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Anthropometric measurements of term singletons at 6 years of age born from fresh and frozen embryo transfer: A multicenter prospective study in Japan

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Abstract

Purpose: The purpose of this study is to compare anthropometric measurements between term singletons conceived via fresh embryo transfer (FreET) and frozen embryo transfer (FET) and those born via natural conception (NC) or fertility treatments milder than assisted reproductive technology (non-ART) at 6 years of age.

Methods: A total of 8149 children were enrolled, and questionnaires about anthropometric measures (weight, height, BMI) were addressed to parents, when the children were 1.5, 3, and 6 years of age. A total of 3299 term singletons were enrolled at birth: 533, 476, 916, and 1374 in the NC, non-ART, FreET, and FET groups, respectively.

Results: A total of 1635 term singletons (290, 176, 467, and 702 in the NC, non-ART, FreET, and FET groups respectively) were enrolled until 6 years of age (follow-up rate, approximately 50%). When non-ART group was used as control, the FreET children were 1.0 cm taller than the non-ART children at 6 years of age, after adjusting for confounding factors. However, no differences were observed in the anthropometric data among the non-ART, ART, and NC children at 6 years of age.

Conclusion: At 6 years of age, term singletons were taller in the FreET group than in the non-ART group, after adjusting for confounders.

Keiko Ueno and Junya Kojima contributed equally to the work and are thus co-first authors.

KEYWORDS
anthropometric measurements, assisted reproductive technology, fresh embryo transfer, frozen–thawed embryo transfer, natural conception

1 | INTRODUCTION

The use of assisted reproductive technology (ART) has increased, and the long-term health effects on children born via ART have become one of the most debatable and challenging topics in reproductive medicine.[1–3]

Development is an important index of the prenatal factors that influence the long-term health of fetuses and children, and several studies have investigated the anthropometric deviation in ART children.[4–6] Consequently, concerns remain about the long-term prospects for the children conceived with this technique and indeed of children conceived from FreET and FET.

Although ART children may be taller than natural conception (NC) children at the age of school entrance (5–6 years of age), the existence of anthropometric deviation remains controversial. Miles et al. reported that fresh embryo transfer (FreET)-conceived children were taller than their NC counterpart,[7] while another study reported that children conceived via FreET were taller than both the NC or frozen–thawed embryo transfer (FET)-conceived children.[8] Contrarily, Hann et al. reported that despite differences in weight, height, and body mass index (BMI) reported in ART children (FET-conceived and FreET-conceived) when compared with NC children at birth, these parameters were similar in all the three groups by the age of 5–7 years.[9] A recent meta-analysis has indicated that in vitro fertilization (IVF)/intra cytoplasmic sperm injection (ICSI) was not associated with long-term deviation in weight and height.[10]

Meanwhile, evidence has shown a significant difference in birth weights among children born via FET, FreET, and NC.[11–13] Elias et al. reported that FreET singletons were associated with small gestational age (SGA), while both FreET and FET singletons were associated with pre-term birth (PTB), and FET singletons were associated with large gestational age (LGA).[14] Hence, long-term health effects should be monitored in children born via FET and FreET.

This study investigated the possibility of deviations in the anthropometric measurements of ART singletons at 6 years of age, considering the fact that their anthropometric measurements significantly vary at birth among children born via FreET and FET.

2 | MATERIALS AND METHODS

2.1 | Study participants

Children born via FreET or FET performed in 2008 were recruited at 23 different ART clinics across Japan. All ART clinics were part of JISART (Japanese Institution of Standardizing Assisted Reproductive Technology),[15] an organization established to set high standards for practice of ART by implementing a quality management system.

Two control groups were examined: NC children and children conceived from infertile parents after fertility treatments milder than ART (non-ART), both born during a similar period as that for the ART groups (between October 2008 and September 2009). NC children were recruited in the large obstetric department of one general hospital in Tokyo, after excluding children born from infertile parents or born through ART. Non-ART children were conceived in 13 JISART clinics, through controlled ovarian stimulation, intrauterine insemination, or just after routine infertility checkup. The categorization of couples as infertile was based on the World Health Organization definition of infertility: failure to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse.[16] In this study, unless there is an obvious cause of infertility that requires any treatment, we used this WHO criteria.

Exclusion criteria were multiple birth, vanishing twin, PTB (<37 weeks of gestational age), post-term birth (≥42 weeks of gestational age), and congenital disease that affects the physical development (such as spina bifida, cardiac malformations, or chromosome abnormality). Notably, the ART clinics involved in this study offered neither single- nor double-gamete donation.

