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## Systemic and Pulmonary Vascular Dysfunction in Children Conceived by Assisted Reproductive Technologies

Urs Scherrer, MD\*; Stefano F. Rimoldi, MD\*; Emrush Rexhaj, MD; Thomas Stuber, MD; Hervé Duplain, MD; Sophie Garcin, MD; Stefano F. de Marchi, MD; Pascal Nicod, MD; Marc Germond, MD; Yves Allemann, MD\*; Claudio Sartori, MD\*

**Background**—Assisted reproductive technology (ART) involves the manipulation of early embryos at a time when they may be particularly vulnerable to external disturbances. Environmental influences during the embryonic and fetal development influence the individual's susceptibility to cardiovascular disease, raising concerns about the potential consequences of ART on the long-term health of the offspring.

**Methods and Results**—We assessed systemic (flow-mediated dilation of the brachial artery, pulse-wave velocity, and carotid intima-media thickness) and pulmonary (pulmonary artery pressure at high altitude by Doppler echocardiography) vascular function in 65 healthy children born after ART and 57 control children. Flow-mediated dilation of the brachial artery was 25% smaller in ART than in control children ( $6.7 \pm 1.6\%$  versus  $8.6 \pm 1.7\%$ ;  $P < 0.0001$ ), whereas endothelium-independent vasodilation was similar in the 2 groups. Carotid-femoral pulse-wave velocity was significantly ( $P < 0.001$ ) faster and carotid intima-media thickness was significantly ( $P < 0.0001$ ) greater in children conceived by ART than in control children. The systolic pulmonary artery pressure at high altitude (3450 m) was 30% higher ( $P < 0.001$ ) in ART than in control children. Vascular function was normal in children conceived naturally during hormonal stimulation of ovulation and in siblings of ART children who were conceived naturally.

**Conclusions**—Healthy children conceived by ART display generalized vascular dysfunction. This problem does not appear to be related to parental factors but to the ART procedure itself.

**Clinical Trial Registration**—URL: [www.clinicaltrials.gov](http://www.clinicaltrials.gov). Unique identifier: NCT00837642.

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**Key Words:** endothelium ■ hypertension, pulmonary ■ reproductive technologies, assisted

In vitro fertilization has been done for 3 decades, and children born after assisted reproductive technology (ART) now make up 1% to 4% of the births in developed countries.<sup>1</sup> Epidemiological work in humans has put forward the hypothesis that environmental influences acting early in life may predispose the children to chronic cardiovascular and metabolic disease in adulthood.<sup>2</sup> Therefore, the safety of ART for long-term health is of utmost importance, but there is little information.<sup>3-6</sup> This may be related, at least in part, to the young age of the progeny because clinically manifest disease may not yet have had time to develop.

### Editorial see p 1832 Clinical Perspective on p 1896

Among the potential long-term consequences of ART, cardiovascular disease is an important concern. Therefore, we

studied the systemic and pulmonary vascular function in healthy children and adolescents conceived by ART and in control children. To assess the systemic circulation, we measured endothelium-dependent and -independent dilation of the brachial artery<sup>7-9</sup> and pulse-wave velocity (PWV), a proxy of elastic artery stiffness.<sup>10</sup> To test for structural alterations, we assessed carotid intima-media thickness.<sup>9</sup> To assess pulmonary vascular function, we used high-altitude exposure (3450 m) because hypoxia induces exaggerated pulmonary hypertension in persons displaying endothelial dysfunction.<sup>11</sup> We found that both systemic vascular function and pulmonary vascular function were defective in ART children and started to test for potential underlying mechanisms. Parent-related factors such as hormonal stimulation to induce hyperovulation in the mother or infertility could be responsible for long-term health problems in the offspring.

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From the Swiss Cardiovascular Center Bern, University Hospital, Bern, Switzerland (U.S., S.F.R., E.R., T.S., S.F.d.M., Y.A., C.S.); Facultad de Ciencias, Departamento de Biología, Universidad de Tarapacá, Arica, Chile (U.S.); Hirslanden Group, Lausanne, Switzerland (U.S., P.N.); Botnar Center for Extreme Medicine and Department of Internal Medicine, CHUV, Lausanne, Switzerland (H.D., S.G., C.S.); and Centre de Procréation Médicalement Assistée, Lausanne, Switzerland (M.G.).

\*Drs Scherrer, Rimoldi, Allemann, and Sartori contributed equally to this article.

