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Impact of suspected preterm labour in foetal cardiovascular and metabolic programming: a prospective cohort study protocol

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ABSTRACT

Introduction Suspected preterm labour (SPL) is an obstetric complication that occurs in 9% of all pregnancies and is the leading cause of antenatal hospital admissions. More than half of women with SPL deliver a premature baby which is a known risk factor for developing cardiovascular and metabolic disorders in childhood and later in adult life. On the other hand, the other half of these women will deliver at term, labelled as 'false preterm labour'. Although this has been thought to be a benign condition, accumulating evidence reported in recent years showed long-term effects for the foetus, neonate and infant even when birth occurs at term. However, the impact of SPL on cardiovascular and metabolic programming has not been studied yet. The aim of this prospective cohort study is to evaluate the impact of SPL on cardiac remodelling and function and on cardiovascular and metabolic profiles independently of gestational age at birth.

Methods and analysis Prospective cohort study of subjects exposed and not exposed to an episode of SPL. Women with singleton pregnancies who are admitted at a tertiary hospital due to SPL and matched controls will be recruited. Evaluation of cardiovascular remodelling by foetal echocardiography will be performed during admission. Cord blood will be collected at birth in order to analyse different metabolomic footprints and several cardiovascular and metabolic risk biomarkers. Moreover, children will undergo an echocardiography 6 months after birth. The relationship between SPL and cardiovascular and metabolic programming will be modelled considering different covariates such as socioeconomic factors, perinatal characteristics, lifestyle, diet and exercise.

Ethics and dissemination Ethical approval was granted in April 2020 from CEIC Aragón (CEICA) (C.P.-C.I. PI20/136). Study outcomes will be disseminated at international conferences and published in peer-reviewed scientific journals.

Trial registration number NCT05670665.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is a well-designed prospective cohort study.
⇒ Due to the difficulty in designing an experimental study of these characteristics and the lack of previous existing studies, we have considered that a prospective cohort study is the best design to achieve our objectives.
⇒ The design of our study will not allow us to examine what biological determinants are the responsible of the insults found.
⇒ Multiple hypothesis testing may increase the risk of type I errors, which could affect the statistical power of the findings.

INTRODUCTION

Suspected preterm labour (SPL) occurs in approximately 9% of all pregnancies[1 2] and is the leading cause of antenatal hospital admission.[3] More than half of pregnant women with SPL deliver a preterm baby. It has been widely demonstrated that children and adults who were born preterm have higher risk of developing cardiovascular and metabolic disorders such as hypertension, insulin resistance and different patterns of cardiac remodelling.[4–8] Moreover, a recent meta-analysis concluded that children and adolescents who were born preterm were at a higher risk of obesity than those who were born at term.[9] Preterm birth occurs at a critical stage of organ development. Maladaptation and direct injury from oxidative stress, sepsis and malperfusion can alter directly key organs such as kidneys, heart or the microvasculature of the brain.[10] It has been observed that neonates born preterm have a metabolomic profile that differs significantly from term neonates,[11] and this

unique metabolomic profile continues being present in young adults born preterm.[12]

On the other hand, up to 44% of the women who have an episode of SPL deliver at term.[2] This phenomenon is known as 'false preterm labour', and for a long time, it was considered a benign entity. Espinoza *et al*[13] were the first to suggest that an episode of false preterm labour was the consequence of an insult not severe enough to trigger preterm delivery but with an increased risk of other complications during pregnancy for the foetus. In their study, Espinoza *et al* found that babies delivered at term after an episode of false preterm labour had a significantly higher risk of being small for gestational age,[13] concluding that false preterm labour is not a benign condition. This has been corroborated in subsequent studies.[14] Paules *et al* found that children born at term after an episode of SPL had a significant risk of neurodevelopmental delay at 2 years of age.[1] Other studies also reported that these children had a higher risk of developing attention deficit hyperactivity disorder and autism.[15–17]

