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

Running title: Scherrer et al.; Vascular dysfunction and in vitro fertilization

Urs Scherrer, MD1,2,3*; Stefano F. Rimoldi, MD1*; Emrush Rexhaj, MD1; Thomas Stuber, MD1; Hervé Duplain, MD4; Sophie Garcin, MD4; Stefano F. de Marchi, MD1; Pascal Nicod, MD3; Marc Germond, MD5; Yves Allemann, MD1*; Claudio Sartori, MD1,4*

1Swiss Cardiovascular Center Bern, University Hospital, Bern, Switzerland; 2Facultad de Ciencias, Departamento de Biología, Universidad de Tarapacá, Arica, Chile; 3Hirslanden Group, Lausanne; 4Botnar Center for Extreme Medicine & Dept of Internal Medicine, CHUV, Lausanne; 5Centre de Procréation Médicalement Assistée, Lausanne, Switzerland
*contributed equally to this work

Correspondence:
Urs Scherrer, MD
Swiss Cardiovascular Center Bern
University Hospital, Bern
Freiburgstrasse, BHH A 143
Bern, 3010, Switzerland
Phone: +41-31- 632 01 02
Fax: +41-31- 632 42 11
E-mail: urs.scherrer2@insel.ch

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Abstract:

**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 regarding 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 percent smaller in ART than in control children (6.7±1.6 vs. 8.6±1.7%, P<0.0001) whereas endothelium-independent vasodilation was similar in the two groups. Carotid-femoral pulse wave velocity was significantly (P<0.001) faster and carotid intima-media thickness significantly (P<0.0001) greater in children conceived by ART than in controls. The systolic pulmonary-artery pressure at high altitude (3450m) was 30 percent higher (P<0.001) in ART than in control children. Vascular function was normal in children conceived naturally during hormonal stimulation of the 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 Information** – clinicaltrials.gov; Unique identifier: NCT00837642.

**Key words:** Endothelial Dysfunction, Pulmonary Hypertension; Assisted Reproductive Technologies.
Introduction

In vitro fertilization has been done for 3 decades and these children now make up for 1 to 4% of the births in developed countries.\textsuperscript{1} Epidemiological work in humans has put forward the hypothesis that environmental influences acting early in life may predispose to chronic cardiovascular and metabolic disease in adulthood.\textsuperscript{2} The safety of ART for long-term health is, therefore, of utmost importance, but there is little information.\textsuperscript{3-6} This may be related, at least in part, to the young age of the progeny, since clinically manifest disease may not yet have had time to develop.

Among the potential long-term consequences of ART cardiovascular disease is an important concern. We, therefore, 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,\textsuperscript{7-9} and we measured pulse wave velocity, a proxy of elastic artery stiffness.\textsuperscript{10} To test for structural alterations, we assessed carotid intima-media thickness.\textsuperscript{9} To assess pulmonary vascular function, we used high-altitude exposure (3450 m), since hypoxia induces exaggerated pulmonary hypertension in persons displaying endothelial dysfunction.\textsuperscript{11} We found that both systemic 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. We, therefore, assessed vascular function in children who were conceived naturally during hormonal stimulation of superovulation in the mother. Moreover, we studied vascular function in sterile and fertile parents and we compared vascular function in pairs of siblings, one being conceived by ART, the other 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 (IVF) and 44 by intracytoplasmic sperm injection (ICSI). 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. 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, the control children were recruited by the families of the ART children. Among the study population there were 5 pairs of siblings, one being conceived by ART (2 males, 3 females), the other naturally (1 male, 4 females); age (ART vs. naturally conceived, 11.0 ± 1.9 vs. 12.8 ± 5.7 years, P=0.23) and BMI (ART vs. naturally conceived, 17.1 ± 1.3 vs. 17.6 ± 1.8 kg/m², P=0.30) were comparable. All children conceived by ART were singletons and were born at term (Table 1). 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 the ovulation in the mother. To assess the potential pathophysiologic importance of this factor, we studied systemic vascular function in a group of 16 children (mean±SD, age 11.8±2.2 years; 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 in 22 sterile (mean±SD, age 43±6 years) and 14 fertile (mean±SD, age 39±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 (Clinical Trials Gov Registration # NCT00837642). All parents provided written informed consent.

