Assisted Reproductive Technologies are Associated with Cardiovascular Remodeling in Utero that Persists Postnatally

Running title: Valenzuela-Alcaraz et al.; Cardiac remodeling in assisted reproductive techniques

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Abstract

**Background**—Assisted reproductive technologies (ART) have shown to be associated with general vascular dysfunction in late childhood. However it is unknown whether cardiac remodeling is also present and if these changes already manifest in prenatal life. Our aim was to assess fetal and infant (6 months of age) cardiovascular function in ART pregnancies.

**Methods and Results**—A prospective cohort study including 100 fetuses conceived by ART and 100 control pregnancies. ART fetuses showed signs of cardiovascular remodeling including a more globular heart with thicker myocardial walls, decreased longitudinal function (tricuspid ring displacement in controls: median 6.5 mm (interquartile range 6.1-7.1) vs. ART: 5.5 mm (5.1-6.1) \( P<0.001 \)), impaired relaxation and dilated atria (atrial area in controls 1.46 cm\(^2\) (1.2-1.5) vs. ART 1.6 cm\(^2\) (1.3-1.8) \( P<0.001 \)). Additionally, ART infants showed persistence of most cardiac changes and a significant increase in blood pressure (systolic blood pressure in controls: 74 mmHg (67-83) vs. ART: 83 mmHg (75-94) \( P<0.001 \)) and aortic intima-media thickness (controls: 0.52 mm (0.45-0.56) vs. ART: 0.64 mm (0.62-0.67) \( P<0.001 \)). We could not demonstrate our findings were directly caused by ART, because of their association to various confounding factors including intrauterine growth restriction or those related to the cause of infertility.

**Conclusions**—Children conceived by ART manifest cardiac and vascular remodeling that is present in fetal life and persists in postnatal life, suggesting opportunities for early detection and potential intervention. The underlying mechanisms and the effect of potential confounders such as growth restriction or prematurity remain to be elucidated.

**Key words:** cardiac remodeling, pregnancy, pediatrics, assisted reproductive technologies, in vitro fertilization.
Introduction

Assisted reproductive technologies (ART), mainly standard in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), permit childbirth in many infertile couples and nowadays represent 1-4% of births in developed countries. Although these technologies are generally considered safe, the potential association of ART with poorer pregnancy outcomes has long been investigated. There is evidence that ART is associated with increased risk for adverse perinatal outcome and congenital malformations. This notwithstanding, it is not possible to separate ART-related risks from those secondary to the underlying reproductive pathology of the infertile couple. In this scenario, preliminary evidence has recently suggested that ART could be associated with long term cardiovascular changes. Celeen et al. first suggested the presence of increased blood pressure in late childhood after ART conception. More recently, another study demonstrated the presence of signs of systemic and pulmonary vascular dysfunction in 12 year-old children conceived by ART.7

Cardiovascular remodeling has previously been described to be associated with low birth weight (LBW) and it is regarded as a manifestation of fetal programming defined as the permanent alteration of tissue structures and functions as a result of fetal environment. It is unknown whether cardiovascular changes in ART children occur already in fetal life. In addition, fetal cardiovascular programming associated with LBW has been demonstrated to be accompanied by cardiac remodeling, which constitutes an additional risk factor in later life. However, the potential association of ART with cardiac structural and functional remodeling was not investigated in previous studies. This information is relevant to advance in the understanding of the long-term impact of ART in cardiovascular function and in the design of preventive strategies.
In the present study we evaluated the hypothesis that pregnancies conceived by ART are associated with both cardiac and vascular remodeling in the offspring, and that changes can be detected already during fetal life. We designed a prospective cohort study including 100 ART and 100 spontaneously conceived fetuses to comprehensively assess cardiac and vascular structure and function in the fetal and postnatal periods.

**Methods**

**Study populations and study protocol**

The study design was a prospective cohort study including 100 singleton pregnancies conceived by IVF and/or ICSI in infertile patients and 100 control pregnancies conceived naturally identified in fetal life and followed up to 6 month of age. The ART group was a consecutive sample of patients with a normal first trimester scan accepting to participate in the study. Cases were considered non eligible if any of the following were present: preimplantation genetic diagnosis, oocyte donation, multiple pregnancies or any maternal medical disease. Likewise, later diagnosis of fetal malformations or any pregnancy complications leading to delivery before 34 weeks of gestation were considered exclusion criteria. The control group was recruited at 28-30 weeks’ gestation among low-risk pregnancies attending our Maternal-Fetal Medicine Unit for normal pregnancy follow-up. Controls were matched for maternal age (±1 year) with cases. Eligibility and exclusion criteria for controls were the same as for cases and they underwent the same study protocol as cases. The study protocol was approved by the IRB at Hospital Clinic, and written parental consent was obtained for all study participants. **Figure 1** shows a flow diagram of the study population. Cardiovascular assessment included echocardiography in fetal life, vascular assessment in the neonatal period and both echocardiography and vascular
assessment at 6 months of age.

Parental baseline and ART characteristics were collected by parental interview and review of medical records at the time of prenatal evaluation. LBW was defined as birth weight below 10\textsuperscript{th} centile.

