Original Paper

Association of Cardiac Development with Assisted Reproductive Technology in Childhood: A Prospective Single-Blind Pilot Study

Jie Zhou^a Hong Liu^b Hai-tao Gu^c Yu-gui Cui^d Nan-nan Zhao^d Juan Chen^d
Li Gao^d Yuan Zhang^d Jia-yin Liu^d

^aThe Department of Echocardiography, ^bDepartment of Cardiothoracic Surgery, ^cDepartment of Pediatric Cardiology, ^dState Key Laboratory of Reproductive Medicine, Clinical Center of Reproductive Medicine; First Affiliated Hospital of Nanjing Medical University, Nanjing, China

Key Words

Assisted reproductive technologies • Echocardiography • Cardiology • Pediatrics

Abstract

Aims: To examine the pattern and extent of cardiovascular developmental alterations among children conceived by assisted reproductive technology (ART) and its association with potential confounders. **Methods:** The present study was a prospective single-blind pilot design lasting 15 months. The ART group was recruited by a non-random, consecutive sample on the basis of the unique personal identification number assigned to ART children, whereas spontaneous conception controls were recruited by a population-based random sample from the same hospital by age. Echocardiography was available for the measurement of 128 ART children and 100 controls with respect to cardiovascular geometric morphology and cardiac function. **Results:** The majority of cardiac geometric morphology parameters were comparable among the study groups ($P > 0.05$), except for significant increases in left ventricular (LV) relative wall thickness ($P = 0.038$), LV mass index ($P = 0.005$) and LV remodeling index ($P = 0.005$) in ART children after adjustment for age, gender, body surface area and heart rate. The results showed similarity in LV systolic function characterized by ejection fraction ($P = 0.140$) and shortening fraction ($P = 0.167$) between the groups. However, ART children had a significant tendency toward a decrease in mitral A ($P = 0.008$) and mitral E' ($P = 0.012$) compared with controls after adjusting for confounders. Additionally, Cox analysis suggested an independent association ($P < 0.05$) of anthropometrics and perinatal outcomes in addition to the ART procedure itself with the differences in cardiac developmental status. **Conclusion:** Our findings support the presence of remodeling in the left cardiac geometric morphology and diastolic dysfunction and the absence of any change to the aortocoronary morphometry or systolic function in ART children compared with controls, which may be independently associated with the anthropometrics and perinatal outcomes in addition to the ART procedure.

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Dr. H Liu and J Zhou contributed equally to this work.

Jia-yin Liu

State Key Laboratory of Reproductive Medicine, Clinical Center of Reproductive Medicine, First Affiliated Hospital of Nanjing Medical University,
340 Guangzhou Road, 210029, Nanjing, Jiangsu (China)
E-Mail jyliu_nj@126.com

Introduction

Since 1978, when the world's first "test tube baby" was born [1], assisted reproductive technology (ART) has extensively allowed for pregnancy in cases of infertile couples, and over five million babies worldwide have been conceived by ART [2], with a reported prevalence ranging from 3.5% to 16.7% in developed countries and from 6.9% to 9.3% in developing countries [3].

While ART is generally considered favorably [4], a considerable number of studies have already suggested the potential association between ART and an increased risk for adverse perinatal outcomes and congenital malformations [5-7], in part due to the changes in the choreography of fertilization compared with spontaneously conceived controls [8, 9], which may give rise to the long-term consequences for the developmental origins of health and disease, as well as growth [10-12].

In addition to neuropsychological development [13-15], concern has been voiced regarding the cardiovascular developmental status of children conceived by ART [16]. Growing evidence has indicated the likely presence of systemic and pulmonary vascular dysfunction [17, 18], as well as elevated blood pressure during childhood and adolescence, among individuals conceived by ART [19]. The latest study supports a possible association between ART and cardiovascular remodeling in the fetal and postnatal periods [20]. Advanced parental age has been suggested to be related to the increased risk of postnatal adverse outcomes [21, 22]; however, it has also been suggested that the underlying pathology of infertility and exposure to ART increase the embryogenic susceptibility to a spectrum of cardiovascular developmental dysfunctions in infancy and childhood. Of particular concern among the potential long-term consequences of ART is cardiac health, but very few studies to date have focused on the possible impact of ART on cardiac geometric morphological and hemodynamic development, which is required to expand our knowledge of the subsequent impact of ART on cardiovascular function and on the design of preventive strategies [20].