A recent study by Villar *et al*[18] proposed a new classification for preterm labour based on 12 different phenotypes, such as placental dysfunction, foetal distress, intra or extrauterine infections and poor maternal conditions. It is worth noting that between 30 and 35% of the women had no characteristics to be classified in any of the phenotypes, remaining the symptoms of preterm labour unexplained. It has been suggested that symptoms of unexplained preterm labour might arise from intraamniotic inflammation, which may not be severe enough to provoke preterm delivery but could lead to a state of chronic intra-amniotic inflammation affecting the foetus.[19–22] Other studies have reported gene expression patterns suggestive of inflammation in human placentas after SPL regardless of whether delivery occurred preterm or at term, suggesting that similar biological mechanisms affect both preterm and term-born infants after SPL.[23]

Still, no studies have evaluated the cardiovascular impact of SPL alone. The high incidence of SPL and its potential impact on cardiometabolic profile during a critical period of development makes it necessary to carry out a more exhaustive study in this cohort of patients. Therefore, our aim is to conduct a prospective cohort study on exposed and non-exposed foetuses to an episode of SPL in order to study its impact on cardiac function and remodelling and on the cardiovascular and metabolic profiles independently of gestational age at birth.

OBJECTIVES

Primary objective

- To evaluate the impact of SPL on cardiovascular and metabolic profile during foetal life and early infancy.

Secondary objectives

- Identify prenatal and postnatal patterns of cardiac remodelling associated with SPL.
- Describe different patterns of growth during foetal life and early infancy associated with SPL.
- Identify different metabolomic footprints in umbilical cord blood associated with SPL.
- Describe the impact of SPL on different biomarkers of cardiometabolic dysfunction in foetal cord blood.

METHODS AND ANALYSIS

Study design

This is a prospective cohort study including pregnant women with a singleton gestation between 24+0 and 34+6 weeks admitted with a diagnosis of SPL. During the admission, foetal echocardiography and foetal growth assessment will be carried out. At the moment of delivery, cord blood will be collected to analyse metabolomics and biomarkers of cardiometabolic (dys)function. At 6 months of age, a paediatric cardiovascular assessment including echocardiography will be performed to all these children.

The study includes a cohort of pregnant women with singleton pregnancies with no episodes of SPL during pregnancy who deliver at term. They will be selected from our general population of low-risk pregnancies and matched with cases by gestational age at the admission of SPL.

SPL will be defined as the presence of regular uterine contractions registered by cardiotocography and cervical shortening (<25 mm)[24] measured by vaginal ultrasound scan in the presence of intact membranes at a gestational age of 24+0 to 34+6 weeks. Pregnancies will be dated according to crown-rump length in the 11–14-week ultrasound scan.[25] Tocolysis with atosiban (Tractocile, Ferring Pharmaceuticals, Madrid, Spain) and lung maturation with intramuscular corticosteroids (betamethasone 12 mg/24 hours, two doses) will be administered for some cases according to international clinical standards and manufacturer recommendations.[26]

Patient recruitment started in June 2020 and is expected to finish in the year 2025.

Participants and recruitment

The study will include two cohorts of patients:

A. Exposed cohort: singleton pregnancies who suffer at least one episode of SPL during pregnancy. According to their gestational age at birth, they will be divided into:
   - Preterm births (delivery between 24+0 and 36+6 weeks of gestation).
   - 'False preterm labour' (full-term births (≥ 37+0 weeks)).
B. Unexposed cohort: low-risk pregnancies with a full-term delivery (≥ 37+0 weeks) without an episode of SPL during pregnancy.

Inclusion and exclusion criteria are detailed in table 1.

Eligible women will be approached by the study researchers, and the study aims and requirements will be discussed in detail. If women express interest, a written informed consent document and an information for

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Age 18 or older	
Singleton pregnancy	Major foetal defects or anomalies
Episode of suspected preterm labour	
Good understanding of Spanish language	Iatrogenic preterm labour

patients' sheet will be provided. After providing informed consent, foetal echocardiography will be performed during the admission in exposed patients. Low-risk women will be randomly selected from the low-risk pregnancy clinics and approached via a phone call. If they express interest, an appointment for a foetal echocardiography will be provided at a matched gestational age with cases. If these women are admitted later on in pregnancy due to a SPL, they will be excluded from the unexposed cohort and approached again to collaborate as part of the exposed cohort. If they agree to participate, foetal cardiac evaluation will be repeated during the admission.