**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 vasodilatation**

Systemic conduit artery endothelial function was assessed by determining the increase of the brachial artery diameter evoked by reactive hyperemia using high-resolution ultrasound and automatic wall tracking software according to International Guidelines and as previously described. Briefly, the brachial artery was identified approximately 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 of brachial artery diameter and flow were continuously measured 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). The coefficient of variation between 2 measurements in the same 30
subjects 24 hours apart was 5.2%. Flow-mediated dilation (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 of the brachial artery diameter evoked by oral glyceryl trinitrate (GTN 250 μg, UCB-Pharma, Bulle, Switzerland).15

**Carotid intima-media thickness**

Carotid intima-media thickness was measured according to recommended guidelines19, 20 using an Acuson Sequoia 512C ultrasound device (Acuson Siemens, Mountain View, Ca) equipped with a 8 to 14Mhz 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 identified. To standardize the transducer angle external landmarks were used. Using 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 two complementary angles (anterior and posterior). Images were stored (Digital Imaging and Communication in Medicine (DICOM) format) for off-line analysis using 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 non-invasively by measuring carotid-femoral pulse wave velocity (PWV) using the Complior® device (Artech Medical, Pantin, France) according to International Guidelines10 as described previously.16, 21 Briefly, carotid and femoral artery waveforms were simultaneously recorded with mechano-transducers directly applied to the skin over the arteries and the mean wave transit time for 10 heart beats was calculated by the system.
software using the foot-to-foot method. To determine the pulse wave velocity, 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, pulse
wave velocity 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, since hypoxia induces exaggerated pulmonary hypertension in persons
displaying endothelial dysfunction. The children ascended to the high-altitude research station
at the Jungfraujoch (Switzerland, 3450 m) by train ride and spent two days and two 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, Mountain View,
Ca) with an integrated color Doppler system and transducers containing crystal sets for 2D-
imaging (5.0 MHz with second harmonic technology) and for continuous-wave Doppler
recording (2.5 MHz). The recordings were stored on magneto-optical discs for off-line analysis
by two investigators who were unaware of the subject’s identity. All reported values represent
the mean of at least three 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. Right ventricular
to right atrial pressure gradient measurements are the standard method for the non-invasive
estimation of pulmonary-artery pressure and have been validated against invasive measurements
at high altitude. We have previously found that in children at this altitude, the intra- and inter-
observer variability for the right ventricular to right atrial pressure gradient measurements was 5.1±4.6% and 6.0±8.6%, respectively (n=30) and for cardiac output measurements 10.7±10.2% and 7.2±4%, respectively (n=30). Cardiac index was calculated by dividing cardiac output (L/min) by the body surface area (m²). 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 of 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**

Trans-cutaneous 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 on heparin, immediately centrifuged at 4°C and the plasma frozen at –80°C. Glucose, insulin, total cholesterol and triglyceride plasma concentration was measured with commercial kits. The insulin resistance was estimated by the homeostatic model assessment (HOMA) using the following formula: fasting serum insulin (μU/L) x fasting plasma glucose (mmol/L)/22.5.

**Statistical analysis**

Statistical analysis was done with the Graphpad Prism 5 software package (GraphPad Software Inc., San Diego CA). Unpaired and paired two-tailed t-tests were used for group comparisons of continuous variables. For comparisons of categorical variables between ART and control groups.
we used Fischer’s exact test. When comparing three groups we performed a one-factor repeated measures analysis of variance 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 based on our previously reported pulmonary artery pressure data at this altitude in healthy children and in children with pulmonary vascular dysfunction. Assuming a 5 mm Hg difference in pulmonary artery pressure between ART and control subjects (SD of pulmonary artery pressure in referents, 7 mm Hg; power > 0.90; α 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, USA). 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, gender, heart rate, birth weight, gestational age, presence of maternal cardiovascular risk factors, maternal age at birth were entered as test variables. In addition, systolic and diastolic blood pressure was tested for FMD and PWV, and brachial artery diameter was tested for FMD. For IMT, ART, age, gender, 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 (\(r^2\)), as well as ANOVA F- and p-values of the overall regression model. Only variables with significant non-zero slopes in the regression equation were considered independent predictors.
A P value of less than 0.05 was considered to indicate statistical significance. Unless otherwise indicated, data are given as means ±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 (HOMA) and glucose tolerance were normal and comparable in children born after ART and controls (Table 1). Birth weight, a possible determinant of vascular function later in life, 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 two groups (Table 2).