On the basis of the hypothesis that at least one of the parental predisposing infertility factors or the ART procedure itself is likely to lead to the poorer cardiovascular developmental status and higher prevalence of cardiovascular abnormalities than spontaneous conception controls, we designed a prospective single-blind pilot study to examine the pattern and extent of cardiovascular developmental adaptations in children conceived by ART, as well as its association with potential confounders.

Materials and Methods

Study design

A historic cohort study was designed with prospective follow-up of ART children born in the Clinical Center of Reproductive Medicine (CCRM) of First Affiliated Hospital of Nanjing Medical University (FAHNMU) from 1 January 2002 to 31 December 2012. The present study was a prospective design during 15 months (1 September 2012 through 31 March 2014) in CCRM. The ART group was recruited by a non-random, consecutive sample on the basis of the unique personal identification number assigned to ART children born in CCRM, whereas the spontaneously conceived controls were selected by age as a population-based random sample from the same hospital. This study specifically focused on the cardiac development of children conceived by ART, and the possible associations with parental infertility and the ART procedure used were determined as well. Figure 1 shows a flow diagram of the study population.

Blinding

A sophisticated sonographer blinded with regard to the mode of conception as well as the the baseline data of all participants was involved in the entire process of echocardiographic measurement to avoid intra-observer bias. The demographics, anthropometrics, perinatal outcomes and ART characteristics of the ART participants were independently collected by a review of medical records in CCRM, and those of the controls were collected by parental questionnaires and real-time measurements, if feasible.

Fig. 1. Flow diagram of the study populations. ART indicates pregnancies conceived by assisted reproductive technologies; Echo is an abbreviation for echocardiography.

Ethics information

The implementation of this study protocol was in accordance with the Declaration of Helsinki and was approved by the Institutional Ethics Committee (IEC) of FAHNMU (20120-SR-048). FAHNMU is qualified and registered with the Association for the Accreditation of Human Research Protection Program (AAHRPP). Written parental informed consent was obtained for all study participants.

Inclusion and exclusion criteria

The inclusion criteria included the capability to perform a complete transthoracic echocardiography examination, as well as the absence of any complex cardiac malformations or systemic diseases. Conversely, the exclusion criteria included a history of cardiovascular or systemic diseases, frequent arrhythmias, critical acquired heart diseases, a familial history of cardiomyopathy, and any limitation to the acquisition of echocardiographic imaging or poor image quality available for assessment.

Measurement and instrument protocol

In adherence to the Task Force Recommendations [23, 24], the transthoracic echocardiography was performed for data collection regarding cardiovascular morphometrics and hemodynamics, which was subsequently used for cardiac assessments following the standardized protocols [25, 26]. Of particular note was the extension of the As Low As Reasonably Achievable (ALARA) principle concerning acoustic exposures during the echocardiographic examinations in case of potential hazards [27, 28]. The diagnostic criteria for cardiac malformations were coded using the WHO International Classification of Diseases, 10th Revision (ICD-10).

A commercial ultrasound machine (No. YZB/USA2489-2011, iE33, Philips Ultrasound Inc., Bothell, USA) was available for echocardiographic examinations using ergonomic xMATRIX transducers with 1-8 MHz 2D phased array probes (S8-3 or S5-1) designed for children. Four consecutive cardiac cycles at a frame rate of 80-100 were acquired for cardiac assessments of all participants.

Measurement for cardiovascular geometric morphology

The left ventricular end-diastolic diameters (LVDD) and end-systolic diameters (LVSD) were measured by M-mode echocardiography at the para-sternal long-axis views. The LV end-diastolic volume (LVEDV) and end-systolic volume (LVESV) were calculated by 2D echocardiography from the apical four-chamber view using the modified *Simpson's* rule. Aortic diameters (AOD) and left atrial diameters (LAD) were measured

using 2D echocardiography at the para-sternal long-axis views. The left and right coronary arteries were measured using 2D echocardiography at the short-axis views. The interventricular septum thickness (IVST) and the left ventricular posterior wall thickness (LVPWT) were measured by M mode from a para-sternal long-axis view. Left ventricular relative wall thickness (LVRWT) was calculated the following equation: $(IVST + PWT) / LVDD$. The left ventricular remodeling index (LVRI) was calculated as follows: $LV \text{ mass} / LVEDV$. LV mass was calculated using *Devereux's* formula [29], and the LV mass index (LVMI) was normalized by body surface area (BSA) and modified according to anthropometric data of the Chinese population [30]. All echocardiographic measurements were based on the average of four consecutive cardiac cycles.

