Intra-amniotic infection and/or inflammation is associated with fetal cardiac concentric hypertrophy and diastolic dysfunction in preterm labor and preterm prelabor rupture of membranes

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CONFLICT OF INTERESTS

The authors report no conflicts of interest.

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CONDENSATON

Fetuses from mothers with preterm labor or preterm prelabor rupture of membranes showed signs of cardiac concentric hypertrophy and subclinical diastolic dysfunction. These changes were more pronounced in fetuses with intra-amniotic infection and/or inflammation.

SHORT VERSION OF TITLE:

Fetal cardiac remodeling in preterm labor and preterm prelabor rupture of membranes.

AJOG AT A GLANCE:

A. Why was this study conducted? Preterm delivery is associated with cardiovascular remodeling and dysfunction in childhood and adulthood. In the animal model, intraamniotic inflammation has been related to cardiac dysfunction. Moreover, few retrospective studies performed in human fetuses exposed to intraamniotic infection have observed cardiac remodeling. However, to our knowledge, there are no prospective data evaluating fetal cardiac structure and function using echocardiography and amniotic fluid biomarkers in fetuses from mothers with preterm labor (PTL) or preterm prelabor rupture of membranes (PPROM), with or without intra-amniotic infection and/or inflammation compared with control fetuses. Whether the reported postnatal cardiac changes are already present at the time of PTL or PPROM diagnosis is unknown and merits evaluation.

B. What are the key findings? Fetuses from mothers with preterm labor or preterm prelabor rupture of membranes showed signs of cardiac concentric hypertrophy and diastolic dysfunction. These changes were more pronounced in fetuses with intra-amniotic infection and/or inflammation.

C. What does this study add to what is already known? This study provides the first complete cardiac study with evidence that fetuses with preterm labor or preterm prelabor rupture of membranes present subclinical cardiac remodeling and dysfunction, suggesting that the effects

observed in childhood and adulthood have, at least in part, a prenatal origin. Identification of fetal cardiac programming represents a unique opportunity to identify a high-risk population that might benefit from early preventive measures to improve their future cardiovascular health.

ABSTRACT

Background: Preterm delivery is associated with cardiovascular remodeling and dysfunction in children and adults. However, it is unknown whether these effects result from the neonatal consequences of preterm birth or are already present in utero.

Objectives: We evaluated fetal cardiac morphology and function in fetuses of mothers admitted for preterm labor (PTL) or preterm prelabor rupture of membranes (PPROM) and the association of these changes with the presence of intra-amniotic infection and/or inflammation (IAI).

Study Design: In this prospective cohort study, fetal echocardiography and amniocentesis were performed at admission in singleton pregnant women with PTL and/or PPROM between 24.0-34.0 weeks, with (IAI group, n=41) and without IAI (no-IAI, n=54). Controls (n=48) were outpatient pregnant women without PTL or PPROM. Intraamniotic infection was defined by a positive amniotic fluid (AF) culture or positive 16S ribosomal RNA gene. Intraamniotic inflammation was defined according to the AF interleukin-6 cut-off levels previously reported by our group, being greater than 1.43 ng/ml in PPROM and greater than 13.4 ng/ml in PTL. Fetal cardiac morphology and function was evaluated by echocardiography, and troponin-I and N-terminal pro-brain natriuretic peptide (NT-proBNP) concentrations were measured in AF from women with PTL/PPROM and compared to 20 AF Biobank samples obtained for reasons other than PTL/PPROM or cardiac pathology. Data was adjusted for the estimated fetal weight below the 10th centile and for PPROM at admission, and also for gestational age at amniocentesis when AF biomarkers were compared.

Results: From 2018-2021, 143 fetuses were included: 95 fetuses were from mothers admitted with a diagnosis of PTL or PPROM: 41 (28.7%) were in the IAI group and 54 (37.8%) in the non-IAI group. 48 (33.6%) fetuses were included in the control group. Fetuses with PTL and/or PPROM had signs of subclinical cardiac concentric hypertrophy (median (25;75 centile) left wall thickness of 0.93 (0.72;1.16) in the IAI group, 0.79 (0.66;0.92) in the no-IAI group and 0.69 (0.56:0.83) in controls, p<0.001) and diastolic dysfunction (tricuspid A duration 0.23 seconds (0.21;0.25), 0.24 (0.22;0.25) and 0.21 (0.2;0.23), p=0.007). Systolic function was similar among

groups. Higher values of AF troponin I (1413 pg/mL (927;2334), 1190 (829;1636) and 841 (671;959), p<0.001) and NT-pro-BNP were detected (35.0%, 17% and 0%, p=0.005) in fetuses with PTL/PPROM compared to the control group. The highest NT-pro-BNP concentrations were found in the IAI group.

Conclusion: Fetuses with PTL/PPROM showed signs of cardiac remodeling and subclinical dysfunction, which were more pronounced in those exposed to IAI. These findings support that the cardiovascular effects observed in children and adults born preterm have, at least in part, a prenatal origin.

KEY WORDS: spontaneous preterm delivery, preterm birth, preterm labor, preterm prelabor rupture of membranes, intra-amniotic infection, intra-amniotic inflammation, fetal cardiac function, fetal cardiac remodeling, cardiac hypertrophy, cardiac dysfunction, diastolic dysfunction, fetal echocardiography, amniotic fluid NT-proBNP, amniotic fluid troponin I, cardiac dysfunction biomarkers, functional echocardiography, amniocentesis

MAIN TEXT

INTRODUCTION

Preterm delivery before 34 weeks affects 9-12% of pregnancies worldwide and is associated with perinatal death and long term sequellae[1]. Several studies have demonstrated that children and adults born preterm have differences in cardiovascular morphology and function. This population has an increased incidence of heart failure[2] and hypertension[3] and have been reported to present a concentric hypertrophy pattern with poorer ventricular diastolic relaxation[4,5] and a reduced ventricular ejection fraction[6,7]. About 40-45% of preterm deliveries occur in association with spontaneous preterm labor (PTL) and 25-30% occur in association with preterm prelabor rupture of membranes (PPROM)[8]. A proportion of these patients have intra-amniotic infection and/or inflammation (IAI), which is associated with poorer outcomes[9-11]. The contribution of the prenatal environment to cardiovascular programming in preterm birth has only been investigated in part.