Correspondence to Urs Scherrer, MD, Swiss Cardiovascular Center Bern, University Hospital, Bern, Freiburgstrasse, BHH A 143, Bern, 3010, Switzerland. E-mail [urs.scherrer2@insel.ch](mailto:urs.scherrer2@insel.ch)

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**Table 1. Participant Characteristics**

Characteristics	Control Children (n=57)	ART Children (n=65)	P
Female/male, n	30/27	27/38	...
Age, y	11.9 (2.3)	11.1 (2.4)	0.06
BMI, kg/m <sup>2</sup>	18.8 (3.0)	17.9 (2.5)	0.10
HR, bpm	71 (9)	71 (9)	0.90
Systolic BP, mm Hg	113 (10)	113 (10)	0.75
Diastolic BP, mm Hg	70 (7)	70 (7)	0.91
Glycemia, mmol/L	4.73 (0.55)	4.54 (0.50)	0.07
Insulinemia, $\mu$ mol/mL	11.94 (6.37)	11.54 (4.28)	0.72
HOMA	56.3 (30.7)	53.0 (22.1)	0.53
Total cholesterol, mmol/L	4.62 (0.72)	4.57 (0.77)	0.76
LDL cholesterol, mmol/L	2.90 (0.57)	2.73 (0.67)	0.17
HDL cholesterol, mmol/L	1.48 (0.29)	1.55 (0.24)	0.19
Triglycerides, mmol/L	0.75 (0.35)	0.73 (0.24)	0.78

ART indicates assisted reproductive technology; BMI, body mass index; HR, heart rate; BP, blood pressure; HOMA, homeostatic model assessment; LDL, low-density lipoprotein; and HDL, high-density lipoprotein. Data are presented as mean (SD).

We therefore assessed vascular function in children who were conceived naturally during hormonal stimulation of super-ovulation in the mother. Moreover, we studied vascular function in sterile and fertile parents and compared vascular function in pairs of siblings in whom one was conceived by ART and the other was conceived naturally.

## Methods

We recruited 65 healthy Swiss children conceived by ART and 57 control children born during the same period for these studies between October 2007 and April 2010 (Table 1).

Among the ART children, 21 were conceived by in vitro fertilization and 44 by intracytoplasmic sperm injection. In 48 cases, fresh embryos were transferred immediately, whereas in the remaining 17, zygotes were kept frozen at the 2-pronuclear stage for transfer at a later time point.<sup>12</sup> The children conceived by ART were contacted by letter (and those who agreed to participate and met the inclusion criteria were recruited) by one of us (M.G) who had performed all the procedures;<sup>13</sup> the control children were recruited by the families of the ART children. Among the study population were 5 pairs of siblings in whom one was conceived by ART (2 boys, 3 girls) and the other was conceived naturally (1 boy, 4 girls); age (ART versus naturally conceived, 11.0 $\pm$ 1.9 versus 12.8 $\pm$ 5.7 years;  $P=0.23$ ) and body mass index (ART versus naturally conceived, 17.1 $\pm$ 1.3 versus 17.6 $\pm$ 1.8 kg/m<sup>2</sup>;  $P=0.30$ ) were comparable. All children conceived by ART were singletons and were born at term (Table 2). None of the children had suffered from perinatal complications or was taking any medication during the time of the study.

ART implies hormonal stimulation of ovulation in the mother. To assess the potential pathophysiological importance of this factor, we studied systemic vascular function in a group of 16 children (age, 11.8 $\pm$ 2.2 years [mean $\pm$ SD]; 10 girls, 6 boys) who were conceived naturally during hormonal stimulation of the ovulation in the mother.

To examine whether sterility in the parents was associated with altered vascular function, we measured flow-mediated dilation (FMD) in 22 sterile (age, 43 $\pm$ 6 years) and 14 fertile (age, 39 $\pm$ 11 years) parents.

The protocol was approved by the institutional review boards on human investigation of the Universities of Bern and Lausanne, both in Switzerland, and was registered ([www.clinicaltrials.gov](http://www.clinicaltrials.gov); registration No. NCT00837642). All parents provided written informed consent.

**Table 2. Children's Characteristics at Birth and Maternal Characteristics at Conception**

Characteristics	Control (n=53)	ART (n=60)	P
Gestational age, wk	39.0 (2.0)	39.2 (1.8)	0.55
Birth weight, g	3371 (419)	3330 (547)	0.56
Maternal age, y	30.1 (4.7)	33.2 (3.9)	<0.001
BMI of the mother, kg/m <sup>2</sup>	21.7 (2.4)	22.4 (2.4)	0.33
Maternal smoking status, n (%)	8 (16)	5 (10)	0.55
Presence of other major cardiovascular risk factor in the mother,* n (%)	2 (4)	2 (4)	1.0

ART indicates assisted reproductive technology; BMI, body mass index. Data are presented as mean (SD).