Measurement of cardiac function

The LV shortening fraction was calculated from the internal ventricular diameters obtained on a para-sternal long-axis view in the M mode using the following equation: $(\text{end-diastolic diameter} - \text{end-systolic diameter}) / \text{end-diastolic diameter}$. LV stroke volumes were calculated as follows: $\pi/4 \times (\text{aortic valve diameter})^2 \times (\text{aortic artery systolic flow velocity} \times \text{time integral})$. The left ejection fraction was calculated as follows: $(\text{end-diastolic volume} - \text{end-systolic volume}) / \text{end-diastolic volume}$. The left cardiac outputs were calculated as stroke volume $\times$ heart rate. The left cardiac indices were normalized as cardiac outputs / BSA.

The mitral inflow velocities, including the peak velocities during early diastole (E) and late diastole (A), were measured using pulsed-wave (PW) Doppler in the apical four-chamber view by performing continuous-wave (CW) Doppler prior to applying the PW Doppler to allow the maximal velocities. The mitral annular velocities, including the early diastolic (E') and late diastolic (A') velocities, were measured from tissue Doppler imaging (TDI) in the apical views at end-expiration to assess LV filling. In addition, the E/A ratio, the E'/A' ratio, and the E/E' ratio were recalculated as predictors in the estimation of LV filling pressures. The pseudo-normal pattern could be distinguished from the true normal pattern by pre-load reduction during the Valsalva maneuver [31].

Sample size and power analysis

Considering the high sensitivity for preclinical cardiac dysfunction, annular peak velocities measured by TDI were chosen to calculate the sample size. On the basis of previous studies measuring cardiac function [20, 24], the sample size was calculated to allow observation of a difference of 25% in mitral E' values for ART children. For a power of 80% and an α risk of 0.05, a minimum of 91 subjects per study group was required. We preliminarily recruited 140 children conceived by ART and 110 conceived naturally in case of the limited power.

Statistical analysis

The results were presented as mean values $\pm$ standard deviations (SD) for continuous data and as percentages for categorical data. The normality test for all of the continuous variables was performed using the *Kolmogorov-Smirnov* test. Continuous data were compared by Student's t test or one-way ANOVA, categorical data by the χ^2 test or the *Fisher's* exact test, and ordinal data by *Wilcoxon's* rank-sum test or *Kruskal-Wallis's* rank-sum test, when appropriate. Univariate regression analysis was performed for the determination of contributing predictors associated with cardiac developmental status by a log-rank test or linear regression analysis, if available. The significant covariates with *p* values less than 0.10 in univariate analyses were then considered for subsequent multivariate analyses to allow the determination of independent predictors associated with cardiac developmental status, using *Cox* proportional hazards regression models by the method of forward likelihood ratio (LR). A *p* value less than 0.05 was considered statistically significant with a 2-tailed test. Statistical analyses were completed in SPSS version 16.0 for windows (SPSS Inc., Chicago, Illinois, USA).

Results

Baseline and perinatal characteristics

A total of 228 patients met the inclusion criteria (128 for ART versus 100 for controls). In terms of baseline anthropometrics, age, gender, height, weight, heart rate, BSA and body mass index (BMI), the characteristics were comparable between the ART

Table 1. Baseline characteristics of the study groups. ART indicates pregnancy conceived by assisted reproductive technologies; †Adverse defined by the presence of bronchopulmonary dysplasia, necrotizing enterocolitis, intraventricular hemorrhage, periventricular leukomalacia, retinopathy, persistent ductus arteriosus, or sepsis. **P* value was calculated by *Student's t* test, *Pearson's χ^2* test or the *Wilcoxon* rank sum test when appropriate