2.2 | Data collection

After obtaining written informed consent, structured questionnaires were sent to all parents when the children were approximately 1.5 years of age; the questionnaire was regarding anthropometric data of the children, social and medical profile of the parents including their age, any complication before and during pregnancy, and the nurturing status of the children. These questionnaires also required parents to transcribe the data of gestational length, body height (crown-heel length), and weight of the children at birth in the Maternal and Child Health HandBook (Boshi Kenko Techo)[17]; these data were measured and recorded by health care providers in the medical institutions.

Information regarding ART, including mother's age at ART performance, ovarian stimulation procedure, type of embryo transfer procedure used (whether FET or FreET), number of embryos replaced, and type of resulting pregnancy (whether singleton or multiple pregnancies) was collected from the ART database in Japan, where all ART clinics must report the result of every ART cycle performed.[18]

Another set of questionnaires were resent to parents who had completed the initial one, when the children were 3 and 6 years of age. Municipal health check-ups in Japan are conducted at 1.5, 3, and 6 (approximately 5–6 months before starting compulsory education) years of age. Parents were asked to report the height and weight of their children, as measured by health care providers at the municipal health check-ups.

2.3 | Statistical analysis

To examine the background characteristics of the three groups (non-ART, FreET, and FET), analysis of variance and chi-square tests were conducted to compare covariates between each group (Table 1). The same analysis of variance and chi-square tests were conducted for the NC, non-ART, FreET, and FET groups (Table S1).

Single regression analysis of the weight, height, and BMI at birth, 1.5, 3, and 6 years of age was conducted for the non-ART, FreET, and FET groups. This analysis also evaluated the effects of factors associated with the anthropometry of infants and children that are considered as potential confounding factors by some previous studies (Table 1).[19–22] BMI was calculated according to the World Health Organization standards (body weight [kg]/height^2 [m^2]). The same single regression model was conducted for the NC, non-ART, FreET, and FET groups. $p < 0.2$ was considered as cutoff value to select variables for single regression model analysis.

Multiple linear regression models were conducted to examine the association of the variables associated with the outcomes of the single regression analysis of the three groups (non-ART, FreET, and FET) at birth, 1.5 years, 3 years, and 6 years (Tables S2, S5, S7, and S9).

Multiple linear regression models were conducted to examine the association of the variables associated with the outcomes of the single regression analysis of the four groups (NC, non-ART, FreET, and FET) at birth, 1.5 years, 3 years, and 6 years (Tables S3, S6, S8, and S10).

The crude results were using the Dunnett method. (Tables 2 and 3; Tables S3 and S11) Finally, each outcome was estimated using the least-squares method, after controlling for the selected covariates, which were the same as those for the multiple regression models. The adjusted outcomes of the FreET and FET groups were compared with the non-ART group using the Dunnett method (Tables 2 and 3). The adjusted outcomes of the non-ART, FreET, and FET groups were compared with NC group using the Dunnett method (Tables S3 and S11).

$p < 0.05$ was considered to be statistically significant.

All statistical analyses were conducted using the IBM Statistical Package for the Social Sciences 28.0 for Mac.

3 | RESULTS

3.1 | Characteristics of the study participants

Figure 1 shows the study population of the ART children. For FreET and FET, a total of 4941 children born via ART were enrolled out of 8149 children whose parents were recruited to participate in the study, and parents of 2991 children completed the initial questionnaire (at children's age of 1.5 years). After excluding cases based on the exclusion criteria, 2290 term singleton children (916 FreET, 1374 FET) were eligible for the analysis. A total of 1169 children (467 FreET, 702 FET) continued and completed the follow-up until 6 years of age.

Table 1 shows parental, gestational, and nursing characteristics, fertility parameters, and birth measurements during the initial questionnaire. Mothers of non-ART children were significantly younger (approximately 2 years). Caesarean section (20.4% and 29.8% for Fre ET and 38.8% for FET, respectively) were higher in both the FreET and FET groups than in the non-ART group. Breastfeeding was more common among the children born from non-ART.

In addition, blastocyst stage embryo was utilized more often in the FET group than in the FreET group (67.0% and 38.5%, respectively) (Table 1). We also analyzed the characteristics of patients among NC, Non-ART, FET, and FreET (Table S1).

3.2 | Comparison of the anthropometric measurements of children at birth

At birth, multiple regression analysis showed that gestational age, sex of children, HDP, maternal diabetes, gestational weight gain, and FET were significantly associated with both the birth weight and height of children among non-ART and ART children. Furthermore, parity and maternal drinking during pregnancy were significantly associated with birth weight (data not shown; Table S2). Meanwhile, parental anthropometric measures—weight, height, or BMI (except paternal BMI)—were associated with those of the children at birth.

