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Growth of twins conceived using assisted reproductive treatments up to 5 years old: a national growth cohort

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STUDY QUESTION: Do twins conceived through assisted reproductive treatments (ART) grow differently from naturally conceived (NC) twins in early life?

SUMMARY ANSWER: Assessments at 6–8 weeks old and at school entry show that ART twins conceived from frozen embryo transfer (FET) grow faster than both NC twins and ART twins conceived from fresh embryo transfer (ET).

WHAT IS KNOWN ALREADY: Singletons born from fresh ET grow more slowly *in utero* and in the first few weeks of life but then show postnatal catch-up growth by school age, compared to NC and FET babies. Evidence on early child growth of ART twins relative to NC twins is inconsistent; most studies are small and do not distinguish FET from fresh ET cycles.

STUDY DESIGN, SIZE, DURATION: This cohort study included 13 528 live-born twin babies conceived by ART (fresh ET: 2792, FET: 556) and NC (10 180) between 1991 and 2009 in Scotland. The data were obtained by linking Human Fertilisation and Embryology Authority ART register data to the Scottish Morbidity Record (SMR02) and Scottish child health programme datasets. Outcome data were collected at birth, 6–8 weeks (first assessment), and school entry (4–7 years old) assessments. The primary outcome was growth, measured by weight at the three assessment points. Secondary outcomes were length (at birth and 6–8 weeks) or height (at school entry), BMI, occipital circumference, gestational age at birth, newborn intensive care unit admission, and growth rates (between birth and 6–8 weeks and between 6–8 weeks and school entry).

PARTICIPANTS/MATERIALS, SETTING, METHODS: All twins in the linked dataset (born between 1991 and 2009) with growth data were included in the analysis. To determine outcome differences between fresh ET, FET, and NC twins, linear mixed models (or analogous logistic regression models) were used to explore the outcomes of interest. All models were adjusted for available confounders: gestational age/child age, gender, maternal age and smoking, Scottish Index of Multiple Deprivation, year of treatment, parity, ICSI, and ET stage.

MAIN RESULTS AND THE ROLE OF CHANCE: In the primary birth weight models, the average birth weight of fresh ET twins was lower [−35 g; 95% CI: (−53, −16)g] than NC controls, while FET twins were heavier [71 g; 95% CI (33, 110) g] than NC controls and heavier [106 g; 95% CI (65, 146) g] than fresh ET twins. However, the difference between FET and NC twins was not significant when considering only full-term twins (≥ 37 weeks gestation) [26 g; 95% CI (−30, 82) g], while it was significantly higher in preterm twins [126 g; 95% CI (73, 179) g]. Growth rates did not differ significantly for the three groups from birth to 6–8 weeks. However, FET twins grew significantly faster from 6 to 8 weeks than NC (by 2.2 g/week) and fresh ET twins (by 2.1 g/week). By school entry, FET twins were 614 g [95%

CI (158, 1070) g] and 581 g [95% CI (100, 1063) g] heavier than NC and fresh ET twins, respectively. Length/height and occipital frontal circumference did not differ significantly at any time point.

LIMITATIONS, REASONS FOR CAUTION: Although the differences between ART and NC reflect the true ART effects, these effects are likely to be mediated partly through the different prevalence of mono/dizygotic twins in the two groups. We could not explore the mediating effect of zygosity due to the unavailability of data. The confounding variables included in the study were limited to those available in the datasets.

WIDER IMPLICATIONS OF THE FINDINGS: Live-born twins from FET cycles are heavier at birth, grow faster than their fresh ET and NC counterparts, and are still heavier at school entry. This differs from that observed in singletons from the same cohort, where babies in the three conception groups had similar weights by school entry age. The results are reassuring on known differences in FET versus fresh ET and NC twin outcomes. However, FET twins grow faster and are consistently larger, and more ART twins depict catch-up growth. These may lead to an increased risk profile for non-communicable diseases in later life. As such, these twin outcomes require careful evaluation using more recent and comprehensive cohorts.

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Key words: assisted reproduction / birth weight / child growth / twins / catch-up growth / catch-down growth

Introduction

ART utilization has increased steadily since 1978 and now accounts for over 10 million babies born worldwide (ESHRE ART Fact Sheet, 2022). Even though most ART babies are born healthy, ART treatments are linked with a higher incidence of poor birth outcomes, including low birth weight (LBW), congenital abnormalities, preterm births (PTB), small for gestational age (<10th birth weight centile of babies with same gestational age), and in some cases perinatal mortality (Kamphuis et al., 2014; Sunde et al., 2016; Qin et al., 2017; Cavoretto et al., 2018; Maheshwari et al., 2018; Castillo et al., 2019; Wennerholm and Bergh, 2020; Xiong et al., 2022). These poor perinatal outcomes are partly the result of restricted intrauterine growth leading to altered growth trajectories, which may predispose babies to adverse later life outcomes (Barker, 1997).

Specific ART procedures, particularly frozen embryo transfer (FET) and fresh embryo transfer (ET), have been linked to differences in outcomes within the ART cohort. Several studies have reported that singleton fresh ET babies are smaller at birth, while FET babies are larger than their naturally conceived (NC) counterparts (Maheshwari et al., 2018; Castillo et al., 2019; Laval et al., 2020; Terho et al., 2021a). Some smaller ART growth studies have reported inconsistent differences in growth for fresh ET, FET, and NC babies (Ludwig et al., 2006; Sakka et al., 2010; Yeung et al., 2016; Magnus et al., 2021; Terho et al., 2021b). Two recent large cohort studies demonstrated that small fresh ET singletons show catch-up growth and weigh the same as FET and NC singletons by ~5 years old or adolescence (Hann et al., 2018; Turner et al., 2020).

Most research on ART-related child growth outcomes has considered only singletons, and data on the development of ART twins are limited. ART is linked to increased rates of multiple births, mainly from the transfer of two or more embryos and a higher incidence of monozygotic twinning relative to NC pregnancies (Vitthala et al., 2009; Hviid et al., 2018; Ikemoto et al., 2018). These twins grow differently *in utero*, have a higher risk for adverse perinatal outcomes, and show different growth patterns in early life compared to their singleton

counterparts (Vitthala et al., 2009; Geisler et al., 2014; Murray et al., 2019; Hirsch et al., 2020).

Despite the known risks when comparing twins to singletons, it is unclear whether twin pregnancies following ART are associated with higher risks of complications compared to NC twin pregnancies. Studies on ART twin growth are few and some report conflicting growth patterns between ART and NC twins in infancy (Koivurova et al., 2003; Lee et al., 2010; van Beijsterveldt et al., 2011; Yeung et al., 2016; Zhang et al., 2021). Other studies have not established significant differences in adverse perinatal outcomes between ART and NC twins (Andrijasevic et al., 2014; Geisler et al., 2014; Pourali et al., 2016; Chen et al., 2019; Murray et al., 2019; Pavoković et al., 2021). However, most of these studies are based on small samples and also do not distinguish FET from fresh ET babies, even though the impact of FET is now well documented in ART.

Although targeted policies such as elective single embryo transfer (eSET) have reduced twin rates to ~10% in the UK and slightly higher elsewhere (Roberts et al., 2010; El-Toukhy et al., 2018), there is still a higher incidence of twin births following ART than NC. In addition, there is a generation of twins born before eSET; hence there is a need to understand how these babies grow and develop to understand risks in current and future twin generations. We hypothesize that similar to the singleton cohort (Hann et al., 2018), ART twins in this cohort may grow differently from their NC counterparts, translating into an increased risk profile for non-communicable diseases in later life. Therefore, this study aimed to compare the child growth outcomes for ART (fresh ET and FET cycles) relative to NC twins from birth to school entry age.

Materials and methods

Dataset

Data on ARTs between 1991 and 2009 from the Human Fertilisation and Embryology Authority (HFEA) register was linked to the Scottish Morbidity Record (SMR02) and Scottish child health programme

datasets held by NHS Scotland (NHS NSS). The HFEA ART Register holds comprehensive records of all women who have used ART since August 1991. The SMR02 contains routinely collected birth records on obstetric outcomes from a woman's period of care in an obstetric clinic. The Scottish child health programme collects child growth data from routine screening programmes. In an earlier study, [Hann et al. \(2018\)](#) first linked the HFEA and SMR02 datasets using probabilistic matching based on maternal names and maternal and child dates of birth, linking maternal ART treatment characteristics to birth outcomes. The individual child-level birth data were then linked to the Scottish child health programme dataset using individual child Community Health Index numbers. The study matched each individual baby conceived by ART to approximately four NC babies based on the mother's age and child's gender, resulting in 8791 ART and 35 100 NC babies (singleton and multiple births) born between 1991 and 2009. The growth data were collected at 6–8 weeks and school entry assessments (4–7 years old). Ethical approval was obtained before accessing and analysing this dataset.