Variables	ART (128)	Control (100)	<i>P</i> * value
Baseline Characteristics			
Gender			
Female	57	41	0.593
Male	72	59	
Age, month	49.67±23.83	47.15±26.70	0.464
Height, cm	106.37±15.50	104.31±19.57	0.375
Weight, Kg	18.64±6.85	177.74±9.12	0.396
Heart rate, bmp	98.5±15.6	101.2±15.4	0.200
Body mass index, Kg/m2	16.0±2.3	15.9±3.8	0.877
Body surface area, m2	0.73±0.18	0.71±0.23	0.447
Perinatal Characteristics			
Pregnancy			
Singleton	91	94	<0.001
Twin	37	6	
Delivery			
Cesarean	100	50	<0.001
Spontaneous	28	50	
Neonatal outcome			
Normal	116	100	0.522
Adverse†	12	10	
Apgar score			
At 1 min	9.85±0.50	9.96±0.20	0.043
At 5 min	9.92±0.32	9.98±0.14	0.094
Amniotic fluid			
Clarity	121	94	0.864
I turbidity	5	4	
II turbidity	1	2	
III turbidity	1	0	
Birth weight, g	3188.3±625.1	3387.0±489.3	0.010
Gestational age at delivery, wk	37.9±2.1	39.3±1.4	<0.001
Cardiac malformations, %	10.2(13/128)	6.0(6/100)	0.260

and control groups, as shown in Table 1. Considering perinatal characteristics, the ART group had a higher occurrence of cardiac malformations and neonatal complications, but the difference failed to achieve statistical significance. Within the ART group, there were 9 patent foramen ovale (PFO: ICD10-Q21.103) with a diameter of 2.20±0.50 mm, 2 atrial septal defects (ASD: ICD10-Q21.102) of 9.84±3.46 mm, 1 ventricular septal defect (VSD: ICD10-Q21.001) of 7.30 mm, and 1 aortopulmonary septal defect (APSD: ICD10-Q21.452) of 8.50 mm. Within the control group, there were 5 PFO (ICD10-Q21.103) of 2.15±0.48 mm and 1 patent ductus arteriosus (PDA: ICD10-Q25.051) of 8.60 mm. In addition, the ART group had an earlier gestational age at delivery, lower birth weights, and a higher occurrences of twins and cesarean deliveries in comparison with the controls. With regard to *Apgar* scores, the 1-min score of the ART neonates was inferior to that of the controls, while the 5-min score was similar between the groups.

Subgroup analysis of baseline ART characteristics

According to the mode of conception, ART was classified as in-vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), IVF+ICSI or artificial insemination (AI), as shown in Table 2. Significant differences among the subgroups were identified by considering the cause of infertility. As affected by infertility cause, the mode of sperm acquisition significantly differed across different subgroups. With respect to hormonal stimulation of ovulation and gamete source, no evidence of significant differences existed among the subgroups. Moreover,

Table 2. Baseline characteristics of the assisted reproductive technology group. IVF, in-vitro fertilization; ICSI, intracytoplasmic sperm injection; AI, artificial insemination; FTET, frozen-thawed embryo transfer; COS, controlled ovarian stimulation. NA, not applicable. **P* value was calculated by the one-way ANOVA or Fisher's Exact Test when appropriate. † Fisher's Exact Test was available for multiple comparisons among the subgroups except AI

Covariates	IVF (86)	ICSI (24)	IVF+ICSI (7)	AI (12)	<i>P</i> value*
Etiological infertility cause					
Primary	51	20	6	10	
Secondary	35	4	1	1	0.027
Parental infertility cause					
Unexplained	4	0	1	1	
Female	52	1	2	6	
Male	17	14	2	2	
Female+male	13	9	2	2	<0.001
Sperm acquisition mode					
Ejaculated extracted sperm	84	16	6	11	
Surgically extracted sperm	2	8	1	0	<0.001
Sperm source					
Husband	84	23	7	11	
Donor	2	1	0	0	0.700
Oocyte source					
Wife	85	24	7	11	
Donor	1	0	0	0	1.000
Ovulation control					
Natural ovulation	6	5	2	2	
COS	49	16	4	5	
Mini-stimulation	4	0	0	0	
Ovulation induction+COS	27	5	1	4	0.515
Embryo transfer modality					
Fresh embryo transfer	67	16	4	NA	
Natural cycle FTET	10	7	1	NA	
Hormone-replacement cycle FTET	9	1	2	NA	0.099†
Embryo transfer stage					
Cleavage	76	17	6	NA	
Blastocyst	10	7	1	NA	0.094†
Transferred embryos, n	2.22±0.80	2.00±0.51	2.00±0.58	NA	0.440

the differences were statistically insignificant regarding embryo transfer modality, stage and number among the subgroups other than AI.