After controlling the effect of all variables in each multiple regression model, birth weights of non-ART, FreET, and FET children were 3023, 3035, and 3122 g, and birth heights were 49.1, 49.0, and 49.3 cm, respectively, indicating that FET babies were significantly heavier and taller than non-ART babies. The result of the birth weights of FET infants, which were significantly higher (by approximately 70 g) than those of FreET or non-ART infants, was concordant with that of the birth weight of ART children in Japan,[23] indicating that our cohort represents the general population of children.

3.3 | Comparison of the anthropometric measurements of children at 1.5, 3, and 6 years of age

At 1.5, 3, and 6 years, multiple regression analysis showed that various confounding factors were significantly associated with both the birth weight and height of children among non-ART, FET, and FreET children (data not shown; Tables S5, S7 and S9; Table 3). Multiple regression analysis showed that parity, parental weight, paternal allergy and asthma, and gestational weight gain were significantly associated with the weight of the children at 6 years of age. Although, breastfeeding for 1 month, sex of children, parental height, and FreET were associated with the height at 6 years of age (data not shown; Table S9).

After controlling the effect of all variables in each multiple regression model, we compared weights, height, and BMI with

TABLE 1 Background characteristics of participating singletons and their parents.

Characteristics	Non-ART (n = 476)	FreET (n = 916)	FET (n = 1374)	p-Value
Children characteristics				
Gestational age (weeks), mean ± SD[a]	39.1 ± 1.2 [n = 476]	39.0 ± 1.2 [n = 916]	39.2 ± 1.3 [n = 1374]	0.001
HBW, n (%)	0 (0)	6 (0.7)	17 (1.2)	0.044
Male, n (%)[a]	239 (50.2) [n = 476]	452 (49.3) [n = 916]	701 (51.0) [n = 1374]	0.73
Only breastfeeding at 1 mo., n (%)[b]	255 (53.9) [n = 473]	349 (38.8) [n = 900]	626 (46.4) [n = 1350]	<0.001
Continuing breastfeeding at 1 y., n (%)[b]	218 (61.6) [n = 354]	321 (52.0) [n = 617]	562 (59.3) [n = 947]	0.004
Parental characteristics				
Maternal age (years), mean ± SD[a]	32.8 ± 3.6 [n = 476]	34.9 ± 3.9 [n = 916]	34.6 ± 3.6 [n = 1374]	<0.001
Primigravida, n (%)[a]	363 (76.3) [n = 476]	736 (80.3) [n = 916]	1038 (75.6) [n = 1373]	0.025
HDP, n (%)[a]	25 (5.3) [n = 476]	33 (3.6) [n = 916]	96 (7.0) [n = 1373]	0.002
GDM, n (%)[a]	5 (1.1) [n = 476]	22 (2.4) [n = 916]	21 (1.5) [n = 1373]	0.13
Caesarean section, n (%)[a]	97 (20.4) [n = 476]	273 (29.8) [n = 916]	533 (38.8) [n = 1373]	<0.001
Maternal disease, n (%)[a]	[n = 476]	[n = 916]	[n = 1373]	
Diabetes	2 (0.4)	2 (0.2)	5 (0.4)	0.77
Thyroid gland disease	16 (3.4)	30 (3.3)	41 (3.0)	0.89
Heart disease	2 (0.4)	1 (0.1)	4 (0.3)	0.51
Kidney disease/hypertension	5 (1.1)	8 (0.9)	10 (0.7)	0.79
Autoimmune disease	3 (0.6)	5 (0.5)	16 (1.2)	0.24
Maternal education[a]	[n = 325]	[n = 646]	[n = 1009]	
College or more, n (%)	109 (33.5)	195 (30.2)	319 (31.6)	0.19
Paternal education[a]	[n = 325]	[n = 646]	[n = 1009]	
College or more, n (%)	190 (58.5)	373 (57.7)	568 (56.3)	0.43
Maternal height (cm), mean ± SD[a]	158.4 ± 5.6 [n = 323]	159.1 ± 5.4 [n = 646]	158.8 ± 5.2 [n = 1009]	0.20
Pre-pregnancy weight (kg), mean ± SD[a]	52.1 ± 8.1 [n = 322]	53.2 ± 8.4 [n = 642]	52.6 ± 7.7 [n = 1006]	0.094
Paternal height (cm), mean ± SD[a]	171.7 ± 5.4 [n = 322]	172.0 ± 5.8 [n = 637]	172.3 ± 5.7 [n = 992]	0.13
Paternal weight (kg), mean ± SD[a]	69.8 ± 11.3 [n = 316]	70.9 ± 10.7 [n = 625]	70.6 ± 10.4 [n = 975]	0.094
Maternal pregnancy drinker, n (%)[a]	7 (2.2) [n = 325]	12 (1.9) [n = 646]	24 (2.4) [n = 1009]	0.34
Maternal pregnancy smoker, n (%)[a]	5 (1.5) [n = 324]	11 (1.7) [n = 646]	18 (1.8) [n = 1009]	0.96
Paternal smoker, n (%)[a]	94 (28.9) [n = 325]	205 (31.7) [n = 646]	299 (29.6) [n = 1009]	0.29
Maternal allergy and asthma, n (%)[a]	144 (44.3) [n = 325]	271 (42.0) [n = 646]	434 (43.0) [n = 1009]	0.90
Paternal allergy and asthma, n (%)[a]	137 (42.2) [n = 325]	240 (37.2) [n = 646]	386 (38.3) [n = 1009]	0.21