\*Presence of at least one of the following: diabetes mellitus, arterial hypertension, or dyslipidemia.

## Assessment of Systemic Vascular Function

Systemic vascular function studies were performed after 15 minutes 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 children.

## Endothelium-Dependent and -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<sup>14</sup> and as previously described.<sup>15,16</sup> Briefly, the brachial artery was identified  $\approx$ 5 cm above the antecubital fossa with a high-resolution ultrasound device (Acuson Sequoia C512, Acuson Siemens, Mountain View, CA) and a high-frequency (7–10 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 minute of baseline measurements, a pressure cuff placed around the forearm was inflated to 250 mm Hg for 5 minutes. After deflation of the cuff, the hyperemia-induced changes in brachial artery diameter and flow were measured continuously for 3 minutes. B-mode ultrasound images were analyzed with a validated system for automatic real-time measurement of the brachial artery diameter (FMD Studio, Computer Vision Group, Pisa, Italy).<sup>17,18</sup> The coefficient of variation between 2 measurements in the same 30 subjects 24 hours apart was 5.2%. FMD was expressed as the maximal percentage change in vessel diameter from baseline.

Endothelium-independent dilation of the brachial artery was assessed by measuring the increase in brachial artery diameter evoked by oral glyceryl trinitrate (250  $\mu$ g; UCB-Pharma, Bulle, Switzerland).<sup>15</sup>

## Carotid Intima-Media Thickness

Carotid intima-media thickness was measured according to recommended guidelines<sup>19,20</sup> with an Acuson Sequoia 512C ultrasound device (Acuson Siemens) equipped with an 8- to 14-MHz linear-array L8 transducer. Briefly, the common carotid artery was scanned 1 to 2 cm proximal to the bulb, and the optimal angle of incidence was identified. To standardize the transducer angle, external landmarks were used. With this procedure, B-mode images (5 beat cine-loop and optimized R-wave-gated still frames) of the left and right carotid arteries were obtained at the optimal and 2 complementary angles (anterior and posterior). Images were stored (digital imaging and communication in medicine [DICOM] format) for offline analysis with a customized border tracing program written on Matlab 7. Reported values represent the mean of 3 measurements of

each carotid artery. The coefficient of variation between 2 measurements in the same 30 subjects 24 hours apart was 4.2%.

### 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<sup>10</sup> as described previously.<sup>16,21</sup> 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. The coefficient of variation between 2 measurements in the same 30 subjects 24 hours apart was 6.3%. For technical reasons, PWV could not be measured in 3 children conceived by ART and 4 control children.

### Pulmonary Vascular Function

Pulmonary artery pressure and cardiac output were assessed at high altitude (3450 m) as previously described<sup>15</sup> because hypoxia induces exaggerated pulmonary hypertension in persons displaying endothelial dysfunction.<sup>11</sup> The children ascended to the high-altitude research station at the Jungfrauoch (Switzerland, 3450 m) by train and spent 2 days and 2 nights at this laboratory. Measurements were performed on the morning before descent. To estimate systolic pulmonary artery pressure and cardiac output, echocardiographic recordings were obtained with a real-time, phased-array sector scanner (Acuson Sequoia 512, Acuson Siemens) with an integrated color Doppler system and transducers containing crystal sets for 2-dimensional imaging (5.0 MHz with second harmonic technology) and for continuous-wave Doppler recording (2.5 MHz). The recordings were stored on magneto-optical disks for offline analysis by 2 investigators who were unaware of the subject's identity. All reported values represent the mean of at least 3 measurements. After tricuspid regurgitation had been localized with Doppler color-flow imaging, the peak flow velocity of the transtricuspid jet was measured with the use of continuous-wave Doppler, and the pressure gradient between the right ventricle and the right atrium was calculated by use of the modified Bernoulli equation.<sup>22,23</sup> Right ventricular to right atrial (RV-RA) pressure gradient measurements, the standard method for the noninvasive estimation of pulmonary artery pressure, have been validated against invasive measurements at high altitude.<sup>24</sup> We have previously found that in children at this altitude, the intraobserver and interobserver variabilities for the RV-RA pressure gradient measurements were  $5.1 \pm 4.6\%$  and  $6.0 \pm 8.6\%$ , respectively ( $n=30$ ), and for cardiac output measurements were  $10.7 \pm 10.2\%$  and  $7.2 \pm 4\%$ , respectively ( $n=30$ ). Cardiac index was calculated by dividing cardiac output (L/min) by the body surface area ( $m^2$ ). For technical reasons, the pressure gradient could not be measured in 3 children conceived by ART and in 5 control children.