Baseline cardiovascular characteristics

Both systolic and diastolic blood pressures were similar between the ART and control groups, as shown in Table 3. The majority of cardiac parameters examined by geometric morphology were comparable among the study groups, except for a significant increase in IVST, LVPWT, RWT, LVMI and LVRI within the ART group, even after adjustment for age, gender, body surface area and heart rate. As desired, the findings suggested the similarity of left systolic function and aortocoronary morphometry among the groups, irrespective of adjustment for confounders. Concerning cardiac diastolic function, although mitral E, mitral A' and the mitral E'/A' ratio were similar between groups, the ART children had a tendency toward decreases in mitral A and mitral E', as well as toward increases in mitral E/A and mitral E/E' ratios compared with controls, among which the tendency remained significant after adjustment for multiple confounders.

Univariate and multivariate analysis of independent predictors

On the basis of the aforementioned differences between the ART and control groups, univariate analysis was used to determine contributing predictors from a series of confounders illustrated in Table 4. Cox proportional hazard analysis suggested that age was an independent predictor for cardiac geometric morphology and diastolic function. Heart

Table 3. Baseline cardiovascular characteristics of the study groups. ART indicates pregnancy conceived by assisted reproductive technologies; LVDD, left ventricular diastolic diameter; LVSD, left ventricular systolic diameter; IVST, interventricular septum thickness; LVPWT, left ventricular posterior wall thickness; LVRWT, left ventricular relative wall thickness; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end systolic volume; LVMI, left ventricular mass index; LVRI, left ventricular remodeling index; LCA, left coronary artery; RCA, right coronary artery; AOD, aortic diameter; E, ventricular inflow in early diastole; A, ventricular inflow during atrial contraction; E', annular peak velocity in early diastole, A', annular peak velocity during atrial contraction. *P value calculated by linear regression adjusted for age, gender, body surface area and heart rate

Covariates	ART (128)	Control (100)	Crude P value	Adjusted P* va
Blood pressure, mmHg				
Systolic blood pressure	100.9±10.9	99.1±8.7	0.173	0.196
Diastolic blood pressure	60.9 ±9.5	60.9±7.6	0.952	0.786
Geometric morphology				
Left atria, mm	17.05±2.80	16.91±2.99	0.722	0.937
LVDD, mm	33.75±4.15	32.83±4.93	0.129	0.210
LVSD, mm	21.16±3.11	20.42±3.32	0.084	0.147
IVST, mm	5.52±1.07	5.12±0.82	0.020	0.002
LVPWT, mm	4.74±0.87	4.37±0.69	0.010	0.001
LVRWT	0.310±0.060	0.29±0.050	0.067	0.038
LVESV, ml	15.26±5.55	17.03±5.07	0.122	0.213
LVEDV, ml	47.71±14.73	45.16±16.97	0.219	0.404
LVMI, g/m ²	55.70±15.14	49.73±9.78	0.001	0.005
LVRI, g/ml	0.86±0.20	0.80±0.14	0.006	0.005
Aortocoronary morphometry				
LCA, mm	2.73±0.35	2.68±0.48	0.380	0.754
RCA, mm	2.38±0.31	2.36±0.37	0.650	0.767
AOD, mm	14.62±1.98	14.45±1.91	0.495	0.912
LCA/AOD ratio	0.188±0.020	0.186±0.028	0.682	0.775
RCA/AOD ratio	0.164±0.018	0.164±0.024	0.836	0.730
Left Systolic function				
stroke volume, ml	32.45±9.97	31.09±11.59	0.342	0.677
cardiac output, mL/min	3.15±0.95	3.05±0.93	0.392	0.294
cardiac index, L/min/m ²	4.41±1.34	4.34±0.88	0.696	0.210
shortening fraction, %	36.90±4.62	37.74±3.56	0.136	0.140
ejection fraction, %	68.03±4.77	69.04±4.30	0.100	0.167
Cardiac Diastolic function				
Mitral E, mm/s	97.70±18.01	97.09±15.46	0.790	0.648
Mitral A, mm/s	59.51±14.91	65.56±15.75	0.003	0.008
Mitral E/A ratio	1.72±0.46	1.56±0.46	0.010	0.015
Mitral E', mm/s	9.66±1.52	10.13±1.76	0.030	0.012
Mitral A', mm/s	6.24±1.58	6.26±1.49	0.892	0.940
Mitral E'/A' ratio	1.63±0.46	1.71±0.52	0.183	0.075
Mitral E/E' ratio	10.36±2.59	9.74±1.73	0.038	0.014