In animal models, fetuses exposed to IAI show altered gene networks programming cardiac development[12], cardiac overload[13], impaired cardiac relaxation[14,15], cardiac dysfunction and elevated brain natriuretic peptide (BNP) mRNA levels[16-17], pulmonary and systemic arterial hypertension[18] and altered cardiac growth and maturation[19]. In human clinical studies, Letti Müller *et al.* reported a longer myocardial performance index in fetuses with PPROM[20]. Romero *et al*.[21] and Di Naro *et al*.[22] reported signs of diastolic dysfunction in fetuses with PPROM and exposed to IAI. However, Aye *et al.* did not find differences in cardiac morphology and function evaluation in a cohort of fetuses born preterm, which included medically-indicated deliveries[23].

The effect of amniotic fluid biomarkers of fetal cardiac dysfunction has been evaluated in different fetal conditions[24,25]. In a normal pregnancy, N-terminal prohormone of BNP (NT-proBNP) concentrations decrease along gestation. Fetuses with heart failure, arrhythmias or congenital heart defects present high concentrations of amniotic fluid NT-proBNP reflecting an increase in cardiac filling pressure[26]. Regarding fetuses with IAI, a prospective cohort study by Irani *et al.*,

found no differences in amniotic fluid NT-proBNP concentrations compared to fetuses without IAI delivered preterm[27].

Troponin I has been scarcely studied as a marker of myocardial injury[28] in amniotic fluid. However, high concentrations of troponin I in the umbilical cord have been related to myocardial stress in fetuses with intra-uterine grow restriction[29], or fetuses with abnormal Doppler findings[30]. To our knowledge there are no data regarding amniotic fluid troponin I concentrations in fetuses exposed to IAI.

Up to now, cardiovascular preventive strategies have been carried out in moderate and extreme premature infants during postnatal life. In this study, we comprehensively evaluated fetal cardiac structure and function using echocardiography and amniotic fluid biomarkers in fetuses from mothers with PTL or PPROM, with or without IAI, compared with control fetuses. Identification of fetal cardiac programming represents a unique opportunity to target a high-risk population that might benefit from earlier preventive strategies to improve their future cardiovascular health.

MATERIAL AND METHODS

Study population and design

Study groups

This was a prospective cohort study including consecutive singleton pregnant women complicated with PTL/PPROM between 23+0 to 34+0 weeks of gestation at BCNatal (Hospital Clínic and Hospital Sant Joan de Déu, Barcelona, Spain) from 2018 to 2021. As part of the local clinical protocol, women with singleton pregnancies admitted with PTL or PPROM below 34 weeks were offered amniocentesis to rule in/out IAI. Eligible cases were those with amniocentesis performed at admission. A group of outpatient singleton pregnant women not diagnosed with PTL or PPROM were also recruited as a control group, matched 2:1 by gestational age at study ultrasound with the PTL/PPROM cases.

Three groups of fetuses were compared: those of mothers admitted for PTL or PPROM with IAI (IAI group), fetuses of mothers presenting PTL/PPROM without IAI (non-IAI group), and the previously described control group.

We also performed a sub-analysis on the IAI group. Thus, the IAI group was divided according to the presence of intra-amniotic infection and inflammation or the presence of intra-amniotic inflammation without infection (known as sterile intra-amniotic inflammation).

Amniocentesis to rule out intraamniotic infection and/or inflammation was not performed in the control group because there was no clinical indication for this purpose. Instead, in order to compare amniotic fluid biomarkers of cardiovascular dysfunction, 20 amniotic fluid samples from our Clinic-IDIBAPS Biobank collected for indications other than PTL/PPROM or cardiac pathology were selected (Biobank amniotic fluid [AF] samples). These amniocenteses were performed in the second or third trimester of pregnancy.

Exclusion criteria

The exclusion criteria were delivery before fetal echocardiography; maternal age below 18 years; multiple gestations; clinical chorioamnionitis[31] at admission; major fetal structural malformations or chromosomal anomalies; and PTL/PPROM cases without amniocentesis at admission.

Definitions

PTL was defined as regular uterine contractions with a cervical length below the 5th centile[32] by transvaginal ultrasound. We only evaluated cervical changes by digital examination if there was a suspicion of imminent delivery. PPROM was diagnosed as leakage of AF confirmed by a positive result in the alpha 1 microglobulin protein test.

Intra-amniotic infection was defined as a positive AF culture for genital Mycoplasma (Mycoplasma IST 2, bioMérieux for *Ureaplasma* spp. or *Mycoplasma hominis*), or aerobic (Chocolate agar) and anaerobic (Schaedler agar for anaerobes and thioglycolate broth) bacteria. IAI was also diagnosed based on specific polymerase chain reaction amplification of the 16S ribosomal RNA

gene using the primers: 5'-AGA GTT TGA TCC TGG CTC AG-3' and 5'-GGA CTA CCA GGG TAT CTA AT at-3' followed by Sanger sequencing. Sequences were identified using the Blast algorithm in the National Center for Biotechnology Information database, with a minimum of 98% of sequence identity.

Intra-amniotic inflammation was defined as high levels of AF interleukin (IL)-6, with a cut-off of 1.43 ng/ml for PPROM cases[33] and 13.4 ng/ml for PTL cases[34] as previously reported by our group. Amniotic fluid IL-6 levels were measured using the enzyme-linked immunoassay (Diasource ImmunoAssays, Louvain-la-Neuve, Belgium). The minimum detection level was 2 pg/mL. The coefficient of variation was 6.23 for a mean concentration of 123.3 pg/mL and 5.18% for a mean concentration of 317.4 pg/mL.

Gestational age in weeks was calculated according to first trimester crown-rump length[35].

Clinical management of PTL/PPROM

Two intramuscular injections of betamethasone 12 mg given 24 hours apart were administered for fetal lung maturation until 34+6 weeks. If there was no clinical contraindication, tocolysis was administered during steroid administration. We only administered broad-spectrum antibiotics to women with PPROM, AF glucose concentrations < 5 mg/dL and/or with microorganisms identified by Gram staining and/or positive AF cultures.

Fetal echocardiography

Fetal echocardiography was performed within 24-72 hours after admission in patients with PTL or PPROM and at a similar gestational age in the asymptomatic group. Echocardiography was done using Voluson E10 Expert and Voluson S8 ultrasound equipment. The sonographers were blinded to AF IL-6 levels or culture results.

The study protocol included the assessment of estimated fetal weight[36], conventional feto-placental Doppler, and a complete two dimensional, M-mode and Doppler echocardiographic

examination to assess cardiac structure, morphometry and function following a strict standardized methodolog[37-40].

