

SPECIAL FOCUS ISSUE: BLOOD PRESSURE

Association of Assisted Reproductive Technologies With Arterial Hypertension During Adolescence



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ABSTRACT

BACKGROUND Assisted reproductive technologies (ART) have been shown to induce premature vascular aging in apparently healthy children. In mice, ART-induced premature vascular aging evolves into arterial hypertension. Given the young age of the human ART group, long-term sequelae of ART-induced alterations of the cardiovascular phenotype are unknown.

OBJECTIVES This study hypothesized that vascular alterations persist in adolescents and young adults conceived by ART and that arterial hypertension possibly represents the first detectable clinically relevant endpoint in this group.

METHODS Five years after the initial assessment, the study investigators reassessed vascular function and performed 24-h ambulatory blood pressure (BP) monitoring (ABPM) in 54 young, apparently healthy participants conceived through ART and 43 age- and sex-matched controls.

RESULTS Premature vascular aging persisted in ART-conceived subjects, as evidenced by a roughly 25% impairment of flow-mediated dilation of the brachial artery ($p < 0.001$) and increased pulse-wave velocity and carotid intima-media thickness. Most importantly, ABPM values (systolic BP, 119.8 ± 9.1 mm Hg vs. 115.7 ± 7.0 mm Hg, $p = 0.03$; diastolic BP, 71.4 ± 6.1 mm Hg vs. 69.1 ± 4.2 mm Hg, $p = 0.02$ ART vs. control) and BP variability were markedly higher in ART-conceived subjects than in control subjects. Eight of the 52 ART participants, but only 1 of the 43 control participants ($p = 0.041$ ART vs. controls) fulfilled ABPM criteria of arterial hypertension ($>130/80$ mm Hg and/or >95 th percentile).

CONCLUSIONS ART-induced premature vascular aging persists in apparently healthy adolescents and young adults without any other detectable classical cardiovascular risk factors and progresses to arterial hypertension. (Vascular Dysfunction in Offspring of Assisted Reproduction Technologies; [NCT00837642](https://doi.org/10.1186/1745-7214-10-11).) (J Am Coll Cardiol 2018;72:1267-74)
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The use of assisted reproductive technologies (ART) has been growing exponentially over the past 2 decades; children born by these techniques now make up 2% to 5% of births in developed countries, and >6 million persons have been conceived by ART (1). There is increasing evidence that ART alters the cardiovascular phenotype in apparently healthy children and mice (2-6). In



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ABBREVIATIONS AND ACRONYMS

ABPM = ambulatory blood pressure monitoring

ART = assisted reproductive technologies

BP = blood pressure

BPV = blood pressure variability

FMD = flow-mediated dilation

IMT = intima-media thickness

PWV = pulse-wave velocity

mice, ART-induced premature vascular aging translates into arterial hypertension (2). Given the young age of the human ART group, the long-term consequences of ART-induced alterations of the cardiovascular phenotype are unknown. We hypothesized that vascular alterations persist in ART-conceived adolescents and that arterial hypertension may represent the first detectable clinically relevant endpoint in humans.

We therefore, 5 years after the initial assessment (4), reassessed vascular function in 54 young, apparently healthy ART-conceived adolescents and 43 age- and sex-matched controls and evaluated 24-h ambulatory blood pressure (BP). To assess vascular function of the systemic circulation, we measured endothelium-dependent and endothelium-independent dilation of the brachial artery (4) and carotid-femoral pulse-wave velocity (PWV), a measure of arterial stiffness (4). To document structural alterations, we assessed carotid intima-media thickness (IMT) (7).