### Estimation of Right and Left Atrial Pressure

Estimation of the right atrial pressure was performed by measuring the respiratory change in the diameter of the inferior vena cava in the subcostal view. The ratio between the early diastolic transmitral peak flow velocity (E) and the septal early diastolic peak velocity of the mitral annulus (E') was calculated (E/E') to estimate the left atrial pressure.

### Arterial Oxygen Saturation and Heart Rate

Transcutaneous arterial oxygen saturation and heart rate were measured at a fingertip with a pulse oximeter (OxiMax N-65, Nellcor, Pleasanton, CA).

### Analytical Methods

After an overnight fast, on the morning of the last day at high altitude, blood samples were taken from children while on heparin and immediately centrifuged at 4°C, and the plasma was frozen at -80°C. Glucose, insulin, total cholesterol, and triglyceride plasma

concentrations were measured with commercial kits. The insulin resistance was estimated by the homeostatic model assessment with the following formula: fasting serum insulin ( $\mu U/L$ ) times fasting plasma glucose (mmol/L) divided by 22.5.

### Statistical Analysis

Statistical analysis was done with the Graphpad Prism 5 software package (GraphPad Software Inc, San Diego, CA). 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 Fischer exact test. When comparing 3 groups, we performed a 1-factor repeated-measures ANOVA and Bonferroni adjustment for multiple comparisons. Relations between variables were analyzed by calculating the Pearson product-moment correlation coefficients.

Power calculation was performed before the study on the basis of our previously reported pulmonary artery pressure data at this altitude in healthy children and in children with pulmonary vascular dysfunction.<sup>15</sup> With a 5-mm Hg difference in pulmonary artery pressure assumed between ART and control subjects (SD of pulmonary artery pressure in referents, 7 mm Hg; power >0.90;  $\alpha=0.05$ ), 42 subjects were needed in each group to address this aim. Multiple regression analysis was performed on Statistica 7.0 (StatSoft Inc, Tulsa, OK).

A standard, multiple linear regression model was chosen to determine the independent predictors of the following vascular parameters: FMD, PWV, RV-RA gradient, and IMT. ART, age, sex, heart rate, birth weight, gestational age, presence of maternal cardiovascular risk factors, and maternal age at birth were entered as test variables. In addition, systolic and diastolic blood pressures were tested for FMD and PWV, and brachial artery diameter was tested for FMD. For IMT, ART, age, sex, presence of maternal cardiovascular risk factors, and maternal age at birth were entered as test variables. Dichotomous variables were used as dummy variables in all multiple regression analyses.