Inclusion–exclusion criteria

We included only twin-baby entries and excluded all singletons and triplets for the current study. We also excluded babies with missing gender and babies without growth data at either the 6–8 weeks and school entry assessments ($n = 1142$ (11%) NC and $n = 393$ (12%) ART). We thus extracted data on 11 993 twin babies for the current study based on the inclusion and exclusion criteria summarized in [Fig. 1](#). In some cases, only one twin was available in the final dataset, as the corresponding twin was excluded. The resultant twin dataset comprised 2955 ART and 9038 NC babies. Data were thus available for ~86% ($n = 10 292$) at 6–8 weeks and 56% ($n = 6709$) at school entry assessments. Lower numbers at 6–8 weeks and school entry assessments were assumed to be random dropouts. A sensitivity analysis of missing data/dropouts revealed that missingness was not associated with any available patient characteristics.

Outcome variables

The primary outcome was growth as measured by weight (g) at the three assessment points after adjustment for age at assessment (gestation at birth). Secondary outcomes were length (measured while lying down at birth and 6–8 weeks) or height (measured while standing at school entry), occipital circumference (OFC) (mm) at birth and 6–8 weeks assessment, BMI (kg/m^2) at school entry, gestational age at birth (weeks), newborn intensive care unit (NICU) admission, and growth rates. Average growth rates were defined as the average change in weight per week (between birth and 6–8 week measurements, and between 6–8 weeks and school entry measurements). We further explored growth rates for discordant twins as a sensitivity analysis. Twin growth discordance was defined as an inter-twin birth weight discordance $> 20\%$: $(\text{larger twin birth weight} - \text{smaller twin birth weight}) / \text{larger twin birth weight} \times 100\%$ ([Miller et al., 2012](#)).

In addition, we explored whether babies with lower or higher birth weights (smaller or larger babies) tended to the median, i.e. catch-up or catch-down growth. We transformed birth weight and school entry weight into deciles of age-standardized z-scores using tables of age and gender-specific L (skew), M (median), and S (coefficient of variation) values ([Cole, 2012](#)). A baby was categorized as smaller or larger at

birth if they fell into the 1st–3rd or 8th–10th deciles, respectively. We defined catch-up or catch-down growth as a baby moving at least one decile up or down on the standardized growth charts from birth to school entry age.

Data analysis

Descriptive statistics are presented as counts (percentages) or mean values (\pm standard deviation). We modelled the outcomes (weight, length, OFC, BMI, gestation, and growth rate) using separate multivariable linear mixed models at each of the three time points (birth, 6–8 weeks, and school entry) with a random effect term (twin-set) to account for correlation within twin pairs or shared parental characteristics. NICU admission was analysed using analogous logistic regression models. We utilized the intraclass correlation coefficient (ICC) to report the correlation in outcomes within a twin set.

For analyses comparing ART (fresh ET and FET cycles) and NC babies, we adjusted for baby gender, age (gestational age at birth, child age at 6–8 weeks, and school entry), deprivation index (assessed from the quintiles of the 2012 Scottish Index of Multiple Deprivation) ([The Scottish Government, 2020](#)), smoking status (self-reported), year of delivery, maternal age, and feeding mode (breast, bottle, both) at 10 days after birth (for child growth outcomes at 6–8 weeks and school entry). Gestational age at birth was fitted as a restricted cubic spline with four degrees of freedom, and maternal age was fitted using a quadratic function. Sensitivity analyses indicated that the selected functional forms adequately captured the age–outcome relationships; additional degrees of freedom for the splines did not improve model fit as assessed using AIC. Similarly, interaction terms between conception groups and gender did not significantly improve the fit. For within ART analyses, we additionally adjusted for the use of ICSI, parity, infertility causes, and IVF treatment centre. As sensitivity analyses, we further explored the weight outcome for twins born at different gestational ages (extremely preterm ($\text{GA} < 28$), preterm ($\text{GA} < 37$), term babies ($\text{GA} \geq 37$)) and growth rates for discordant twins.

All statistical analyses were performed in Stata (version 16; Stata Corporation, College Station, TX, USA), and 95% confidence intervals for the estimates are presented.

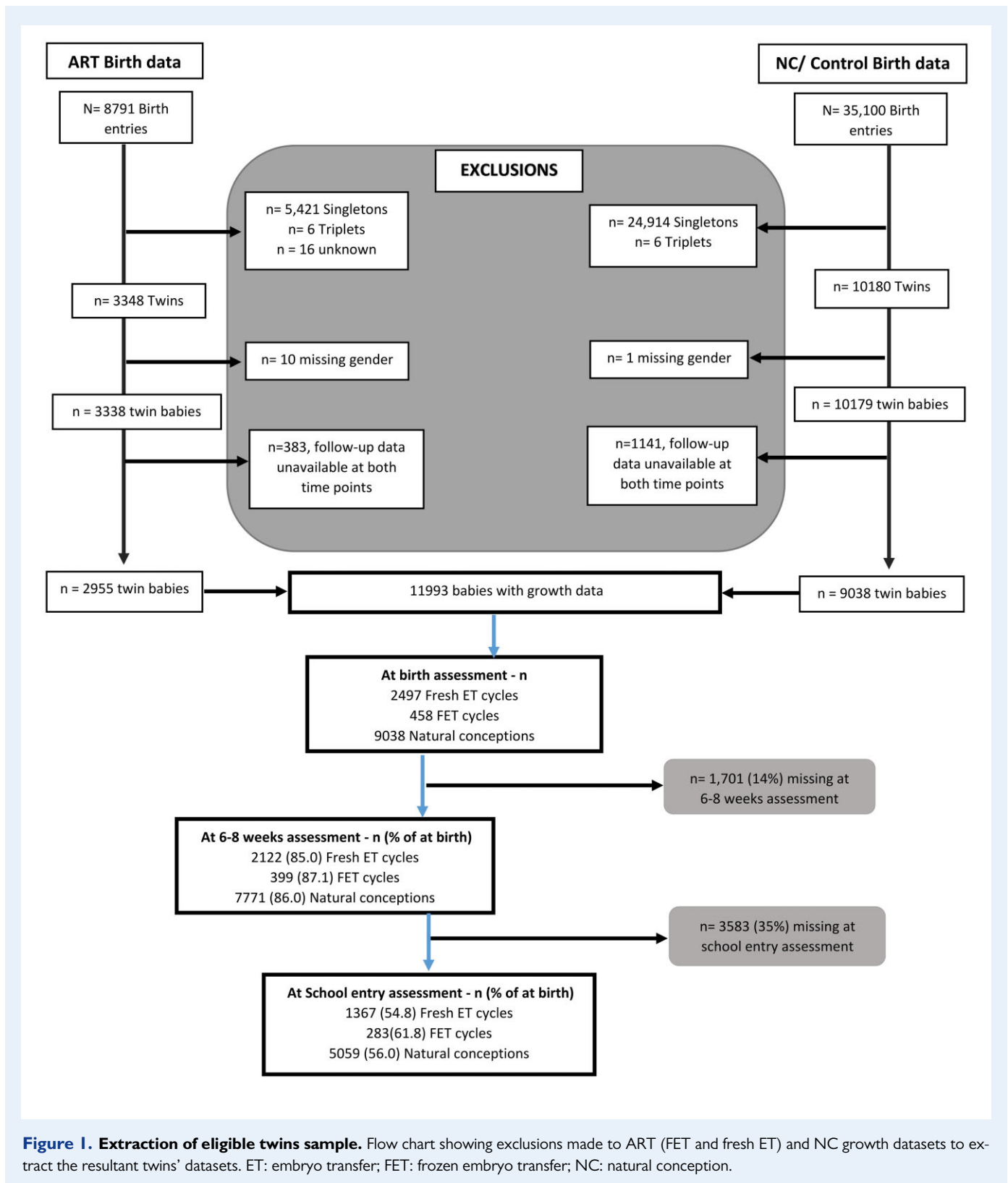
Results

Data summaries

The primary and secondary outcome variables are presented in [Table 1](#). Other summary statistics (characteristics of mothers, babies, and ART treatment information) are presented in [Supplementary Table S1](#). Overall, the proportion of infants within particular subgroups of characteristics did not vary substantively across conception groups or at different assessment points ([Supplementary Table S11](#)).

Child weight at all assessment points: ART versus NC

The average unadjusted birthweight was 2399 g, 2395 g, and 2575 g for NC, fresh ET, and FET twins, respectively ([Table 1](#)). Compared to NC twins, birth weight was significantly lower in fresh ET twins [-35 g; 95% CI ($-53, -16$)] and higher in FET twins [71 g; 95% CI (33, 110)]



in the fully adjusted model (Table II). Additional sensitivity analyses showed that the difference between FET and NC twins was not significant when considering only full-term babies (GA \geq 37 weeks) [26 g; 95% CI (-30, 82)], while it was significantly higher in preterm babies

(GA < 37 weeks) [126 g; 95% CI (73, 179)]. In contrast, differences between NC and fresh ET babies persisted for both preterm and full-term babies. At 6–8 weeks, fresh ET twins were still smaller than NC twins [-59 g; 95% CI (-103, -16)], while FET twins though slightly

Table 1 Summaries of outcome variables at the assessment points by conception type.