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METHODS

All participants of our initial study were invited to participate in the follow-up. A total of 54 ART-conceived participants (mean age, 16.5 ± 2.3 years) and 43 control participants (mean age, 17.4 ± 2.7 years) agreed to be restudied. Control subjects were recruited by the families of the ART-conceived children among school friends of their children. The rationale was to recruit control participants with the best possible match for socioeconomic background, physical activity, and interests in sports, potential confounding factors. Eleven of the 65 ART participants and 14 of the 57 control participants were not restudied. The reasons were as follows: 4 ART subject and 2 control subjects had moved and could not be contacted, 7 ART subject and 9 controls refused to participate without giving any reason. One control subject had social problems, and 2 had left Switzerland; 15 ART participants were conceived by in vitro fertilization and 39 by intracytoplasmic sperm injection. In 43 cases, fresh embryos were transferred immediately, whereas in the remaining 11, zygotes were kept frozen at the 2-pronuclear stage for transfer at a later time point. None of the participants was born prematurely, mean gestational age was comparable in the 2 groups, and there were no pregnancy or perinatal complications. All participants were singletons, were born at term, and had a normal birth weight (Table 1). Five control subjects and 3 ART

subjects were taking noncardiac medication during the time of the study. One ART subject was treated with insulin for type 1 diabetes (Table 1).

The protocol was approved by the Institutional Review Board on Human Investigation of the University of Bern, Bern, Switzerland, and was registered. All participants (and the parents of participants <16 years of age) provided written informed consent.

ASSESSMENT OF SYSTEMIC VASCULAR FUNCTION.

Studies were performed after 15 min of rest in the supine position in a temperature-controlled room (22°C). The operators who performed and the observers who measured the vascular function tests were blinded to the mode of conception of the participant.

ENDOTHELIUM-DEPENDENT AND ENDOTHELIUM-INDEPENDENT VASODILATION.

Systemic conduit artery endothelial function was assessed by determining the increase of the brachial artery diameter evoked by reactive hyperemia with high-resolution ultrasound and automatic wall tracking software according to international guidelines (8) and as previously described (4,9). Briefly, the brachial artery was identified ≈ 5 cm above the antecubital fossa with a high-resolution ultrasound device (Esaote MyLab30 Gold, Esaote SpA, Genoa, Italy) and a high-frequency (7 to 10 MHz) linear-array probe. The ultrasound probe was then fixed in a stereotactic clamp with micrometer movement capabilities (AMC Vascular Imaging, Amsterdam, the Netherlands), and Doppler flow was recorded continuously throughout the study. After 1 min of baseline measurements, a pressure cuff placed around the forearm was inflated to 250 mm Hg for 5 min. After deflation of the cuff, the hyperemia-induced changes in brachial artery diameter and flow were measured continuously for 4 min. Images of the artery were analyzed using edge detection software with a system for real-time measurement of the brachial artery diameter in B-mode ultrasound images (Cardiovascular Suite, Quipu, Pisa, Italy) for determination of the baseline diameter of the vessel (10-12). We previously found that the coefficient of variation between 2 measurements in the same 30 subjects 24 h apart was 5.2% (4). Flow-mediated dilation (FMD) was expressed as the maximal percentage change in vessel diameter from baseline. For technical reasons, FMD could not be measured in 3 control participants.

Endothelium-independent dilation of the brachial artery was assessed by measuring the increase in brachial artery diameter evoked by oral glyceryl trinitrate (50 μ g; UCB-Pharma, Bulle, Switzerland) (9).

CAROTID INTIMA-MEDIA THICKNESS. Carotid IMT was measured according to recommended guidelines (13,14) and as previously reported (7,15). Briefly, after identification of the carotid bulb, the segments of the right and left common carotid artery 1 to 2 cm proximal to the bulb were scanned to identify the optimal angle of incidence. Carotid IMT was measured using radiofrequency signals with a 21- μ m resolution (RF QIMT, Esaote SpA) (16). After scanning the vessel, each radiofrequency line was automatically analyzed forward and backward in real time by the ultrasonography device. Radiofrequency-based echo-tracking systems are considered reference techniques with very high precision and reproducibility (17).

LARGE-ARTERY STIFFNESS. Large-artery stiffness was assessed noninvasively by measuring carotid-femoral PWV with the Complior device (Artech Medical, Pantin, France) according to international guidelines (17) and as previously described (4,18). Briefly, carotid and femoral artery waveforms were simultaneously recorded with mechanotransducers applied directly to the skin over the arteries, and the mean wave transit time for 10 heartbeats was calculated by the system software with the foot-to-foot method. To determine the PWV, the surface distance between the recording sites was measured. We previously found that the coefficient of variation between 2 measurements in the same 30 subjects 24 h apart was 6.3% (4). For technical reasons, PWV could not be measured in 3 control and 1 ART participants.