TABLE 1 (Continued)

Characteristics	Non-ART (*n* = 476)	FreET (*n* = 916)	FET (*n* = 1374)	*p*-Value
GWG (kg), mean ± SD[a]	9.2 ± 3.4 [*n* = 316]	9.4 ± 3.7 [*n* = 630]	9.2 ± 3.6 [*n* = 983]	0.75
Fertility parameters				
Infertility period (months), mean ± SD[a]	27.1 ± 25.3 [*n* = 476]	40.1 ± 31.9 [*n* = 916]	43.4 ± 34.3 [*n* = 1374]	<0.001
Non-ART treatment	[*n* = 427]			
Timing, *n* (%)	170 (39.8)			
Intrauterine insemination, *n* (%)	211 (49.4)			
With controlled ovarian stimulation	[*n* = 264]			
Clomiphene, *n* (%)	129 (48.9)			
HMG-HCG, *n* (%)	81 (30.7)			
Infertility diagnosis		[*n* = 916]	[*n* = 1374]	<0.001
Tubal factor, *n* (%)		203 (22.2)	354 (25.8)	
Endometriosis, *n* (%)		83 (9.1)	117 (8.5)	
Male factor, *n* (%)		376 (41.0)	486 (35.4)	
Unexplained, *n* (%)		253 (27.6)	429 (31.2)	
Other, *n* (%)		122 (13.3)	193 (14.0)	
ART characteristics				
Ovarian stimulation		[*n* = 748]	[*n* = 547]	<0.001
Agonist, *n* (%)		470 (62.8)	115 (21.0)	
Antagonist, *n* (%)		193(25.8)	111(20.3)	
Number of embryos transferred, mean ± SD		1.5 ± 0.60 [*n* = 814]	1.4 ± 0.54 [*n* = 1322]	<0.001
Blastocyst, *n* (%)		332 (38.5) [*n* = 863]	886 (67.0) [*n* = 1323]	<0.001

Note: Children characteristics, parental characteristics (maternal age, primigravida, HDP, GDM, caesarean section, maternal disease), infertility period, and non-ART treatment were obtained from the first questionnaire at 1.5 years of age. The other parental characteristics were obtained from the second and third questionnaires at 3 and 6 years of age.

Abbreviations: %, the percentage among responders; 1 mo., one-month; 1 y., one-year; FET, frozen embryo transfer; Fresh ET, fresh embryo transfer; GDM, gestational diabetes mellitus; GWG, gestational weight gain; HBW, high birth weight (≥4000 g); HCG, human chorionic gonadotropin; HDP, hypertensive disorders of pregnancy; HMG, human menopausal gonadotropin; *n*, number of responders; non-ART, non-assisted reproductive technology; SD, standard deviation.

[a]Candidate variables in multiple linear regression models at birth, 1.5, 3, and 6 years of age.

[b]Candidate variables in multiple linear regression models at 1.5, 3 and 6 years of age.

non-ART, FET, and FreET. FET children were significantly heavier at 1.5 years of age and much BMI at 1.5 and 3 years of age. Also, FreET children were taller at 6 years of age (Table 3).

There was significant difference in the height at 6 years of age in boys, and no different in girls when analyzed separately (data not shown).

3.4 | Comparison of the anthropometric measurements of children at birth and 1.5, 3, and 6 years of age

Next, we compared NC, non-ART, and ART children using NC children as control. At birth, multiple regression analysis revealed that gestational age, sex of children, HDP, maternal diabetes, gestational weight gain, and FET were significantly associated with both the birth weight and height of children among non-ART, ART, and NC children. Furthermore, parity, maternal education background, maternal drinking during pregnancy, and maternal smoking during pregnancy were significantly associated with birth weight (data not shown; Table S3). Meanwhile, parental anthropometric measures, weight, height, or BMI (except paternal BMI) were associated with those of the children at birth (Table S3).

After controlling the effect of all variables in each multiple regression model, birth weights of NC, non-ART, FreET, and FET children were 3054, 3029, 3042, and 3128 g, and birth heights were 48.7, 49.1, 49.0, and 49.3 cm, respectively, indicating that FET babies were significantly heavier and taller than NC babies (Table S3).