On the same day of the foetal echocardiography, participants will be asked to complete a validated Mediterranean dietary questionnaire, the State-Trait Anxiety Inventory (STAI)[27 28] and the Perceived Stress Scale (PSS).[29]

During third stage of labour, before the expulsion of the placenta, samples of cord blood will be collected and stored. Six months after birth (6 months of corrected age in the case of premature neonates), participants will be contacted again in order to perform an echocardiography. Clinical data will also be collected at the time of the postnatal appointment. The study design is summarised in figures 1 and 2.

Setting

This study includes pregnant women who are admitted to the obstetrics ward due to an episode of SPL and pregnant women who attend the low-risk antenatal clinic and plan to deliver at Hospital Clinico Universitario Lozano Blesa. Foetal cardiovascular assessment will be performed at the Fetal Medicine Unit. Postnatal cardiovascular assessment will be performed at the Paediatric Cardiology Unit.

Patient and public involvement

Patients were not involved in the design and conduct of this research. During the recruitment stage, patients will be informed, and research questions, choice of outcome measures and methods of recruitment will be discussed. Once the results are published, participants will be informed and will receive the details of the results.

Primary outcome measurement

- Foetal cardiovascular and metabolic remodelling including cardiac morphometry and cardiac function: transverse and longitudinal ventricular and atrial diameters, left and right sphericity indices, ventricular and atrial areas, ejection fraction, ejection volume, filling and ejection time fractions, cardiac output mitral and tricuspid annular plane systolic excursion (MAPSE and TAPSE), E/A ratio, and isovolumetric relaxation time; foetal growth; foetal Doppler (umbilical artery pulsatility index (PI), medial cerebral artery PI and systolic peak velocity (SPV), ductus venosus PI and mean uterine arteries PI); cord blood metabolomic and lipidomic profiles; cord blood biomarkers of cardiac dysfunction (brain natriuretic peptide (BNP), troponin I and cystatin C).
- Children structural and functional cardiac remodelling: including left ventricle walls thickness, left ventricular end-systole and end-diastole diameters, left ventricular ejection fraction, global longitudinal strain, global longitudinal strain rate and mitral annulus tissue doppler velocities.

Secondary outcome measurement

There are multiple underlying factors that could potentially affect foetal metabolic or cardiovascular health; therefore, the following covariates will be actively evaluated:

1. Maternal adherence to Mediterranean diet will be evaluated with the Prevention with Mediterranean Diet validated 14-item questionnaire[30] during the admission or at the time of the foetal ultrasound evaluation.
2. Stress and anxiety levels will be evaluated with the PSS[29] and STAI[27] during the admission or at the time of the foetal ultrasound evaluation.
3. Sociodemographic data and perinatal and postnatal outcomes will also be collected. Sociodemographic parameters include maternal age, ethnicity, education level, occupation and consumption of alcohol, tobacco or other drugs. Relevant medical history will capture any pre-existing clinical variables and the existence of previous premature offspring. Data regarding any pregnancy complications that might appear after inclusion will be collected. Details about tocolysis, progesterone use, antibiotic treatment, corticoid treatment, number of admissions due to an episode of SPL and results of posterior investigations will be collected for the exposed cohort. Perinatal outcomes include diagnosis of gestational diabetes, preeclampsia or foetal growth restriction, type of onset of labour and mode of delivery. Neonatal parameters include gestational age at birth, birth weight, gender, Apgar scores, umbilical arterial blood pH, admission to neonatal intensive care unit and length of hospital stay. Postnatal variables include any relevant medical events and breastfeeding duration. We will also collect anthropometric parameters (weight, height and head circumference) at 1, 3 and 6 months after birth from paediatric medical records.