Fetal cardiac morphometry included the cardio-thoracic areas and sphericity, right and left ventricular areas and sphericities, auricular areas, septal and right and left free wall myocardial wall thickness, and right and left relative wall thicknesses by M-Mode.

Fetal cardiac systolic function was evaluated using tricuspid and mitral annular plane systolic excursion (TAPSE, MAPSE), the left shortening fraction, right fractional area change, stroke volumes and cardiac outputs, and left ejection and isovolumetric contraction time.

Fetal cardiac diastolic function was evaluated by atrioventricular peak velocities at early diastole (E) and atrial contraction (A), E/A ratios, tricuspid and mitral inflow time fraction, and left isovolumetric relaxation time.

Details of each measurement are described in Supplementary Material (Table S1).

Cardiac biomarkers in amniotic fluid

A total of 500 µl of AF was collected and frozen at -80ºC. All samples were thawed and immediately centrifuged at 16,000 x g for 4 minutes prior to use or dilution. Total protein concentrations were evaluated with the Pierce™ BCA Protein Assay Kit (Thermo Scientific™, ref: 23225) to estimate the appropriate dilution factor for Luminex assays. Samples were analyzed in duplicate and diluted as follows: 1/1 and 1/2 for NT-proBNP; 1/5 and 1/10 for troponin I.

The NT-proBNP DuoSet assay (DY3604-05) was used to detect NT-proBNP and the Magnetic Luminex® performance assay Human Discovery assay (LXSAHM-04) to detect troponin I (manufactured by R&D systems™). LX100 (LX10010187403) device and XPonent software were used for 96-well MagPlex analyses with a minimum threshold of 50 events. Seven standards with a 1/3 dilution factor were used to perform the calibration curve from a stock solution of 12,150 pg/mL for troponin I and 10,000 pg/mL for NT-proBNP. The lower limit of NT-proBNP detection was 250 mg/ml.

All procedures were performed following the manufacturer's recommendations.

Statistical analysis

Encoded information was processed using an Access database. Qualitative variables were described in tables as absolute frequency and relative percentage and quantitative variables as median and 25th percentile-75th percentile.

For the baseline, fetal, perinatal and admission characteristics of the study population, univariate analysis was performed using the Chi-square or Fisher's exact test for comparison of qualitative variables. For quantitative variables, the Student's T-test was used for independent samples if normal distribution was assessed by the Shapiro-Wilk test and homoskedasticity by the Levene test. The Wilcoxon-W test was applied in variables with a non-normal distribution.

Regarding the analysis of echocardiographic and AF biomarkers, multivariate analysis was performed using multiple linear regression (continuous variables) or logistic regression (categorical variables) controlling for possible confounding factors, which were: estimated fetal weight below 10th centile and PPROM at admission for the echocardiographic evaluation; and estimated fetal weight below the 10th centile, gestational age at amniocentesis, and PPROM at admission for the AF biomarker concentrations. A linearity trend analysis was also performed among the three groups (IAI – non-IAI – control).

Pearson’s correlation was performed to analyze IL-6 concentrations and echocardiographic parameters and AF biomarkers that significantly differed between groups.

z-scores previously published[39] and percentage of cases below 5th percentile or above 95th percentile have been calculated in those variables statistically different among groups.

The data were analyzed using STATA for MAC (version 15.1 StataCorp LP). p values ≤0.05 were considered statistically significant.

Ethics

The Research Ethics Committees of the Hospital Clinic and Sant Joan de Déu reviewed and approved the study (HCB/2018/0567 and PIC 150-19, respectively) and all participants were informed and provided signed written consent.

RESULTS

Study population

During the study period, 154 women with singleton pregnancies were admitted for PTL or PPROM between 23+0 to 34+0 weeks. Of these, 116 were eligible for the study (with an amniocentesis at admission) and 95 were finally included (Figure 1). Approximately 80% of these women with PTL/PPROM had previously been included in other non-interventional studies[41,42].

Of the pregnancies included in the study, 34 (23.8%) were complicated with PPROM, 61 (42.7%) with PTL and 48 (32.7%) were controls (without PTL or PPROM). IAI was present in 41 women: 28/41 (68.3%) had PPROM and 13/41 (31.7%) had PTL, being much more frequent in women with PPROM (82%; 28/34) compared to those with PTL (21%; 13/61).

There were no significant differences in maternal characteristics (Table 1). Only one woman presented chronic hypertension and another preeclampsia, both being in the non-IAI group. There were no cases of pre-gestational diabetes or human immunodeficiency virus positivity.

Table 2 shows the admission characteristics of the PTL/PPROM group. Twenty percent of the total AF cultures were positive and the microorganisms isolated are shown in Table S2. Antenatal corticosteroids for lung maturation were administered in 100% of patients with PTL/PPROM and in 0% of the control group. Thus, adjustment for this parameter was not required. Fifty-seven percent of our patients diagnosed with PTL finally delivered at term.

Table 3 shows the fetal ultrasound findings. There were no differences among groups in gestational age, estimated fetal weight (EFW) or fetal-placental Doppler at ultrasound evaluation. However, we found a lower EFW centile and a higher percentage of fetuses under the 10^{th} centile in the IAI group (Table 3).

Table S3 describes the gestational age and the indication for amniocentesis in the Biobank AF samples selected to compare AF biomarkers. Since gestational age at amniocentesis significantly differed among groups, AF cardiac dysfunction biomarker concentrations were adjusted for

gestational age. The results of all the genetic and infection studies performed in these samples were normal. These Biobank patients did not present risk factors for cardiac remodeling and healthy offspring were confirmed in all these cases.

Fetal echocardiographic results

Table 3 shows the fetal echocardiographic results in the three groups. Fetuses in the IAI group showed signs of myocardial hypertrophy defined by a thicker myocardium without cardiomegaly. The observed hypertrophy is concentric as relative wall thickness is increased. Thus, the heart was of normal size but the ventricular areas were reduced because the myocardium was growing "inwards". These fetuses also showed a preserved stroke volume and cardiac output with an increased (compensatory) left shortening fraction and a shorter ejection time. They also presented signs of impaired relaxation with prolonged tricuspid A duration and left isovolumic relaxation time. All these functional changes are in line with myocardial hypertrophy, since a heart with more muscle can pump harder and faster but takes more time to relax. Indeed, impaired relaxation together with the faster ejection leads to preserved (compensated) cardiac output.