Birth outcomes	Naturally conceived n = 9038	ART fresh ET n = 2497	ART frozen ET n = 458
Raw birth weight (g) ^a	2399 [571]	2395 [572]	2575 [510]
Raw birth weight ^b			
<2500 g	4767 (52.7)	1322 (52.9)	197 (43.0)
≥2500 g and ≤4000 g	4252 (47.1)	1173 (47.0)	261 (57.0)
>4000 g	7 (0.1)	2 (0.1)	0 (0.0)
Crown–heel length (cm) ^a	47.2 [3.4]	47.4 [3.2]	47.9 [3.1]
OFC (cm) ^a	4034 (44.6)	1154 (46.2)	195 (42.4)
	32.90 [1.73]	33.0 [1.74]	33.30 [1.46]
	6082 (67.3)	1683 (67.4)	322 (70.1)
NICU admission ^b			
No	5353 (59.2)	1437 (57.6)	318 (69.4)
Yes	3592 (39.7)	1031 (41.3)	135 (29.5)
Gestation (weeks) ^a	35.7 [2.7]	35.7 [2.8]	36.1 [2.3]
	9036 (99.2)	2497 (100.0)	458 (100.0)
Gestation (weeks) ^b			
Full term (gestation ≥ 37)	4393 (48.6)	1279 (51.2)	254 (55.5)
Preterm (gestation < 37)	4643 (51.4)	1218 (48.8)	204 (44.5)
6–8 week assessment^a	7771 (86.0)	2122 (85.0)	399 (87.1)
Weight (g)	4464 [817]	4422 [937]	4538 [770]
	7771 (86.0)	2122 (85.0)	399 (87.1)
OFC (cm)	37.95 [2.19]	38.08 [2.48]	38.09 [1.53]
	7664 (84.8)	2098 (84.0)	399 (83.1)
Length (cm)	54.4 [3.3]	54.4 [3.4]	55.0 [3.0]
	7649 (84.6)	2089 (83.7)	396 (86.5)
School entry assessment^a	5059 (56.0)	1367 (54.8)	283 (61.8)
Weight (g)	19 806 [3047]	19 939 [2906]	20 203 [3284]
	5059 (56.0)	1367 (54.8)	283 (61.8)
Height (cm)	112.3 [5.4]	113.1 [4.9]	113.1 [6.0]
	5059 (56.0)	1367 (54.8)	283 (61.8)
Body mass index (kg/m ²)	16.1 [24.3]	15.5 [1.6]	15.8 [1.7]
	5059 (56.0)	1367 (54.8)	283 (61.8)

^aContinuous variables are presented as mean [standard deviation] and cell count (percentage of the sample at birth).

^bCategorical variables are presented as cell count (column percentage). ET: embryo transfer; OFC: occipital frontal circumference; NICU: neonatal intensive care unit.

heavier, did not differ significantly from NC twins (Table II and Supplementary Table SIII). FET twins were 614 g and 581 g heavier than NC and fresh ET twins at school entry, respectively.

The ICC values show that outcomes for babies born to the same mother (twin sets and the very few sibling twin sets) were highly correlated, suggesting that some proportion of variance in twin sets was associated with shared maternal/paternal factors. Further analysis also revealed that only 17% (n = 2038 babies) of the twins in the study were discordant twins (highly divergent from one another).

Child weight at all assessment points and ART factors: FET versus fresh ET

Within the ART cohort, we explored whether other ART factors (infertility diagnosis, treatment centre, ICSI, and parity) accounted for the

observed associations between FET and fresh ET outcomes. Adjusting for extra ART factors (Table III), FET twins were consistently heavier at birth [104 g; 95% CI (63, 145)] and school entry assessment [by 601 g; 95% CI (119, 1083)] than fresh ET twins. None of the other explored ART factors significantly affected the outcome estimates (Table III).

Secondary birth and growth outcomes

In the adjusted model, gestation was slightly longer in FET twins by 0.32 weeks (~3 days) (Supplementary Table SIV). These results correspond to the higher rates of PTB observed in fresh (49%) and NC (51%) twins compared to FET (44%) twins. Babies conceived from fresh ET were 23% more likely, while FET babies were 26% less likely to be admitted to the NICU. OFC and crown–heel length did not

Table II Weight at the three assessment points for ART versus NC twins.

	Unadjusted BW N = 11 982	Gestation- adjusted BW N = 11 946	Weight at 6–8 weeks N = 9798	Weight at school entry N = 5814
Effect size [95% CI] (g)				
Type of conception				
ART—fresh ET vs NC	–9 [–42 to 25]	–35 [–53 to –16]	–59 [–103 to –16]	32 [–203 to 268]
ART—frozen ET vs NC	196 [127–266]	71 [33–110]	58 [–30 to 147]	614 [158–1070]
Frozen ET vs fresh ET	205 [131–279]	105 [65–146]	118 [25–211]	581 [100–1063]
ICC^a	0.76 [0.75–0.77]	0.38 [0.36–0.41]	0.41 [0.38–0.43]	0.51 [0.49–0.53]

^aIntraclass correlation coefficient. Multiple linear regression results for models (except unadjusted birthweight) adjusted for child gender and age (gestation at birth, child age at 6–8 weeks and school assessment), maternal age, Scottish Index of Multiple Deprivation, smoking status, feeding mode (at 6–8 weeks and school assessment) and year of birth. Full model results are presented in [Supplementary Table SIII](#). ET: embryo transfer; BW: Birthweight; NC: natural conception.

Table III Effect of patient and ART treatment factors on the twin weights at the three assessment points for fresh and frozen ET twins.

	Birth weight N = 2892	Weight at 6–8 weeks N = 2360	Weight at school entry N = 1429
Effect size [95% CI] (g)			
Embryo transfer type			
ART—fresh cycle ^a	Ref	Ref	Ref
ART—frozen cycle	104 [63–145]	95 [–12 to 202]	601 [119–1083]
ICSI			
Yes ^a	Ref	Ref	Ref
No	16 [–27 to 59]	–58 [–171 to 55]	–163 [–717 to 391]
Infertility cause^b			
Endometrial	17 [–39 to 74]	–62 [–209 to 86]	–25 [–708 to 1658]
Male factor	31 [–17 to 80]	28 [–104 to 160]	263 [–388 to 912]
Ovulatory	15 [–41 to 71]	–57 [–208 to 94]	–282 [–1019 to 456]
Fallopian tube	22 [–29 to 72]	20 [–116 to 156]	264 [–360 to 889]
Unknown	36 [–16 to 88]	8 [–131 to 148]	219 [–443 to 881]
Previous births			
None ^a	Ref	Ref	Ref
1	38 [–15 to 90]	87 [–50 to 224]	150 [–482 to 782]
2+	397 [19 to 776]	225 [–691 to 1142]	–
ICC^c	0.26 [0.21–0.31]	0.29 [0.23 to 0.35]	0.39 [0.33 to 0.46]

^aReference group.

^bPatients can have multiple infertility causes.

^cIntraclass correlation coefficient. Multiple linear regression results for weight (fresh ET vs frozen ET) at the three time points, adjusted for the variables shown and additionally for child gender, age (gestation at birth, child age at 6–8 weeks, and school entry), Scottish Index of Multiple Deprivation, maternal age, year of delivery, smoking, treatment centre, feeding mode (6–8 weeks and school entry only). Effect estimate for 2+ previous births is not available at school entry due to the unavailability of data. ET: embryo transfer.

differ significantly between NC babies and babies born from FET or fresh transfers.

At the 6–8 weeks assessment, OFC did not differ significantly between ART and NC twins, while FET twins were longer than NC twins [by 0.5 cm; 95% CI (0.15, 0.85)] and fresh ET twins [by 0.61 cm; 95% CI

(0.24, 0.97)] ([Supplementary Table SV](#)). At school entry assessment, FET twins were slightly taller than NC twins [1.02 cm; 95% CI (0.25, 1.80)], while BMI did not differ significantly between ART and NC twins.

Considering fresh ET versus FET babies and adjusting for ART factors, FET babies were slightly taller at 6–8 weeks [by 0.72 cm; 95% CI

(0.32, 1.10)] and school entry [by 0.87 cm; 95% CI (0.04, 1.70)]. OFC at birth was 0.22 cm longer [95% CI (0.01, 0.42)] in FET twins, while there were no differences in OFC at 6–8 weeks and BMI at school entry between fresh and FET twins (Supplementary Table SVI).

Growth rates

Average growth rates did not differ significantly between NC and ART twins from birth to 6–8 weeks. From 6–8 weeks to school entry, FET twins grew significantly faster than their NC counterparts [by 2.17 g/week; 95% CI (0.54, 3.82)] (Table IV). A sensitivity analysis indicated that the difference in growth rates was mainly confined to term babies [2.8 g/week; 95% CI (0.74, 4.88)] compared to preterm babies [1.2 g/week; 95% CI (−1.44, 2.83)]. No differences were observed when comparing fresh ET and NC twins.

A total of 2038 (17%) babies were categorized as birthweight-discordant twins, of which 1503 (17%), 453 (18%), and 82 (18%) were NC, fresh ET, and FET, respectively. Overall, growth rates between 6–8 weeks and school entry did not differ when comparing smaller to larger twins from discordant twin sets, while smaller twins grew slightly faster between birth and 6–8 weeks assessment [by 80.1 g/week; 95% CI (71.6, 88.6)] (Supplementary Table SVII).