AMBULATORY BLOOD PRESSURE MONITORING. The 24-h ambulatory BP monitoring (ABPM) was performed using validated recorders (Spacelabs model 90217, Spacelabs Healthcare, Snoqualmie, Washington) during usual daily activities as previously described (15). The arm cuff was programmed to inflate every 20 and 60 min during the day and night, respectively. Patients completed a diary for the identification of activity, and sleep and wake periods. In 2 ART participants 24-h ambulatory BP could not be measured for technical reasons.

SHORT-TERM BLOOD PRESSURE VARIABILITY. Short-term BP variability (BPV) on the 24-h ABPM was assessed as previously reported (19,20) by calculating the average real variability (ARV) index using the following formula:

$$ARV = \frac{1}{n-1} \sum_{k=1}^{n-1} (BP(k+1) - BP(k))$$

where *n* denotes the number of valid BP measurements in the 24-h ABPM data corresponding to a given subject.

TABLE 1 Subject Characteristics

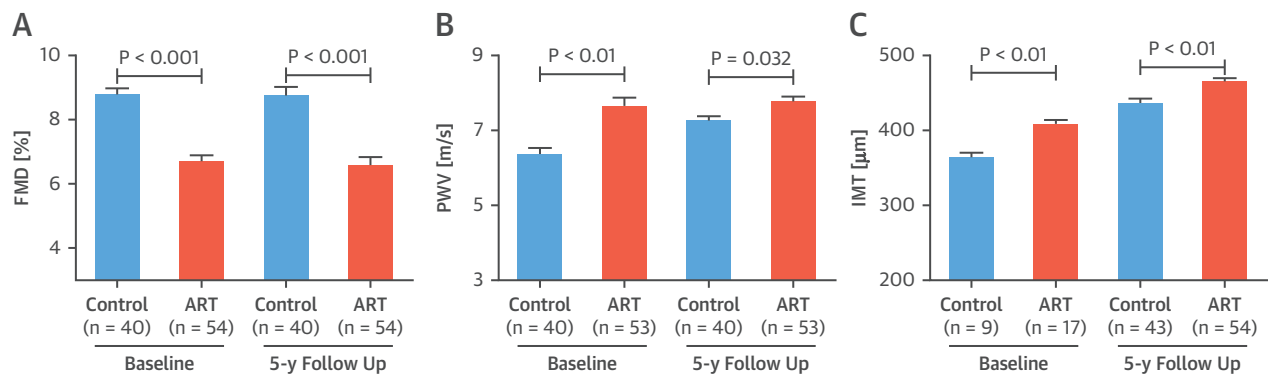
	Control (n = 43)	ART (n = 54)	p Value
Age, yrs	17.4 ± 2.7	16.5 ± 2.3	0.11
Female	24 (55)	27 (50)	0.68
BMI, kg/m ²	21.3 ± 3.0	21.7 ± 3.4	0.61
Birth weight, g	3342.2 ± 456.5	3357.4 ± 556.7	0.89
Gestational age, week	38.9 ± 2.1	39.3 ± 1.9	0.45
Maternal BMI, kg/m ²	21.8 ± 2.8	22.5 ± 2.5	0.43
Maternal age, yrs	28.6 ± 5.5	29.1 ± 5.1	0.64
Maternal history of hypertension	1	2	—
Drugs*	5 (11)	3 (6)	0.46
Contraception	6 (25)	7 (26)	1.0
Smoking	7 (16)	2 (4)	0.07
Diabetes	0	1	—
Sodium, mmol/l	141.3 ± 1.4	141.6 ± 1.4	0.35
Potassium, mmol/l	4.1 ± 0.2	4.0 ± 0.2	0.46
Creatinine, μ mol/l	71.0 ± 11.9	72.2 ± 13.4	0.65
hsCRP, mg/l	1.4 ± 1.9	1.1 ± 3.5	0.90
Cholesterol, mmol/l	4.3 ± 0.6	4.1 ± 0.7	0.25
LDL cholesterol, mmol/l	2.6 ± 0.7	2.4 ± 0.6	0.19
HDL cholesterol, mmol/l	1.4 ± 0.3	1.4 ± 0.3	0.65
Triglycerides, mmol/l	1.4 ± 0.6	1.3 ± 0.7	0.47