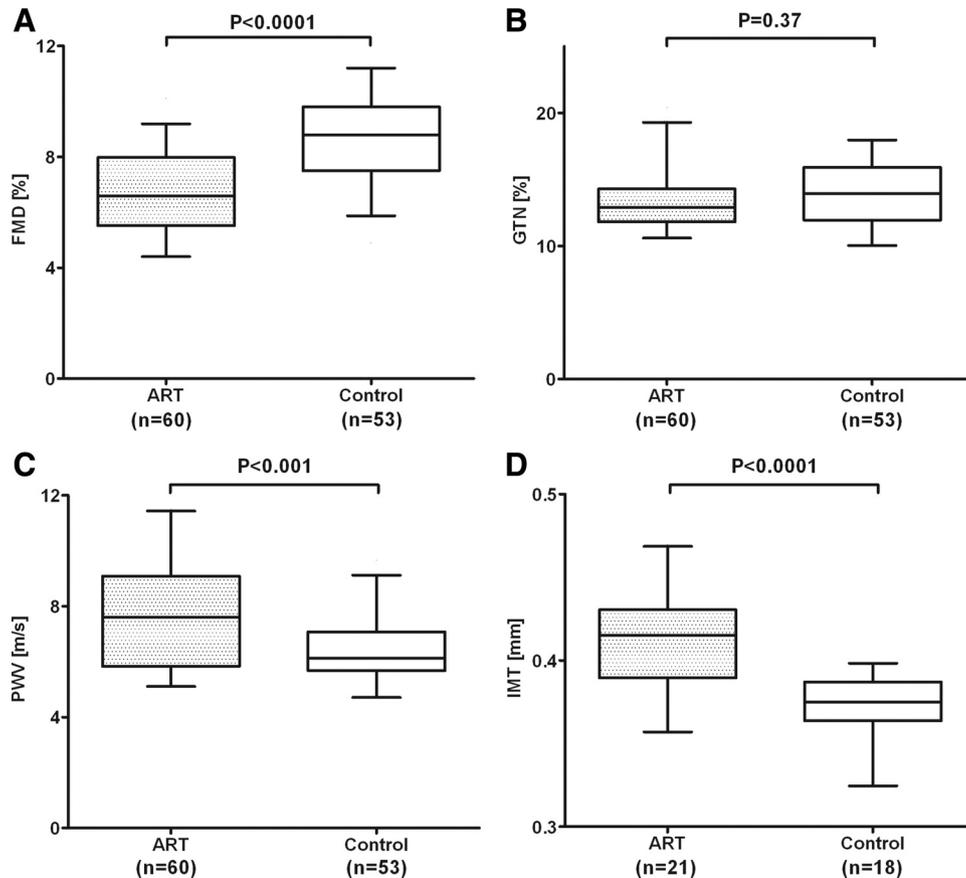
Results of the regression fittings were calculated, including the intercept and standardized coefficients of the multiple regression equation ( $\beta$ ), coefficient of determination ( $r^2$ ), and ANOVA *F* and *P* values of the overall regression model. Only variables with significant nonzero slopes in the regression equation were considered independent predictors.

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

## Results

Arterial blood pressure and body mass index were comparable in ART and control children (Table 1). Lipid, glucose, and insulin plasma concentration, as well as the insulin resistance index (homeostatic model assessment) and glucose tolerance, were normal and comparable in children born after ART and control children (Table 1). Birth weight, a possible determinant of vascular function later in life,<sup>25</sup> was similar in ART and control children (Table 2). Moreover, gestational age, maternal body mass index, maternal smoking status, and maternal cardiovascular risk profile were comparable in the 2 groups (Table 2). None of the children suffered from structural heart disease (as assessed by echocardiography).

Baseline brachial artery diameter (ART versus control,  $3.1 \pm 0.3$  versus  $3.2 \pm 0.5$  mm;  $P=0.58$ ) and the ischemia-induced increase in blood flow ( $520 \pm 180\%$  versus  $490 \pm 130\%$ ;  $P=0.57$ ) were similar in the 2 groups. FMD of the brachial artery was roughly 25% smaller in children conceived by ART than in control children ( $6.7 \pm 1.6$  versus  $8.6 \pm 1.7\%$ ;  $P < 0.0001$ ; Figure 1A), whereas endothelium-independent vasodilation evoked by nitroglycerine was similar in the 2 groups ( $13.5 \pm 2.3\%$  versus  $13.9 \pm 2.5\%$ ;  $P=0.37$ ;



**Figure 1.** Flow-mediated dilation (FMD) of the brachial artery (**A**), endothelium-independent nitroglycerine (GTN)-induced dilation of the brachial artery (**B**), carotid-femoral pulse-wave velocity (PWV; **C**), and intima-media thickness (IMT) of the carotid artery (**D**) in children conceived by assisted reproductive technology (ART) and in control children. Horizontal lines represent the median; boxes, 25th to 75th percentiles; and T bars, 5th and 95th percentiles. The number of subjects is indicated in parentheses.