**Fetal assessment**

All pregnancies underwent ultrasonographic examination at 28-30 weeks of gestation using a Siemens Sonoline Antares (Siemens Medical Systems, Malvern, PA, USA) with 6–4-MHz linear curved-array and 2-10 MHz phased-array probes including the assessment of estimated fetal weight, feto-placental Doppler and fetal echocardiography.

*Feto-placental Doppler* assessment included pulsatility index (PI) measurement of umbilical artery and middle cerebral artery according to a previously published methodology.

*Fetal echocardiography* included a comprehensive examination to assess structural heart integrity and morphometry, and also systolic and diastolic function parameters. Cardiac dimensions were measured on 2D images from an apical four-chamber view. Left and right atrial areas were taken at maximum point of atrial distension and ventricular base-to-apex lengths and basal diameters at end-diastole. Left and right ventricular sphericity indexes were calculated as base-to-apex / basal diameter.\textsuperscript{10} Ventricular end-diastolic septal and free wall thicknesses were measured by M-mode from a transverse four-chamber view. Left and right ventricular ejection fraction (%) was obtained from M-mode long-axis transverse four chamber views using the Teichholz's formula. Left and right stroke volumes were calculated as \( \pi / 4 \times (aortic \ or \ pulmonary \ valve \ diameter)^2 \times (aortic \ or \ pulmonary \ artery \ systolic \ time–velocity \ integral) \).\textsuperscript{11} Then, left and right cardiac outputs were calculated as left/right stroke volume x heart rate.\textsuperscript{11} Mitral/tricuspid annular displacement (MAPSE/TAPSE) were assessed by M-mode from an
apical or basal four chamber view. Tissue Doppler was applied to record systolic peak velocities (S’) at mitral and tricuspid lateral annuli from an apical or basal four-chamber view, and measured in real time during the echocardiographic study. Right and left E/A ratios were estimated by calculating the ratio between early ventricular filling (E) to late ventricular filling (A). Deceleration time of the E wave was measured from mitral and tricuspid inflow velocities from an apical four-chamber view. Tissue Doppler was applied to record early diastolic (E’) peak velocity at mitral and tricuspid lateral annuli from an apical or basal four-chamber view. Left IRT was measured from the closure of the aortic valve to the opening of the mitral valve.

Neonatal assessment

Neonatal vascular assessment was performed within the first month of life, including the measurements of blood pressure and vascular intima-media thickness (IMT). Blood pressure centiles were calculated according to standard normograms.

Carotid and aorta ultrasound assessment was performed by skilled sonographers using a Siemens Sonoline Antares (Siemens Medical Systems, Malvern, PA, USA). Longitudinal clips of the far wall of both carotid arteries and abdominal aorta were obtained using a linear-array transducer. Carotid and aorta IMT measurements were performed offline according to a standardized protocol based on a trace method with the assistance of a computerized program (Siemens Syngo Arterial Health Package). IMT results were normalized by neonatal weight.

Assessment at 6 months of age

Infants’ follow-up evaluation was scheduled at 6 months of age including anthropometric data, echocardiography and vascular assessment. Anthropometric data included the infants’ height and weight, measured at the time of the examination.

Echocardiography was performed following a standardized protocol using a Vivid q
(General Electric Healthcare, Norway) with 2-10 MHz phased-array transducer. Infants were studied when resting quietly or asleep. A complete echocardiography was performed initially to assess structural heart integrity. Left and right atrial planimetric areas were measured on a 2D image from an apical four-chamber view at end-systole (greatest dimension, just before mitral or tricuspid valve opening). Ventricular base-to-apex length and transverse diameter were measured on a 2D image from an apical four-chamber view at end-diastole. Left and right ventricular sphericity indexes were calculated as base-to-apex length/mid-transverse diameter. Ventricular end-diastolic wall thicknesses were measured by M-mode from a parasternal long-axis view.\textsuperscript{15, 16} Left shortening fraction was calculated from internal ventricular diameters obtained from a parasternal long-axis view by M-mode, using the equation (end-diastolic – end-systolic)/end-diastolic dimensions. Left and right stroke volumes were calculated as π/4 x (aortic or pulmonary valve diameter)\textsuperscript{2} x (aortic or pulmonary artery systolic flow velocity-time integral). Left and right cardiac outputs were calculated as stroke volume*heart rate TAPSE was measured real time in an apical four-chamber view, by placing the M-mode cursor at the atrioventricular junction, marked by the tricuspid valve rings at the right free wall. Maximum amplitude of motion was taken as the extent of displacement between end-systole and end-diastole, and measured in millimeters. Tissue Doppler was applied at mitral and tricuspid lateral annuli from an apical four-chamber view, to record S’. E/A ratios were calculated. E deceleration time was measured as the time from the maximum mitral/tricuspid velocity to the baseline. Tissue Doppler was applied at mitral and tricuspid lateral annuli from an apical four-chamber view, to record E’. Left IRT was obtained from the pulsed Doppler waveform of the aortic blood flow, from the end of the aortic wave to the beginning of the mitral early filling wave.

\textit{Vascular assessment} included blood pressure and aortic wall thickness by ultrasound.
Aortic IMT measurement involved obtaining longitudinal clips of the far wall of the proximal abdominal aorta in the upper abdomen with a 10-MHz linear probe. Aortic IMT measurements were performed offline according to a standardized protocol based on a trace method with the assistance of a computerized program (GE EchoPAC PC 108.1.x, General Electric Healthcare). IMT results were normalized by infant weight.

