Vascular Health of Children Conceived via In Vitro Fertilization

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Objective To evaluate whether in vitro fertilization (IVF) has an effect on the cardiovascular health of offspring.

Study design This was a cross-sectional pilot study. We performed vascular health assessment for 17 children aged 10-14 years who were conceived via IVF with autologous oocytes at Stanford University. Carotid artery ultrasound evaluated intima-media thickness and stiffness, carotid-femoral pulse wave velocity determined segmental arterial stiffness, and endothelial pulse amplitude testing assessed endothelial function. We compared IVF offspring with control adolescents assessed in the same laboratory, with all comparisons adjusted for age, sex, and race/ethnicity.

Results All participants had normal body mass index and blood pressure. Compared with controls, IVF children had thicker common carotid artery intima-media thickness (0.44 ± 0.03 mm vs 0.38 ± 0.03 mm; $P < .01$), higher elastic modulus (395.29 ± 185.33 mm Hg vs 242.79 ± 37.69 mm Hg; $P = .01$), higher $\beta_{stiffness}$ (2.65 ± 0.38 vs 2.28 ± 0.23; $P < .01$), and higher peak velocity (142.29 ± 31.62 cm/s vs 117.71 ± 32.69 cm/s; $P = .04$). The mean endothelial pulse amplitude testing reactive hyperemia index was not significantly different between IVF and controls. The mean pulse wave velocity was 4.69 ± 0.51 m/s compared with the controls 4.60 ± 0.57 m/s ($P = .11$), with 8 (47%) having abnormal values.

Conclusion In an assessment of endothelial function and arterial properties of children conceived via IVF, we found that children conceived via IVF seem to have evidence of abnormal vascular health. Further studies with larger sample size and long-term follow-up are warranted. *(J Pediatr 2019;214:47-53)*.

The first live birth with in vitro fertilization (IVF) occurred in 1978, and since then it has become a commonly used procedure with >6 million babies born worldwide.[1] Although the rate of IVF use is continuing to grow, the long-term health of IVF offspring is still largely unknown.

There are biologically plausible reasons to hypothesize that IVF may have an adverse effect on child health, because conception begins in conditions which are not physiologic for the mother or embryo. Maternal levels of hormones such as estradiol and progesterone are supraphysiologic owing to ovarian stimulation protocols used to mature multiple oocytes, and in vitro culture does not ideally replicate the in vivo environment during which critical epigenetic reprogramming occurs.[2-4] Because the heart is one of the earliest organs to form, it may be particularly sensitive to perturbations during the periconceptual period.[2] Abnormalities in epigenetic control may result in abnormal cardiovascular development and susceptibility to disease.

A meta-analysis of international literature found a 1.8% incidence of cardiac malformations with IVF compared with 0.4% in the general population, and data from California confirm a higher risk.[5,6] These higher rates of malformation raise the concern that other, more subtle adverse effects of IVF on the cardiovascular system could be occurring. Limited available clinical data from Switzerland, the Netherlands, Spain, and China suggest that IVF may be associated with a higher risk of cardiovascular dysfunction in offspring, but no studies examining the cardiovascular health of IVF offspring have been performed in the US where IVF protocols and IVF laboratory conditions may differ from other parts of the world.[7-12] Thus, we conducted the first study in the US to compare endothelial function and arterial wall properties of IVF adolescents with those of non-IVF adolescents.

BMI	Body mass index
CCA	Common carotid artery
EndoPAT	Endothelial pulse amplitude testing
DBP	Diastolic blood pressure
IMT	Intima-media thickness
IVF	In vitro fertilization
PWV	Pulse wave velocity
SBP	Systolic blood pressure

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Methods

We identified 10- to 14-year-old children conceived via IVF using a Stanford database listing all patients who had undergone IVF at Stanford from 2004 to 2008. Recruitment is detailed in the Figure 1. The database included 693 former patients who conceived via IVF with autologous oocytes who also had contact information in their records. Of those, 45 lived out of state and were excluded owing to the low likelihood that they would return to California for testing. Recruitment letters were mailed to 648 mothers. For those who had email accounts in our electronic health record system, an email message was sent. All letters and emails were also followed up by a phone call. For many of those who we attempted to contact, addresses or phone numbers listed in our record were no longer accurate. A total of 40 parents replied to a letter, email, or phone call expressing initial interest in enrolling their child. Before phone screening for eligibility, 22 were either unavailable to bring in their child for the vascular testing or were no longer interested in enrolling their child after learning more about the study. Thus, 18 children were ultimately screened for eligibility. All 18 children were eligible, enrolled in our study, and consented to the study visit. At the study visit, 1 participant was excluded from our analysis owing to ineligible body mass index (BMI). Therefore, data from 17 children were ultimately included.