Values are mean ± SD, n (%), or n. *Controls (iron and folic acid [n = 1], bilastin [n = 1], etilefrine [n = 1], citalopram [n = 1], and wala Akne-Kapseln [n = 1]); ART (methylphenidate and isotretinoin [n = 2], insulin [n = 1]).
 ART = assisted reproductive technologies; BMI = body mass index; HDL = high-density lipoprotein; hsCRP = high-sensitivity C-reactive protein; LDL = low-density lipoprotein.

ANALYTICAL METHODS. Blood samples from participants were obtained on heparin and immediately centrifuged at 4°C, and the plasma was frozen at -80°C. Total cholesterol and triglyceride plasma concentrations were measured with commercial kits.

STATISTICAL ANALYSIS. The primary study endpoint was ambulatory BP reading. The secondary endpoints were FMD, PWV, and IMT. Statistical analysis was performed with the GraphPad Prism 5 software package (GraphPad Software, San Diego, California). Unpaired and paired 2-tailed *t*-tests were used for group comparisons of continuous variables. For comparisons of categorical variables between the ART and control groups, we used the Fisher exact test. The correlations between variables were analyzed by calculating Spearman correlation coefficients.

Power calculation was performed before the study on the basis of our preliminary data, and assuming that a 4 mm Hg difference in 24-h systolic ambulatory BP between ART and control participants was clinically relevant, an SD of 6 mm Hg, and considering an alpha error of 5% and a desired statistical power of 80%, we calculated that the minimal sample size of each group should be *n* = 37 subjects.

FIGURE 1 Premature Vascular Aging in ART Children Persists Into Young Adulthood

(A) Flow-mediated dilation (FMD), (B) pulse wave velocity (PWV), and (C) intima-media thickness (IMT) at baseline (mean \pm SD age, control 11.8 ± 2.2 years; assisted reproductive technologies [ART] 10.9 ± 2.4 years) and at 5-year follow-up (mean age; control 17.6 ± 2.7 years; assisted reproductive technologies 16.6 ± 2.4 year) in subjects conceived through assisted reproductive technologies and naturally conceived control subjects. Premature vascular aging, as evidenced by impaired flow-mediated dilation and by increased pulse wave velocity and intima-media thickness, persists at 5-years follow up in subjects conceived through assisted reproductive technologies. Data are shown as mean \pm SD.

A value of $p < 0.05$ was considered to indicate statistical significance. Unless otherwise indicated, data are given as mean \pm SD.

RESULTS

Body mass index (21.7 ± 3.4 kg/m² vs. 21.3 ± 3.0 kg/m², $p = 0.61$ ART vs. controls) was comparable in ART and control participants. Lipid, creatinine, and electrolyte plasma concentrations, as well as high-sensitivity C-reactive protein, were normal and comparable in ART and control subjects (Table 1). Birth weight, a possible determinant of vascular function later in life (21), was similar in ART and control subjects (Table 1). Moreover, gestational age, maternal body mass index, maternal smoking status, and maternal cardiovascular risk profile were comparable in the 2 groups (Table 1). None of the participants had structural heart disease (as assessed by echocardiography) (4).