rate, BMI and BSA were independent predictors for geometric morphology, as shown in Table 5. Among the perinatal covariates, neonatal outcome and birth weight were independent predictors for geometric morphology, whereas delivery was independently associated with diastolic function. Embryo transfer stage was an independent predictor for geometric morphology, while the number of embryos transferred was a predictor for diastolic function. The association between hormonal stimulation and geometric morphology was statistically independent as well.

Discussion

This study demonstrates the presence of remodeling in the left cardiac geometric morphology and diastolic dysfunction but the absence of adaption in the aortocoronary morphometry or systolic function among children of pregnancies obtained by ART. In addition, our findings suggested a potential association among the anthropometrics of participants (i.e., age, heart rate, BMI and BSA), perinatal outcomes (i.e., delivery, birth weight, and neonatal complications), and the ART procedure itself (i.e., hormonal stimulation of ovulation and stage and number of embryos transferred) as independent predictors for the differences in cardiac developmental statuses between ART and control children.

Table 4. Univariate analysis of contributing predictors in terms of differences between ART and controls. ART indicates pregnancy conceived by assisted reproductive technologies; IVST, interventricular septum thickness; LVPWT, left ventricular posterior wall thickness; LVMI, left ventricular mass index; LVRI, left Ventricular Remodeling Index; A, ventricular inflow during atrial contraction; E', annular peak velocity in early diastole; NC, Natural conception; IVF, in-vitro fertilization; ICSI, intracytoplasmic sperm injection; AI, artificial insemination; COS, controlled ovarian stimulation; MS, mini-stimulation; OI, ovulation induction; FET, fresh embryo transfer; NF-TET, natural cycle frozen-thawed embryo transfer; HF-TET, hormone-replacement cycle frozen-thawed embryo transfer. *P value was calculated by the log-rank test or linear regression analysis when appropriate

Confounders	IVST (P value)	LVPWT (P value)	LVMI (P value)	LVRI (P value)	Mitral A (P value)	Mitral E' (P value)
Age	<0.001	<0.001	0.602	0.003	<0.001	<0.001
Gender	0.555	0.497	0.277	0.022	0.983	0.260
Heart rate	0.075	0.015	0.016	0.399	<0.001	0.025
Body mass index	0.089	0.486	0.024	0.070	0.336	0.056
Body surface area	<0.001	<0.001	0.814	0.030	<0.001	<0.001
Pregnancy (Singleton/ Twin)	0.356	0.361	0.240	0.314	0.050	0.142
Delivery	0.252	0.645	0.261	0.194	0.005	0.019
Neonatal outcome (Normal/ Adverse)	<0.001	<0.001	<0.001	<0.001	0.030	0.302
Apgar score						
At 1 min	0.757	0.173	0.248	0.280	0.361	0.642
At 5 min	0.149	0.718	0.121	0.953	0.138	0.772
Amniotic fluid (Clarity/ Turbidity I/ II/ III)	0.746	0.964	0.608	0.666	0.012	0.513
Birth weight	0.006	0.013	0.001	0.043	0.242	0.864
Gestational age	0.862	0.424	0.072	0.655	0.022	0.015
Etiological infertility cause (Normal/ Primary/ Secondary)	0.004	<0.001	<0.001	0.009	0.014	0.027
Parental infertility cause (Normal/ Female/ Male/ Female+male/ Unexplained/)	0.011	<0.001	0.002	0.007	0.047	0.058
Reproductive technique (NC/ IVF/ ICSI/ IVF+ICSI/ AI)	0.012	<0.001	0.001	0.013	0.031	0.072
Sperm acquisition mode (IES/ EES/ SES)	0.003	<0.001	<0.001	0.007	0.003	0.020
Sperm origin (Husband/ Donor)	0.629	0.732	0.122	0.807	0.176	0.852
Ovulation control (NC/COS/MS/OI+COS)	<0.001	<0.001	0.011	<0.001	0.001	0.054
Embryo transfer modality (NC/FET/NFTET/HFTET)	0.004	<0.001	<0.001	0.007	0.028	0.077
Embryo transfer stage (NC/ Early cleavage/ Blastocyst)	0.002	<0.001	<0.001	0.003	0.016	0.098
Embryos transferred number	0.006	<0.001	<0.001	0.012	<0.001	0.037