**Statistical analysis**

SPSS Statistics 19 (IBM) was used for the statistical analysis. The study outcome was fetal and postnatal cardiovascular assessment. The independent variable of interest was the type of conception (natural or ART) and the covariates were birth weight centile, gestational age at delivery and the presence of preeclampsia. Annular peak velocities by tissue Doppler were chosen to calculate sample size because of their high sensitivity for preclinical cardiac dysfunction in fetuses and children. Based on previous studies measuring fetal cardiac function, sample size was calculated to enable to observe a difference of 25% in tricuspid E’ values in ART fetuses. For a power of 80% and alpha risk of 0.05, a minimum of 91 subjects per study group was required. We decided to include 100 fetuses in each study group. Data are presented as median (interquartile range) or as percentage, as appropriate. Statistics for baseline and perinatal data included comparison of means by Student’s t-test or proportions by Pearson Chi-Square test. In order to evaluate the influence of covariates, comparisons of the cardiovascular parameters between the study and control groups were adjusted for association to preeclampsia, birth weight centile and gestational age at delivery by linear regression. All reported P-values are two-sided. All feto-placental Doppler and cardiac parameters are shown as crude values (main document) and also normalized into z-scores by previously published reference values or adjusted by heart size, estimated fetal weight (in fetuses) or body surface area (in infants).
(supplementary material).

Results

Baseline and perinatal characteristics

Baseline and perinatal characteristics of the study population are shown in Tables 1 and 2. The study groups were similar in terms of maternal and paternal baseline characteristics, as compared to controls (Table 1). As expected, ART pregnancies had a higher occurrence of pregnancy complications, mainly a higher prevalence of LBW and a tendency to increased preeclampsia incidence (Table 2). Delivery and perinatal characteristics were similar among the study groups with the exception of an earlier gestational age at delivery and lower birthweight centile in ART as compared to controls. Maternal blood pressure values were similar among the study groups.

Fetal assessment

Results are shown in Table 3. Gestational age at evaluation, estimated fetal weight and fetoplacental Doppler were similar among groups. As illustrated in figure 2, fetuses conceived by ART showed increased atrial size and myocardial wall thickness together with lower ventricular sphericity indices as compared to controls. While cardiac output was similar among groups, ART fetuses showed a significant decrease of left ejection fraction, ring displacement, tricuspid E’, E deceleration time and IRT as compared to controls. Most cardiovascular changes in ART fetuses remained significant after adjustment by gestational age at delivery, birthweight centile and association to preeclampsia.

Neonatal assessment

Results are displayed in Table 4 and Figure 3. Systolic blood pressure was similar among the study groups, while diastolic blood pressure centile was significantly higher after ART
pregnancy as compared to controls. Aorta and carotid IMT were significantly increased in ART children, even after normalizing for neonatal weight and adjusting by gestational age at delivery, birthweight centile and association to preeclampsia.

**Assessment at 6 months of age**

Follow-up characteristics and cardiovascular results are shown in Table 5. ART infants showed similar anthropometric results at the time of evaluation as compared with controls. ART infants showed increased right atrial size together with lower right sphericity index and thicker right ventricle wall. Although cardiac output was similar among the study groups, ART infants showed a significantly decreased shortening fraction and increased heart rate. ART cases also showed signs of both systolic and diastolic dysfunction as measured by significant decreases in ring displacement, E deceleration time and tissue Doppler velocities, and a significant increase in IRT. Most cardiac changes remained significant even after adjustment by gestational age at delivery, birthweight centile and preeclampsia.

Blood pressure was significantly higher in the ART group as compared to controls. Aortic IMT was also significantly increased, even after normalizing by infant weight and adjustment by gestational age at delivery, birthweight centile and preeclampsia.

**Discussion**

This study demonstrates the presence of cardiac and vascular remodeling in fetuses and infants of pregnancies obtained by ART. These findings are consistent with previous reports demonstrating signs of vascular dysfunction in children conceived by ART\(^6,7\) and provide evidence for the existence of fetal cardiovascular programming in these pregnancies. We could not determine causality of our findings were caused by ART itself, by intrauterine growth
restriction or prematurity in ART pregnancies, or by other confounders related to the indications for ART.

Fetuses from pregnancies conceived by ART showed more globular hearts together with increased myocardial wall thickness, decreased right longitudinal function, impaired relaxation and dilated atria. The differences persisted after birth and were more prominent in the right heart as compared to the left. The cardiac findings are consistent with experimental data showing an increased heart weight in an IVF bovine model. From a pathophysiological viewpoint, more globular and hypertrophic ventricles with decreased longitudinal function are the usual ventricular response to pressure overload. Therefore, fetal observations are in line with post-natal findings of elevated blood pressure and increased IMT. In addition, cardiac remodeling described in our ART population resembles other fetal conditions with known pressure overload such as twin-to-twin transfusion syndrome or ductus arteriosus restriction. These clinical entities and experimental models of systemic pressure loading have been reported to show more pronounced changes in the right heart. This might reflect the dominance of right heart during fetal life together with a higher susceptibility to pressure overload of the right as compared with the left ventricle. The dilated atria and impaired relaxation (decrease in E’ and E deceleration time) could be explained by a decrease in ventricular compliance leading to higher end-diastolic pressures and increased atrial pressures. Finally, the changes described in vascular function and structure in neonates and infants reproduce the findings of previous reports in late childhood and support the development and presence of these differences from early life.