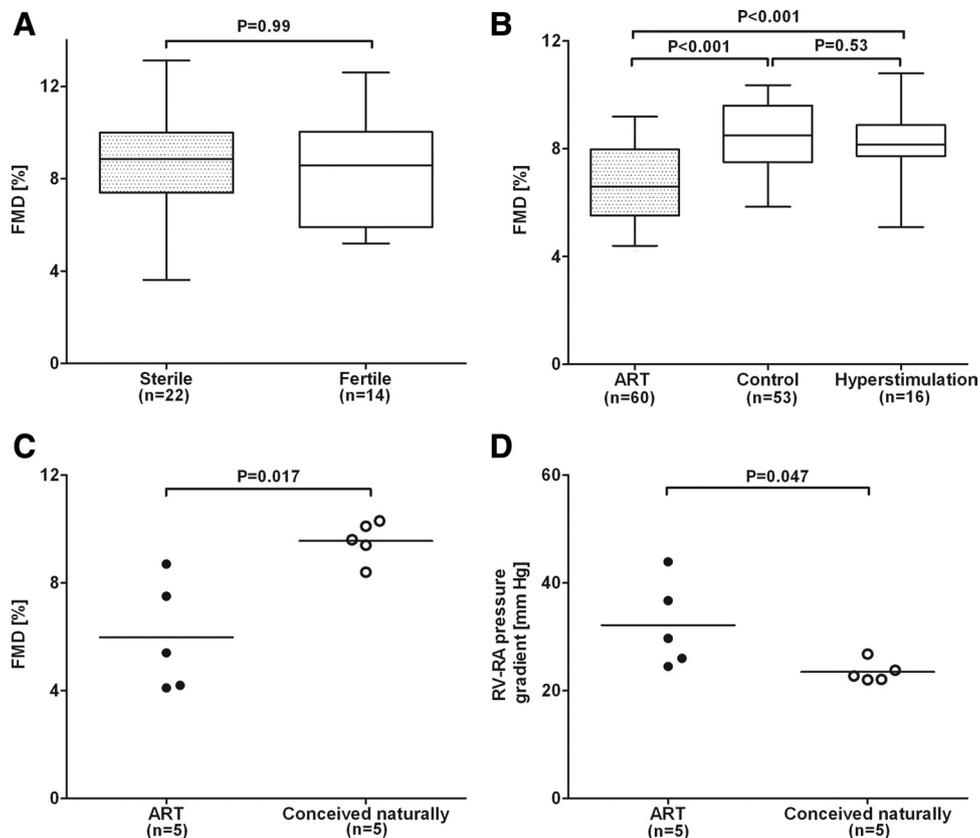
Figure 1B). Carotid-femoral PWV was significantly faster in children conceived by ART than in control children ( $7.8 \pm 2.4$  versus  $6.5 \pm 1.3$  m/s;  $P < 0.001$ ; Figure 1C). Carotid intima-media thickness was significantly greater in ART than in control children ( $410 \pm 30$  versus  $370 \pm 20$   $\mu\text{m}$ ;  $P < 0.0001$ ; Figure 1D).

To assess pulmonary vascular function, we used high-altitude exposure (3450 m). At high altitude, arterial oxygen saturation of hemoglobin was similar in the 2 groups ( $90 \pm 2\%$  versus  $90 \pm 2\%$ ;  $P = 0.70$ ). The systolic pulmonary artery pressure was 30% higher in children conceived by ART than in control children ( $39 \pm 11$  versus  $30 \pm 9$  mm Hg;  $P < 0.0001$ ), whereas the cardiac index was similar in ART and control children ( $2.92 \pm 0.48$  versus  $2.90 \pm 0.52$   $\text{L} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$ ;  $P = 0.86$ ). The mean diameter of the inferior vena cava (ART versus controls,  $1.05 \pm 0.39$  versus  $0.99 \pm 0.32$  cm;  $P = 0.48$ ) and its respiratory change (ART versus controls,  $48 \pm 9\%$  versus  $47 \pm 8\%$ ;  $P = 0.41$ ), as well as the E/E' ratio (ART versus controls,  $5.3 \pm 1.1$  versus  $5.5 \pm 1.1$ ;  $P = 0.38$ ), were comparable in the 2 groups, suggesting that the RV-RA pressure increase in the ART children was due to pulmonary vascular and not cardiac dysfunction. A significant inverse relationship existed between pulmonary artery pressure and FMD ( $r = -0.30$ ,  $P = 0.001$ ).

Systemic and pulmonary vascular dysfunction was similar in children born after in vitro fertilization or intracytoplasmic

sperm injection (FMD in in vitro fertilization versus intracytoplasmic sperm injection,  $6.1 \pm 1.4\%$  versus  $7.0 \pm 1.6\%$ ;  $P = 0.08$ ; RV-RA gradient in in vitro fertilization versus intracytoplasmic sperm injection,  $37 \pm 14$  versus  $41 \pm 9$  mm Hg;  $P = 0.22$ ) and in children whose zygotes were kept frozen at the pronuclear stage for later transfer and those whose fresh embryos were transferred immediately (FMD, frozen versus fresh,  $6.4 \pm 1.5\%$  versus  $6.8 \pm 1.6\%$ ;  $P = 0.40$ ; RV-RA gradient, frozen versus fresh,  $40 \pm 10$  versus  $39 \pm 11$  mm Hg;  $P = 0.85$ ).