Adjusting for additional ART variables, FET twins grew faster than fresh ET twins between 6–8 weeks and school entry [2.09 g/week; 95% CI (0.43, 3.74)]. Though still higher in FET twins, the growth rates did not differ significantly from birth to 6–8 weeks [5.8 g/week; 95% CI (−5.7, 17.2)] (Supplementary Table SVII).

Catch-up/catch-down growth

Table V summarizes and compares babies showing catch-up or catch-down growth between ART and NC babies. With marginal statistical significance, slightly more fresh ET babies [43%, OR: 1.32 (1.01, 1.73)] showed catch-up growth from birth to school entry compared to FET babies (37%). The catch-up growth proportions did not significantly differ when comparing NC to fresh ET or FET babies, even though fresh ET babies were more likely [63%, OR: 1.31 (1.08, 1.57)] to depict catch-up growth than NC babies (57%) when considering the smaller babies. There were no differences in catch-down growth between NC and fresh ET or FET babies. However, slightly fewer fresh ET babies [OR: 0.71 (0.53, 0.97)] showed catch-down growth than

FET babies (27% FET vs 21% fresh). A sensitivity analysis looking at growth rates for babies categorized as smaller or larger (in Table V) revealed that smaller babies grew slightly slower [−2.47 g/week; 95% CI (−2.96, −1.97)] between 6–8 weeks and school entry, while larger babies grew slightly faster [by 2.32 g/week; 95% CI (1.61, 3.02)] (Supplementary Figures S1 and S2).

Discussion

The results from our large national cohort study suggest that newborn twins conceived by fresh ET have a significantly lower birth weight than NC twins, whereas FET twins are heavier, consistent with singleton studies (Hann *et al.*, 2018; Maheshwari *et al.*, 2018; Laval *et al.*, 2020; Terho *et al.*, 2021b). Contrary to what was observed in singletons from the same cohort (Hann *et al.*, 2018), FET twins grow faster than NC and fresh ET twins after the first assessment, and by school entry, FET twins are heavier than NC and fresh ET twins. Of the ART treatment factors explored, only embryo transfer type (fresh ET/FET) significantly impacted the studied outcomes. The use of ICSI, infertility diagnosis, and previous pregnancies did not pose any strong confounding effects in the within-ART analyses.

Compared to NC twins, FET twins have a slightly longer average gestation (by ~3 days) than fresh ET twins. This may partly explain why FET twins have a higher unadjusted BW; however, FET twins remain significantly heavier even following gestation length adjustment. In addition, sensitivity analyses revealed that birth weight differences between FET and NC twins were only observed in preterm babies (gestation < 37 weeks), suggestive of possible differences in embryo growth emerging after 37 weeks gestation, where FET twins presumably grew more slowly. The mechanisms behind these suggested foetal growth differences remain unclear. However, some researchers linked the differences to varying epigenetic changes and differences in gene expression in placentas and children from pregnancies originating after FET, fresh ET, and NC (Estill *et al.*, 2016; Lee *et al.*, 2019). Others have linked oestradiol and progesterone concentrations following ovarian stimulation with an increased risk of LBW in fresh ET babies (Järvelä *et al.*, 2014).

Growth rates between birth and 6–8 weeks did not differ significantly between ART and NC twins. At school entry, FET twins were

Table IV Growth rates of twin births from fresh or frozen ET and NC twins between birth and 6–8 weeks, and 6–8 weeks and primary school entry (4–7 years).

	Birth to 6–8 weeks (grams per week)		6–8 weeks to school entry (grams per week)	
	Unadjusted (N = 10 254)	Adjusted (N = 10 231)	Unadjusted (N = 5675)	Adjusted (N = 5663)
Type of conception		Ref		Ref
ART—fresh ET vs NC	−5.03 [−9.50 to −0.60]	−3.60 [−8.21 to 1.01]	0.14 [−0.69 to 0.97]	0.11 [−0.76 to 1.03]
ART—frozen ET vs NC	2.50 [−6.86 to 11.85]	3.11 [−6.23 to 12.41]	2.07 [0.45–3.69]	2.17 [0.54–3.82]
Frozen vs fresh ET	7.52 [−2.41 to 17.46]	6.71 [−3.10 to 16.51]	1.93 [0.20–3.66]	2.05 [0.32–3.79]
ICC^a	0.22 [0.20–0.25]	0.20 [0.17–0.23]	0.54 [0.52–0.57]	0.54 [0.51–0.57]

^aIntraclass correlation coefficient. Linear regression models for growth rates between birth and 6–8 weeks, and 6–8 weeks and primary school entry (average weekly growth rates), adjusting for type of conception, gender, Scottish Index of Multiple Deprivation, maternal age and smoking status during pregnancy, feeding mode, and the year of delivery.

Table V Comparisons of proportions of catch-up or catch-down growth between birth and school entry for twins conceived by NC, fresh ET, or frozen ET.

	Catch-up growth				Catch-down growth			
	All babies (n = 6707)		Smaller babies (n = 3109)		All babies		Larger babies (n = 974)	
	n (%)	OR (95% CI)	n (%)	OR (95% CI)	n (%)	OR (95% CI)	n (%)	OR (95% CI)
NC^a	2119 (42%)	—	1360 (57%)	—	1165 (23%)	—	438 (61%)	—
Fresh ET	595 (43%)	1.06 (0.94–1.20)	400 (63%)	1.31 (1.08–1.57)	292 (21%)	0.91 (0.78–1.05)	101 (56%)	0.83 (0.59–1.17)
Frozen ET	105 (37%)	0.82 (0.63–1.05)	64 (58%)	1.07 (0.72–1.63)	78 (27%)	1.27 (0.96–1.67)	39 (55%)	0.79 (0.47–1.34)
Frozen^b vs fresh	—	1.32 (1.01–1.73)	—	1.21 (0.78–1.87)	—	0.71 (0.53–0.97)	—	1.05 (0.58–1.89)

^aReference group for raw odds ratios comparing NC with fresh ET twins, NC with frozen ET twins.

^bReference group comparing Frozen ET with Fresh ET twins. Catch-up or catch-down growth is defined as a baby crossing one decile upwards or downwards on the age/gender-adjusted growth charts between birth and school entry. Smaller babies are defined as those in the 1st–3rd deciles at birth, while larger babies are those in the 8th–10th deciles. Proportions are presented for all twins experiencing catch-up growth for smaller babies or catch-down growth for larger babies. NC: natural conception; ET: embryo transfer; OR: odds ratio.

heavier than NC and fresh ET twins, which is consistent with higher growth rates observed in the FET group after 6–8 weeks. One study (Yeung et al., 2016) reported similar results of slightly rapid infant weight gain (adjusted OR: 1.08; 95% CI: 1.00, 1.16) among twins conceived by ART only up to 9 months. Afterwards, no significant differences in growth through to 3 years of age were observed between the ART and NC groups. Contrary to our weight findings, a study (van Beijsterveldt et al., 2011) investigating the growth of ART twins from birth to 12 years of age reported similar weight and height growth patterns between IVF and NC twins. Similarly, an earlier Finish study of 100 IVF twins matched to control twins until age 3 years found no evidence of differences in growth measures between IVF and NC twins (Koivurova et al., 2003), consistent with a study comparing 157 ART to 549 NC twins from birth and up to age 18 months in Taiwan (Lee et al., 2010). It is noteworthy that the above studies were small, and the ART groups did not distinguish fresh ET from FET cycles, which could potentially mask the differences as the literature is now clear and consistent on differences in outcomes between these two ART groups. A sensitivity analysis of our data comparing the two groups (ART vs NC twins) could only detect minor differences in birthweight whilst masking the reported differences between NC and ART (FET) cycles at school entry. Additionally, our results show that the effects differ in magnitude and often direction between fresh ET and FET twins. Based on these results, it is clear that we would have reported similar findings to the above studies if we did not account for the use of FET.

Unlike in singletons (Hann et al., 2018), where fresh ET infants notably catch up more, nearly half of the babies in each of the three groups depict catch-up growth by school entry. The results further suggest that slightly more FET twins show catch-up growth. In contrast, larger fresh ET and NC twins had catch-down growth, probably further explaining why we observed higher weight averages in the FET group at school entry. It is well-documented from historical studies that twins are generally smaller than singletons at birth (Wilson, 1979; Chaudhari et al., 1997; Buckler and Green, 2004). In most cases, they experience restricted intrauterine growth starting from ~27 weeks gestation (Hirsch et al., 2020), often corrected postnatal by altered/accelerated

growth trajectories, evidenced by ~40% of all babies in our study experiencing catch-up growth. A study comparing growth in NC singletons and twins suggested fast weight gain in twins immediately after birth which slowed down after 2 years old (Chaudhari et al., 1997; Buckler and Green, 2004), consistent with other historical studies that reported catch-up growth by 8 years old (Wilson, 1979; Morley et al., 1989). The differences between fresh ET and NC singletons in Hann's et al. (2018) study can be attributed to fresh ET babies, on average, being smaller than NC babies, hence the observed catch-up. However, it is unclear why FET twins appear to grow faster than the smaller NC and fresh ET twins. Perhaps the generally small size of twins implies that all twin babies, regardless of conception type, are susceptible to some form of catch-up in growth or grow faster after birth. It can be argued that the observed growth patterns in both ART and NC twin babies are a compensatory process where the infants attempt to reach their genetic growth potential after a period of prenatal growth restraint. The evidence from NC twin studies suggests that twins slow down in growth after ~2 years old and slowly catch up to their singleton counterparts by 8 years old. It is possible that by school entry age (5–7 years old), both NC and ART twins in this cohort are still catching up in growth; hence, the differences observed in growth may be attributed to known growth patterns in twins and not only ART factors. Perhaps a more extended period would provide information about whether twins in the three conception groups reach similar weights at a later age.