Fetal cardiovascular programming has previously been described in fetuses and children who suffered LBW. LBW is associated with globular hearts and longitudinal dysfunction in utero, and these changes, accompanied by increased blood pressure and vascular wall
thickness, have been described to persist into childhood in humans and to adulthood in animal models. Direct cardiac effects of fetal growth restriction have been proposed to provide a link to explain the long described epidemiological association of this prenatal condition with increased cardiovascular mortality in adults. Due to the high and expected prevalence of LBW in ART cases, it has been suggested that fetal growth restriction could be a potential confounder for cardiovascular remodeling in ART offspring. However, we believe that the results of this study strongly support a direct effect of ART on fetal and infant cardiovascular changes. Firstly, ART fetuses and infants presented changes that have not previously been reported in LBW, such as myocardial hypertrophy and increased atrial size. Secondly, most cardiovascular changes in ART remain significant even after adjustment by birthweight centile. Finally, the differences between ART pregnancies and controls remained virtually unchanged after excluding LBW pregnancies from the study group (supplementary data).

The mechanisms driving fetal and postnatal cardiovascular remodeling in ART pregnancies remain to be elucidated. Parental predisposing factors, epigenetic changes secondary to the early embryo manipulation, hormonal effects and postnatal environmental factors have been postulated as potential factors. Changes in fetuses and infants in this study were similar to those described in late childhood. Consequently, the role of postnatal environment as a potential factor determining long term vascular dysfunction in ART children is possibly negligible. Advanced maternal age in ART has been proposed as a major contributor of childbearing. In this study, cases and controls were matched by maternal age, however we acknowledge that other parental factors related to their subfertility could still play a role.

Concerning epigenetic mechanisms, there is clinical and mainly experimental evidence that the processes involved in egg manipulation might be associated with epigenetic changes,
mainly mediated by changes in the DNA methylation pattern. The majority of the changes
described affect imprinted genes, which have mainly been involved with fetal and placental
growth.\textsuperscript{25-27} However, it has been suggested that methylation might be relevant for other
functions as yet not characterized.\textsuperscript{26} The importance of DNA methylation in the regulation of
vascular endothelial function is being increasingly demonstrated, including nitric oxide
expression and synthesis, and endothelial angiogenesis. As indirect evidence, experimental
models suggest that fetal cardiovascular programming occurring in LBW is associated with
specific epigenetic signatures involving abnormal methylation.\textsuperscript{28} Therefore, molecular pathways
involved in cardiovascular regulation deserve further research to ascertain their potential
involvement in the vascular changes described in ART pregnancies. However, due to the
variability in ART protocols and the rarity of imprinting disorders, it can be challenging to
determine reliably the causative relationship between and increased risk for imprinting disorders
and ART exposure.\textsuperscript{29} 

Concerning hormonal factors, the effect on supra-physiological estradiol levels on the
outcome of IVF-embryo transfer and subsequent pregnancies is a matter of controversy in the
literature. Estradiol concentrations are not correlated with oocyte yield and quality,
embryological outcome, implantation and pregnancy rates, abortion rate, congenital
malformations and birth weight.\textsuperscript{30} However, associations with pregnancy complications related
to abnormal placentation such as LBW, preeclampsia and abnormal implantation of the placenta
have been reported.\textsuperscript{31} The relationship between estradiol levels in ART and long-term
cardiovascular function is unknown. As indirect evidence, a recent study reported no association
between ovarian hyperstimulation, a condition associated with a dramatic increase in estrogen
levels, with neuromotor development at 3 months of age, but, again, a potential independent
effect of a history of subfertility was suggested.32 Progesterone, another important hormone in human reproduction, has not been shown to have effects on fetal placental circulation or any association with the presence of LBW.

There are several limitations and considerations with regard to the present study. The changes here reported are subclinical with most cardiovascular indices lying within normal ranges. While these differences are recognized as potential cardiovascular risk factors, their long-term persistence and association with adult cardiovascular function and disease remains to be proven. Therefore, longer follow up of these ART pregnancies to ascertain whether ART pregnancy remains a risk factor in later life is crucial. We acknowledge that several potential confounders could have interfered in our results. However, cases and controls were matched by maternal age, and twin pregnancies and mothers with medical diseases were excluded. The analysis was adjusted for other potential influences including prematurity, birthweight centile and preeclampsia. Additionally, other potential confounders, such as gender, ethnicity, cardiovascular history, socioeconomic status, parity and parental smoking were similar among study groups. However, we acknowledge that analysis correcting for birth weight percentile may inadequately control for the differences in etiology as children conceived by ART may be more likely to have in-utero growth restriction from placental failure. In addition, there is increasing evidence that current definitions of fetal growth restriction most likely do not detect all instances of true restriction.33 Consequently, one could argue that by the same token we cannot exclude that the whole distribution of fetal weights in our population was shifted to the left, reflecting a more general effect on fetal growth in ART fetuses. If this was the case, there would be forms of true fetal growth restriction that have been missed because of the lack of sensitivity of currently used definitions, and therefore the impact of fetal growth restriction on our result would be
greater than it is now apparent, because hidden forms of growth restriction not detected by
conventional criteria \(^{33}\) might have affected the cardiac outcome of the ART pregnancies. In
conclusion, we fully acknowledge that prematurity and fetal growth restriction may have
significantly contributed to the cardiac findings, rather than ART via a mechanism of altered
fetal programming. This concept deserves further clarification in future studies. Finally, we
acknowledge that future studies might unveil non-obvious confounders not considered in the
design of the study, which might have affected the present results.