Multiple regression analysis showed various factors were associated with the weight, height, and BMI at 1.5 and 3 years of age (data not shown; Tables S6 and S8).

TABLE 2 Crude and adjusted mean weight, height, and BMI at birth between the ART and non-ART groups.

	Crude			Adjusted			
	n	Mean	*p*-Value	*n*	Mean	SE	*p*-Value
At birth							
Weight		(g)			(g)		
non-ART	476	3023.6	(control)	311	3023.9 a	18.3	(control)
FreET	916	3044.8	0.33	613	3035.5 a	12.9	0.61
FET	**1374**	**3125.4**	**<0.001**	**953**	**3122.7 a**	**10.3**	**<0.001**
Height		(cm)			(cm)		
non-ART	473	49.0	(control)	312	49.1 b	0.097	(control)
FreET	899	49.1	0.55	619	49.0 b	0.068	0.77
FET	**1349**	**49.3**	**0.004**	**958**	**49.3 b**	**0.055**	**0.044**
BMI		(kg/m^2)			(kg/m^2)		
non-ART	473	12.6	(control)	308	12.5 c	0.062	(control)
FreET	899	12.6	0.34	609	12.6 c	0.044	0.35
FET	**1349**	**12.8**	**<0.001**	**942**	**12.8 c**	**0.035**	**<0.001**

Note: *p*-Values were calculated using Dunnett's test by least squares mean adjustment.

Abbreviations: BMI, body mass index; FET, frozen embryo transfer; FreET, fresh embryo transfer; GWG, gestational weight gain; HDP, hypertensive disorders of pregnancy; non-ART, non-assisted reproductive technology; SE, standard error.

a: Gestational age, sex of children, maternal age, parity, HDP, mode of delivery, maternal diabetes and autoimmune disease, parental weight, maternal pregnancy drinker, paternal allergy and asthma, GWG, and infertility period were adjusted.

b: Gestational age, sex of children, HDP, mode of delivery, maternal diabetes, maternal education background, parental height, paternal allergy and asthma, GWG, and infertility period were adjusted.

c: Gestational age, sex of children, maternal age, parity, HDP, maternal autoimmune disease, parental BMI, maternal pregnancy drinker, and GWG were adjusted.

Statstical significances were presented in bold values.

FET children were heavier and more BMI than NC at 1.5 years of age (Table S11).

No significant differences were observed in weight, height, and BMI among the non-ART, ART, and NC children at 6 years of age (data not shown; Table S11).

4 | DISCUSSION

Our data showed a significant difference in the anthropometric measures among children between non-ART, FET, and FreET children. At birth, FET children were larger in height, weight, and BMI. At 1.5 years of age, the FET children were larger in both weight and BMI. At 3 years of age, the FET children had a larger BMI. At 6 years of age, FreET children were taller.

Earlier studies have reported no significant difference in the anthropometric measures of ART children after 5 years of age,[4–6] but failed to show the proportion of FET children included in the studies, since these studies were conducted before reports of birth weight differences between children born via FET and FreET.[11–13] Consequently, the difference in anthropometric measurements between ART and NC children might not have been detected due to the different proportions of FreET and FET children.

Miles et al. reported that FreET-conceived full-term, singleton children (4–10 years of age) were taller than their NC counterparts and showed higher levels of growth factors (IGF-I and IGF-II) and a favorable lipid profile [less triglyceride and less high-density lipoprotein (HDL) cholesterol].[7] Another reported that children (3.5–11 years of age) conceived via FreET, especially girls, were taller than both the NC controls and FET-conceived children, showed higher IGF-I and HDL levels and lower triglyceride levels.[8] Magnus et al. also reported in their population-based cohort study that children conceived by ART were smaller at birth and achieved a rapid "catch-up" growth. Accelerated postnatal "catch-up" growth is a common compensatory mechanism for low birth weight.[24]

"Catch-up" growth was thought to be beneficial for the individual in the short term, but may be related to health problems such as cardiometabolic disease in the long term.[25] Catchup was defined as a change of more than one decile in the standardized growth charts (between birth and primary school entry).[9] ART children were largely similar in anthropometric measurements to NC children at school age. The authors also showed differences in growth between fresh and frozen embryo transfer (FET) children up until school age.[26] Most recently, another within-sibship large-scale cohort study showed that FET was associated with increased birth weight and the risk of LGA, whereas FreET was associated with the opposite result.[27] Taller

TABLE 3 Crude and adjusted mean weight, height, and BMI at 1.5, 3, and 6 years of age between the ART and non-ART groups.