Fetuses in the non-IAI group showed similar, albeit less pronounced, findings as compared to the IAI group. Thus, they also presented signs of concentric myocardial hypertrophy, preserved cardiac output but increased TAPSE, right atrial area, tricuspid inflow time fraction and A duration and lower tricuspid E/A ratio. The linearity trend analysis of the three groups, showed that fetuses in the non-IAI group seemed to be an intermediate cardiac remodeling group.
Figures 2 and 3 show the cardiac phenotypes among groups.

Z-scores from echocardiographic parameters different among groups are presented in table S4a. Table S4b shows that up to 80% of patients in the IAI group had concentric cardiac hypertrophy defined as myocardial thickness higher than 95th percentile, being the more frequent the septal hypertrophy. Given that the cardiac functional changes are subclinical (compensated), despite there were significant differences among groups, most of the systolic and diastolic parameters are in the normal range (between the 5th and 95th percentile).

Cardiac biomarkers in amniotic fluid

NT-proBNP was detectable in 35% of women in the IAI group (14/40) and in 17% (9/53) in the non-IAI group. Moreover, patients with detectable NT-proBNP values in the IAI group presented significantly higher concentrations compared to the no-IAI group (median 5750 vs. 2662 pg/ml, respectively). To the contrary, AF NT-proBNP values were below detection levels in all the pregnancies from the Biobank AF samples.

Amniotic fluid troponin I concentrations were significantly higher in the IAI and non-IAI group than in the Biobank AF samples (Table 4, Figure 4).

We also observed a linear correlation between AF interleukin-6 concentrations and biomarker concentrations in the AF (Table S5)

Sub-analysis according to the presence of intra-amniotic infection and inflammation vs. sterile intra-amniotic inflammation

The perinatal outcomes of patients with intra-amniotic infection with inflammation and patients with sterile intra-amniotic inflammation were similar (Tables S6 and S7). All patients with infection had inflammation. The fetal cardiac morphometry, diastolic function and AF biomarker concentrations (Table S8 and S9) were similar in cases with and without infection. However, fetuses with intra-amniotic infection and inflammation showed an increased cardiac output compared to those with sterile inflammation (Table S8) most likely reflecting the increased need for compensation.

COMMENT

Principal findings

This study provides evidence that fetuses with spontaneous PTL or PPROM have echocardiographic and biomarker signs of subclinical cardiac remodeling and dysfunction, supporting a prenatal origin of cardiovascular programming described in children and adults born preterm. In general, most of the changes observed were more pronounced in PTL/PPROM fetuses with IAI, supporting an etiopathogenic role of inflammation/infection in the genesis of cardiovascular programming in prematurity.

Results in the context of what is known

Previous postnatal studies report that children and adults born preterm have an increased cardiac mass with reduced ventricular internal diameters and systolic and diastolic dysfunction[4-7]. These findings are in line with the present study, in which fetuses with PTL/PPROM presented myocardial concentric hypertrophy (thicker myocardium at the expense of smaller ventricular cavities as the myocardium 'grows' inwards) and subclinical dysfunction (mainly impaired cardiac filling) with preserved cardiac output. Romero *et al.* and Di Naro *et al*. described fetal diastolic impairment in fetuses with PPROM exposed to intra-amniotic infection[21,22], with increased ventricular relaxation, similar to adult septic cardiac changes[43]. While we found similar changes, impaired diastolic function was less pronounced, which may be explained by the inclusion of cases with clinical chorioamnionitis, a late severe stage of infection, in the former studies. Differences in study population and echocardiographic techniques could also explain the differences between our study and that of Di Naro. As compared with previous studies in fetuses, our findings of a shorter ejection time and longer myocardial performance index are in line with those of Letti Müller in fetuses with PPROM and IAI[20]. Aye *et al.* reported no differences in cardiac structure and function of fetuses subsequently born preterm[23]. However, this study included both spontaneous and iatrogenic preterm delivery. In addition, the mean gestational age at delivery was 34 weeks, when IAI is uncommon[10,11]. Concerning cardiac biomarkers in AF, Irani *et al*. reported no differences in AF NT-proBNP in fetuses born preterm with or without IAI[27]. Some differences with this study may explain the positive findings in our study, including a different definition of IAI, their smaller number of patients, their lack of a control non-preterm group and a

different NT-proBNP analysis technique. Regarding troponin I, to our knowledge, this study is the first to report amniotic fluid troponin I concentrations in fetuses with PTL or PPROM.

From a pathophysiological perspective, IAI could plausibly be related to a myocardial insult and subsequent cardiovascular dysfunction and remodeling. This notion is supported by animal studies describing altered gene networks programming cardiac development in a primate model[12], and acute generation of proinflammatory cytokines in the myocardium as well as fetal cardiovascular compromise after animal intra-amniotic lipopolysaccharide injection[12,16,17,44] . We hypothesize that IAI might directly insult the myocardium and cause a compensatory cardiac hypertrophy, which allows maintaining normal stroke volume and cardiac output allowing normal tissue perfusion. In experimental studies, IAI has also been associated with increased placental resistance and a higher afterload[13-15], which could also contribute to the concentric hypertrophy with normal cardiac size observed in this study. Likewise, a similar concentric hypertrophy has been described in other conditions with a persistent pressure overload[45]. The hypothesis is that IAI overloads the fetal heart which responds by increasing myocardial mass without cardiomegaly -concentric hypertrophy-. This hypertrophy implies that the heart can pump harder and faster but takes more time to relax and thus, ventricular filling is impaired. Nevertheless, the fetal cardiac changes observed can compensate for this situation as the final cardiac output - blood volume ejected each minute - is preserved. The echocardiographic findings are consistent with results of biomarkers as increased NT-pro-BNP and troponin values reflect fetal cardiac overload[26,28,29]. Strikingly, the cardiac findings correlate with AF fluid IL-6 levels, suggesting the higher the inflammation the greater the need for cardiac adaptation. Interestingly, the cardiac findings in IAI were remarkably different from those previously reported in fetal growth restriction [46], which is mainly characterized by larger and globular hearts and decreased longitudinal motion. While hypertrophy may occasionally be present in severe fetal growth restriction, it is normally associated with cardiomegaly and ventricular dilation, which were not observed in IAI fetuses[45]. Thus, these findings support a specific mechanism for cardiovascular programming in IAI, which would be different from that caused by the undernutrition and hypoxia occurring in placental insufficiency. Likewise, the findings in this study are different from the fetal cardiac changes described in fetuses conceived by assisted reproductive techniques[47,48] or exposed to antiretroviral drugs[49].