Foetal cardiovascular assessment

Foetal ultrasound examinations will be performed using a Voluson E8 ultrasound system (GE Healthcare, Chicago, USA) equipped with a 2–6-MHz convex transducer and

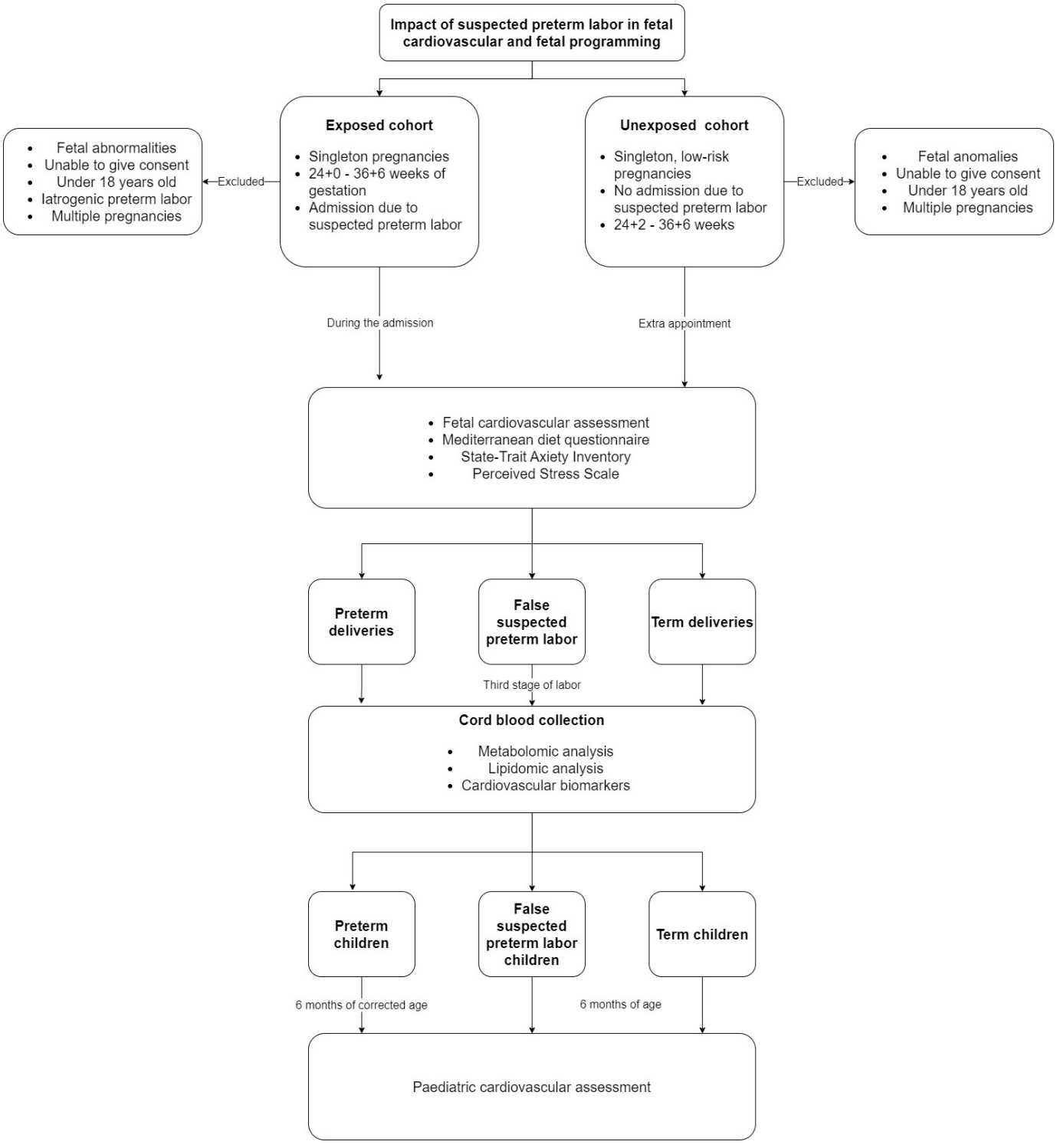

Figure 1 Study flowchart.

will be performed by Maternal-Fetal Medicine experts trained in foetal cardiac ultrasound, foetal biometry and foetal Doppler examination following the International Society of Ultrasound in Obstetrics and Gynecology current guidelines.[31–34] Ultrasound examinations will be performed during the admission in exposed patients or at a separate appointment in non-exposed patients.