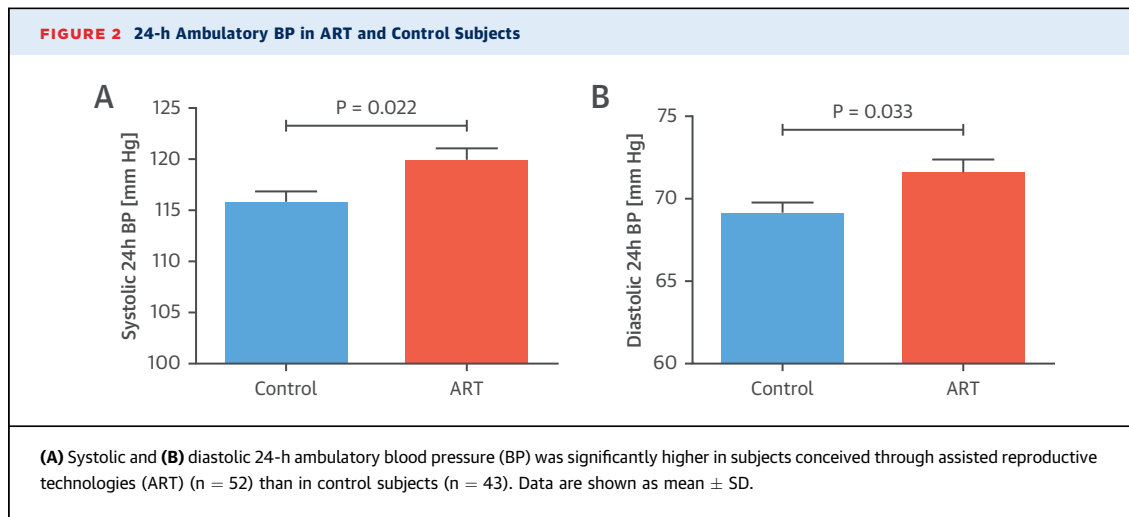
PREMATURE VASCULAR AGING. Baseline brachial artery diameter (ART vs. control, 3.2 ± 0.4 mm vs. 3.2 ± 0.5 mm, $p = 0.50$) and the ischemia-induced increase in blood flow ($695.8 \pm 268.5\%$ vs. $652.7 \pm 341.1\%$; $p = 0.51$) were similar in the 2 groups. Figures 1A to 1C show that premature vascular aging persisted in ART-conceived adolescents. At 5-year follow up, FMD remained roughly 25% smaller in the ART group than in controls ($6.5 \pm 2.1\%$ vs. $8.6 \pm 2.2\%$; $p < 0.001$ ART vs. control). This problem appears to be related to endothelial dysfunction because endothelium-independent vasodilation evoked by nitroglycerin

was similar in the 2 groups ($11.2 \pm 3.4\%$ vs. $11.3 \pm 3.5\%$; $p = 0.89$ ART vs. control). Similarly, carotid-femoral PWV (7.7 ± 1.2 m/s vs. 7.2 ± 0.9 m/s, $p = 0.032$ ART vs. control) (Figure 1B) and carotid IMT (463.7 ± 45.2 μ m vs. 435.0 ± 49.5 μ m, $p < 0.01$ ART vs. control) remained significantly increased in the ART group compared with controls. There were no significant relationships among FMD, PWV, and IMT (all $p > 0.1$).

ARTERIAL HYPERTENSION. Figures 2A and 2B show that 24-h systolic as well as diastolic BP was markedly higher in the ART group than in control subjects (systolic BP, 119.8 ± 9.1 mm Hg vs. 115.7 ± 7.0 mm Hg, $p = 0.02$; diastolic BP, 71.4 ± 6.1 mm Hg vs. 69.1 ± 4.2 mm Hg, $p = 0.03$ ART vs. control).

Most importantly, 8 of the 52 ART participants fulfilled the criteria for the diagnosis of arterial hypertension ($>130/80$ mm Hg and/or >95 th percentile) whereas only 1 of the 40 control participants fulfilled these criteria ($p = 0.041$ ART vs. controls). There was a positive correlation between systolic BP and IMT ($r = 0.22$, $p = 0.03$) and PWV ($r = 0.26$, $p = 0.012$), respectively, and between diastolic BP and PWV ($r = 0.26$, $p = 0.014$). There was no correlation between systolic BP and FMD and diastolic BP and FMD and IMT (all $p > 0.4$).

BLOOD PRESSURE VARIABILITY. The 24-h systolic BPV (9.9 ± 1.7 mm Hg vs. 8.7 ± 1.6 mm Hg, $p = 0.001$ ART vs. control), diastolic BPV (8.7 ± 1.8 mm Hg vs. 7.9 ± 1.4 mm Hg, $p = 0.03$ ART vs. control) and mean BPV (8.2 ± 1.6 mm Hg vs. 7.5 ± 1.5 mm Hg, $p = 0.02$ ART vs. control) were increased in ART participants compared with control participants.



