solely based on register data. In Norway, ethical approval was given by the Regional Committee for Medical and Health Research Ethics (REK-Nord, 2010/1909). In Sweden, approval was obtained from the Ethical committee in Gothenburg, Dnr 214-12, T422-12, T516-15, T233-16, T300-17, T1144-17 and T121-18 and the Swedish Ethical Review Authority 2019-02347.

In addition, data retrieval for CoNARTaS was approved by the register-keeping authorities in each country (Denmark: Region H 22-06-2016, DT-journal number 2012-58-0004, local journal number AHH-2016-033, I-suite number: 04790; Norway: Norwegian Institute of Public Health, Statistics Norway, Norwegian Directorate of Health and the Cancer Registry of Norway; Sweden: National Board of Health and Welfare, Statistics Sweden) and several National Quality Registries for additional data on morbidity.

Outcomes and statistical analyses

Mean birth weights and the percentages of SGA, AGA and LGA for each GW at birth were compared between singletons born after FET, fresh ET and NC, stratified by offspring sex. Chi-square test and test for relative proportions were used to compare categorical variables and Student's *t*-test to compare continuous variables. Adjusted odds ratios (aORs) for LGA and SGA with 95% CIs were calculated using logistic regressions first for the whole population and next categorized into extremely preterm (GW 22–27), very preterm (GW 28–31), moderate/late preterm (GW 32–36), term (GW 37–41) and post-term (GW 42–45) subgroups. Adjustments were made for year of birth, maternal age, BMI, parity, smoking, any diabetes mellitus, chronic hypertension and offspring sex. A subanalysis was performed for the moderate/late preterm, term and post-term subgroups as well as the whole population adjusting also for embryo culture time.

Sensitivity analyses were performed excluding induced births (Model 1) and excluding both induced births and births by caesarean section (Model 2) from the analyses.

All statistical analyses were performed using SAS statistical software, version 9.4. (SAS Institute).

Results

Background factors

A description of the study population is presented in Table I. There were statistically significant differences between FET and fresh ET groups for all background factors except for maternal BMI. The incidence of hypertensive disorders in pregnancy was 7.5% among FET, 5.9% among fresh ET and 4.4% among NC ($P < 0.001$ for FET versus fresh ET).

Term and preterm births and birth weights

The largest proportions of preterm (GW 22–36) births were seen among children born after fresh ET (8.4% for boys and 7.2% for girls). The corresponding proportions among children born after FET were 6.6% for boys and 6.4% for girls. In the NC group, the proportions were 5.1% for boys and 4.6% for girls ($P < 0.001$ for FET versus fresh ET) (Table I).

Mean birth weights were significantly higher for boys born after FET starting from GW 33 (range from 75 g to 228 g by week), and for girls born after FET starting from GW 34 (range from 90 g to 236 g by week), compared to fresh ET (Fig. 1a and b).

Figure 1. Mean birth weights for each gestational week for children born after frozen embryo transfer (FET), fresh embryo transfer (fresh ET) and natural conception (NC) stratified by sex of child. (a) Mean weights, boys. (b) Mean weights, girls.

Proportions of LGA (>90 percentiles) and SGA (<10 percentiles)

Boys born after FET had significantly higher proportions (11.0–15.1%) of LGA at birth compared to those born after fresh ET (7.1–9.4%) between GW 36 and 42 (range from $P < 0.001$ to $P = 0.048$ by week). Girls born after FET had significantly higher proportions of LGA (10.6–13.4%) compared to those born after fresh ET (6.6–8.0%) between GW 37 and 42 (range from $P < 0.001$ to $P = 0.009$ by week). Compared to children born after NC, significantly more LGA (12.3–15.1%) was seen for boys born after FET between GW 38 and 41 ($P < 0.001$) and for girls born after FET (12.6–13.4%) between GW 37 and 40 (range from $P < 0.001$ to $P = 0.018$ by week) (Table II).

Table II Proportions of large-for-gestational-age (LGA, >90 percentiles) birth weights for boys and girls for each gestational week (GW) among frozen embryo transfer (FET), fresh embryo transfer (fresh ET) and naturally conceived (NC) pregnancies.