None of the children suffered from structural heart disease (as assessed by echocardiography).

Baseline brachial artery diameter (ART vs. control, 3.1±0.3 vs. 3.2±0.5 mm, P=0.58) and the ischemia-induced increase in blood flow (520±180 vs.490±130%, P=0.57) were similar in the two groups. Flow-mediated dilation of the brachial artery was roughly 25 percent smaller in children conceived by ART than in controls (6.7±1.6 vs.8.6±1.7%, P<0.0001, Figure 1A) whereas endothelium-independent vasodilation evoked by nitro-glycerine was similar in the two groups (13.5±2.3 vs.13.9±2.5%, P=0.37, Figure 1B). Carotid-femoral pulse wave velocity was significantly faster in children conceived by ART than in controls (7.8±2.4 vs.6.5±1.3 m/s, P<0.001, Figure 1C). Carotid intima-media thickness was significantly greater in ART than in control children (410±30 vs.370±20μm, 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 two groups (90±2 vs.
90±2%, P=0.70). The systolic pulmonary-artery pressure was 30 percent higher in children conceived by ART than in controls (39±11 vs. 30±9 mm Hg, P<0.0001) whereas the cardiac index was similar in ART and control children (2.92±0.48 vs. 2.90±0.52 L/min/m², P=0.86). The mean diameter of the inferior vena cava (ART vs. controls, 1.05±0.39 vs. 0.99±0.32 cm, P=0.48) and its respiratory change (ART vs. controls, 48±9 vs. 47±8 %, P=0.41), as well as the E/E’ ratio (ART vs. controls, 5.3±1.1 vs. 5.5±1.1, P=0.38), were comparable in the two groups suggesting that the RV-RA pressure increase in the ART children was due to pulmonary vascular and not cardiac dysfunction. There existed a significant inverse relationship between pulmonary-artery pressure and flow-mediated dilation (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 IVF vs. ICSI, 6.1±1.4 vs. 7.0±1.6 %, P=0.08; RV-RA gradient in IVF vs. ICSI, 37±14 vs. 41±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 vs. fresh, 6.4±1.5 vs. 6.8±1.6 %, P=0.40; RV-RA gradient, frozen vs. fresh, 40±10 vs. 39±11 mm Hg, P=0.85). Vascular function in sterile and fertile parents was normal and comparable (P=0.90, Figure 2A).

While 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 following hormonal stimulation of the ovulation in the mother (FMD, P=0.53 vs. controls, Figure 2B), RV-RA gradient (stimulation vs. control, 29±7 vs. 30±9 mm Hg, P=0.68), PWV (stimulation vs. control, 6.3±1.3 vs. 6.5±1.3 m/s, P=0.65), and IMT (stimulation vs. control, 371±23 vs. 370±20 μm, P=0.87). Among 5 pairs
of siblings, one being conceived by ART (2 males), the other naturally (1 male), FMD was significantly smaller (P=0.017, Figure 2C), pulmonary-artery pressure was significantly higher (P=0.047, Figure 2D), and PWV (ART vs. naturally conceived, 6.6±1.8 vs. 6.1±1.9 m/s, P=0.007) and IMT (ART vs. naturally conceived, 420±50 vs. 360±60 μm, 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 (i.e. FMD, PWV, IMT and RV-RA gradient).

Discussion

The steadily increasing use of assisted reproductive technologies (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.3, 5, 6 Here we show that healthy children and adolescents conceived by ART display marked vascular dysfunction of the systemic as well as of the pulmonary circulation. This problem does not appear to be related to parental factors or hormonal stimulation of the ovulation in the mother but to the ART procedure itself.

In the systemic circulation, flow-mediated dilation of the brachial artery was roughly 25 percent smaller in ART children than in control children. Defective flow-mediated dilation was related to endothelial dysfunction, because endothelium-independent vasodilation evoked by nitroglycerine was similar in the two groups. Endothelial dysfunction in ART children was not limited to the systemic circulation, since pulmonary-artery pressure at high altitude was 30
percent higher in children conceived by ART than in controls. This problem was not related to more severe altitude-induced hypoxemia, since arterial oxygen saturation was comparable in the 2 groups. Endothelial dysfunction in the pulmonary and systemic circulation was a robust finding since there existed a significant inverse relationship between pulmonary artery pressure and flow-mediated dilation.