Foetal growth will be estimated by transabdominal ultrasound using the Hadlock formula.[35] Foetal weight centiles will be calculated using local customised standards. Umbilical artery PI, medial cerebral artery PI, ductus venosus PI, and right and left uterine arteries PI will be measured with pulsed-wave Doppler. Foetal cardiac anatomy and structure will be evaluated using 2D imaging. Ventricular morphometry evaluation will include longitudinal diameters, basal and mid-transverse diameters, septal wall thickness, and right and left ventricular wall thickness. Atrial morphometry evaluation will

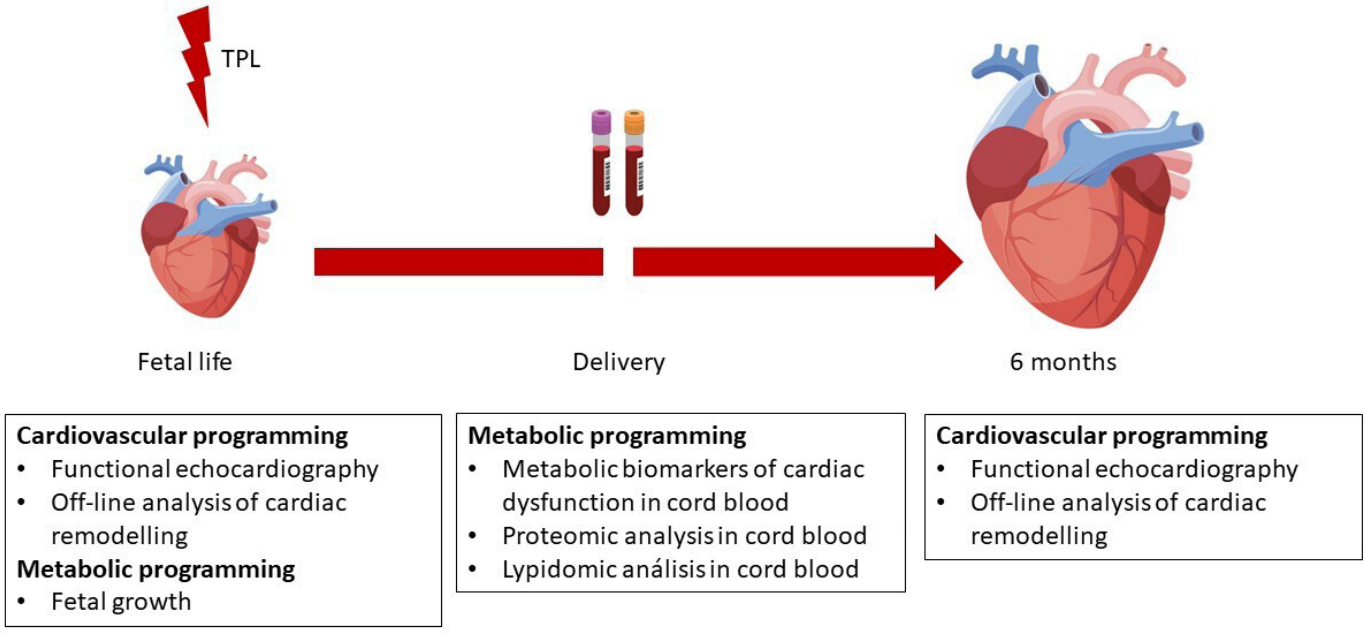

Figure 2 Study chronology.

include transverse and longitudinal diameters. Foetal cardiac function assessment will include diastolic (E/A ratios), systolic (pulmonary and aortic ejection fractions and velocity, stroke volume, cardiac output, and tricuspid and mitral annular plane systolic excursion) and global parameters (myocardial performance index) measured with conventional Doppler and M-mode.[36 37] Foetal cardiac function variables are described in table 2.