To be eligible to be included in the IVF group, subjects had to meet all of the following criteria: age of 10-14 years at the time of study; conceived via IVF with autologous oocytes; no known cardiovascular risk factors such as hypertension (systolic blood pressure [SBP] and/or diastolic blood pressure [DBP] <95th percentile for sex, age, and height), congenital heart defect, hyperlipidemia, overweight or obesity (inclusion criteria was a BMI <85th percentile), diabetes mellitus, or vascular diseases; no other significant medical conditions; and no regular medications other than over-the-counter pain relievers or asthma medication.[13-15] Although older children (>15 years) and young adults could be studied, the practice of IVF has evolved significantly over the past 10-15 years, and thus a study of vascular health for older offspring could be of questionable significance to the contemporary practice of IVF. The Institutional Review Board of Stanford University approved the study protocol. Written informed consent was obtained from parents or guardians, and assent was obtained for each participant.

Normative Controls

Controls were adolescents of similar age to the IVF group who had been previously recruited from the general pediatric population to serve as normal healthy controls.[14-16] All controls had a normal BMI and were normotensive. The controls underwent assessment for carotid intima-media thickness (IMT), elastic modulus, $\beta_{stiffness}$, peak velocity (n = 22), pulse wave velocity (PWV; n = 30), and endothelial function (n = 42) in the same Stanford Pediatric Vascular Research Laboratory as the IVF offspring (see detailed description of Vascular Health Assessments).

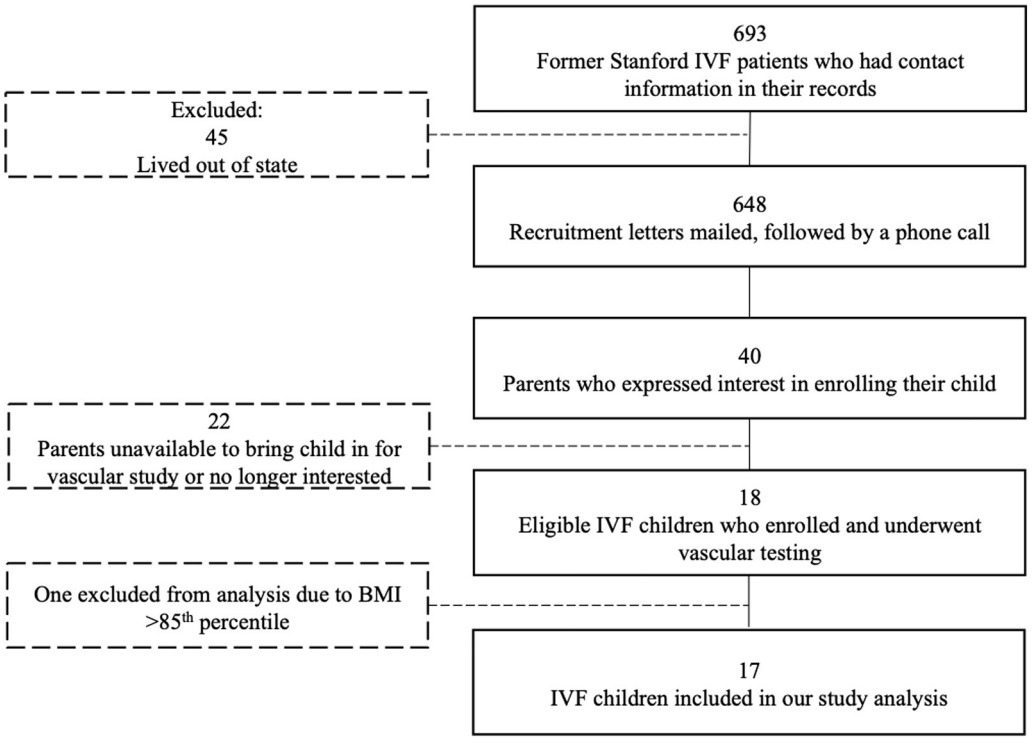

Figure 1. Flow diagram of study recruitment.