In addition to the increased IVST, LVPWT and RWT, the significantly increasing tendency of ART children toward both LVMI and LVRI compared with controls suggested the possibility of LV remodeling. In line with previous clinical [20] and experimental research [32] demonstrating patterns of remodeling in ART subjects, our findings provide additional evidence for the extent of remodeling in ART children. From an etiological perspective, increasingly hypertrophic ventricles accompanied by a reduction in longitudinal function usually develop in response to hemodynamic overload in either physiological or pathological conditions [33]. However, the results revealed an insignificant elevation of systolic and diastolic blood pressures, alterations in aortocoronary morphometry, and dilation in the left chambers among the ART group compared with the controls, which differs from previous reports indicating the potential dysfunction of vascular structures and function in ART children [17-20]. Considering the unlikely attribution of cardiac remodeling to pressure and volume overload, further Cox analysis suggested the independent association of perinatal outcomes (i.e., delivery, birth weight, and neonatal complications) and the ART procedure itself (i.e., hormonal stimulation and stage and number of embryos transferred) with LV remodeling in addition to the anthropometrics of participants. Given that subsequent changes in remodeling from childhood to adolescence and adulthood may progress to ventricular hypertrophy, depressed systolic function and overt heart failure [34, 35], it is of great value to distinguish those ART children at high risk of cardiac remodeling and identify

Table 5. Cox proportional hazard analysis of independent predictors associated with cardiac developmental health. ART indicates pregnancy conceived by assisted reproductive technologies; IVST, interventricular septum thickness; LVPWT, left ventricular posterior wall thickness; LVMI, left ventricular mass index; LVRI, left ventricular remodeling index; A, ventricular inflow during atrial contraction; E', annular peak velocity in early diastole; NC, natural conception; IVF, in-vitro fertilization; ICSI, intracytoplasmic sperm injection; AI, artificial insemination; COS, controlled ovarian stimulation; MS, mini-

Confounders	IVST (P† value)	LVPWT (P† value)	LVMI (P† value)	LVRI (P† value)	Mitral A (P† value)	Mitral E' (P† value)
Age	0.790	<0.001	NA	0.182	<0.001	<0.001
Heart rate	0.056	0.029	0.127	NA	0.083	0.413
Body mass index	<0.001	NA	0.010	0.050	NA	0.423
Body surface area	<0.001	0.804	NA	0.625	0.271	0.973
Delivery (Cesarean/ Spontaneous)	NA	NA	NA	NA	<0.001	0.205
Neonatal outcome (Normal/ Adverse)	<0.001	<0.001	<0.001	<0.001	0.150	NA
Amniotic fluid (Clarity/ Turbidity I/ II/ III)	NA	NA	NA	NA	0.063	NA
Birth weight, g	0.056	0.342	0.006	0.099	NA	NA
Gestational age at delivery	NA	NA	0.776	NA	0.081	0.555
Etiological infertility cause (Normal/ Primary/ Secondary)	0.470	0.439	0.651	0.679	0.765	0.649
Parental infertility cause (Normal/ Female/ Male/ Female+male/ Unexplained/)	0.218	0.112	0.417	0.218	0.541	0.616
Reproductive technique (NC/ IVF/ ICSI/ IVF+ICSI/ AI)	0.998	0.911	0.937	0.450	0.521	0.392
Sperm acquisition (IES/ EES/ SES)	0.834	0.791	0.790	0.568	0.082	0.845
Hormonal stimulation (NC/COS/MS/OI+COS)	0.091	0.046	0.202	0.001	0.362	0.790
Embryo transfer modality (NC/FET/NFTET/HFTET)	0.883	0.981	0.955	0.411	0.968	0.586
Embryo transfer stage (NC/ Early cleavage/ Blastocyst)	0.001	0.007	<0.001	0.259	0.987	0.602
Embryos transferred number	0.947	0.932	0.548	0.282	<0.001	0.035

stimulation; OI, ovulation induction; FET, fresh embryo transfer; NFTET, natural cycle frozen-thawed embryo transfer; HFTET, hormone-replacement cycle frozen-thawed embryo transfer; NA, not applicable. † indicates Cox proportional hazards analysis by method of forward likelihood ratio (LR)

risk factors leading to the progression of remodeling at the early stages, when the changes remain reversible and systolic function is preserved [36, 37]. It is also important to evaluate the cardiac geometric morphology among infants and children, which contributes to possible early interventions for the at-risk population.