DISCUSSION

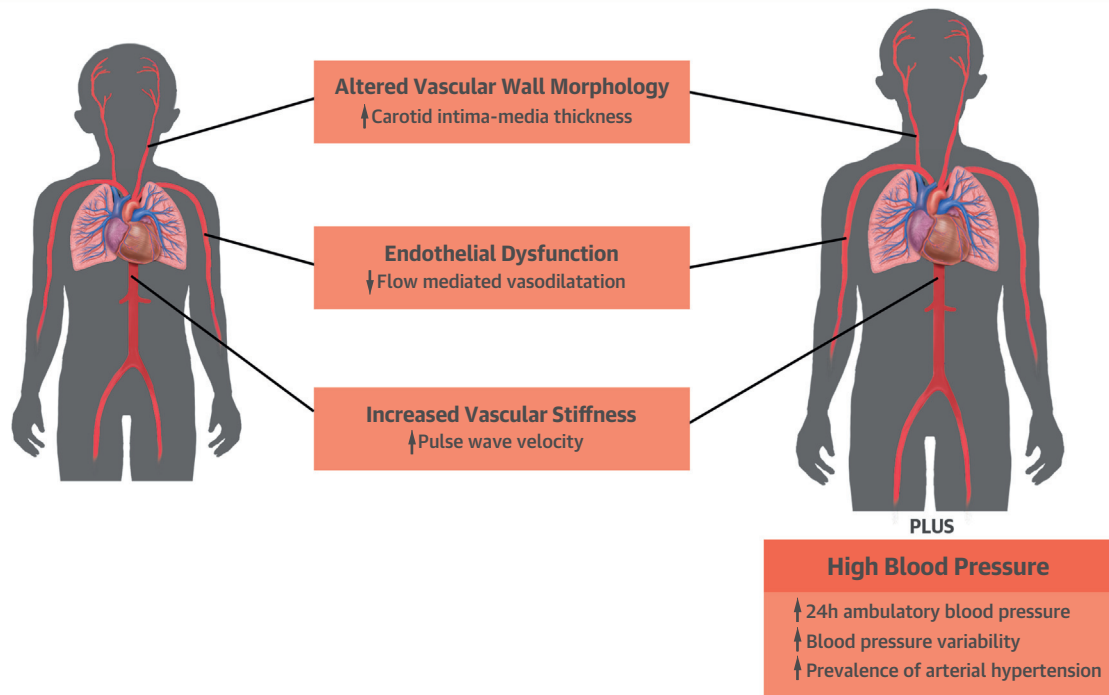
ART has allowed millions of infertile couples to have children. However, this success may have come at a price because evidence is accumulating indicating that ART alters the cardiovascular and metabolic phenotype in mice and humans (Central Illustration) (2-6,22-24). In line with this concept, we demonstrated that ART causes premature vascular aging in young, apparently healthy children (4). However, given the young age of this population, it is not known how this problem will persist and whether it will evolve into clinically manifest cardiovascular disease. Here, we found that ART-induced premature vascular aging persisted over a 5-year period in apparently healthy adolescents and young adults without any detectable classical cardiovascular risk factor and progressed to increased arterial BP and BPV, as assessed by 24-h ABPM. These data underscore the potential of ART to increase cardiovascular risk in this rapidly growing group.

We had studied the participants 5 years earlier and found that ART-conceived children displayed premature vascular aging as evidenced by a roughly 25% smaller FMD in the brachial artery, increased PWV (a measure of vascular stiffness), and increased carotid IMT compared with control children, whereas arterial BP, as assessed by office and central BP measurements, was not different between the 2 groups (4). Five years later, the participants now being adolescents or young adults, we found that differences in vascular function between ART and control participants persisted and were of similar magnitude as those observed during childhood 5 years earlier.

Impaired FMD and increased arterial stiffness and carotid IMT are independent cardiovascular risk factors in children (17,25-28), and arterial hypertension is expected to represent an early cardiovascular endpoint in this group. Indeed, we found that in adolescent and young adult ART subjects the presence of these risk factors evolved into increased arterial BP and a significant increase in the prevalence of established arterial hypertension. In line with this concept, we found significant relationships between systolic BP and IMT and PWV, respectively, and between diastolic BP and PWV. Moreover, we found that BPV, a measure of autonomic control of the circulation (29) and an independent predictor of cardiovascular risk (30-33), was significantly increased in ART participants compared with control participants.