Vascular Health Assessments

Vascular health was assessed by 3 modalities: (1) carotid artery ultrasound examination to assess carotid IMT and carotid artery stiffness, (2) arterial tonometry to assess carotid-femoral arterial stiffness by PWV, and (3) endothelial pulse amplitude testing (EndoPAT) to assess endothelial function. These vascular testing modalities are feasible for the pediatric population with consistently high inter-observer reproducibility.[14-16] Additionally, they are included as standard research assessment tools for children and adolescents in a consensus statement from the American Heart Association.[17] These noninvasive measures are sensitive markers of arterial health in children and cardiovascular risk in adults, and thus may serve as surrogate markers of cardiovascular health in the general population.[18-29] All tests for both the IVF and controls groups were performed by the same professional technicians and sonographers within the Pediatric Vascular Research Laboratory; one author trained all technicians and performed extensive quality control for both the IVF and control groups. The same study protocol and vascular testing equipment were used for both groups, and the intraclass correlation coefficient for carotid IMT testing within the laboratory was 0.86.

The vascular testing duration was 2-3 hours. Before testing, participants were asked to fast overnight, for ≥10 hours, with exception only for consumption of water, vitamins, or over-the-counter medication. Furthermore, participants were not tested if they had any recent illnesses. On the testing date, the participant's height and weight were measured (Seca stadiometer, Columbia, Maryland; Scaletronix scale, White Plains, New York), and 4 sets of resting BPs were measured on the dominant arm with the oscillometric method (Dinamap-GE, Waukesha, Wisconsin) after 5 minutes of rest; the last 3 BP measurements were averaged. The testing room environment was set up to provide a quiet and peaceful ambiance with minimal disturbance, and the room was temperature controlled to 72°F-75°F for all participants. All tests were conducted while participants were resting comfortably in the supine position.

Carotid Artery Ultrasound Examination. A linear array probe (L11-3 MHz, iE33 Ultrasound System; Philips Medical Systems, Andover, Massachusetts) was placed at the anterior, posterior, and lateral angulations over a 1-cm segment located at the distal aspect of the right and left common carotid artery (CCA), just proximal to its bifurcation into the internal and external carotid arteries. Three 10-second loops were obtained, and the carotid IMT of the far wall of the CCA was measured at end-diastole. Measurements were performed using QLAB semiautomated edge detection software (Philips Medical System) and were included in our study analysis only if the edge detection software was able to measure 95% of the 1-cm arterial segment. Anterior, posterior, and lateral IMT measurements were obtained for both the right and left common carotid arteries, then averaged for right and left CCA. Functional arterial wall properties were expressed by 2 derived measures: stiffness index β and elastic modulus.[29]

$$\text{Elastic modulus} = (\text{SBP} - \text{DBP})/[(\text{AA}_{\max} - \text{AA}_{\min}) \times /\text{AA}_{\min}][\text{mm hg}]$$

$$\beta_{\text{stiffness}} = [\ln(\text{SBP}/\text{DBP})]/[(\text{AA}_{\max} - \text{AA}_{\min})/\text{AA}_{\min}]$$

where ln is the natural log and AA is the arterial area.

PWV. PWV was obtained to measure participants' arterial stiffness using a pressure tonometer (SphygmoCor, AtcorMedical, Sydney, Australia). The distance from the carotid artery to the sternal notch and the distance from sternal notch to femoral artery pulse were measured; the pressure tonometer was subsequently placed on the carotid artery and then the femoral artery to obtain arterial waveforms gated to R-wave. PWV was obtained by the manufacturer's software and is calculated as the relative difference in path length between the carotid and femoral sites divided by the transit time difference. PWV measurements were obtained in triplicate and averaged for each participant. Per prior publications studying the feasibility and reproducibility of measuring PWV, a PWV value of ≥4.5 m/s for 8- to 14-year-old was defined as abnormal.[15]