With regard to cardiac function, the reduction in mitral A and mitral E', as well as an increase in mitral E/A and mitral E/E' ratios, suggested signs of cardiac diastolic dysfunction in ART children compared with controls, while no evidence supported the direct impact of ART on the changes in systolic function during childhood. In contrast to the latest study [20], in which ART children had signs of both cardiac systolic and diastolic dysfunctions, our findings may be attributed to the dominance of diastolic function during childhood and a higher susceptibility of diastolic function to contributing confounders compared with systolic function [38, 39]. Among the ART children, the impaired relaxation could be explained by a decrease in ventricular compliance partly due to LV remodeling. Furthermore, Cox multivariate analysis suggested that age, delivery and the number of embryos transferred are independently associated with the risk of diastolic dysfunction with ART. Numerous clinical studies and experimental models have demonstrated that diastolic dysfunction is involved in the increasing risk and frequency of deterioration of systolic dysfunction, especially in subjects with LV remodeling with had preserved LVEF [40]. Consequently, appropriate recognition of the presence of preclinical diastolic dysfunction and early identification of

the risk factors of diastolic dysfunction in ART children relative to controls is advisable to prevent further progression to systolic dysfunction.

The mechanisms dominating cardiac remodeling and diastolic dysfunction in ART children require further elaboration, despite their extensive discussion in the literature. Considerable evidence suggests a possible association between parental predisposing factors and epigenetic alterations secondary to ART procedures and postnatal environmental confounders with such changes [41, 42]. Not taking into consideration the causes of parental infertility in present study, it is likely that the other parental covariates irrelevant to infertility mediate and affect the emergence, development and progression of remodeling and dysfunction [43]. Our findings from Cox analysis approximately reproduce these consequences and support the development and presence of remodeling and diastolic dysfunction from early life [44]. Despite the gradual certainty of the potential involvement of epigenetic changes in cardiovascular regulation among ART pregnancies, it remains challenging to ascertain the causative association of incremental risks for epigenetic disorders and ART exposure due to the complexity of ART procedures and the instability of epigenetic disorders [45, 46]. Although we determined the existence of potential confounders affecting cardiac developmental health, it can hardly be denied that postnatal environmental factors also likely affect the postnatal development of the heart [47]. Consequently, the potential impact of the postnatal environment by driving progressive changes in cardiovascular development during aging requires further study.

Inevitably, our study has several limitations and considerations. Because of the dramatic alteration of cardiovascular development in cardiac geometric morphology and hemodynamics during childhood, as well as the scarcity of recognized standards designed for children, our findings should be interpreted cautiously in this context. Cases and controls were considered by their age when initially recruited; however, the uncontrollable differences produced by ART (e.g., the tendency towards twins in pregnancy) make it more difficult to examine the ART signatures compared with controls, even after adjustment for other potential confounders. In addition, in our study, the children's blood pressures were assessed over a wide age range, from 2 to 6 years old, and this limitation may provide insufficient power to detect a potentially significant change in blood pressure in young ART children, which may contribute to the early hypertrophy phenotype occurring at 2-6 years of age in ART children [20]. Considering the focus of our present pilot study on left cardiac geometric morphology and function that limits the generalizability of our findings, the global cardiac characteristics and other potential confounders not considered in the design but likely affecting the final results deserve further attention.

Conclusions

In summary, our study supports the potential association between ART exposure and ART derived risk factors with cardiac remodeling and diastolic dysfunction. Further Cox analysis suggests that the anthropometrics and perinatal outcomes in addition to ART procedure are independently associated with the changes in abnormal cardiac development among ART children compared with controls. On the basis of prophylaxis, our findings also account for the possibility and feasibility of early identification of ART children at risk for cardiovascular abnormalities, and they provide opportunities for further interventions and the prevention of progression to enable better cardiovascular development in the relevant at-risk population of ART children [48]. However, future studies should further elucidate the underlying mechanisms of potential confounders and follow the long-term cardiac impact on ART children.

Disclosure Statement

The authors have no conflicts of interest.

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