Sub-analysis of the IAI group: Effect of intra-amniotic infection with inflammation and sterile intra-amniotic inflammation on fetal cardiac remodeling.

Sub-analysis of intra-amniotic infection and inflammation versus sterile inflammation showed augmented cardiac outputs in the infection group most likely reflecting a greater insult and greater need for compensation. Interestingly, these finding were due to augmented stroke volumes, while the heart rate was similar in both groups.

To our knowledge, this is a novel finding and could translate some degree of fetal systemic response against the infection.

Clinical implications

Prenatal cardiac changes seem to contribute to cardiac remodeling and cardiovascular risk in children and adults born preterm. Thus, our work opens a window of opportunity to study early (prenatal) identification of fetuses that will present postnatal cardiovascular risk, allowing targeting a high-risk cardiovascular population and performing preventive actions in the first months/years of life. The amniocentesis performed to rule in/out IAI helped to target a higher cardiovascular risk group of neonates that merits further follow-up. This finding might highlight the importance of performing amniocentesis in these women, and particularly in those complicated with PPROM due to the high proportion of IAI identified in this group.

Future research

Future studies are needed to assess postnatal changes in the same cohort and also to identify possible prenatal predictors of cardiovascular risk in order to target prenatal high cardiovascular risk. Prenatal cardiovascular risk evaluation could be tremendously important, given the increased risk of heart failure[2] and hypertension[3] reported in young adults born preterm.

Strengths and limitations

The main strengths of this study are its prospective design and the recruitment of a well-characterized cohort with data on AF analyses, being the first cohort published with a complete fetal cardiovascular evaluation. Another strength is the evaluation of both echocardiography and cardiovascular AF biomarkers.

However, we have to acknowledge some limitations. The sample size was relatively limited. We excluded cases with imminent delivery in whom fetal echocardiography was not performed, as well as cases of anhydramnios without amniocentesis. It remains unknown whether these individuals might have different profile from those studied. Molecular techniques might increase the detection of intra-amniotic infection. Thus, it is possible that some of the patients may possibly have had a positive culture for fungi or viruses which was not detected. Another limitation was the lack of a clear universal definition of intra-amniotic inflammation. In our study inflammation was defined according to amniotic fluid IL-6 concentrations but other authors include other inflammatory biomarkers to define intra-amniotic inflammation[50,51]. Finally, another limitation was that a postnatal follow-up was not included. Further research is warranted to prospectively evaluate cardiovascular changes postnatally in these cohorts before changing our clinical management in these patients.

Conclusions

Our findings suggest that cardiovascular changes including concentric cardiac hypertrophy and diastolic impairment observed in premature infants and adults might already be present, at least in part, at the time that symptoms of PTL and/or PPROM occur, and are more pronounced when IAI is present.

The observation of elevated of AF NT-proBNP and troponin I concentrations strengthen the echocardiographic findings. These results open an opportunity for early detection of cases that might benefit from preventive measures to improve future cardiovascular health later in life.

495 **TABLES**

TABLE 1

Baseline, fetal and perinatal characteristics of the study population.

Variables	Group 1: PTL/PPROM-IAI (n= 41)	Group 2: PTL/PPROM no-IAI (n=54)	Group 3: Control (n=48)	p1 *(1 vs. 2)*	p2 *(1 vs. 3)*	p3 *(2 vs. 3)*
Maternal characteristics						
Maternal age (years)	33.5 (30.2-37.5)	33.1 (28.8-34.9)	33.6 (30.3-36.9)	0.154	0.634	0.078
BMI (Kg/m^2)	23.0 (20.2-27.8)	22.5 (20.3-26.8)	21.8 (20.8-27.1)	0.899	0.776	0.673
Race						
Caucasian, n (%)	29/38 (76.3)	37/50 (74.0)	41 (85.4)			
Maghrebi, n (%)	3/38 (7.9)	0	1 (2.1)			
Hispanic, n(%)	4/38 (10.5)	6/50 (12.0)	6 (12.5)	0.356	0.337	0.109
Asian, n(%)	1/38 (2.6)	4/50 (8.0)	0			
Other, n(%)	1/38 (2.6)	3/50 (6.0)	0			
Maternal smoking, n(%)	2/39 (5.1)	7 (13.0)	5/47 (10.6)	0.295	0.448	0.719
Nulliparity, n(%)	19 (46.3)	34/52 (65.4)	27 (56.3)	0.066	0.351	0.349
Assisted Reproductive Technology, n(%)	3/34 (8.8)	4/46 (8.7)	6/48 (12.5)	1.000	0.729	0.740
Pregnancy and delivery data						
GA at inclusion (weeks)	28.9 (26.0-30.4)	27.7 (26.0-30.7)	29.4 (27.0-30.9)	0.367	0.259	0.714
Time from inclusion to delivery (days)	11 (6-17)	57 (33-74)	72 (59-81)	**<0.001**	**<0.001**	**<0.001**
Neonatal male sex, n(%)	26 (63.4)	32 (59.3)	22 (45.8)	0.681	0.097	0.175
GA at delivery (weeks)	31.1 (27.7-32.4)	37.4 (34.3-39.3)	39.6 (38.9-40.3)	**<0.001**	**<0.001**	**<0.001**
Birthweight (g)	1557 (1080-2166)	2600 (2010-3110)	3270 (3000-3510)	**<0.001**	**<0.001**	**<0.001**