Elastic artery stiffness represents an independent predictor of cardiovascular outcome in subjects at risk.\textsuperscript{10,26} Here, we found that pulse wave velocity, a widely used proxy of arterial stiffness,\textsuperscript{10} 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 hyper-ovulation in the mother could be responsible for long-term health problems in the offspring.\textsuperscript{4} These possibilities are unlikely. The prevalence of hypertension and diabetes 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, since, in accordance with findings in the offspring of superovulated mice,\textsuperscript{27} vascular function was normal in children who were conceived naturally following
hormonal stimulation of the ovulation in the mother. Finally, and most importantly, 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. The present data in humans suggest that vascular dysfunction does not appear to depend on the technique used for ART, since it was similar in children born after in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) and in children born following 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. 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. The epigenome undergoes a series of changes during gametogenesis, fertilization and early embryo development, 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 IGF2 gene, and ART is associated with a higher than expected frequency of rare imprinting disorders. 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.27
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 known yet
how this dysfunction will evolve. The systemic vascular dysfunction is of similar magnitude as
the one described in children suffering from type 1 diabetes,35 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.5,6 We speculate that ART
children represent a unique opportunity for cardiovascular risk modeling.

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**Conflict of Interest Disclosures:** None.
References:


Table 1. Participant characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control (n=57)</th>
<th>ART (n=65)</th>
<th>P Value</th>
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<td>27/38</td>
<td>-</td>
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<td>Age, years</td>
<td>11.9 (2.3)</td>
<td>11.1 (2.4)</td>
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<td>BMI, kg/m²</td>
<td>18.8 (3.0)</td>
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<td>HR, beats/Min</td>
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<td>71 (9)</td>
<td>0.90</td>
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<td>Systolic BP, mm Hg</td>
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<td>113 (10)</td>
<td>0.75</td>
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<td>Diastolic BP, mm Hg</td>
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<td>70 (7)</td>
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<td>4.54 (0.50)</td>
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<td>Insulinemia (μM/mL)</td>
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<td>53.0 (22.1)</td>
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<td>Total cholesterol (mmol/L)</td>
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<td>LDL cholesterol (mmol/L)</td>
<td>2.90 (0.57)</td>
<td>2.73 (0.67)</td>
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<td>HDL cholesterol (mmol/L)</td>
<td>1.48 (0.29)</td>
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<td>Triglycerides (mmol/L)</td>
<td>0.75 (0.35)</td>
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HR, heart rate; BP, blood pressure; BMI, body mass index; HOMA: homeostatic model assessment. LDL: low-density lipoprotein; HDL: high-density lipoprotein. Data are presented as mean (SD).

Table 2. Children characteristics at birth and maternal characteristics at conception

<table>
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<th>Control (n=53)</th>
<th>ART (n=60)</th>
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<td>Gestational age, weeks</td>
<td>39.0 (2.0)</td>
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<td>Birth weight, grams</td>
<td>3371 (419)</td>
<td>3330 (547)</td>
<td>0.56</td>
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<tr>
<td>Maternal age, years</td>
<td>30.1 (4.7)</td>
<td>33.2 (3.9)</td>
<td>&lt;0.001</td>
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<td>BMI of the mother, kg/m²</td>
<td>21.7 (2.4)</td>
<td>22.4 (2.4)</td>
<td>0.33</td>
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<td>Maternal smoking status, n (%)</td>
<td>8 (16%)</td>
<td>5 (10%)</td>
<td>0.55</td>
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<tr>
<td>Presence of other major cardiovascular risk factor in the mother*, n (%)</td>
<td>2 (4%)</td>
<td>2 (4%)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

BMI, body mass index; *Presence of at least one of the following: diabetes mellitus, arterial hypertension, dyslipidemia. Data are presented as mean (SD).
Figure Legends:

**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 concived by ART and in control children. Horizontal lines represent the median; boxes, 25th to 75th percentiles; and T bars, 5-th and 95-th percentiles. The number of subjects is indicated in parenthesis.

**Figure 2.** Flow-mediated dilation (FMD) in sterile and fertile parents (A), FMD in children concived by ART, in children conceived naturally following hormonal stimulation of the ovulation in the mother, and in 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 one being conceived by ART, the other naturally. Horizontal lines represent the median; boxes, 25th to 75th percentiles; and T bars, 5-th and 95-th percentiles. The number of subjects is indicated in parenthesis.
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