	Crude			Adjusted			
	N	Mean	*p*-Value	*n*	Mean	SE	*p*-value
1.5 years of age							
Weight		(kg)			(kg)		
non-ART	439	10.3	(control)	215	10.3 a	67.8	(control)
FreET	**857**	**10.5**	**0.005**	407	10.4 a	49.2	0.46
FET	**1270**	**10.5**	**<0.001**	**614**	**10.6 a**	**39.9**	**0.004**
Height		(cm)			(cm)		
non-ART	436	80.3	(control)	215	80.3 b	0.17	(control)
FreET	852	80.3	0.63	409	80.2 b	0.12	0.57
FET	1267	80.5	0.072	621	80.5 b	0.1	0.25
BMI		(kg/m^2)			(kg/m^2)		
non-ART	436	15.9	(control)	213	16.0 c	0.082	(control)
FreET	**852**	**16.2**	**<0.001**	404	16.2 c	0.058	0.19
FET	**1266**	**16.2**	**<0.001**	**611**	**16.3 c**	**0.047**	**0.007**
3 years of age							
Weight		(kg)			(kg)		
non-ART	303	14.1	(control)	289	14.2 d	0.089	(control)
FreET	617	14.3	0.13	583	14.3 d	0.063	0.84
FET	**967**	**14.4**	**0.036**	906	14.4 d	0.05	0.17
Height		(cm)			(cm)		
non-ART	303	95.1	(control)	212	95.6 e	0.25	(control)
FreET	615	95.3	0.47	407	95.3 e	0.18	0.22
FET	964	95.1	0.80	641	95.1 e	0.14	0.067
BMI		(kg/m^2)			(kg/m^2)		
non-ART	303	15.6	(control)	290	15.6 f	0.065	(control)
FreET	615	15.7	0.16	585	15.7 f	0.045	0.24
FET	**964**	**15.8**	**<0.001**	**912**	**15.9 f**	**0.036**	**<0.001**
6 years of age							
Weight		(kg)			(kg)		
non-ART	170	20.0	(control)	165	20.2 g	0.21	(control)
FreET	**455**	**20.5**	**0.034**	441	20.4 g	0.13	0.25
FET	684	20.4	0.06	658	20.5 g	0.10	0.13
Height		(cm)			(cm)		
non-ART	169	114.3	(control)	164	114.5 h	0.35	(control)
FreET	**448**	**115.6**	**0.004**	**439**	**115.4 h**	**0.22**	**0.034**
FET	**675**	**115.3**	**0.018**	656	115.3 h	0.018	0.058
BMI		(kg/m^2)			(kg/m^2)		
non-ART	168	15.3	(control)	162	15.3 i	0.11	(control)
FreET	448	15.3	0.82	435	15.3 i	0.066	0.81
FET	675	15.3	0.74	650	15.4 i	0.054	0.63

Note: *p*-Values were calculated using Dunnett's test by least squares mean adjustment.

Abbreviations: BMI, body mass index; FET, frozen embryo transfer; FreET, fresh embryo transfer; GWG, gestational weight gain; HDP, hypertensive disorders of pregnancy; non-ART, non-assisted reproductive technology; SE, standard error.

a: Breastfeeding at 1 month, breastfeeding at 1 year, sex of children, maternal kidney disease/hypertension, parental weight, maternal pregnancy drinker, paternal allergy and asthma, and GWG were adjusted.

b: Breastfeeding at 1 month, breastfeeding at 1 year, gestational age, sex of children, parity, parental height, maternal pregnancy drinker, paternal allergy and asthma, and GWG were adjusted.

c: Breastfeeding at 1 month, breastfeeding at 1 year, sex of children, HDP, maternal autoimmune disease, parental BMI, paternal allergy and asthma, GWG, and infertility period were adjusted.

d: Breastfeeding at 1 month, sex of children, parity, maternal heart disease, paternal educational background, parental weight, parental allergy and asthma, and GWG were adjusted.

e: Breastfeeding at 1 month, breastfeeding at 1 year, gestational age, sex of children, parity, maternal heart disease, paternal educational background, parental height, paternal allergy and asthma, and GWG were adjusted.

f: Mode of delivery, parental BMI, maternal pregnancy drinker, maternal allergy and asthma, GWG, and infertility period were adjusted.

g: Breastfeeding at 1 month, sex of children, parity, parental weight, paternal allergy and asthma, and GWG were adjusted.

h: Breastfeeding at 1 month, gestational age, sex of children, parental height, parental allergy and asthma, and GWG were adjusted.

i: Breastfeeding at 1 month, parity, maternal thyroid gland disease, parental BMI, paternal smoking during pregnancy, paternal allergy and asthma, GWG, and infertility period were adjusted.

Statstical significances were presented in bold values.