Variables	*Group 1: PTL/PPROM-IAI (n= 41)*	*Group 2: PTL/PPROM no-IAI (n=54)*	*Group 3: Control (n=48)*	*p1 (1 vs. 2)*	*p2 (1 vs. 3)*	*p3 (2 vs. 3)*
Birthweight centile	20.5 (5.5-47)	32 (9-59)	48 (19-76)	0.301	**0.002**	**0.047**
Clinical chorioamnionitis at delivery	7 (17.1)	3 (5.6)	0	0.115	**0.004**	0.085
Cesarean section, n(%)	18 (43.9)	10 (18.5)	11 (22.9)	**0.019**	**0.035**	0.805
Induction of labor, n(%)	7 (17.1)	9 (16.7)	27 (56.3)	0.837	**<0.001**	**<0.001**
Non cephalic presentation, n(%)	14 (34.1)	3 (5.6)	3 (6.3)	**0.001**	1.000	**0.001**
APGAR <7 at 5 min, n(%)	3/37 (8.1)	0/43	0/45	0.095	0.088	NA
pH umbilical artery	7.27 (7.18-7.34)	7.24 (7.19-7.30)	7.21 (7.15-7.27)	0.378	0.119	0.551
Placental histology						
Acute chorioamnionitis without funisitis, n(%)	12 (29.3)	4/26 (15.4)	1/20 (5)	**0.023**	**<0.001**	0.165
Acute chorioamnionitis + funisitis, n(%)	19 (46.3)	7/26 (26.9)	2/20 (10)			
Neonatal outcome						
NICU admission n(%)	26/38 (68.4)	11/45 (24.4)	0/46 (0)	**<0.001**	**<0.001**	**<0.001**
Major neonatal morbidity[1] or mortality, n(%)	14/38 (36.8)	3/45 (6.7)	0/46 (0)	**0.001**	**<0.001**	0.075

Data are presented as number (percentage) for qualitative variables or median (25th centile-75th centile) for quantitative variables. IAI, intra-amniotic infection or inflammation; BMI, body mass index; ART, artificial reproduction technique; GA, gestational age; NICU, neonatal intensive care unit.

[1]Major complications defined by the presence of bronchopulmonary dysplasia, necrotizing enterocolitis, intraventricular hemorrhage, periventricular leukomalacia, retinopathy or early onset sepsis.

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TABLE 2

Admission characteristics

Variables	PTL/PPROM-IAI (n= 41)	PTL/PPROM no-IAI (n=54)	p
Maternal/pregnancy data			
PPROM at admission, n(%)	28 (68.3)	6 (11.1)	**<0.001**
Latency from admission to ultrasound (days)	2 (1-3)	2 (1-2)	0.104
C-reactive protein (mg/L)	1.06 (0.56-2.2)	0.51 (0.28-1.06)	**0.021**
White blood cell count ($x10^9$/L)	11300 (8670-14490)	11235 (9470-13900)	0.388
Neutrophils (%)	81 (74-89)	78 (73-84)	0.756
Cervical length (mm)	20.5 (12.0-31.5)	13 (10-18)	**<0.001**
Amniotic fluid results			
AF glucose (mg/dL)	22 (11-35)	41 (31-52)	**<0.001**
AF Positive Gram stain, n(%)	3 (7.3)	0	
AF interleukin-6 (pg/mL)	32090 (7111.3-73038.3)	1171.5 (728.5-1918.8)	**<0.001**
Positive AF culture, n(%)	19 (46.3)	0	**<0.001**

Data are presented as number (percentage) for qualitative variables or median (25^{th} centile-75^{th} centile) for quantitative variables.

IAI, intra-amniotic infection or inflammation; PPROM, preterm premature rupture of membranes; AF, amniotic fluid

TABLE 3

Fetal echocardiographic results.

Variables	Group 1: PTL/PPROM IAI (n= 41)	Group 2: PTL/PPROM no-IAI (n=54)	Group 3: Control (n=48)	p1 *(1 vs. 2)*	p2 *(1 vs. 3)*	p3 *(2 vs. 3)*	p4 *(linearity trend among the three groups)*
Fetal-placental parameters							
GA at ultrasound (weeks)	27.9 (28.7-31.1)	29.6 (27-31.2)	29.4 (26.6 – 31.3)	0.380	0.481	0.900	0.462
EFW at ultrasound (gr)	1150 (864-1547)	1362.5 (1066.5 -1741.5)	1380.5 (1088 - 1705)	0.141	0.122	0.870	0.085
EFW centile	23 (9.5 – 60.5)	53.5 (22.5 – 70)	58.5 (27.5 – 75)	**0.0139**	**0.0022**	0.553	**0.004**
Small for gestational age (EFW< 10th centile)	11 (26.8%)	7 (13.0%)	4 (8.3%)	0.088	**0.020**	0.452	**0.018**
Umbilical artery PI	0.92 (0.72-1.09)	0.99 (0.84-1.11)	1.03 (0.82-1.17)	0.208	0.168	0.603	0.083
Middle cerebral artery PI	1.82 (1.56-2.12)	1.76 (1.59-2.05)	2 (1.71-2.25)	0.171	0.888	0.137	0.303
Ductus venosus PI	0.41 (0.31-0.54)	0.46 (0.34-0.57)	1.93 (1.65-2.43)	0.206	0.112	0.803	0.243
Uterine arteries mean PI	0.76 (0.63-0.96)	0.78 (0.64-0.95)	0.72 (0.64-0.89)	0.108	0.619	0.084	0.309
Cardiac morphometry							
Cardiothoracic ratio[1] (%)	29.2 (25.4-33.2)	27.2 (24.5 – 30.8)	28.7 (26.1-31.5)	0.625	0.448	0.563	0.297
Cardiac sphericity index[2]	1.14 (1.07–1.20)*	1.16 (1.08 – 1.21)	1.2 (1.1 – 1.3)	0.814	**0.010**	0.538	**0.001**