In conclusion, this study provides evidence that the use of ART in infertile couples is
associated with fetal and postnatal cardiovascular remodeling, suggesting prenatal exposure to
pressure overload. From a clinical perspective, and regardless of the need to clarify the specific
mechanisms, the existence of fetal programming in these infants opens important opportunities
to improve cardiovascular health in a relevant proportion of the population. Nowadays one to
four percent of all newborns in developed countries are conceived by ART \(^{1}\) and therefore the
findings of this study involve thousands of children yearly. The importance of early
identification and the impact of interventions in pediatric risk factors for cardiovascular disease
is now well recognized. \(^{34,35}\) Moreover, ART in infertile patients should be regarded as a
potential cardiovascular risk factor and strategies to detect and improve cardiovascular
remodeling could be explored in children conceived with ART. The underlying mechanisms and
the effect of the potential confounders in the primary observation here reported remain to be
e lumidated in future research.

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**Conflict of Interest Disclosures:** The authors do not have any commercial interest or other association that might pose a conflict of interest, and they are independent from funders and sponsors.

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Table 1. Baseline characteristics of the study groups.

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<th>Characteristic</th>
<th>Controls (N=100)</th>
<th>ART (N=100)</th>
<th>P-Value*</th>
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<td>36 (35-38)</td>
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<td>BMI (kg/m²)†</td>
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<td>23 (21-25)</td>
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<td>¶</td>
<td>18</td>
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Data are median (interquartile range) or percentage.
ART = pregnancies conceived by assisted reproductive technologies. BMI = body mass index. IVF = in vitro fertilization. ICSI = intracytoplasmic sperm injection.
* P-value calculated by Student’s t-test or Pearson Chi-Square test.
† BMI calculated as weight in kilograms divided by the square of the height in meters.
††Early cardiovascular disease defined by the presence of congenital heart disease, coronary disease, hypertension, diabetes or hypercholesterolemia in male <55 years and female <65 years.
¶ Not applicable.
Table 2. Perinatal characteristics of the study groups.

<table>
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<th>Controls (N=100)</th>
<th>ART (N=100)</th>
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<tr>
<td>Vanishing twin (%)</td>
<td>0</td>
<td>8</td>
<td>0.012</td>
</tr>
<tr>
<td>Preeclampsia (%)</td>
<td>0</td>
<td>4</td>
<td>0.123</td>
</tr>
<tr>
<td>Low birth weight (%)</td>
<td>1</td>
<td>17</td>
<td>0.001</td>
</tr>
<tr>
<td>Spontaneous preterm delivery (%)</td>
<td>2</td>
<td>5</td>
<td>0.442</td>
</tr>
<tr>
<td>Gestational diabetes (%)</td>
<td>4</td>
<td>6</td>
<td>0.746</td>
</tr>
<tr>
<td>Placenta previa (%)</td>
<td>0</td>
<td>3</td>
<td>0.245</td>
</tr>
<tr>
<td>Obstetric cholestasis (%)</td>
<td>0</td>
<td>0</td>
<td>1.000</td>
</tr>
<tr>
<td>Prenatal corticoid exposure (%)</td>
<td>2</td>
<td>1</td>
<td>1.000</td>
</tr>
<tr>
<td>Delivery data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)</td>
<td>40 (39-40)</td>
<td>38 (37-39)</td>
<td>0.032</td>
</tr>
<tr>
<td>Maternal systolic blood pressure (mmHg)</td>
<td>111(110-115)</td>
<td>114(111-116)</td>
<td>0.437</td>
</tr>
<tr>
<td>Maternal diastolic blood pressure (mmHg)</td>
<td>70(68-71)</td>
<td>71(65-72)</td>
<td>0.891</td>
</tr>
<tr>
<td>Cesarean section (%)</td>
<td>24</td>
<td>31</td>
<td>0.342</td>
</tr>
<tr>
<td>Male (%)</td>
<td>49</td>
<td>51</td>
<td>0.888</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>3355 (3020-3550)</td>
<td>2740 (2585-2920)</td>
<td>0.022</td>
</tr>
<tr>
<td>Birthweight centile</td>
<td>49 (31-73)</td>
<td>26 (5-55)</td>
<td>0.033</td>
</tr>
<tr>
<td>5 minutes Apgar score</td>
<td>10 (9-10)</td>
<td>10 (9-10)</td>
<td>0.102</td>
</tr>
<tr>
<td>Umbilical artery pH</td>
<td>7.2 (7.1-7.2)</td>
<td>7.2 (7.2-7.3)</td>
<td>0.920</td>
</tr>
<tr>
<td>Neonatal outcome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Admission to neonatal intensive care unit (%)</td>
<td>1</td>
<td>1</td>
<td>0.477</td>
</tr>
<tr>
<td>Major neonatal morbidity (%)†</td>
<td>0</td>
<td>0</td>
<td>1.000</td>
</tr>
<tr>
<td>Perinatal mortality (%)</td>
<td>0</td>
<td>0</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Data are median (interquartile range) or percentage.
ART = pregnancies conceived by assisted reproductive technologies.
* P-value calculated by Student’s t-test or Pearson Chi-Square test.
† Major neonatal morbidity defined by the presence of bronchopulmonary dysplasia, necrotizing enterocolitis, intraventricular hemorrhage, periventricular leukomalacia, retinopathy, persistent ductus arteriosus or sepsis.
Table 3. Fetal assessment in the study groups.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls (N=100)</th>
<th>ART (N=100)</th>
<th>Crude P-value</th>
<th>Adjusted P-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at scan (weeks)</td>
<td>29 (28-29)</td>
<td>28 (28-29)</td>
<td>0.062</td>
<td>0.130</td>
</tr>
</tbody>
</table>