Several additional points need to be mentioned. ART is associated with an increased prevalence of pathological events during fetal life (e.g., preeclampsia) that are known to increase cardiovascular risk in naturally conceived humans (3) and are associated with premature vascular aging during adolescence (9). These problems did not play a confounding role in the present studies because none of the participants was born after a pregnancy complicated by preeclampsia, and birth weight and gestational age were normal and comparable in ART and control participants.

Moreover, we previously demonstrated that the alterations of the cardiovascular phenotype in the ART participants cannot be attributed to parent-related factors or to hormonal stimulation of ovulation in the mother, a finding suggesting that the cardiovascular alterations are related to the procedure itself (4). In line with this speculation, studies in normal mice show that ART causes premature vascular aging and arterial hypertension in the

CENTRAL ILLUSTRATION Assisted Reproductive Technologies-Induced Alterations of the Cardiovascular Phenotype**Children**
Premature Vascular Aging**Young Adults**
Premature Vascular Aging Persists

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Premature vascular aging detected in children conceived by assisted reproductive technologies (ART) persists in young adults and evolves into arterial hypertension.

offspring (2). Collectively, these observations suggest that the altered cardiovascular phenotype in offspring conceived by ART is related to the procedure itself. Finally, a recent meta-analysis did not find definitive evidence for an increased cardiovascular risk in mothers undergoing fertility treatment (34). This finding is consistent with our previous data showing that ART-treated mothers do not display endothelial dysfunction (4), a problem that may increase cardiovascular risk in the mother or may be transmitted by ART to the child.

Comparable signs of premature vascular aging have been found in young children of mothers who had preeclampsia (9). In children of mothers with preeclampsia, the prevalence of arterial hypertension is also increased later in life (35,36) and may contribute to the increased prevalence of stroke reported in this group (37). Thus, the consequences for the cardiovascular phenotype and cardiovascular risk

later in life may be similar regardless of whether pathological events occurred during the early embryonic period or the late fetal period.

STUDY LIMITATIONS. A final important issue is whether participants in the present study are representative of the overall ART and control groups. The ambulatory BP values of our control subjects were very similar to published normal values (38) by height and sex for this age group (delta between BP in control participants and the published population norm: 0.43 ± 6.6 mm Hg and 1.75 ± 4.0 mm Hg for systolic and diastolic BP, respectively). Prematurity, low birth weight, and preeclampsia (i.e., factors known to alter the cardiovascular phenotype and increase cardiovascular risk in naturally conceived children [3]), were exclusion criteria in our study. The prevalence of these complications is markedly higher in ART-conceived pregnancies than in naturally conceived

pregnancies and is further increased in twin (and multiples) pregnancies, which are also significantly more frequent with ART. For this latter reason, we studied singletons only. Thus, the exclusion criteria used in our study may have resulted in a “low cardiovascular risk” group of ART participants compared with the overall ART group. Along the same lines, ART participants were recruited from a single procreation center. The demonstration, by several groups of independent investigators, of ART-induced alteration of the cardiovascular phenotype in humans (4,5,24,39,40) and mice (2,41,42) strongly suggests that ART-induced alterations of the cardiovascular phenotype are ubiquitous.

CONCLUSIONS

We found that ART-induced premature vascular aging persists in apparently healthy young adults without any detectable classical cardiovascular risk factor and progresses to arterial hypertension. There is increasing evidence that in experimental animals epigenetic mechanisms contribute to ART-induced alteration of the cardiovascular phenotype (2,3,23,42-44). Translation of this mechanistic insight gained in mice to humans may allow preventing

ART-induced alterations of the cardiovascular phenotype and cardiovascular risk in the millions of future children expected to be conceived by these methods.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Individuals conceived by ART are prone to developing hypertension during early adulthood and are at elevated risk of cardiovascular events.

TRANSLATIONAL OUTLOOK: Long-term cohort studies are needed to define optimum strategies for prevention and treatment of cardiovascular disease in this population.

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