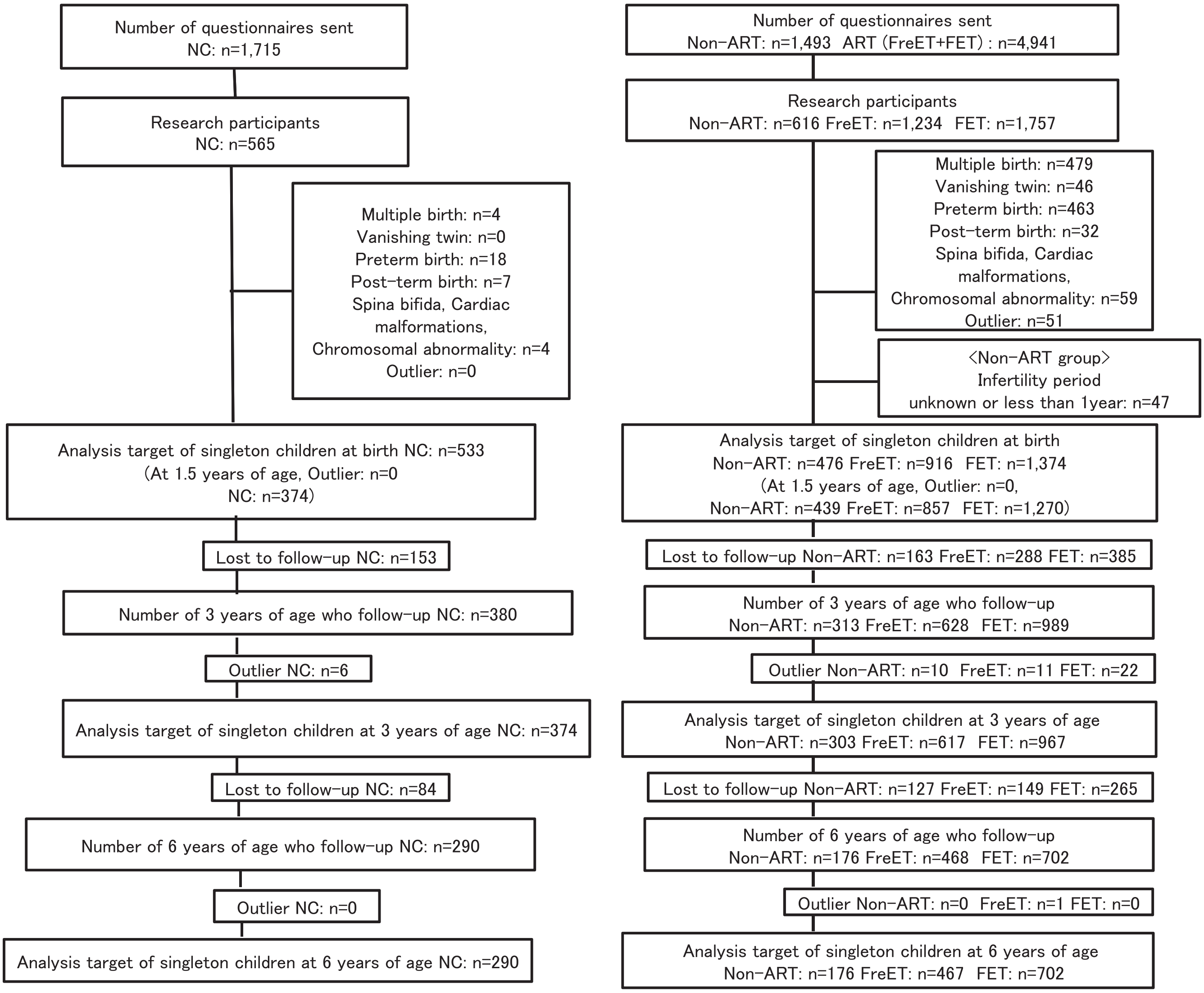

FIGURE 1 Flow diagram of follow-up children until 6 years of age.

stature has also been reported in very low-birth-weight children conceived via IVF and born prematurely.[28] Evidently, the patient backgrounds of the NC and ART groups are dissimilar. Subfertile women tended to be older, obese, and more often nulliparous than fertile ones.[29,30] It indicates that characteristics associated with subfertility possibly also play a role. In our study, our control group of children was set primarily to subfertile couples, which minimizes the role of some potential confounders, and the comparison ART and non-ART reflects children that of the in vitro procedure.

Different from many previous studies in that the control is subfertile women, our anthropometric findings were in line with these previous studies, only the FET term children had higher birth weights at birth.

Conversely, Hann et al. reported that, despite the weight, height, and BMI differences at birth, these parameters were similar in all three groups by the age of 4–7 years in singleton pregnancy.[9] Their results differ from our findings in two aspects: (1) occipito-frontal circumference and crown-heel length were smaller in babies born via FreET but larger than in those born via FET at birth; (2) no difference was found in any anthropometric measures at 5 years of age. The difference in the conclusions of Hann et al. and ours might be attributed to the different study populations, since their study included significant numbers of children born pre-term and very preterm (FreET 8%/1.3%, FET 7%/1% and NC 6%/0.9% respectively), while our study, and Miles's and Green's only included term children.