	Boys				Girls			
	LGA FET n = 1190	LGA fresh ET n = 2708	LGA NC n = 167 559	P-value ^a	LGA FET n = 1030	LGA fresh ET n = 2416	LGA NC n = 156 190	P-value ^a
GW	n (%)	n (%)	n (%)		n (%)	n (%)	n (%)	
22–25	<5	9	181	NA	<5	<5	154	NA
26	– (0)	7 (17.9)	90 (9.8)	0.169 ^b ; 0.324 ^c	<5 (14.3)	<5 (9.8)	78 (9.7)	0.717 ^b ; 0.681 ^c
27	– (0)	7 (14.9)	110 (9.5)	0.274 ^b ; 0.390 ^c	– (0)	<5 (9.5)	81 (8.8)	0.310 ^b ; 0.327 ^c

	Boys				Girls			
	LGA FET n = 1190	LGA fresh ET n = 2708	LGA NC n = 167 559	P-value ^a	LGA FET n = 1030	LGA fresh ET n = 2416	LGA NC n = 156 190	P-value ^a
GW	n (%)	n (%)	n (%)		n (%)	n (%)	n (%)	
28	<5 (8.3)	7 (9.0)	124 (9.2)	0.932 ^b ; 0.918 ^c	– (0)	<5 (7.7)	109 (9.7)	0.417 ^b ; 0.355 ^c
29	<5 (28.6)	9 (12.9)	156 (9.1)	0.138 ^b ; 0.012 ^c	<5 (16.7)	7 (11.3)	131 (9.8)	0.602 ^b ; 0.426 ^c
30	<5 (5.6)	11 (9.3)	216 (9.9)	0.600 ^b ; 0.539 ^c	<5 (22.2)	7 (8.3)	174 (9.9)	0.085 ^b ; 0.083 ^c
31	<5 (9.1)	8 (7.2)	297 (10)	0.760 ^b ; 0.882 ^c	<5 (5.3)	5 (6.3)	155 (6.6)	0.871 ^b ; 0.819 ^c
32	5 (22.7)	15 (8.4)	429 (10)	0.034 ^b ; 0.047 ^c	– (0)	8 (6.0)	348 (10.0)	0.229 ^b ; 0.110 ^c
33	6 (12.0)	23 (9.8)	660 (10)	0.638 ^b ; 0.637 ^c	5 (11.4)	15 (7.0)	527 (10.1)	0.325 ^b ; 0.783 ^c
34	13 (19.4)	19 (4.9)	1133 (10.1)	<0.001 ^b ; 0.012 ^c	7 (10.6)	24 (7.7)	911 (10.0)	0.433 ^b ; 0.876 ^c
35	11 (9.1)	46 (7.9)	1817 (10)	0.671 ^b ; 0.745 ^c	10 (8.8)	31 (6.1)	1547 (10.1)	0.277 ^b ; 0.654 ^c
36	33 (14.9)	97 (9.4)	3488 (10)	0.015 ^{b,c}	19 (9.5)	57 (6.7)	3062 (10.0)	0.166 ^b ; 0.783 ^c
37	61 (12.4)	176 (8.3)	8135 (10)	0.004 ^b ; 0.067 ^c	57 (13.4)	152 (7.7)	7650 (10.0)	<0.001 ^b ; 0.018 ^c
38	193 (15.1)	365 (7.1)	21 879 (9.9)	<0.001 ^{b,c}	148 (12.6)	388 (7.8)	21 583 (9.8)	<0.001 ^b ; 0.002 ^c
39	269 (15.0)	584 (7.6)	37 612 (9.9)	<0.001 ^{b,c}	229 (12.9)	512 (6.6)	34 606 (9.0)	<0.001 ^{b,c}
40	277 (12.3)	692 (7.6)	45 662 (9.7)	<0.001 ^{b,c}	278 (12.8)	636 (6.9)	46 539 (10.0)	<0.001 ^{b,c}
41	239 (12.5)	474 (7.4)	33 167 (9.9)	<0.001 ^{b,c}	195 (10.6)	433 (7.0)	29 564 (10.0)	<0.001 ^b ; 0.340 ^c
42	71 (11.0)	154 (8.4)	11 961 (10)	0.048 ^b ; 0.415 ^c	68 (11.6)	122 (8.0)	8647 (9.9)	0.009 ^b ; 0.150 ^c
43–44	<5	5	442	NA	<5	<5	324	NA

NA, not applicable. **Bold** indicates statistical significance.

a Test for relative proportions.

b FET versus fresh ET.

c FET versus NC.