Vascular function in sterile and fertile parents was normal and comparable ( $P = 0.90$ ; Figure 2A). Whereas as expected maternal age was slightly but significantly higher in ART children (Table 2), there existed no relationship between maternal age and vascular dysfunction in the progeny (FMD,  $r = -0.07$ ,  $P = 0.43$ ; PWV,  $r = -0.01$ ,  $P = 0.85$ ; IMT,  $r = 0.17$ ;  $P = 0.30$ ). Vascular function was normal in children who were conceived naturally after hormonal stimulation of ovulation in the mother (FMD,  $P = 0.53$  versus control children; Figure 2B), RV-RA gradient (stimulation versus control,  $29 \pm 7$  versus  $30 \pm 9$  mm Hg;  $P = 0.68$ ), PWV (stimulation versus control,  $6.3 \pm 1.3$  versus  $6.5 \pm 1.3$  m/s;  $P = 0.65$ ), and IMT (stimulation versus control,  $371 \pm 23$  versus  $370 \pm 20$   $\mu\text{m}$ ;  $P = 0.87$ ). Among 5 pairs of siblings in whom one was conceived by ART (2 boys) and the other was conceived naturally (1 boy), FMD was significantly smaller ( $P = 0.017$ ;



**Figure 2.** Flow-mediated dilation (FMD) in sterile and fertile parents (A) and in children conceived by assisted reproductive technology (ART), children conceived naturally after hormonal stimulation of ovulation in the mother, and control children (B). Dot plots of FMD (C) and systolic right ventricular to right atrial (RV-RA) pressure gradient (D) in 5 pairs of siblings in which one was conceived by ART and the other was conceived naturally. Horizontal lines represent the median; boxes, 25th to 75th percentiles; and T bars, 5th and 95th percentiles. The number of subjects is indicated in parentheses.

Figure 2C), pulmonary artery pressure was significantly higher ( $P=0.047$ ; Figure 2D), and PWV (ART versus naturally conceived,  $6.6\pm 1.8$  versus  $6.1\pm 1.9$  m/s;  $P=0.007$ ) and IMT (ART versus naturally conceived,  $420\pm 50$  versus  $360\pm 60$   $\mu\text{m}$ ,  $P=0.01$ ) were significantly greater in the siblings who were conceived by ART than in those who were conceived naturally.

### Multivariate Analysis

Multivariate analysis revealed ART as an independent predictor of all vascular parameters (ie, FMD, PWV, IMT, and RV-RA gradient).

### Discussion

The steadily increasing use of ART has allowed millions of infertile couples to have children. There are concerns regarding the potential consequences of ART on the long-term health of the offspring, but there is little information.<sup>3,5,6</sup> Here, we show that healthy children and adolescents conceived by ART display marked vascular dysfunction of the systemic and pulmonary circulation. This problem does not appear to be related to parental factors or hormonal stimulation of ovulation in the mother but to the ART procedure itself.

In the systemic circulation, FMD of the brachial artery was roughly 25% smaller in ART children than in control chil-

dren. Defective FMD was related to endothelial dysfunction because endothelium-independent vasodilation evoked by nitroglycerine was similar in the 2 groups. Endothelial dysfunction in ART children was not limited to the systemic circulation because pulmonary artery pressure at high altitude was 30% higher in children conceived by ART than in control children. This problem was not related to more severe altitude-induced hypoxemia because arterial oxygen saturation was comparable in the 2 groups. Endothelial dysfunction in the pulmonary and systemic circulation was a robust finding because a significant inverse relationship existed between pulmonary artery pressure and FMD.

Elastic artery stiffness represents an independent predictor of cardiovascular outcome in subjects at risk.<sup>10,26</sup> Here, we found that PWV, a widely used proxy of arterial stiffness,<sup>10</sup> was significantly faster in ART than in control children. To further test for potential structural alterations of the vasculature, we measured carotid intima-media thickness and found that it was significantly increased in ART children. Taken together, these data demonstrate generalized vascular dysfunction in young, apparently healthy children conceived by ART.