We are aware of complexities associated with twin pregnancies, such as differences in twin pregnancy outcomes due to zygosity–chorionicity. Buckler and Green (2008) reported that dizygotic twins had slightly better outcomes than monozygotic twins. Therefore, some of the observed differences between ART and NC twins in the current study may be associated with single ET ART pregnancies resulting in more monozygotic twins than NC pregnancies (Sobek et al., 2016). Although it would be informative to explore the impact of zygosity on the outcomes, our primary comparisons of ART and NC twins remain valid. As a sensitivity analysis, we replicated the analyses on a subset of girl–boy twin sets that were assumed to be dizygotic. The differences in child outcomes and growth rate estimates among conception

groups were similar to what is reported in the main analysis. In addition, we acknowledge the use of singleton growth references for centile calculations whose application in twins has been disputed; hence, we cautiously interpret the results that focus on smaller versus larger twin catch-up or catch-down comparisons. We are also mindful that gestation and feeding mode can be considered mediators instead of confounders. However, we treated feeding mode as more of a maternal characteristic (certain groups of mothers are likely to breastfeed or not) and a decision likely to have been taken before birth or even conception. We adjusted for gestation length as child growth (size for age) needs to be adjusted for time to grow (gestational age *in utero*). In the main, we were interested in the effect of ART on outcomes and hence adjusted for gestation and feeding, which are known to be associated with these outcomes; and this is in line with most studies on ART growth.

Mainly, these data include babies born before the introduction of twin reduction policies and changes in recent treatment trends; thus, outcomes in more recent data may differ. In addition, the introduction of consent for disclosure allowing the use of HFEA data for similar linkage studies may lead to serious biases due to low and potentially non-random consent rates in the post-2009 cohort. However, our study is one of the few large national follow-up ART twin cohorts with a reasonable sample size to distinguish fresh ET from FET cycles in the analysis. Even though we acknowledge that more comprehensive data on confounders would be preferred, we managed to adjust for some important confounding factors; hence, we believe that the results are informative.

The results of our study are somewhat reassuring. However, the observed small differences at the child level between ART and NC twins do translate into small but not insignificant population-level differences. In addition, these minor differences in birth outcomes (suggestive of altered foetal growth) and growth outcomes (suggestive of catch-up/down growth) after birth could still potentially lead to a higher risk of cardiovascular diseases (Ceelen *et al.*, 2009) and metabolic diseases, including obesity and Type 2 diabetes in later life (Ceelen *et al.*, 2009; Kerkhof and Hokken-Koelega, 2012; Martin *et al.*, 2017). The higher proportion of twins showing catch-up growth implies that these risks may be more pronounced in twins. Since twin rates are higher in ART, it implies more adverse outcomes; hence, it is reasonable to advance twinning preventative measures in ART.

Conclusion

This UK national cohort study is one of the few large ART twin growth studies showing differences in birth weight and growth trajectories between ART (separating fresh ET from FET cycles) and NC twins between birth and school entry age (4–7 years old). We have linked the ART treatment (fresh ET/FET) to the weight outcome and have demonstrated that compared to fresh ET and NC twins, FET twins differ significantly in birth weight, grow faster, and remain heavier by school entry age. Treatments are constantly changing, and this cohort ends in 2009; hence there is a need to replicate these in more recent cohorts to capture changes in ART practice. Future research should explore more recent ART cohorts with more extended follow-up periods and collect more detailed data for more comprehensive adjustments for confounding.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

The data underlying this article were provided by the Human Fertilisation and Embryology Authority and NHS Public Health Scotland under licence/by permission. The data are not publicly accessible, but may be accessed after ethics approvals.

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Authors' roles

F.J.M.K. performed all the data manipulation and cleaning, designed and conducted the formal analyses, and drafted the manuscript. Expert methodological advice was provided by S.A.R. and M.G., with expert clinical input from D.R.B., P.C., and S.W.D.S. D.R.B. and S.A.R. obtained the funding and conceptualized the study. S.A.R., D.R.B., M.G., and P.C. co-supervised the project and reviewed the analysis. All authors contributed to the preparation and critical review of the manuscript.

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Conflict of interest

The authors have no competing interests.

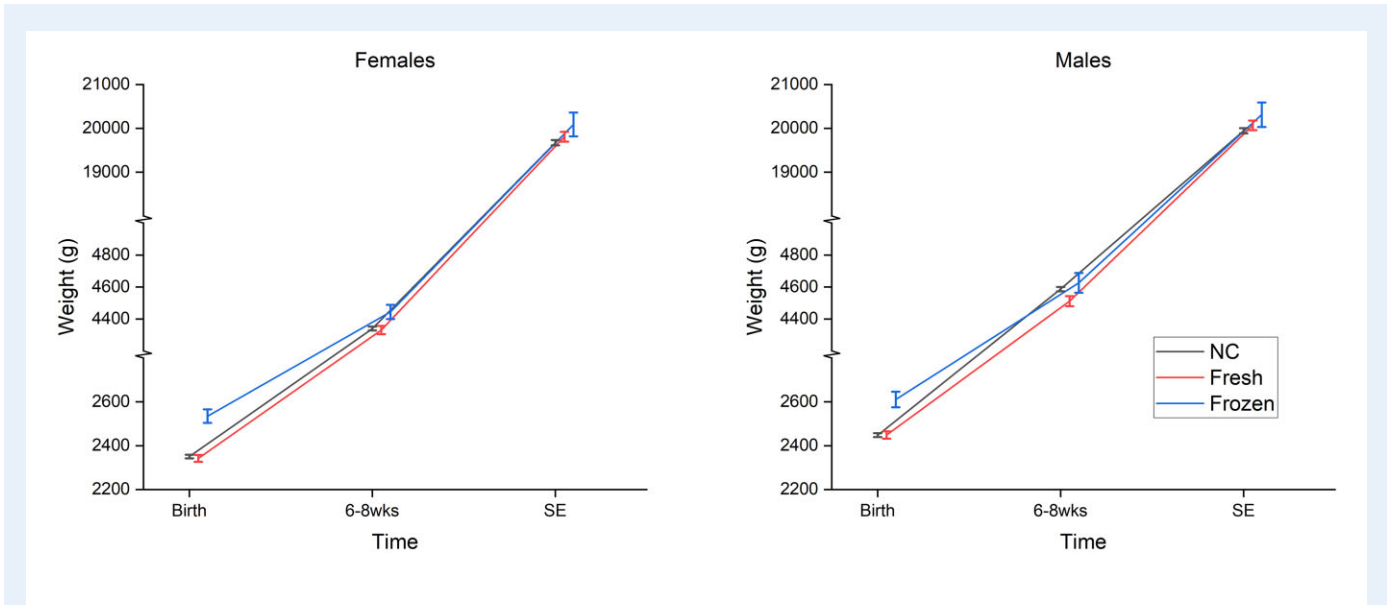
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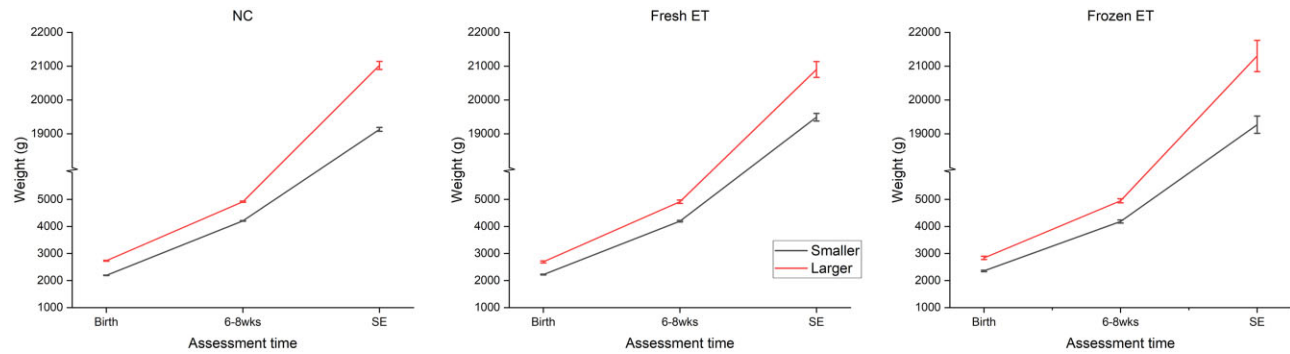
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human reproduction SUPPLEMENTARY DATA



Supplementary Figure S1. Mean infant weights for male and female twins at the three assessment points: birth, 6–8 weeks and school entry (SE) for ART-conceived babies following fresh and frozen embryo transfers and naturally conceived (NC) controls.