The proportion of SGA was significantly lower among boys born after FET (7.6–8.7%) compared to those born after fresh ET (11.9–13.6%) between GW 36 and 42 (range from $P < 0.001$ to $P = 0.016$ by week). For girls born after FET, the difference was seen between GW 38 and 42 (7.0–9.3%) compared to those born after fresh ET (13.0–14.6%) ($P < 0.001$). Compared to children born after NC, significantly more SGA was seen for boys born after fresh ET (11.9–13.4%) between GW 38 and 39 (range from $P = 0.002$ to $P = 0.015$ by week) and for girls born after fresh ET (13.2–13.6%) between GW 38 and 40 (range from $P = 0.003$ to $P = 0.015$ by week) (data not shown).

Adjusted odds ratios for LGA (>90 percentiles) and SGA (<10 percentiles)

All singletons born after FET had a significantly greater risk of being LGA (crude odds ratio (OR) 1.82, 95% CI 1.73–1.92; aOR 1.87, 95% CI 1.76–1.98) compared to fresh ET. When gestational age at birth was categorized into extremely preterm (GW 22–27), very preterm (GW 28–31), moderate/late preterm (GW 32–36), term (GW 37–41) and post-term (GW 42–45) subgroups, statistically significant differences in the aOR for LGA were found for moderate/late preterm (aOR 1.84, 95% CI 1.40–2.40), term (aOR 1.91, 95% CI 1.79–2.04) and post-term (aOR 1.53, 95% CI 1.20–1.96) FET versus fresh ET subgroups (Table III). Adjusting for embryo culture time did not change the results (data not shown).

Table III Crude odds ratio (OR) and adjusted odds ratio (aOR) for large-for-gestational-age (LGA, >90 percentiles) categorized by gestational age for frozen embryo transfer (FET) versus fresh embryo transfer (fresh ET).

FET versus Fresh ET				
GW	Crude OR 95% CI		aOR 95% CI	
22–27	0.77	0.31–1.90	0.32	0.07–1.41
28–31	1.45	0.79–2.65	1.39	0.66–2.92
32–36	1.62	1.29–2.04	1.84	1.40–2.40
37–41	1.88	1.78–1.99	1.91	1.79–2.04
42–45	1.39	1.13–1.72	1.53	1.20–1.96
All	1.82	1.73–1.92	1.87	1.76–1.98

Logistic regression, crude and adjusted OR (year of birth, maternal age, parity, BMI, chronic hypertension, diabetes, smoking and sex of child). **Bold indicates statistical significance.**

GW, gestational week.

When stratified by sex, the risks of LGA associated with FET were similar for boys (crude OR 1.84, 95% CI 1.71–1.98; aOR 1.84, 95% CI 1.69–2.01) and girls (crude OR 1.80, 95% CI 1.66–1.94; aOR 1.90, 95% CI 1.73–2.07) compared to fresh ET (data not shown).

Also compared to NC, the risk of LGA was greater among children born after FET (crude OR 1.34, 95% CI 1.28–1.40; aOR 1.28, 95% CI 1.22–1.35). Categorized by gestational age at birth, significant differences were found for moderate/late preterm (aOR 1.37, 95% CI 1.09–1.74) and term (aOR 1.30, 95% CI 1.23–1.37) FET versus NC sub-groups (Table IV).

Table IV Crude odds ratio (OR) and adjusted odds ratio (aOR) for large-for-gestational-age (LGA, >90 percentiles) categorized by gestational age for frozen embryo transfer (FET) versus natural conception (NC).