Paediatric cardiovascular assessment

Echocardiographic examinations will be performed using a Siemens Acuson SC2000 ultrasound system (Siemens Healthcare, Erlangen, Germany) equipped with an 8-MHz sector transducer. Two paediatric cardiologists will perform all the echocardiographic examinations, following current guidelines. Image acquisition procedures will be harmonised before the study starts. Optimal frame rate will be used to optimise myocardial deformation analysis. Three ECG-guided 2D cardiac-cycle loops will be systematically recorded in the following views: long axis parasternal view and four-chamber apical view. Pulse-wave Doppler, tissue Doppler, M-mode and speckle tracking will be used to analyse cardiac function and heart flows. The following left ventricle measurements and function parameters will be studied: end-diastolic

Table 2 Foetal cardiac variables

Systolic variables	Diastolic variables	Global cardiac function variables	Morphologic variables
Aortic ejection velocity	Mitral E and A waves velocity	Heart rate	Aortic valve diameter
Pulmonary ejection velocity	Tricuspid E and A waves velocity	Myocardial performance index	Pulmonary valve diameter
Aortic velocity time integral	Left ventricle filling time	Cardiac output	Atrial transverse and longitudinal diameters
Pulmonary velocity time integral	Right ventricle filling time	Combined cardiac output	Ventricular basal, mid-transverse and longitudinal diameters
Aortic stroke volume			Atrial areas
Pulmonary stroke volume			Ventricular areas
Aortic ejection fraction			Interventricular septum thickness
Pulmonary ejection fraction			Ventricular wall thickness
Left ventricle ejection time			Cardiac longitudinal and transverse diameter
Right ventricle ejection time			Cardiac area
Tricuspid annular plane systolic excursion			
Mitral annular plane systolic excursion			

Table 3 Paediatric cardiac function variables

Systolic variables	Diastolic variables	Global variables	Morphological variables
Left ventricle ejection fraction	Mitral E/A waves ratio	Heart rate	Left ventricle end-diastolic diameter
Left ventricle strain	Lateral mitral annulus E′ and A′ waves velocity (tissue doppler)	Myocardial performance index	Left ventricle end-systolic diameter
Left ventricle strain rate			Interventricular septum thickness
Lateral mitral annulus S′ wave velocity (tissue Doppler)			Left ventricle posterior wall thickness

and end-systolic diameter; septum and posterior wall thickness; heart rate; ejection fraction (by Teicholz and Simpson methods); mitral E and A wave velocities (pulse-wave Doppler); lateral mitral annulus S′, E′ and A′ wave velocities (tissue Doppler); TEI index (tissue doppler); and left ventricle strain and strain rate at four chamber apical views (speckle tracking). Paediatric cardiac function variables are described in table 3.

Sample collection and processing

Cord blood will be obtained during third stage of labour. Blood will be collected into dry tubes and EDTA tubes for collection of serum and plasma. Cord blood will be centrifugated (3000 g for 10 min, 4°C) and supernatant stored at −80°C until analysis.

Metabolomics in umbilical cord plasma

Lipidomic and metabolomic analysis will be addressed by two methods: methanol extraction and chloroform/methanol extraction (Folch method). Methanol extraction will be performed by adding 4 volumes of methanol to the sample and incubating 30 min at −20°C. The mixture will be centrifuged at 1300 g for 20 min at 4°C. Then, the supernatant will be collected and filtered. Folch method will be carried out by adding 4 vol of chloroform/methanol (2:1) and incubating for 30 min at −20°C. Then, 1 vol of 0.8% NaCl will be added, and the mixture will be centrifuged at 13 000 g for 20 min 4°C. The upper phase (methanol) will be collected, filtered and stored at −20°C. The lower phase will be collected, dried in a vacuum concentrator and reconstituted with 1 vol of butanol/methanol (1:1), filtered and stored at −20°C.