The exact reason why only the FreET children who compared non-ART children were taller at 6 years of age in our study remains to be elucidated. Small for dates (SFD), which is more frequent in FreET children, might not be the only reason of this anthropometric deviation, since our cohort study subjects children for a small proportion of SFD and there is no difference among the three groups (non-ART 1.7%, FreET 1.8%, FET 1.6%; data not shown). Pre- or early implantation factors might have contributed to the taller stature.[28]

Evidence suggests that the uterine environment in the peri-implantation period might be responsible for the observed differences in rates of SGA among FreET children. Karla et al. found no difference in the incidence of SFD among children born from donor-oocyte embryos transferred fresh or frozen/warmed in which uterine preparation was similar, but found a higher incidence of SFD among children from fresh than among those from frozen/warmed cycles among women using autologous oocytes.[31] Recently, Weinerman et al. reported that mouse pups born to recipients exposed to superovulation prior to implantation had lower birth weight and altered placenta vasculature, regardless of whether the pups were derived from blastocysts that had been frozen/thawed or transferred fresh.[32] We analyzed the data by ovarian stimulation (data not shown). Height was higher at age 6 years of age in freET compared with non-ART when only agonist was used. However, due to the small number of responses, it is difficult to be precise. From sonographic fetometry, FET fetus may become larger than FreET or NC fetus from early stages of pregnancy.[33–35] Considering our results, it may also account for that ovarian stimulation protocols vary widely in Japan, and the proportion of mild ovarian stimulation using oral drugs such as clomiphene citrate would be very large compared with other countries.

Certain driving forces of growth, such as increases in growth factors and growth suppressive effects derived from alternative peri-implantation in the uterine environment, may be present in FreET children at birth. This force would maintain a persistent taller stature in FreET children until 6 years of age. In this context, long bone development assessment in fetuses from FreET, FET, and non-ART as well as assessment of birth weight and height and childhood development is warranted.

It has been reported that the physical development of fetuses and children is correlated with cardiometabolic diseases later in life,[36] and ART children with rapid weight gain during early childhood have a higher risk of developing hypertension and elevated blood glucose in late childhood.[37–40]

A large scale, prospective study comparing anthropometric change among children born via FreET, FET, and non-ART, while considering the effect of controlled ovarian stimulation, is warranted.

Compared with previous findings, this study has several strengths including: (1) a prospective cohort study design, which makes the effect of unknown confounding factor to be relatively small; (2) age homogeneity of children in each cohort, which shows that puberty commencement or developmental spurt had not occurred, while the heterogeneity of the ART techniques remains limited; (3) information availability regarding the possible confounding factors of childhood growth, anthropometric data of both parents,[41,42] sex of the children,[43] maternal age,[44] HDP,[45] gestational diabetes mellitus,[46] parity, maternal smoking and alcohol intake,[47] and children's age in days from birth; (4) having both NC and non-ART children as control groups, which made it possible to determine the cause of the anthropometric differences. The non-ART data were within 50%–75% tile of the height and weight data for Japanese children.

Conversely, this study has some limitations, including: (1) the anthropometric data at 6 years of age were not collected from an official database wherein the data are recorded by healthcare providers; (2) whether the transplanted embryos from FET were generated via IVF or ICSI was not described, although it was generally accepted that there was no significant difference between the weights of ART children born via IVF or ICSI[48]; (3) this study did not take into account the different ART procedures, which might have influenced the anthropometric measurements, such as ovarian stimulation method, culture media,[49] and hormone replacement method for FET[50]; (4) ART and non-ART parents were recruited nationwide, NC group was recruited from a single institution; and (5) there might be selection biases caused by unfollowed-up participants and missing data in the statistical models. Regarding the selection bias of this study, the follow-up results could be associated with the difference of characteristics between the participants who could be followed up, and they who could not. Because the number of participants was decreased from the baseline survey, there might be type II error about the follow-up results. Nevertheless, at 6 years of age, there was a significant difference of height between non-ART and FreET groups. Thus, this difference might be statistically robust.

In conclusion, among three-group comparison (non-ART, FreET, and FET), the FET group is larger in height, weight, and BMI at birth, at 1.5 years of age, the FET group is larger in both weight and BMI. At 3 years of age, the FET group has a higher BMI. FreET children were slightly, yet significantly, taller than at 6 years of age.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ETHICS STATEMENT

This study has been approved by the ethical committee of the Tokyo Medical University (SH2666).

HUMAN RIGHTS STATEMENTS AND INFORMED CONSENT

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and its later amendments. Informed consent was obtained from all patients for being included in the study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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