FET versus NC				
GW	Crude OR 95% CI		aOR 95% CI	
22–27	0.76	0.33–1.74	0.41	0.10–1.67
28–31	1.37	0.80–2.36	1.56	0.82–2.95
32–36	1.19	0.98–1.46	1.37	1.09–1.74
37–41	1.37	1.31–1.44	1.30	1.23–1.37
42–45	1.17	0.98–1.39	1.16	0.96–1.41
All	1.34	1.28–1.40	1.28	1.22–1.35

Logistic regression, crude and adjusted OR (year of birth, maternal age, parity, BMI, chronic hypertension, diabetes, smoking and sex of child). **Bold indicates statistical significance.**

GW, gestational week.

All singletons born after FET had a significantly lower risk of being SGA (crude OR 0.60, 95% CI 0.56–0.63; aOR 0.61, 95% CI 0.57–0.65) compared to fresh ET. Also compared to NC, singletons born after FET had a slightly lower risk of being SGA (OR 0.82, 95% CI 0.78–0.87; aOR 0.91, 95% CI 0.85–0.96) (data not shown).

Sensitivity analyses

To test the robustness of the findings, we performed sensitivity analyses first excluding induced births (Model 1) and next excluding both induced births and births by caesarean section (Model 2), mainly to exclude cases with iatrogenic preterm or term birth (e.g. induced birth because of pregnancy complications). There were 4446 inductions and 5016 caesarean sections in the FET group. The respective numbers for fresh ET were 13 726 and 17 685 (Table I). The final group sizes in Model 2 were 9309 for FET, 41 544 for fresh ET and 2 362 812 for NC. The differences between FET and fresh ET remained unchanged in the moderate/late preterm, term and post-term groups, and be-

tween FET and NC in the moderate/late preterm and term groups. For all FET versus fresh ET, the aOR for LGA was 1.90, 95% CI 1.77–2.04 in Model 1. In Model 2, aOR for FET versus fresh ET was 1.89, 95% CI 1.73–2.07. For FET versus NC, the aOR for LGA was 1.31, 95% CI 1.23–1.38 in Model 1 and 1.22, 95% CI 1.13–1.31 in Model 2.

Discussion

The main finding in this large Nordic population-based cohort study is that the differences in birth weight between children born after FET and fresh ET become apparent and statistically significant in the last trimester of pregnancy from GW 33 onwards when comparing mean birth weights. Considering the risk of LGA, the differences show later on, starting from GW 36, becoming significant slightly earlier for boys than girls.

To date, our study is the largest study on birth weights among preterm and term ART singletons with an NC control group. The population-based design adds to the reliability of these results. In addition, the Nordic registers have high coverage, and linking of information is possible because of the individual national identity numbers ([Opdahl et al., 2019](#)).

As a limitation to our study, there lies a possible bias in using birth weight as the study outcome: LGA or other growth deviations will only be picked up among the children who are born at a certain GW, not among those who carry on growing in utero. Several causes of spontaneous preterm birth or iatrogenic preterm delivery may influence foetal growth and as such, birth weight. Unfortunately, we had no information on the reasons for preterm births in our study. In the sensitivity analyses, we aimed to test our results excluding induced births and births by caesarean section, thus excluding iatrogenic or spontaneous births caused by severe pregnancy complications and found that the results remain similar. Another limitation is that, within this study design, we have no information on the growth between implantation and GW 22. Considering the time span and different registration practices in the four Nordic countries, unfortunately, the CoNARTaS data do not include information on the embryo culture media that have been used. It also needs to be taken into account that even in a large study cohort, the number of children born in the extremely preterm or post-term weeks is quite low. As an observational cohort, there may also be residual confounding that we could not control for. However, the study design that we are presenting is the easiest way to look at this phenomenon in a large population as foetal ultrasound weight estimate comparison is prone to error ([Salomon et al., 2019](#)) and also laborious to carry out.

Previously, birth weights of preterm and term ART children have been described in an Australian cohort where birthweight for gestational age percentile charts were developed for singleton births following ART treatment. Previously published general population growth charts acted as reference in this study of about 70 000 live born ART singletons from years 2002 to 2010. The proportion of LGA (>90 percentiles) for boys born after FET was significantly higher than that of the general population between GW 33 and 41. For girls born after FET, this difference was seen between GW 34 and 42. Boys and girls born after fresh ET had significantly more SGA (<10 percentiles) at GW 41–42 and 40–41, respectively ([Li et al., 2014](#)). Compared to our study, the risk of LGA associated with FET compared to general population became statistically significant earlier in the pregnancy. In the study by [Li et al. \(2014\)](#), FET was not compared to fresh ET, and no crude or background-aORs for LGA or SGA were calculated.