**Feto-placental data**

- Estimated fetal weight at scan (g) 1375 (1205-1508) 1300 (1173-1446) 0.061 0.078
- Estimated fetal weight centile at scan 53 (26-80) 54 (29-69) 0.710 0.656
- Umbilical artery PI 1.10 (0.98-1.25) 1.09 (0.95-1.25) 0.979 0.706
- Middle cerebral artery PI 2.18 (1.8-1.3) 2.0 (1.5-2.3) 0.961 0.806

**Fetal echocardiography**

**Cardiac morphometry**

- Left atrial area (cm²) 1.35 (1.1-1.4) 1.48 (1.2-1.7) <0.001 <0.001
- Right atrial area (cm²) 1.46 (1.2-1.5) 1.60 (1.3-1.8) 0.002 <0.001
- Left sphericity index 1.77 (1.61-1.92) 1.71 (1.54-1.78) 0.002 <0.003
- Right sphericity index 1.58 (1.4-1.72) 1.37 (1.25-1.5) <0.001 <0.001
- Left free wall thickness (mm) 2.7 (2.4-3) 2.9 (2.7-3.1) 0.001 0.068
- Interventricular septum thickness (mm) 2.4 (2.4-2.8) 2.7 (2.4-2.9) <0.001 <0.001
- Right free wall thickness (mm) 2.8 (2.6-3.2) 3.2 (2.9-3.3) 0.026 0.038

**Systolic function**

- Left ejection fraction (%) 69 (63-73) 63 (57-68) <0.001 <0.001
- Right ejection fraction (%) 68 (63-73) 67 (60-73) 0.657 0.659
- Heart rate (bpm) 140 (136-148) 143 (136-150) 0.836 0.749
- Left cardiac output (mL/min) 25.6 (20-30) 25.5 (21-30) 0.838 0.798
- Right cardiac output (mL/min) 31 (26-37) 33 (28-38) 0.385 0.727
- Mitral ring displacement (mm) 4.7 (4.2-5.3) 4.2 (3.7-4.9) <0.001 <0.001
- Tricuspid ring displacement (mm) 6.5 (6.1-7.1) 5.5 (5.1-6.1) <0.001 <0.001
- Mitral S' (cm/s) 6.9 (6-7.4) 6 (6-7) 0.031 0.038
- Tricuspid S' (cm/s) 7.9 (6.7-8.8) 7 (6-8) 0.148 0.179

**Diastolic function**

- Mitral E/A ratio 0.71 (0.66-0.76) 0.74 (0.67-0.78) 0.681 0.320
- Tricuspid E/A ratio 0.80 (0.70-0.90) 0.80 (0.72-0.91) 0.806 0.810
- Mitral E deceleration time (ms) 73 (55-91) 63 (51-78) 0.003 0.002
- Tricuspid E deceleration time (ms) 64 (51-77) 51 (44-66) <0.001 <0.001
- Mitral E' (cm/s) 7.6 (6.9-8) 7 (6-8) 0.061 0.049
- Tricuspid E' (cm/s) 8.3 (7.9-9.1) 8 (7-9) 0.003 0.002
- Left isovolumic relaxation time (ms) 30 (42-52) 48 (41.5-54.5) 0.031 0.003

Data are median (interquartile range).
ART = pregnancies conceived by assisted reproductive technologies. PI = pulsatility index. S' = systolic annular peak velocity. E = ventricular inflow in early diastole. A = ventricular inflow during atrial contraction. E' = annular peak velocity in early diastole.