Variables	*Group 1: PTL/PPROM IAI (n= 41)*	*Group 2: PTL/PPROM no-IAI (n=54)*	*Group 3: Control (n=48)*	*p1 (1 vs. 2)*	*p2 (1 vs. 3)*	*p3 (2 vs. 3)*	*p4 (linearity trend among the three groups)*
Right atrial area to heart ratio[3]	0.16 (0.14-0.20)	0.19 (0.16-0.20)	0.16 (0.13-0.18)	0.754	0.060	**0.003**	0.124
Right ventricular area to heart ratio[3]	0.20 (0.16-0.24)	0.23 (0.21-0.28)	0.25 (0.21-0.28)	0.266	**0.010**	0.564	**0.004**
Right ventricular sphericity index[2]	1.62 (1.36-1.82)	1.57 (1.45-1.81)	1.74 (1.56-2.0)	0.640	**0.005**	**0.012**	**0.011**
Right ventricle free wall thickness (mm)	3.4 (3.1-4.2)	3.0 (2.5-3.7)	3.0 (2.5-3.3)	0.178	<**0.001**	0.066	**<0.001**
Right relative wall thickness[4]	1.0 (0.88-1.17)	0.72 (0.64-0.85)	0.65 (0.56-0.81)	**<0.001**	**<0.001**	**0.023**	**<0.001**
Left atrial area to heart ratio[3]	0.15 (0.14-0.19)	0.16 (0.14-0.19)	0.14 (0.13-0.18)	0.927	0.242	0.111	0.384
Left ventricular area to heart ratio[3]	0.22 (0.19-0.26)	0.26 (0.24-0.31)	0.27 (0.22-0.30)	**0.029**	**0.005**	0.568	**0.004**
Left ventricular sphericity index[2]	1.69 (1.52-1.91)	1.68 (1.55-1.87)	1.79 (1.60-1.91)	0,438	0.879	0.424	0.149
Left ventricle free wall thickness (mm)	3.3 (2.8-4.2)	3.4 (2.6-4.0)	2.8 (2.4-3.3)	0.850	**0.002**	**0.012**	**0.003**
Left relative wall thickness[4]	0.93 (0.72-1.16)	0.79 (0.66-0.92)	0.69 (0.56-0.83)	0.271	**<0.001**	**0.042**	**<0.001**
Septal wall thickness (mm)	4.1 (3.6-4.9)	3.7 (3.1-4.2)	3.5 (3.1-4.0)	0.109	**<0.001**	0.075	**<0.001**
Systolic function							
TAPSE	6.1 (5.3-7)	6.4 (5.7-7.0)	6.1 (5.4-6.7)	0.066	0.197	**0.020**	0.523
MAPSE	4.4 (3.6-5)	4.2 (3.8-4.7)	4.3 (3.8-5.2)	0.927	0.910	0.397	0.919

Variables	*Group 1: PTL/PPROM IAI (n= 41)*	*Group 2: PTL/PPROM no-IAI (n=54)*	*Group 3: Control (n=48)*	*p1 (1 vs. 2)*	*p2 (1 vs. 3)*	*p3 (2 vs. 3)*	*p4 (linearity trend among the three groups)*
Septal thickening[7]	0.24 (0.18-0.30)	0.21 (0.13-0.27)	0.20 (0.12-0.27)	0.068	0.097	0.738	0.109
Right fractional area change area (FAC) (%)[8]	31.8 (23-41.5)	34.1 (30.2-41.9)	36.0 (27.6-47.9)	0.773	0.180	0.979	0.183
Left ventricular shortening fraction (%)[9]	50.0 (42.1-58.6)	43.5 (35.6-54.2)	40.4 (33.7-51.3)	0.269	**0.007**	0.276	**0.007**
Left Isovolumic contraction time (ICT, %)[6]	12.9 (11.5-14.5)	11.7 (10.5-13.1)	11.9 (10.3-13.0)	0.510	**0.049**	0.860	**0.041**
Left ejection time (ET, %)[6]	39.0 (37.6-41.1)	39.6 (37.6-41.6)	39.9 (38.5-42.4)	0.648	**0.046**	0.242	**0.028**
Heart rate (bpm)	145 (138-153)	143.5 (137.2-152)	144 (139-152)	0.777	0.940	0.398	0.963
Pulmonary artery diameter (mm)	5.9 (5-6.7)	6.1 (5.5-6.8)	5.6 (5.2-6.8)	0.078	0.871	0.209	0.715
Right stroke volume (ml/kg)[10]	1.9 (1.5-2.5)	1.9 (1.4-2.4)	1.8 (1.5-2.0)	0.523	0.110	0.112	0.177
Right cardiac output (ml/min/kg)[11]	276 (210-370)	281 (218-348)	255 (211-296)	0.504	0.096	0.127	0.108
Aortic diameter (mm)	4.4 (4-5.2)	4.8 (4.2-5.3)	4.9 (4.4-5.4)	**0.019**	**0.048**	0.547	0.072
Left stroke volume (ml/kg)[10]	1.1 (0.9-1.7)	1.2 (1.0-1.5)	1.3 (1.0-1.6)	0.991	0.176	0.553	0.312
Left cardiac output (ml/min/kg)[11]	168 (127-244)	183 (136-214)	186 (158-237)	0.926	0.181	0.410	0.310

Variables	*Group 1: PTL/PPROM IAI (n= 41)*	*Group 2: PTL/PPROM no-IAI (n=54)*	*Group 3: Control (n=48)*	*p1 (1 vs. 2)*	*p2 (1 vs. 3)*	*p3 (2 vs. 3)*	*p4 (linearity trend among the three groups)*
Cardiac index (ml/min/kg)[12]	476.1 (307.1-567.6)	466.6 (370.2-546.5)	438.3 (383.4-507.7)	0.558	0.586	0.530	0.316
Diastolic function							
Tricuspid E/A ratio	0.71 (0.65-0.76)	0.67 (0.63-0.74)	0.71 (0.65-0.81)	0.518	0.113	**0.029**	0.253
Tricuspid inflow time fraction[5]	0.39 (0.36-0.42)	0.39 (0.37- 0.43)	0.38 (0.34-0.41)	0.260	0.201	**0.008**	0.150
Tricuspid A duration fraction[5]	0.23 (0.21-0.25)	0.24 (0.22-0.25)	0.21 (0.20-0.23)	0.114	**0.020**	**0.001**	**0.007**
Mitral E/A ratio	0.71 (0.62-0.80)	0.71 (0.67-0.75)	0.73 (0.66-0.79)	0.763	0.219	0.314	0.440
Mitral inflow time fraction[5]	0.42 (0.39-0.46)	0.43 (0.40-0.46)	0.43 (0.40-0.45)	0.858	0.554	0.948	0.939
Mitral A time fraction[5]	0.22 (0.20-0.24)	0.23 (0.21-0.24)	0.22 (0.21-0.24)	0.754	0.964	0.519	0.713
Left isovolumic relaxation time (IRT, %)[6]	12.9 (11.5-14.5)	11.7 (10.5-13.1)	11.9 (10.3-13.0)	0.510	**0.049**	0.860	**0.028**
Combined systolic and diastolic function							
Left MPI index[13]	0.55 (0.47-0.65)	0.52 (0.45-0.57)	0.49 (0.43-0.56)	0.868	**0.016**	0.276	**0.018**

Data are presented as number (percentage) for qualitative variables or median (25th centile-75th centile) for quantitative variables. ***p1*** *was adjusted for estimated fetal weight <10th centile and PPROM at admission.* ***p2 and p3*** *were adjusted for estimated fetal weight <10th centile.*

IAI, intra-amniotic infection or inflammation; GA, gestational age; EFW, estimated fetal weight; PI, pulsatility index; MPI: Myocardial performance index.