EndoPAT. We followed the EndoPAT protocol provided by the test manufacturer (Itamar Medical Ltd, Caesarea, Israel).[30] Noninvasive pneumatic probes were placed on the index fingers of both hands, probes were confirmed to have appropriate fit, continuous recording of pulse wave amplitude was initiated, the brachial artery of 1 arm was occluded for 5 minutes by a blood pressure cuff (to around 200 mm Hg), and the EndoPAT data was then automatically analyzed by the software. The EndoPAT reactive hyperemia index is defined as the ratio of average pulse amplitude over a 1-minute period (beginning after exactly 90 seconds of brachial artery occlusion) to the average pulse amplitude during a 210-second pre-occlusion baseline. Using data from prior publications studying the feasibility and reproducibility of EndoPAT in adolescents, we defined an EndoPAT reactive hyperemia index value of <1.9 as abnormal for this age group.[14]

Lipid Profile. After the carotid artery ultrasound examination, PWV, and EndoPAT testing, participants underwent a blood draw for a lipid panel, because fasting lipid levels in adolescents have been shown to predict later cardiovascular health.[31-35] Fasting lipid panel assays were performed through Lucile Packard Children's Hospital Core Laboratory at Stanford. The reference range for abnormal values were also based on the Stanford Children's hospital-wide references: normal was considered <170 mg/dL for total cholesterol, <150 mg/dL for triglyceride, >40 mg/dL for high-density lipoprotein cholesterol, and <130 mg/dL for low-density lipoprotein cholesterol.[36]

Statistical Analyses

Study data were captured and managed in Stanford's REDCap electronic data tool.[37] BMI percentiles were calculated for the participants' age and sex using Centers for Disease Control's online BMI Percentile Calculator for Child and Teen.[38] Similarly, BP percentiles were calculated for the participants' age and sex using Baylor College of Medicine's "Age-based Pediatric Blood Pressure Reference Charts" online calculator, which is based on the National Heart, Lung, and Blood Institute's normative blood pressure levels for children and adolescents.[13,39]

Statistical analyses of the raw data were performed by a professional biostatistician within Stanford Medicine's Quantitative Sciences Unit; the biostatistician was not a part of the study design or data collection. Linear regression was used to compare the IVF group with the controls to assess the association with each of the outcome variables: CCA IMT, CCA elastic modulus, CCA $\beta_{stiffness}$, CCA peak velocity, PWV, and EndoPAT reactive hyperemia index, adjusting for age, sex, and race/ethnicity. Owing to the small sample size, race/ethnicity was coded as a binary variable with 2 categories: white and others. For the low level of missing data (2 PWV values missing in the IVF cohort), we used mean imputation. All statistical analyses were conducted using R statistical software 3.5.1.[39] The *P* values generated from linear regression models were 2 sided, and a *P* value of $< .05$ was considered statistically significant.

Results

Demographic data for the study cohort is shown in Table I. There were 18 adolescents (ages 10-14 years) initially enrolled, but one was excluded owing to an ineligible BMI of 24.14 on the day of the study visit (88th percentile for age and sex). There was 1 set of fraternal twins (1 female, 1 male); all other adolescents were singletons. The IVF cohort was younger than the normative control cohort (12.1 ± 1.0 years vs 15.3 ± 2.2 years). All IVF offspring had normal BMI (mean 18.5 ± 2.1), mean systolic BP (104.4 ± 5.9 mm Hg), and mean diastolic BP (64.7 ± 4.9 mm Hg). Only 1 participant had a borderline lipid profile, with a low-density lipoprotein cholesterol level of 132 mg/dL.

Unadjusted mean and SD of all outcome variables, as well as beta coefficients and *P* values obtained from multivariable linear regression models are displayed in Table II and Figure 2. Compared with normative controls (n = 22), IVF adolescents had significantly thicker CCA IMT (0.44 ± 0.03 mm vs 0.38 ± 0.03 mm; $P < .01$), higher elastic modulus (395.29 ± 185.33 mm Hg vs 242.79 ± 37.69 mm Hg; $P = .01$), higher $\beta_{stiffness}$ (2.65 ± 0.38 vs 2.28 ± 0.23; $P < .01$), and higher CCA peak velocity (142.29 ± 31.62 cm/s vs 117.71 ± 32.69 cm/s in control; $P = .04$) (Table II). There were no statistically significant differences in mean PWV (4.69 ± 0.51 in IVF cohort vs 4.60 ± 0.57 in control) or EndoPAT (1.66 ± 0.53 in IVF cohort vs 1.78 ± 0.58 in control) compared with normative controls. A varying number of controls was used depending on the availability of measurements in the controls.[2]