The present data also provide information on potential underlying mechanisms. Birth weight and gestational age were similar in the 2 groups. Vascular dysfunction in ART children was not related to dyslipidemia or altered glucose

homeostasis. Parent-related factors such as concomitant diseases, infertility, maternal age, or hormonal stimulation to induce hyperovulation in the mother could be responsible for long-term health problems in the offspring.<sup>4</sup> These possibilities are unlikely. The prevalence of hypertension and diabetes mellitus was low and similar in sterile and fertile parents. Sterility was not associated with vascular dysfunction in the parents that could have been transmitted to the offspring by ART, and increasing maternal age was not associated with more severe vascular dysfunction in the progeny. Hormonal stimulation did not appear to play a role because, in accordance with findings in the offspring of superovulated mice,<sup>27</sup> vascular function was normal in children who were conceived naturally after hormonal stimulation of ovulation in the mother. Finally, and most important, vascular function was normal in siblings of ART children who were conceived naturally.

Collectively, these observations provide no evidence that parent-related factors play an important role and suggest that vascular dysfunction in offspring of ART is related to the procedure itself. In line with this speculation, studies in normal mice show that ART causes vascular dysfunction and arterial hypertension in the offspring.<sup>27</sup> The present data in humans suggest that vascular dysfunction does not appear to depend on the technique used for ART because it was similar in children born after in vitro fertilization and intracytoplasmic sperm injection and in children born after the transfer of frozen zygotes or the transfer of fresh embryos.

Vascular dysfunction has also been found in offspring of mothers with preeclampsia and young adults who had suffered from transient perinatal hypoxia.<sup>15,23</sup> These observations suggest that pathological events occurring during fetal life or shortly after birth may have similar long-term consequences for the circulation.

Epigenetic mechanisms have been proposed to play a key role in the developmental origins of adult disease.<sup>28,29</sup> The epigenome undergoes a series of changes during gametogenesis, fertilization, and early embryo development,<sup>30,31</sup> suggesting that these stages are particularly vulnerable to epigenetic dysregulation. In line with this speculation, offspring of parents exposed periconceptually to famine show altered methylation of the insulin-like growth factor-2 gene,<sup>32</sup> and ART is associated with a higher-than-expected frequency of rare imprinting disorders.<sup>33,34</sup> This suggests that epigenetic mechanisms may underpin the vascular dysfunction in children conceived by ART. In line with this hypothesis, we found that in mice, ART alters the methylation pattern of genes in the vasculature and induces vascular dysfunction, a problem that can be prevented by modification of the culture media used for ART.<sup>27</sup>

Translation of this mechanistic insight gained in mice to humans may allow prevention of ART-induced vascular dysfunction in future children conceived by this method.

### Conclusions

This study shows for the first time that ART induces generalized vascular dysfunction in the offspring. In the pulmonary circulation, this dysfunction predisposes to exaggerated hypoxic pulmonary hypertension already at a young

age. In the systemic circulation, it is not yet known how this dysfunction will evolve. The systemic vascular dysfunction is similar in magnitude to that described in children suffering from type 1 diabetes mellitus,<sup>35</sup> a disease associated with an increased risk of premature cardiovascular morbidity, and may offer a mechanism for the recently reported increase of arterial blood pressure in ART children.<sup>5,6</sup> We speculate that ART children represent a unique opportunity for cardiovascular risk modeling.

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### Disclosures

None.

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### CLINICAL PERSPECTIVE

Assisted reproductive technology (ART) has been used for 3 decades, and the children born after ART now make up for 1% to 4% of the births in developed countries. ART involves the manipulation of early embryos at a time when they may be particularly vulnerable to external disturbances. Environmental influences during the embryonic and fetal development influence the individual's susceptibility to cardiovascular disease, raising concerns about the potential consequences of ART on the long-term health of the offspring. Here, we show for the very first time that apparently healthy children born after ART show systemic and pulmonary vascular dysfunction, as evidenced by endothelial dysfunction, increased arterial stiffness, and carotid intima-media thickness in the systemic circulation and exaggerated hypoxic pulmonary hypertension during short-term high-altitude exposure. This vascular dysfunction does not appear to be related to parental factors or hormonal stimulation of the ovulation in the mother but to the ART procedure itself. For the practicing physician, this study indicates that ART children who live at high altitude or suffer from diseases associated with chronic hypoxia are at risk for exaggerated pulmonary hypertension and need to be monitored for this problem. In the systemic circulation, it is not known yet how this vascular dysfunction, which is similar in magnitude to that in children suffering from type 1 diabetes mellitus, will evolve. Although future mechanistic studies in ART mice may reveal possibilities for targeted intervention to improve or prevent ART-induced vascular dysfunction in humans, avoiding additional cardiovascular risk factors in this population appears to be important now.