Ultra-performance liquid chromatography-mass spectrometry analysis will be performed on a Bruker Elute Chromatograph coupled to a Trapped Ion Mobility Spectrometry-Time-of-Flight (TIMS-TOF) Ion Mobility-Q-TOF Mass Spectrometer (Bruker, Bremen, Germany) in both positive and negative electrospray ionisation mode using an Agilent Zorbax Eclipse Plus C18 column (1.8 µm, 2.1 mm × 100 mm).

For the metabolite extracts, a gradient elution with 0.1% formic acid in water (phase A) and 0.1% formic acid in acetonitrile (phase B) with 0.5 mL/min flow rate will be used, and the mass spectrometer will be operated with predefined '3D-Metabolomics' methods consisting in data-dependent MS2 Analysis.

The lipid extracts will be analysed using a gradient method with 0.1% formic acid and 10 mM ammonium formate in 60:40 acetonitrile/water (phase A) and 0.1% formic acid and 10 mM ammonium formate in 90:10 isopropanol/acetonitrile (phase B) with a 0.4 mL/min flow rate. The mass spectrometer will be used predefining '4D-Lipidomics' methods that combine ion mobility separation and determination of collisional cross-sections (CCS) with a data-dependent MS2 analysis.

After acquiring data in the Bruker spectrometer, we will align chromatograms, extract the features and annotate metabolites and lipids using the Metaboscape Software (Bruker, Bremen, Germany)

Regarding identification, only compounds that meet all the criteria will be noted: exact mass, correct isotopic distribution, MS2 spectrum and in the case of lipids also CCS (ionic mobility). Annotation will be performed using several databases: Bruker HMDB, Mass Bank of America, MS-Dial public MS2, MS-Dial LipidBlast and Bruker-MetaboScape lipid annotation.

Biomarkers of metabolic and cardiac dysfunction

Serum total cholesterol (enzymatic, colorimetric method), triglycerides (TG) (enzymatic colorimetric method), high density lipoprotein (HDL) (homogeneous enzymatic colorimetric method) and glucose (enzymatic reference method with hexokinase) will be tested using Roche Cobas c503 (Roche Diagnostics, Switzerland). Serum apolipoprotein B (immunoturbidimetric method) and cystatin C (particle-enhanced immunoturbidimetric assay) levels will be measured using the same Cobas c503 platform. Serum insulin, C-peptide, troponin I and N-terminal prohormone of BNP (NT-proBNP) will be determined by electrochemiluminescence immunoassay in a Cobas c801 (Roche Diagnostics, Switzerland).

For all assays, the same analyser, the same lot of reagents and an identical lot of commercial control materials will be used throughout the study. Moreover, quality controls will be performed in accordance with an internal laboratory operating procedure. The measurements will be performed at Clinical Biochemistry department of Hospital Universitario Lozano Blesa (Zaragoza, Spain).

Some biomarkers will be estimated by equations. Low-density lipoprotein-cholesterol (LDL-C) will be calculated by Friedewald or Sampson formula (34). Very low-density lipoprotein-cholesterol (VLDL-C) will be estimated by TG/5. Finally, indirect indicators of insulin resistance will be evaluated by the homeostatic model assessment (HOMA) from HOMA-IR=Insulin* Glucose/22.5 and the

Quantitative Insulin Sensitivity Check Index (QUICKI) by QUICKI=1/(log insulin+log glucose).

We also analyse different protein levels for obesity and inflammatory markers: adiponectin, adipsin, leptin, resistin, serpin E1/plasminogen activator inhibitor-1PAI-1;, interleukin- 6, IL-interleukin 8 and tumour necrosis factorTNF- alpha using the Human Luminex Discovery Assay (8-Plex), (RYD-LXSAHM-08, R&D Systems). Each serum sample will be analysed in duplicate according to the manufacturer's recommendations, and fuorescence will be acquired using the Luminex200 platform (Luminex Corporation). In order to calculate protein concentrations, a standard curve will be generated by serially diluting reconstituted standards. Data will be collected and analysed with Luminex xPONENT 3.1 software (Luminex Corporation). Grade 3 curve fitting will be applied, adding point (0, 0) to obtain protein concentrations (pg/ml).