In a recent French retrospective cohort of 560 singleton pregnancies, foetal ultrasound growth estimates were compared to reference curves in each trimester. Four groups were compared: pregnancies originated from IVF, ICSI, FET and IUI. At the first (GW 11–13) and second (GW 21–23) trimester US examinations, the estimated foetal sizes (mean *z* scores) for all groups were significantly larger than the 0 SD reference curve. At the third trimester US examination (GW 31–33), foetal growth had slowed down for the IVF, ICSI and IUI groups, but not for FET fetuses, which remained significantly larger than the 0 SD reference curve ([Ginod et al., 2018](#)). This difference in the growth between FET and fresh ET (IVF and ICSI) developing by the last trimester seems compatible with our results.

In a previous study by the CoNARTaS group, the aOR of LGA was 1.45 (95% CI 1.27–1.64) for children born after FET compared to fresh ET ([Wennerholm et al., 2013](#)). It should be noted that LGA was classified as birth weight ≥ 2 SD and adjustments were made for maternal age, parity, year of birth, offspring sex and country of origin. In our study, a larger and more recent (but partly overlapping) study population was studied, with a less restrictive definition of LGA (birth weight ≥ 90 percentiles), and the aOR for LGA was 1.87, 95% CI 1.76–1.98 (adjusted for year of birth, maternal age, parity, BMI, chronic hypertension, diabetes, smoking and offspring sex) for singletons born after FET compared to fresh ET. During the study period of [Wennerholm et al. \(2013\)](#) mostly cleavage stage slow-frozen embryos were used in FET, which in recent years has been replaced with more vitrification and blasto-

cyst transfer. It has been suggested that extended embryo culture time might be associated with higher birthweights (Zhu *et al.*, 2014; Martins *et al.*, 2016; Zhang *et al.*, 2019). In a subanalysis of our study cohort, adjusting for embryo culture time did not significantly change the results on the risk of LGA. Furthermore, in a recent CoNARTaS study by Ginström Ernstad *et al.* (2019a), no difference was found in the risk of LGA between children born after vitrified blastocyst transfer and slow-frozen cleavage stage embryo transfer. Since vitrification was introduced simultaneously with blastocyst transfers, it was not possible to investigate the impact of freezing method and culture time separately (Ginström Ernstad *et al.*, 2019a). In a previous Finnish study, the freezing method did not impact the risk of LGA (Kaartinen *et al.*, 2016).

There is a wide physiological range in human foetal growth (Gardosi *et al.*, 2018). Male foetus, advanced maternal age, height, weight and parity all have a positive effect on foetal growth estimated from ultrasound measurement. Also, ethnicity has a substantial role on foetal growth (Kiserud *et al.*, 2017). In our study, we were able to control for offspring sex, maternal age, BMI and parity, and also smoking, chronic hypertension and diabetes. Information on ethnicity was not available, but since the majority of the Nordic population is of Caucasian origin, this is not likely to be a major confounding factor in our study. Unfortunately, we had no information on gestational diabetes. Our data on diabetes included any diabetes due to different registration practices in the study countries. However, in the meta-analysis by Sha *et al.* (2018), no difference in the amount of gestational diabetes was shown between pregnancies originating after FET and fresh ET.

Several factors may contribute to foetal growth differences between children born after FET, fresh ET and NC. The underlying cause for infertility/subfertility of the parents, laboratory procedures, embryo culture medium, culture length and patient or embryo selection through the freeze–thaw process may have an influence. Placentation may be altered due to the supraphysiological blood concentrations of oestradiol and progesterone following ovarian stimulation and fresh ET, lasting at least through the first trimester (Järvelä *et al.*, 2014) and possibly causing increased risk of low birth weight (Pereira *et al.*, 2017). Varying epigenetic changes and differences in gene expression in placentas and children from pregnancies originating after FET, fresh ET and NC have been discovered (Estill *et al.*, 2016; Choux *et al.*, 2018; Lee *et al.*, 2019). It has also been suggested that the effect of ART on DNA methylation in placenta is genotype specific (Marjonen *et al.*, 2018).