Supplementary Figure S2. Mean infant weights at the three assessment points: birth, 6–8 weeks and school entry (SE) for ART (fresh and frozen ET) and naturally conceived (NC) twins categorized as smaller or larger babies in the catch-up/catch-down growth analysis. ET: embryo transfer. Smaller babies are defined as those in the 1st–3rd deciles at birth, while larger babies are those in the 8th–10th deciles on the age and gender-adjusted LMS growth charts.

human reproduction **SUPPLEMENTARY DATA**

Supplementary Table SI Characteristics of mothers, babies, and ART treatments in the analysis datasets.

Variables of interest	Controls N (%) 9038 (75.4)	ART Fresh N (%) 2497 (20.8)	ART Frozen N (%) 458 (3.8)
<i>n (%)</i> , [<i>n missing</i> / %]			
Child			
Gestation			
≥37 weeks	4393 (48)	1279 (51)	254 (55)
<37 and ≥32 weeks	3927 (43)	1005 (40)	185 (40)
<32 weeks	716 (8)	213 (9)	19 (4)
Gender			
Male	4522 (50.0)	1230 (49.3)	239 (52.2)
Female	4516 (50.0)	1267 (50.7)	219 (47.8)
Discharge mode			
Dead	12 (0.13)	6 (0.24)	0 (0)
Home	5826 (64.4)	1583 (63.4)	346 (75.6)
NICU	2106 (23.3)	575 (23.0)	75 (16.4)
NICU then home	786 (8.7)	234 (9.4)	26 (5.7)
Remain in hospital	300 (3.3)	95 (3.8)	11 (2.4)
Foster home	10 (0.1)	0 (0)	0 (0)
<i>Mean (Min, Max)</i> , [<i>n missing</i> / %]			
Gestation at birth (weeks)	35.7 (21, 42) [2]	35.7 (23, 41) [0]	36.1 (26, 42) [0]
Age at 6–8-week assessment (days)	66.2 (10, 421) [1123/12.4]	65.8 (24, 185) [335/13.4]	63.0 (12, 150) [51/11.1]
Age at pre-school assessment (years)	5.6 (1.3, 7.3) [3979/44]	5.6 (4.1, 6.5) [1130/45]	5.5 (4.6, 6.5) [175/38]
Mother			
Previous IVF births	Data not available		
0		2342 (93.8)	353 (77.1)
1		155 (6.2)	101 (22.1)
2		0 (0) [0]	4 (0.9) [0]
Smoking			
No	6284 (69.5)	2107 (84.4)	366 (80.1)
Yes	1911 (21.1)	164 (6.6)	36 (7.8)
Unknown	843 (9.3)	226 (9.1)	56 (12.1)
Lifetime smoking			
Never smoked	5106 (56.5)	1708 (68.4)	320 (70.1)
Continued smoking	1812 (20)	148 (6)	32 (6.9)
Stopped smoking	764 (8.5)	220 (8.8)	20 (4.3)
Unknown	1356 (15)	421 (16.9)	86 (18.6)

(continued)

Supplementary Table SI Continued

Variables of interest	Controls N (%)	ART Fresh N (%)	ART Frozen N (%)
	9038 (75.4)	2497 (20.8)	458 (3.8)
Infertility cause (Can be multiple causes)	Not applicable		
Fallopian Tubal		571 (22.9)	106 (23.1)
Endometriosis		252 (10.1)	42 (9.2)
Ovulatory disorders		213 (8.5)	58 (12.7)
Male factor		1187 (47.5)	213 (46.5)
Unknown		743 (29.8)	124 (27.1)
Scottish Index of Multiple Deprivation			
1 (most deprived)	2304 (25.5)	289 (11.6)	68 (14.7)
2	1800 (19.9)	423 (16.9)	74 (16.2)
3	1528 (16.9)	433 (17.3)	95 (20.7)
4	1616 (17.9)	555 (22.2)	103 (22.5)
5 (least deprived)	1770 (19.6)	787 (31.5)	114 (24.9)
	[20/0.2]	[10/0.4]	[4/0.9]
Mode of feeding at discharge			
Breast	1669 (18.5)	542 (21.7)	80 (17.5)
Bottle	5030 (55.7)	1156 (46.3)	260 (56.8)
Both	1303 (14.4)	509 (20.4)	73 (15.9)
	[1036/11.5]	[290/11.6]	[45/9.8]
	<i>Mean (Min, Max), [n missing/%]</i>		
Age at childbirth (Mother)	30.4 (15, 52)	33.4 (20, 43)	33.6 (21, 45)
	[2/0.02]	[0]	[0]
Age at childbirth (Father)	Data unavailable	35.7 (20, 67)	35.4 (21, 51)
		[6/0.24]	[0]
	ART treatment factors		
Embryo duration in culture (days)	Not applicable		Data not available
0–2		1537 (61.6)	
3		632 (25.3)	
4		14 (0.6)	
5		28 (1.1)	
		[286/11.5]	
Specific treatment type	Not applicable		
IVF		1525 (61.1)	334 (72.9)
ICSI		972 (38.9)	124 (27.1)
		[0]	[0]

NICU: newborn intensive care unit.

Supplementary Table SII Summaries of available data for selected variables at the three assessment points.

	Birth ^a			6–8 weeks ^b			School entry ^b		
	N			n (% of N at birth)					
	Natural conception	ART fresh	FET	Natural conception	ART fresh	FET	Natural conception	ART fresh	FET
Child gender									
Male	4520	1231	237	3907 (86.5)	1064 (86.2)	207 (87.3)	2497 (55.1)	680 (55.0)	145 (60.3)
Female	4518	1266	221	3864 (85.5)	1058 (83.8)	192 (86.9)	2562 (56.9)	687 (54.5)	138 (63.3)
Scottish IMD									
1 (most deprived)	2304	289	68	2021 (87.7)	259 (89.6)	60 (88.2)	1140 (49.5)	130(45.0)	40 (58.8)
2	1800	423	74	1576 (87.6)	355 (83.9)	67 (90.5)	1039 (57.7)	248 (58.6)	42 (56.8)
3	1528	433	95	1274 (83.4)	366 (84.5)	79 (83.2)	922 (60.3)	273 (63.0)	69 (72.6)
4	1616	555	103	1366 (84.5)	473 (85.2)	87 (84.5)	944 (58.4)	305 (55.0)	55 (53.4)
5 (least deprived)	1770	787	114	1514 (85.5)	660 (83.9)	102 (89.5)	1004 (56.7)	411 (52.2)	75 (65.8)
Unknown	20	10	4	20 (100)	9 (90)	4 (100)	10 (50)	0 (0)	2 (50)
Maternal lifetime smoking status									
Never smoked	5106	1708	320	4391 (85.9)	1452 (85.0)	277 (86.6)	2850 (55.8)	954 (55.9)	207 (64.7)
Continued	1812	148	32	1533 (84.6)	119 (80.4)	31 (96.9)	1008 (55.6)	66 (44.6)	10 (31.3)
Stopped	764	220	20	662 (86.6)	185 (84.1)	20 (100)	422 (55.2)	120 (54.5)	11 (55.0)
Unknown	1356	421	86	1185 (87.4)	366 (86.9)	71 (82.6)	779 (57.4)	227 (53.9)	55 (64.0)
Maternal age at birth									
N (%)	9038	2497	458	7771 (85.9)	2122 (84.9)	399 (87.1)	5059 (56.0)	1367 (54.8)	283 (61.8)
[Mean (Min, Max)]	[30.4 (15, 52)]	[33.4 (20, 43)]	[33.6 (21, 45)]	[30.4 (15, 52)]	[33.4 (20, 43)]	[33.6 (21, 45)]	[30.3 (15, 50)]	[33.4 (20, 43)]	[33.8 (21, 41)]
Total	9038	2497	458	7771 (85.9)	2122 (84.9)	399 (87.1)	5059 (56.0)	1367 (54.8)	283 (61.8)

^aNumber of babies in each category at birth.

^bNumber of babies at a time point and the percentage of babies at birth. IMD: index of multiple deprivation.

Supplementary Table SIII Further details of the fitted models for weight outcomes at birth, at 6–8 weeks and school entry assessment for ART (fresh/frozen ET) versus NC twin babies—an extension of manuscript Table II.