Table I. Demographic and clinical characteristics of participants: Children conceived via IVF with autologous oocytes vs normative controls

Characteristics	IVF (n = 17)	Controls (n = 42)
Age, years	12.1 ± 1.0	15.3 ± 2.2
Sex		
Female	6 (35)	20 (47)
Male	11 (65)	22 (53)
Race/ethnicity		
White	10 (59)	34 (81)
Asian	2 (12)	4 (10)
Hispanic/Latino	2 (12)	1 (2)
Mixed race	3 (17)	0 (0)
Black	0 (0)	3 (7)
Mode of conception		
Fresh embryo transfer	15 (88)	NA
Frozen embryo transfer	2 (12)	
Maternal age at conception, years	35.6 ± 3.3	NA
Height (cm)	159.3 ± 12.0	167.8 ± 9.0
Weight (kg)	47.5 ± 10.6	56.9 ± 8.8
BMI (kg/m^2)	18.5 ± 2.1	19.9 ± 3.0
BMI percentile for age and sex	49.5 ± 25.0	48.3 ± 23.7
SBP (mm Hg)	104.4 ± 5.9	113.7 ± 9.4
DBP (mm Hg)	64.7 ± 4.9	69.4 ± 7.1
Total cholesterol, mg/dL	147.8 ± 25.7	NA
LDL, mg/dL	79.3 ± 17.8	NA
HDL, mg/dL	58.3 ± 16.2	NA
Triglyceride (TG), mg/dL	50.8 ± 22.9	NA
TG/HDL ratio	0.9 ± 0.5	NA

HDL, high-density lipoprotein; *LDL*, low-density lipoprotein; *NA*, not applicable; *TG*, triglycerides.
Data are shown as mean ± SD or number (%).

Discussion

Our cohort of IVF adolescents had significantly higher CCA IMT and stiffness (measured by elastic modulus, $\beta_{stiffness}$, and peak velocity) when compared with healthy, non-IVF controls.

Our study adds to the limited clinical data regarding the cardiovascular health of IVF children. Worldwide, investigators have published data regarding the cardiovascular health of a total of 432 IVF children since 2008.[7-12] A Dutch study reported higher SBP and diastolic BP and higher fasting glucose levels in IVF children.[10] A 2012 Swiss study reported that children conceived via assisted reproductive technology had greater PWV and carotid IMT when compared with healthy, non-IVF controls.[7] Meister et al followed-up with this same cohort of assisted reproductive technology children 5 years later and found evidence that those initial signs of premature vascular aging not only persisted, but also may have translated into arterial hypertension.[9] Examining early development, a 2013 Spanish study found signs of cardiovascular remodeling in fetuses conceived with assisted reproductive technology, with abnormalities that persisted into infancy.[11]

Table II. Ultrasound arterial wall measurements and CCA functional properties of IVF adolescents compared with normative values

Variables	IVF (n = 17)	Controls	Beta coefficient (95% CI)	*P* value*
CCA IMT (mm)	0.44 ± 0.03	0.38 ± 0.03 (n = 22)	0.05 (0.03 to 0.07)	<.01
CCA elastic modulus (mm Hg)	395.29 ± 185.33	242.79 ± 37.69 (n = 22)	137.08 (33.39 to 240.78)	.01
CCA $\beta_{Stiffness}$	2.65 ± 0.38	2.28 ± 0.23 (n = 22)	0.43 (0.18 to 0.68)	<.01
CCA peak velocity (cm/s)	142.29 ± 31.62	117.71 ± 32.69 (n = 22)	27.62 (0.94 to 54.29)	.04
PWV (m/s)	4.69 ± 0.51	4.60 ± 0.57 (n = 30)	0.26 (−0.06 to 0.58)	.11
EndoPAT Index (RHI)	1.66 ± 0.53	1.78 ± 0.58 (n = 42)	−0.20 (−0.62 to 0.22)	.34
Natural log of EndoPAT Index (lnRHI)	0.45 ± 0.36	0.53 ± 0.31 (n = 42)	−0.10 (−0.35 to 0.14)	.40

RHI, reactive hyperemia index.
Data are shown as unadjusted mean ± SD.
**P* values are adjusted for age, sex, and race/ethnicity.