Sample size and statistical analysis

The sample size has been defined considering the main objective of the study and the magnitude of the change in the main variable. In this project, different variables are relevant in this regard; therefore, a simulation of the sample size calculation has been carried out in reference to three variables of interest: birth weight, myocardial performance index and NT-ProBNP.

For type I error, α (the probability of incorrectly rejecting the null hypothesis), we have assumed a value of 0.05. For type II error, β (the probability of incorrectly accepting the alternative hypothesis), we have assumed a frequent value of 20%; β=0.2; therefore, a statistical power of 0.8.

To perform the calculations, the G*Power tool (V.3.1.9.2 for Mac) was used, applying the sample size test for Student's t tests for two independent samples, since it was intended to compare differences between groups. To calculate this, we have used the values of α and β, previously defined, and Cohen's d value for each of the variables to measure the effect size. Reviewing previous studies, it is observed that the differences in the variables reach effect sizes of 0.6 SD for the birth weight (13), 0.98 for myocardial performance index[38] and 1.09 for the proBNP.[39] Taking as a reference for the study the highest value of the three variables, the total size of the sample would be n=98; 49 participants in each group. But the number of subjects to be recruited also depends on the possible losses, N′= N/(1-p), so that if we consider the percentage of losses of 20%, the number of participants to be recruited would be N′ = 123.

As a general rule, qualitative variables will be described as absolute frequencies and relative percentages and quantitative variables as means and medians for the assessment of central tendency and SD and IQR for the assessment of dispersion. In the case of ordinal variables, the description of both forms will be evaluated. A univariate analysis will be carried out for the comparison of two qualitative variables using the χ^2 test or Fisher's exact test. When the variables are quantitative, Student's t-test for independent samples or the Mann-Whitney U test will be used if the applicability criteria are not met. Multivariate analysis will be performed by means of multiple (continuous variables) or logistic linear regression (categorical variables) controlling the possible confounding factors. For statistical analysis, values of p<0.05 will be considered as statistically significant.

In order to analyse metabolomics footprints, mean differences among the three study groups were also analysed using a one-way analysis of variance (ANOVA). The obtained p values will be adjusted using the Benjamini-Hochberg method, with a significance threshold set at p<0.05. The most significant ANOVA results will be visually represented using a heatmap, constructed with the R pheatmap package. Clustering will be performed using the Euclidean method. Finally, a multivariate analysis will be conducted using principal component analysis (PCA, unsupervised method) and partial least squares discriminant analysis (PLS-DA, supervised method). PCA will be performed using the base R function prcomp, while PLS-DA will be carried out using the mixOmics package.

The data will be analysed with the SPSS programme (V.20.0, IMB or newer) and the base functions of R (V.4.3.2).

Ethics and dissemination

The study will follow the ethical standards of the Nuremberg Code of 1947 and the Declaration of Helsinki of 1964 as revised Fortaleza (2013). The confidentiality and anonymity of the study participants will be guaranteed. In the case of detecting any abnormal result in any of the evaluated variables, the participants will be informed, and if necessary, they will be assessed by the obstetric or paediatric specialist.

Participant information sheets and informed consent have been approved by the research ethics committee (C.P.-C.I. PI20/136), and they will be delivered and explained to each of the participants. Study outcomes will be disseminated at international conferences and published in peer-reviewed scientific journals. Lay reports will be made available to study participants on request.

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Correction notice This article has been corrected since it was first published. Author name 'Jon Schoorlemmer' has been updated.

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Contributors CP, DO, DL and SR-M conceived and supervised the project. CP, JS, MF, MC-A, AA, AM-M and LY designed the experiment. NA-C, CP, SR-M, DO, DL, PM-Q, JR-C, JG, MC-A and AA participated in the inclusion of women and performed the follow-up of the children. CP, NA-C, AM-M, MF, LY, JL-I and JS analysed and interpreted the data. NA-C, SR-M, CP, DO, JL-I, PM-Q, MF and JG co-wrote and revised the manuscript. NA-C is the guarantor. All authors have read and agreed to the published version of the manuscript.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, conduct, reporting or dissemination plans of this research.

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