	Gestation adjusted birthweight (g) N = 11 946	Unadjusted weight at 6–8 weeks (g)	Weight at 6–8 weeks (g) N = 9798	Unadjusted weight at school entry (g)	Weight at school entry (g) N = 5814
Effect size [95% CI] (g)					
Type of conception					
Naturally conceived	Ref		Ref		Ref
ART—fresh cycle	−34.5 [−53.3, −15.7]	−41.7 [−92.1, 8.7]	−59.3 [−103.0, −15.7]	130.1 [−93.6, 353.7]	32.3 [−203.4, 268.0]
ART—frozen cycle	71.1 [32.5, 109.8]	86.9 [−17.7, 191.6]	58.4 [−29.9, 146.7]	468.6 [23.5, 913.7]	613.7 [157.6, 1069.8]
Fresh vs frozen	105.7 [65.0, 146.3]	128.6 [17.7, 239.6]	117.7 [24.7, 210.8]	338.5 [−136.1, 813.2]	581.4 [100.2, 1062.6]
Gender					
Male	Ref		Ref		Ref
Female	−99.3 [−111.5, −87.1]		−155.3 [−183.3, −127.2]		−81.5 [−223.1, 60.2]
Age at measurement					
			22.0 [21.0, 23.0]		4.8 [4.1, 5.4]
Deprivation index					
1 (most deprived)	−45.1 [−68.7, −21.5]		−27.1 [−82.1, 27.9]		−196.3 [−504.2, 111.5]
2	−23.3 [−46.7, 0.10]		3.95 [−50.6, 58.5]		−113.0 [−406.2, 180.3]
3	−14.1 [−37.8, 9.64]	NA	0.41 [−54.9, 55.8]	NA	−85.1 [−379.9, 209.6]
4	−1.02 [−23.9, 21.9]		25.1 [−28.2, 78.4]		52.4 [−238.3, 343.1]
5 (least deprived)	Ref		Ref		Ref
Smoking					
No	Ref		Ref		Ref
Yes	−101.0 [−119.3, −82.7]		−102.2 [−145.0, −59.4]		159.2 [−73.5, 391.9]
Unknown	−23.0 [−44.1, −1.89]		−4.95 [−53.6, 43.7]		−57.1 [−313.8, 199.6]
Feeding mode					
Breast			Ref		Ref
Bottle	NA		180.9 [137.9, 223.9]		65.0 [−164.9, 294.9]
Breast and bottle			41.8 [−10.6, 94.2]		31.3 [27.3, 598.0]
ICC^a	0.38 [0.36, 0.41]		0.41 [0.38, 0.43]		0.51 [0.48, 0.53]

Mixed linear regression results for models adjusted for child gender and age (gestation at birth, child age at 6–8 weeks and school assessment), maternal age, Scottish Index of Multiple Deprivation, smoking status, feeding mode (at 6–8 weeks and school assessment), and year of birth. ^aIntraclass correlation.

human reproduction **SUPPLEMENTARY DATA**

Supplementary Table SIV Secondary birth outcomes for ART (fresh/frozen ET) versus NC twin babies.

	Birth outcomes			
	Birth length (cm) N = 5359	Birth OFC (cm) N = 8062	Gestation (weeks) N = 11 957	NICU N = 13 347
	Effect size (95% CI)			OR [95% CI]
Type of conception				
Naturally conceived	Ref	Ref	Ref	Ref
ART—fresh cycle	−0.09 [−0.33, 0.14]	−0.003 [−0.10, 0.09]	−0.14 [−0.31, 0.04]	1.23 [1.09, 1.39]
ART—frozen cycle	0.22 [−0.28, 0.73]	0.17 [−0.02, 0.36]	0.32 [0.01, 0.64]	0.74 [0.58, 0.95]
Fresh ^a vs frozen	0.32 [−0.21, 0.84]	0.18 [−0.02, 0.38]	0.46 [0.12, 0.80]	0.61 (0.47, 0.79)
Gender				
Male	Ref	Ref	Ref	Ref
Female	−0.60 [−0.75, −0.45]	−0.48 [−0.54, −0.42]	−0.01 [−0.12, 0.10]	1.03 [0.93, 1.13]
Deprivation index				
1 (most deprived)	−0.36 [−0.67, −0.05]	−0.12 [−0.24, 0.001]	−0.23 [−0.44, 0.01]	1.12 [0.96, 1.30]
2	−0.16 [−0.45, 0.14]	−0.14 [−0.25, −0.02]	−0.23 [−0.44, −0.01]	1.00 [0.86, 1.16]
3	−0.35 [−0.64, −0.06]	−0.04 [−0.15, 0.08]	0.06 [−0.15, 0.27]	1.21 [1.04, 1.40]
4	−0.23 [−0.51, −0.05]	−0.06 [−0.17, 0.05]	−0.03 [−0.24, 0.17]	0.96 [0.84, 1.11]
5 (least deprived)	Ref	Ref	Ref	Ref
Smoking				
No	Ref	Ref	Ref	Ref
Yes	−0.67 [−0.89, −0.44]	−0.34 [−0.43, −0.25]	−0.10 [−0.27, 0.06]	1.06 [0.94, 1.19]
Unknown	−0.01 [−0.33, −0.31]	−0.09 [−0.20, −0.023]	−0.47 [−0.68, −0.27]	0.80 [0.70, 0.91]
ICC^b	0.49 [0.46, 0.52]	0.37 [0.34, 0.40]	NA	NA

NICU: newborn intensive care unit; OFC: occipitofrontal circumference.

^aReference group comparing fresh to frozen.

^bIntraclass correlation is only presented for length and OFC. Gestation and NICU models were fixed effects models.

Results from mixed linear regression models for Length, OFC, gestation, and NICU admission. Models were adjusted for the variables shown and additionally adjusted for gestation (except the gestation model), maternal age, and birth year. Effect sizes represent the difference from the indicated reference group.

Supplementary Table SV Secondary growth outcomes at 6–8 weeks and school entry for ART (fresh/frozen ET) compared to NC twins.

	Length or height (cm)		OFC (cm)	BMI (kg/m ²)
	Effect size [95% CI]			
	6–8 weeks	School entry	6–8 weeks	School entry
Type of conception				
Naturally conceived	Ref	Ref	Ref	Ref
ART—fresh cycle	−0.10 [−0.27, 0.07]	0.38 [−0.03, 0.78]	0.072 [−0.04, 0.19]	−0.75 [−2.23, 0.74]
ART—frozen cycle	0.50 [0.15, 0.85]	1.02 [0.25, 1.80]	0.09 [−0.13, 0.33]	−0.98 [−3.86, 1.91]
Gender				
Male	Ref	Ref	Ref	Ref
Female	−0.46 [−0.57, −0.35]	−0.13 [−0.36, 0.09]	−0.43 [−0.50, −0.35]	−0.42 [−1.58, 0.73]
Maternal age (linear)	−0.01 [−0.01, 0.02]	0.01 [−0.04, 0.05]	−0.001 [−0.01, 0.01]	0.20 [0.04, 0.37]
Deprivation index				
1 (most deprived)	−0.21 [−0.43, 0.01]	−1.33 [−1.85, −0.80]	−0.07 [−0.21, 0.08]	0.34 [−1.59, 2.28]
2	−0.04 [−0.25, 0.18]	−0.92 [−1.42, −0.42]	0.07 [−0.08, 0.22]	1.66 [−0.18, 3.51]
3	−0.16 [−0.38, 0.06]	−0.54 [−1.04, −0.04]	0.10 [−0.05, 0.25]	0.17 [−1.68, 2.02]
4	−0.09 [−0.30, 0.12]	−0.18 [−0.68, 0.31]	0.05 [−0.46, 0.26]	0.75 [−1.07, 2.58]
5 (least deprived)	Ref	Ref	Ref	Ref
Smoking				
No	Ref	Ref	Ref	Ref
Yes	−0.55 [−0.72, −0.38]	−0.52 [−0.99, −0.05]	−0.24 [−0.36, −0.13]	0.09 [−1.37, 1.56]
Unknown	−0.10 [−0.29, 0.09]	0.06 [−0.38, 0.50]	−0.14 [−0.27, −0.01]	2.34 [0.72, 3.96]
Feeding mode				
Breast	Ref	Ref	Ref	Ref
Bottle	0.31 [0.14, 0.48]	0.04 [−0.35, 0.43]	0.12 [0.004, 0.24]	0.73 [−0.77, 2.22]
Breast and bottle	−0.08 [−0.28, 0.13]	0.41 [−0.07, 0.89]	0.040 [−0.10, 0.18]	0.32 [−1.59, 2.23]
ICC^a	0.42 [0.39, 0.44]	0.59 [0.56, 0.61]	0.23 [0.20, 0.26]	<0.001
N	9656	5814	9674	5814

^aIntraclass correlation coefficient. OFC: occipitofrontal circumference. Linear mixed model results adjusted for the variables shown and additionally for child age at 6–8 weeks and school entry, maternal age, and year of delivery. Effect sizes represent the difference from the indicated reference group.

human reproduction **SUPPLEMENTARY DATA**

Supplementary Table SVI Length at 6–8 weeks and height at school entry for fresh and frozen ET twins showing the relationships with patient and ART treatment parameters.