The mechanism by which IVF may affect vascular health of offspring is not known, and it is quite possible that multiple factors are involved. Animal models including data from sheep, cattle, and rodents implicate both ovarian stimulation and in vitro culture environment.[2,40,41] Ovarian stimulation is routinely used in human IVF. To maximize the chance of pregnancy, it is typical for physicians in the US to use high doses of gonadotropin for ovarian stimulation, often retrieving 15-25 eggs, resulting in high maternal levels of estradiol, progesterone, and relaxin.[42,43] Xu et al found an association between cardiovascular dysfunction and ovarian hyperstimulation syndrome, suggesting that the underlying mechanism of dysfunction may involve supraphysiologic levels of estradiol and progesterone.[12]

IVF may also adversely affect cardiovascular health owing to the fact that in vitro culture does not ideally replicate the in vivo environment.[2,40,41] Critical epigenetic reprogramming occurs in the first 3-5 days during which the embryo

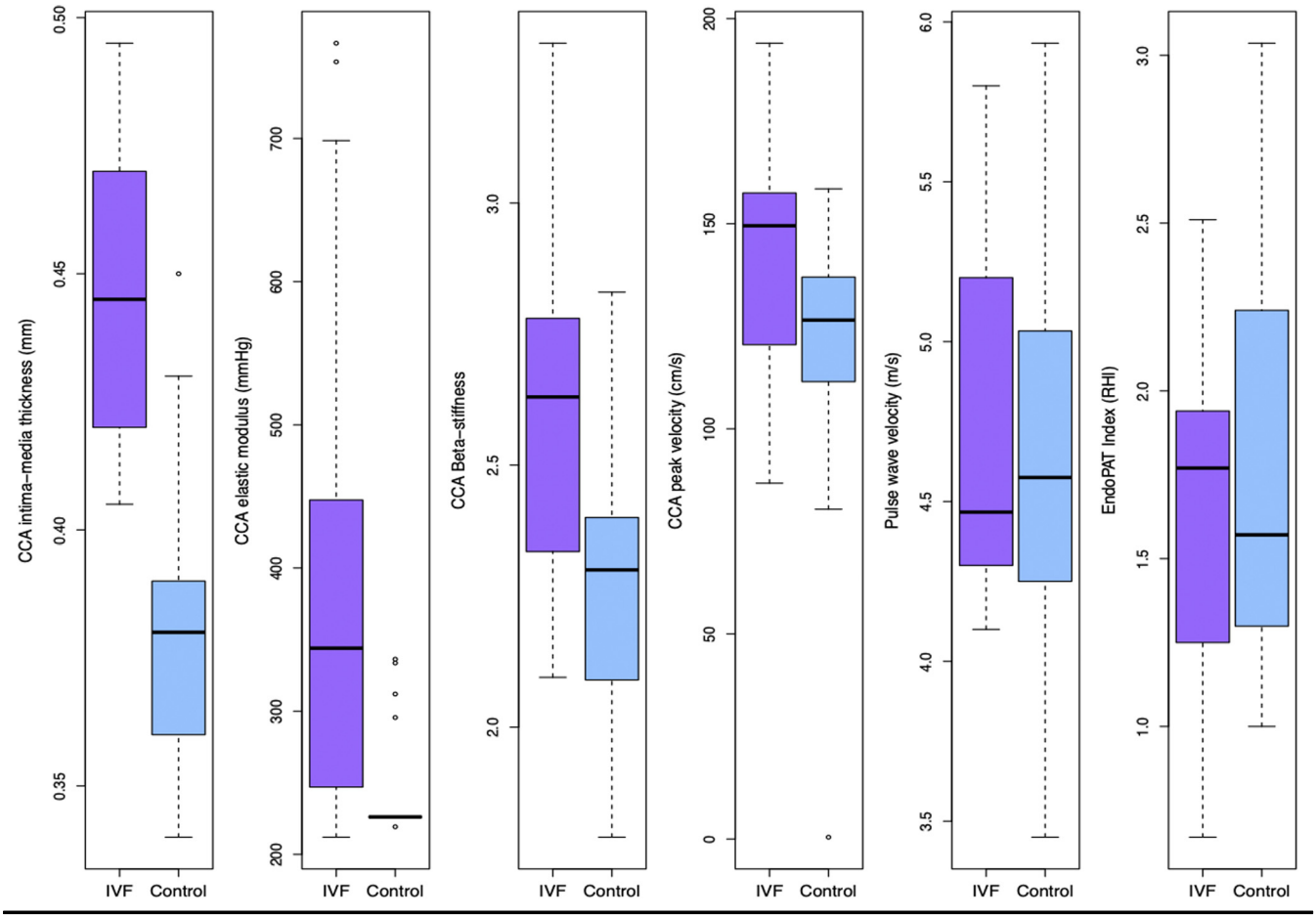

Figure 2. Boxplots of all outcome variables in IVF and control cohorts.

develops within an in vitro culture, and some evidence suggests that IVF is associated with an increased risk of epigenetic disorders.[44] IVF, particularly frozen embryo transfer and more recently pre-implantation genetic testing, has been associated with an increased risk for preeclampsia.[45-51] Preeclampsia in turn has been associated with a higher risk of cardiovascular disease in the offspring. Finally, the population of couples undergoing IVF differ from those who conceive spontaneously in ways that could theoretically affect health of offspring. For example, couples undergoing IVF are often older and are more likely to be obese compared with the general population of couples who conceive spontaneously.[52,53] Further study is required to determine whether the IVF procedure itself or factors related to infertility could explain our findings.

This study has some strengths. We compared the endothelial function and arterial wall properties of IVF adolescents with that of non-IVF adolescents in the US. The IVF offspring and controls were all assessed in the same cardiovascular laboratory under a rigorous protocol. The IVF offspring were recruited from the total population of children who had been conceived by IVF, not specifically from an academic pediatric practice.