The consequences of these changes on the long-term health of the ART offspring are still largely unclear. It is known that the perinatal health of children originating after ART has improved in the past years, greatly explained by the reduced amount of multiple gestations after introduction of FET and eSET (Henningesen *et al.*, 2015). It is noteworthy, that the risk of LGA is pronounced in FET pregnancies, even as the risk of hypertensive disorders is also higher compared to fresh ET and NC pregnancies (Opdahl *et al.*, 2015; Maheshwari *et al.*, 2018; Sha *et al.*, 2018). The absence of a corpus luteum in programmed FET cycles has been shown to be associated with higher risk of hypertensive disorders in pregnancy and neonatal macrosomia (birth weight > 4500 g) compared to FET in natural or stimulated cycles (Ginström Ernstad *et al.*, 2019b). In our study, we unfortunately had no data on the different FET cycle modalities.

A question has been raised about whether the effects of the freeze–thaw process depend on the offspring sex. In our study, we found the weight difference to become statistically significant slightly earlier in boys than girls, in accordance with Li *et al.* (2014). The observed gender difference may, however, also be caused by statistical power, since there are more boys than girls among preterm births. In a study by Kaartinen *et al.* (2015), male sex was found to be more affected by extended embryo culture times as regards to LGA birth weight. In a recent single-centre study of 1295 singletons, term boys born after FET from blastocyst transfers in artificial cycles were found to have significantly greater risk of LGA compared to girls (Coetzee *et al.*, 2020), and in a nationwide cohort from the US, consisting of 180 184 singletons (Litzky *et al.*, 2018), FET had a slightly stronger association with larger birthweights of term boys compared to girls. Our results somewhat contradict these findings, as there were no sex-dependent differences in the aOR for LGA in the whole study population or in the term-born subgroup.

It is unlikely that small differences in birth weight should cause any problems for the offspring. In addition, there is evidence that the ponderal index of FET newborns is in line with those of fresh ET and NC newborns, suggesting that FET children are not ‘fat’, just large in size (Pelkonen *et al.*, 2010). Still, concerning LGA birthweight, clear consequences have been shown. LGA at term is associated with certain perinatal risks for both the mother and the neonate, such as birth trauma (Khambalia *et al.*, 2017; Doty *et al.*, 2019). A recent study also suggests that pregnan-

cies complicated by LGA are at increased risk of stillbirth at or beyond GW 36, independent of maternal diabetic status (Carter *et al.*, 2019). On the other hand, extremely or very premature LGA infants (GW 22–29) had better in-hospital outcomes compared to AGA infants in a recently published retrospective cohort (Boghossian *et al.*, 2018). Children who are born LGA have been shown to have a higher risk for obesity later in childhood, school-age and adulthood (Taal *et al.*, 2013; Kapral *et al.*, 2018; Derraik *et al.*, 2020), which may lead to elevated cardiometabolic risks later in life.

In conclusion, the freeze–thaw process seems to be associated with excess foetal growth, leading to higher birth-weights and greater risk of LGA at least in the last trimester of pregnancy. This is a major aspect of the safety profile of ART treatments. Our results add to the understanding of the timing of these differences. While FET and eSET have been important steps forward in reducing the risks associated with multiple gestations, more research is needed on the possible risks associated with LGA birth weights, especially combined with preterm birth and long-term outcomes of these ART children.

Data availability

The Nordic register keepers have given permission for this specific study, and the data cannot be shared without authorization from the register keepers. Interested research collaborators can contact the CoNARTaS researchers at www.conartas.com.

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Authors' roles

All authors contributed to the obtaining and preparing the data. A.M.T., S.P., A.T. and M.G. participated in the study design. M.G. analysed the data. A.M.T., S.P., M.G. and A.T. drafted the manuscript. All authors contributed in the revisions of the manuscript and approved the final version.

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Conflict of interest

None reported.

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