* P-value calculated by linear regression adjusted by gestational age at delivery, birthweight centile and preeclampsia.
Table 4. Neonatal vascular outcome of the study groups within the first month of life.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls (N=75)</th>
<th>ART (N=60)</th>
<th>Crude P-value</th>
<th>Adjusted P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood pressure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>82 (75-90)</td>
<td>84 (78-91)</td>
<td>0.163</td>
<td>0.489</td>
</tr>
<tr>
<td>Systolic blood pressure centile</td>
<td>47 (28-74)</td>
<td>54 (30-79)</td>
<td>0.892</td>
<td>0.614</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>47 (39-57)</td>
<td>53 (46-61)</td>
<td>0.548</td>
<td>0.256</td>
</tr>
<tr>
<td>Diastolic blood pressure centile</td>
<td>55 (21-85)</td>
<td>71 (44-91)</td>
<td>0.004</td>
<td>0.042</td>
</tr>
<tr>
<td><strong>Vascular wall thickness†</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aortic mean IMT (mm)</td>
<td>0.45 (0.36-0.51)</td>
<td>0.55 (0.53-0.61)</td>
<td>$&lt;0.001$</td>
<td>0.035</td>
</tr>
<tr>
<td>Aortic mean IMT / weight (mm/kg)</td>
<td>0.12 (0.10-0.14)</td>
<td>0.16 (0.14-0.18)</td>
<td>$&lt;0.001$</td>
<td>0.011</td>
</tr>
<tr>
<td>Aortic maximum IMT (mm)</td>
<td>0.57 (0.47-0.64)</td>
<td>0.65 (0.62-0.66)</td>
<td>$&lt;0.001$</td>
<td>0.002</td>
</tr>
<tr>
<td>Aortic maximum IMT / weight (mm/kg)</td>
<td>0.14 (0.13-0.17)</td>
<td>0.19 (0.16-0.22)</td>
<td>0.001</td>
<td>0.024</td>
</tr>
<tr>
<td>Carotid mean IMT (mm)</td>
<td>0.24 (0.21-0.27)</td>
<td>0.28 (0.25-0.30)</td>
<td>$&lt;0.001$</td>
<td>0.091</td>
</tr>
<tr>
<td>Carotid mean IMT / weight (mm/kg)</td>
<td>0.06 (0.05-0.07)</td>
<td>0.07 (0.07-0.08)</td>
<td>0.001</td>
<td>0.035</td>
</tr>
<tr>
<td>Carotid maximum IMT (mm)</td>
<td>0.29 (0.20-0.33)</td>
<td>0.32 (0.31-0.33)</td>
<td>$&lt;0.001$</td>
<td>0.005</td>
</tr>
<tr>
<td>Carotid maximum IMT / weight (mm/kg)</td>
<td>0.07 (0.05-0.08)</td>
<td>0.09 (0.07-0.09)</td>
<td>0.003</td>
<td>0.018</td>
</tr>
</tbody>
</table>