This study has some limitations, with the most important being the small sample size, recruitment from a single university IVF practice, and the use of normative data for the controls rather than the recruitment of a new control group. Additionally, our analysis was not adjusted for multiple comparisons. Recruitment was very challenging as outlined in the Methods, exposing the selection of participants to voluntary response bias and nonresponse bias. Recruitment from a single university practice limits the generalizability of our findings because IVF protocols vary from institution to institution, and restriction of the study to children aged 10-14 years limits applicability to older IVF progeny because protocols have evolved significantly over the past decades. Funding was not available to recruit a new control group, and the use of previously assessed normative controls prevented blinding during the assessment of IVF offspring. However, the same technicians performed vascular tests on both the IVF and control groups using identical study protocols and equipment. Finally, because these conceptions occurred more than a decade ago, we did not have access to detailed records regarding parental health at the time of pregnancy and the course of pregnancy.

The cross-sectional nature of this study limits the ability to investigate temporal relationships and form casual inferences, making it difficult to know if the findings have clinical significance for later development of cardiovascular dysfunction. Thicker IMT and abnormal arterial stiffness in apparently healthy children has been associated with increased risk of cardiovascular disease later in life.[19-23] Therefore, it is possible that, if our findings are confirmed, IVF offspring could potentially face an increased risk of cardiovascular dysfunction in adulthood. If future studies corroborate our findings of higher cardiovascular dysfunction, primary prevention strategies could be developed for IVF children to include more intensive screening and preventive care, because many cardiovascular disease processes are most reversible in childhood.[26] If the nonphysiologic hormonal milieu is determined to be responsible for cardiovascular dysfunction, or if any other aspects of the IVF treatment are found culpable, practical changes in clinical practice have the potential to improve child health.

In our cohort of adolescents conceived via IVF, there is evidence of abnormal vascular health. Additional studies are needed to replicate or refute these findings, further investigate the pathophysiology leading to these vascular changes, and identify modifiable aspects of the IVF process to minimize any adverse effects. Furthermore, future studies should assess the long-term cardiovascular health of IVF offspring as they reach adulthood. ■

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