	Length or height (cm)			OFC (cm) and BMI (kg/m ²)		
	Birth N = 1318	6–8 weeks N = 2328	School entry N = 1429	OFC @ birth N = 1957	OFC @ 6–8 weeks N = 2340	BMI @ School entry N = 1429
	Coefficient [95% CI]			Coefficient (95% CI)		
Embryo transfer type						
ART—fresh cycle ^a	Ref	Ref	Ref	Ref	Ref	Ref
ART—frozen cycle	0.48 [−0.04, 1.00]	0.72 [0.32, 1.10]	0.87 [0.04, 1.70]	0.22 [0.01, 0.42]	0.03 [−0.22, 0.31]	0.22 [−0.04, 0.48]
ICSI						
No ^a	Ref	Ref	Ref	Ref	Ref	Ref
Yes	0.26 [−0.26, 0.79]	0.06 [−0.35, 0.48]	0.24 [−0.71, 1.19]	0.01 [−0.21, 0.23]	−0.03 [−0.33, 0.26]	−0.19 [−0.48, 0.11]
Infertility Cause^b						
Endometrial	0.70 [−0.06, 1.47]	−0.20 [−0.74, 0.34]	0.64 [−0.54, 1.82]	0.37 [0.07, 0.68]	0.03 [−0.36, 0.42]	−0.20 [−0.56, 0.17]
Male factor	0.42 [−0.18, 1.01]	−0.14 [−0.62, 0.34]	0.20 [−0.92, 1.32]	0.22 [−0.03, 0.47]	0.17 [−0.17, 0.51]	0.16 [−0.18, 0.51]
Ovulatory	−0.31 [−0.97, 0.35]	−0.14 [−0.69, 0.41]	−0.83 [−2.10, 0.45]	0.12 [−0.18, 0.41]	−0.11 [−0.49, 0.28]	0.03 [−0.36, 0.42]
Fallopian tube	0.37 [−0.29, 1.03]	0.27 [−0.23, 0.77]	0.43 [−0.65, 1.51]	0.07 [−0.20, 0.34]	0.17 [−0.19, 0.53]	0.12 [−0.21, 0.45]
Unknown	0.24 [−0.40, 0.87]	0.07 [−0.44, 0.58]	0.54 [−0.60, 1.68]	0.11 [−0.16, 0.38]	0.11 [−0.25, 0.48]	0.05 [−0.31, 0.40]
Previous births						
None ^a	Ref	Ref	Ref	Ref	Ref	Ref
1	−0.13 [−0.76, 0.50]	−0.11 [−0.60, 0.38]	−0.38 [−1.46, 0.71]	0.050 [−0.22, 0.32]	0.04 [−0.36, 0.36]	0.24 [−0.10, 0.58]
2+	0.55 [−4.75, 5.85]	0.02 [−3.29, 3.33]		−0.80 [−3.61, 2.02]	−0.43 [−2.82, 1.97]	

^aReference group for a categorical variable.

^bPatients can have multiple infertility causes. OFC: occipitofrontal circumference. Multiple linear regression results for length at birth, height at 6–8 weeks and school entry, OFC at birth, and 6–8 weeks, and BMI at school entry age. Regression results additionally adjusted for child gender and age (gestation at birth and child age at 6–8 weeks and school entry), maternal age, smoking status, Scottish index of multiple deprivation, feeding mode after birth, year of delivery, and Treatment centre. Effect estimate for 2+ previous births is not available at school entry due to the unavailability of data.

Supplementary Table SVII Growth rates for ART (fresh/frozen ET) vs NC twins for the full sample (an extension of manuscript Table IV), for the discordant twin's sample sub-analysis, and the Fresh vs Frozen ET twins comparison.

	Growth rates (g/week)					
	Fresh/frozen ET vs NC (full sample)		Fresh/frozen ET vs NC (discordant twins) ^a		Fresh vs frozen ET	
	Birth to 6–8 weeks N = 10 221	6–8 weeks to school entry N = 5663	Birth to 6–8 weeks N = 1739	6–8 weeks to school entry N = 938	Birth to 6–8 weeks N = 2466	6–8 weeks to school entry N = 1373
Type of conception						
Naturally conceived ^b	Ref	Ref	Ref	Ref		
ART—fresh cycle	−3.60 [−8.15, 0.95]	0.11 [−0.74, 0.97]	−0.54 [−11.31, 10.24]	0.76 [−1.28, 2.80]		
ART—frozen cycle	3.11 [−6.20, 12.41]	2.17 [0.52, 3.81]	26.44 [4.07, 48.82]	5.75 [2.05, 9.45]	NA	NA
Fresh^b vs frozen	6.71 [−3.10, 16.51]	2.05 [0.32, 3.79]	27.0 [3.52, 50.44]	4.99 [1.08, 3.91]	5.76 [−5.67, 17.17]	2.09 [0.43, 3.74]
Discordant set						
Bigger twin ^b			Ref	Ref		
Smaller twin	NA	NA	80.09 [71.57, 88.61]	0.34 [−0.61, 1.28]	NA	NA
Gender						
Male ^b	Ref	Ref	Ref	Ref	Ref	Ref
Female	−9.00 [−12.28, −5.73]	0.23 [−0.27, 0.73]	−16.28 [−24.86, −7.69]	−0.14 [−1.39, 1.10]	−5.80 [−13.59, 1.97]	−0.01 [−0.98, 0.96]
Deprivation index						
1 (most deprived)	−1.60 [−7.35, 4.15]	−0.47 [−1.58, 0.65]	−4.65 [−18.17, 8.88]	1.14 [−1.52, 3.81]	−1.97 [−16.45, 12.52]	0.56 [−1.77, 2.88]
2	−0.80 [−6.49, 4.89]	−0.52 [−1.58, 0.54]	−1.42 [−14.93, 12.09]	−0.64 [−3.17, 1.89]	−5.23 [−18.04, 7.58]	0.50 [−1.47, 2.46]
3	2.98 [−2.82, 8.78]	0.15 [−0.91, 1.22]	−4.88 [−18.78, 9.02]	0.07 [−2.44, 2.59]	−1.33 [−13.77, 11.10]	1.93 [0.07, 3.79]
4	−0.34 [−5.91, 5.23]	0.22 [−0.82, 1.26]	−13.96 [−27.81, −0.11]	2.08 [−0.43, 4.58]	−3.94 [−15.34, 7.47]	1.01 [−0.76, 2.78]
5 (least deprived) ^b	Ref	Ref	Ref	Ref	Ref	Ref
Smoking						
No ^b	Ref	Ref	Ref	Ref	Ref	Ref
Yes	0.33 [−4.14, 4.79]	0.60 [−0.24, 1.45]	9.70 [−0.91, 20.32]	1.35 [−0.75, 3.44]	7.25 [−4.98, 19.48]	0.64 [−1.28, 2.56]
Unknown	−2.27 [−7.34, 2.80]	−0.06 [−1.00, 0.88]	4.90 [−7.74, 17.54]	0.57 [−1.75, 2.89]	4.00 [−7.13, 13.14]	−0.01 [−1.72, 1.70]
Feeding mode						
Breast ^b	Ref	Ref	Ref	Ref	Ref	Ref
Bottle	23.65 [19.00, 28.29]	−0.29 [−1.12, 0.54]	15.56 [3.50, 27.61]	−0.76 [−2.84, 1.32]	24.19 [13.73, 34.65]	−0.63 [−2.13, 0.87]
Breast and bottle	11.99 [6.26, 17.72]	0.99 [−0.04, 2.03]	6.57 [−8.30, 21.45]	−1.16 [−3.67, 1.34]	3.45 [−8.65, 15.54]	0.21 [−1.61, 2.02]
Unknown	4.96 [−3.95, 13.87]	−0.91 [−2.45, 0.63]	−15.36 [−36.06, 5.35]	−3.20 [−6.88, 0.48]	11.79 [−9.00, 32.58]	−1.35 [−4.52, 1.82]
ICSI						
No ^b	NA	NA	NA	NA	Ref	Ref
Yes					−3.80 [−16.10, 8.51]	−0.26 [−2.21, 1.69]

(continued)

Supplementary Table SVII Continued

	Growth rates (g/week)					
	Fresh/frozen ET vs NC (full sample)		Fresh/frozen ET vs NC (discordant twins) ^a		Fresh vs frozen ET	
	Birth to 6–8 weeks N = 10 221	6–8 weeks to school entry N = 5663	Birth to 6–8 weeks N = 1739	6–8 weeks to school entry N = 938	Birth to 6–8 weeks N = 2466	6–8 weeks to school entry N = 1373
<hr/>						
Infertility cause ^c						
Endometrial					3.96 [–19.81, 11.89]	0.74 [–1.70, 3.17]
Male factor	NA	NA	NA	NA	0.92 [–13.20, 15.04]	0.75 [–1.51, 3.02]
Ovulatory					–10.22 [–26.69, 6.24]	–0.42 [–3.03, 2.20]
Fallopian tube					7.90 [–6.77, 22.58]	0.79 [–1.43, 3.01]
Unknown					–0.19 [–15.27, 14.89]	1.10 [–1.24, 3.44]
ICC ^d	0.45 [0.44, 0.46]	0.51 [0.51, 0.51]	0.42 [0.39, 0.45]	0.47 [0.46, 0.48]	0.51 [0.51, 0.51]	0.50 [0.50, 0.51]

^aDiscordance is defined as an intertwin birthweight discordance/difference greater than 20%: (larger twin birth weight – smaller twin birthweight)/larger twin birth weight × 100%.

^bReference group.

^cPatients can have multiple infertility causes.

^dIntraclass correlation coefficient. Mixed linear regression models for growth rates (NC vs ART, NC vs ART for discordant twins, and Fresh vs Frozen cycle ART babies) between birth and 6–8 weeks. Models are adjusted for presented variables, year of delivery, and Treatment centre (for the fresh vs frozen ET models).