Data are median (interquartile range).
ART = pregnancies conceived by assisted reproductive technologies. IMT = intima-media thickness.
* P-value calculated by linear regression adjusted gestational age at delivery, birthweight centile and preeclampsia.
† Vascular intima-media thickness normalized by neonatal weight.
Table 5. Anthropometric data and cardiovascular assessment at 6 months of age.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls (N=50)</th>
<th>ART (N=50)</th>
<th>Crude P-value</th>
<th>Adjusted P-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometric data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>68 (65-69)</td>
<td>66 (65-68)</td>
<td>0.302</td>
<td>0.794</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>7650 (7170-8000)</td>
<td>7600 (6995-8200)</td>
<td>0.772</td>
<td>0.806</td>
</tr>
<tr>
<td><strong>Infant echocardiography</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac morphometry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left atrium area (cm²)</td>
<td>2.71 (2.6-3)</td>
<td>2.75 (2.6-3.1)</td>
<td>0.782</td>
<td>0.574</td>
</tr>
<tr>
<td>Right atrium area (cm²)</td>
<td>2.50 (2.2-2.9)</td>
<td>2.70 (2.5 - 3.2)</td>
<td>0.018</td>
<td>0.005</td>
</tr>
<tr>
<td>Left sphericity index</td>
<td>1.81 (1.7-1.8)</td>
<td>1.83 (1.7-1.9)</td>
<td>0.271</td>
<td>0.650</td>
</tr>
<tr>
<td>Right sphericity index</td>
<td>1.91 (1.8-2)</td>
<td>1.82 (1.5-2)</td>
<td>0.021</td>
<td>0.010</td>
</tr>
<tr>
<td>Left ventricular wall thickness (mm)</td>
<td>4.80 (4.4-5.4)</td>
<td>4.58(4.5-5.2)</td>
<td>0.880</td>
<td>0.908</td>
</tr>
<tr>
<td>Septum thickness (mm)</td>
<td>4.15 (3.4 - 4.5)</td>
<td>3.60(3.3-4.1)</td>
<td>0.555</td>
<td>0.605</td>
</tr>
<tr>
<td>Right free wall thickness (mm)</td>
<td>2.59 (2.3-3.2)</td>
<td>3.21(2.9-3.5)</td>
<td>0.009</td>
<td>0.019</td>
</tr>
<tr>
<td><strong>Systolic function</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left shortening fraction (%)</td>
<td>36(32-40)</td>
<td>29(26-35)</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>132 (124-144)</td>
<td>141 (131- 148)</td>
<td>0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>Left cardiac output (mL/min)</td>
<td>25 (21.9-30)</td>
<td>25 (21.29-7)</td>
<td>0.744</td>
<td>0.204</td>
</tr>
<tr>
<td>Right cardiac output (mL/min)</td>
<td>32 (25-38)</td>
<td>33 (28-38)</td>
<td>0.208</td>
<td>0.587</td>
</tr>
<tr>
<td>Mitral ring displacement (mm)</td>
<td>10.8(10.1-11.8)</td>
<td>9.4(7.4-10.3)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tricuspid ring displacement (mm)</td>
<td>16.3(15.1-17.2)</td>
<td>13.1(11.9-14.1)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mitral S’ (cm/s)</td>
<td>7.7(7-8.9)</td>
<td>6.9(5.7-7.5)</td>
<td>0.062</td>
<td>0.339</td>
</tr>
<tr>
<td>Tricuspid S’ (cm/s)</td>
<td>11.5(10.9-13.2)</td>
<td>10.9(9.7-13.3)</td>
<td>0.462</td>
<td>0.381</td>
</tr>
<tr>
<td><strong>Diastolic function</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitral E/A ratio</td>
<td>1.3 (1.2-1.4)</td>
<td>1.2 (1.1-1.3)</td>
<td>0.374</td>
<td>0.133</td>
</tr>
<tr>
<td>Tricuspid E/A ratio</td>
<td>1 (0.8-1.1)</td>
<td>1 (1-1.3)</td>
<td>0.014</td>
<td>0.132</td>
</tr>
<tr>
<td>Mitral E deceleration time (ms)</td>
<td>66 (52-90)</td>
<td>63 (49-78)</td>
<td>0.068</td>
<td>0.014</td>
</tr>
<tr>
<td>Tricuspid E deceleration time (ms)</td>
<td>62 (51-77)</td>
<td>52 (44-66)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mitral E’ (cm/s)</td>
<td>13.7 (12 to 14)</td>
<td>12.2 (10 to 13)</td>
<td>0.016</td>
<td>0.207</td>
</tr>
<tr>
<td>Tricuspid E’ (cm/s)</td>
<td>15 (14-17)</td>
<td>13 (11-16)</td>
<td>0.015</td>
<td>0.077</td>
</tr>
<tr>
<td>Left isovolumic relaxation time (ms)</td>
<td>50 (41-59)</td>
<td>63 (55-67)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Vascular assessment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Blood pressure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>74 (67-83)</td>
<td>83 (75-94)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>50 (49-59)</td>
<td>50.5 (50-62)</td>
<td>0.070</td>
<td>0.214</td>
</tr>
<tr>
<td><strong>Aortic wall thickness†</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aortic mean IMT (mm)</td>
<td>0.52(0.45-0.56)</td>
<td>0.64(0.62-0.67)</td>
<td>&lt;0.001</td>
<td>0.003</td>
</tr>
<tr>
<td>Aortic mean IMT/weight (mm/kg)</td>
<td>1.4 (1.2-1.5)</td>
<td>1.8 (1.60-1.9)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aortic maximum IMT (mm)</td>
<td>0.60(0.52-0.64)</td>
<td>0.72(0.68-0.75)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aortic maximum IMT/ weight (mm/kg)</td>
<td>1.6 (1.4-1.8)</td>
<td>2.0 (1.9-2.1)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are median (interquartile range).
ART = pregnancies conceived by assisted reproductive technologies. S’ = systolic annular peak velocity. E = ventricular inflow in early diastole. A = ventricular inflow during atrial contraction. E’ = annular peak velocity in early diastole. IMT = intima-media thickness
* P-value calculated by linear regression adjusted by gestational age at delivery, birthweight centile and preeclampsia.
† Aortic wall thickness normalized by infant weight.
Figure Legends:

**Figure 1.** Flow diagram of the study populations. ART denote pregnancies conceived by assisted reproductive technologies.

**Figure 2.** Echocardiographic images in fetuses conceived naturally (control) and by assisted reproductive technologies (ART). The images are 2D apical four-chamber views at end-diastole illustrating larger atria and a more globular right ventricular shape in ART as compared to controls.

**Figure 3.** Ultrasound carotid images in neonates conceived naturally (control) and by assisted reproductive technologies (ART) illustrating the increase of carotid intima-media thickness (cIMT) in ART as compared to controls.
Figure 1

- 257 ART cases eligible at first trimester scan
  - Refuse to participate (146)
  - 100 normal pregnancies recruited during third trimester
  - 111 ART cases Recruited at first trimester scan
    - Exclusions (11)

- CONTROLS N=100
  - Fetal echocardiography: cardiac morphometry, systolic and diastolic function
  - Perinatal data
  - Neonatal vascular assessment: blood pressure, aortic and carotid artery ultrasound
  - Infant cardiovascular assessment: cardiac morphometry, systolic and diastolic function, blood pressure and aortic artery ultrasound

- 28-30 weeks of gestation
- Delivery
- First month of life
- 6 months of age
Figure 2
Control

clMT = 0.282 mm

ART

clMT = 0.339 mm

Figure 3
Assisted Reproductive Technologies are Associated with Cardiovascular Remodeling in Utero that Persists Postnatally
Brenda Valenzuela-Alcaraz, Fátima Crispi, Bart Bijnens, Monica Cruz-Lemini, Montserrat Creus, Marta Sitges, Joaquim Bartrons, Salvadora Cívic, Juan Balasch and Eduard Gratacós

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