[1] **Cardio-thoracic ratio**: (cardiac area(cm^2)/thoracic area (cm^2))*100; [2] **sphericities**: cardiac, auricular or ventricular longitudinal diameter (mm)/ transverse diameter (mm); [3] **Areas to heart ratio**: right or left auricular or ventricular areas (cm^2) / cardiac area (cm^2); [4] **Relative wall thickness (by M-Mode)**: (septal wall thickness (mm) + right or left free wall thickness (mm))/ right or left cavity diameter (mm); [5] **Diastolic times fraction**: tricuspid or mitral inflow or A duration (ms) / total cycle duration (ms); [6] **IRT, ICT, ET**: (IRT, ICT or ET (ms) / total cycle duration (ms))*100; [7] **Septal Thickening**: (End-systolic septal thickness (mm) – end-diastolic septal thickness (mm))/ End-systolic septal thickness (mm); [8] **FAC**: ((End-diastolic right ventricular area (cm^2) – End-systolic right ventricular area (cm^2)) / End-diastolic right ventricular area (cm^2))*100; [9] **Left ventricular shortening fraction** (by M-Mode): ((End-diastolic ventricular diameter (mm) - End-systolic ventricular diameter (mm)) / End-diastolic ventricular diameter (mm))*100; [10] **Stroke volumes**: (Pulmonary or aortic area (cm^2)* pulmonary or aortic VTI (cm/contraction))/EFW (Kg); [11] **Cardiac outputs index:** (Pulmonary or aortic stroke volume (ml/contraction) * heart rate (bpm)) / EFW (Kg); [12] **Cardiac index:** (Right cardiac output (ml/min)+ left cardiac output (ml/min)) / EFW (Kg); [13] **MPI**: (ICT (ms) + IRT (ms)) / ET (ms)

Table 4

Cardiac biomarkers in amniotic fluid.

Variables	Group 1: PTL/PPROM-IAI (n= 41)	Group 2: PTL/PPROM no-IAI (n=54)	Biobank samples (n=20)	p1 *(1 vs. 2)*	p2 *(1 vs. Biobank)*	p3 *(2 vs. Biobank)*	p4 *(linearity trend among the three groups)*
GA at amniocentesis (weeks)	28.9 (26.0-30.4)	27.7 (26.0-30.7)	29.5 (28.3-32.0)	0.367	0.193	**0.034**	**0.009**
EFW at ultrasound (gr)	1150.0 (864.0-1547.0)	1362.5 (1066.5–1741.5)	1495 (1191.5-2011.5)	0.141	**0.006**	**0.039**	**0.005**
Neonatal male sex, n(%)	26 (63.4)	32 (59.3)	12 (60.0%)	0.681	0.915	0.967	
EFW centile	23.0 (9.5 – 60.5)	53.5 (22.5 – 70.0)	56.5 (32.0-77.5)	**0.014**	**0.015**	0.584	**0.008**
Detectable NT-proBNP, n (%)	14/40 (35.0)	9/53 (17.0)	0	0.079[1]	**0.032[1]**	0.383[1]	**0.005**
NT-proBNP (pg/ml)[2]	5750 (1565.5-10300)	2662 (480.6-3391)	NA	**0.012[1]**	NA	NA	NA
Troponin-I (pg/ml)	1413.6 (927.3-2334.1)	1190.9 (829.3-1635.9)	841.3 (671.7-959.9)	0.435[1]	**0.007[1]**	**0.037[1]**	**<0.001**

Data are presented as number (percentage) for qualitative variables or median (25th centile; 75th centile) for quantitative variables. ***p1*** *was adjusted for estimated fetal weight <10th centile, gestational age at amniocentesis and PPROM at admission.* ***p2 and p3*** *were adjusted for estimated fetal weight <10th centile and gestational age at amniocentesis.*

[1]Adjusted for gestational age at amniocentesis and estimated fetal weight centile.

[2]Including only the sample levels of detectable NT-proBNP.

IAI, intra-amniotic infection or inflammation; AF, amniotic fluid; BNP, brain natriuretic peptide

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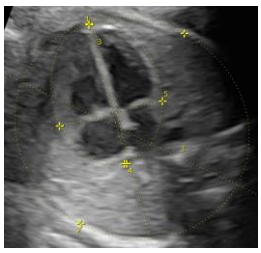
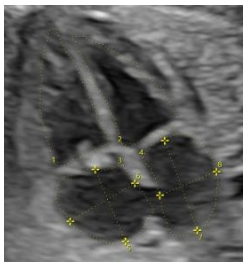
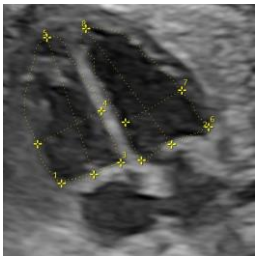
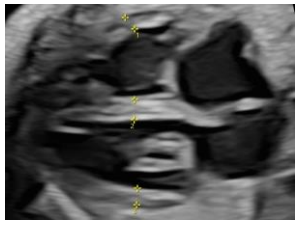
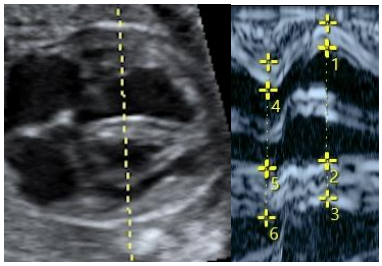
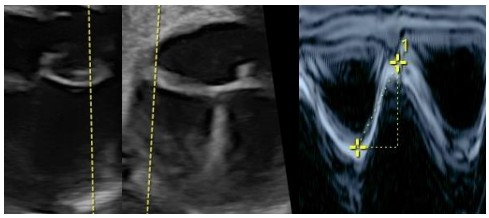
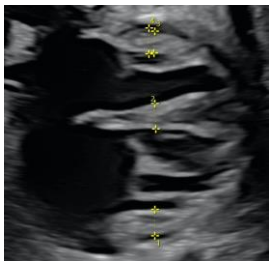
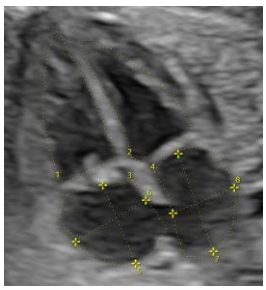
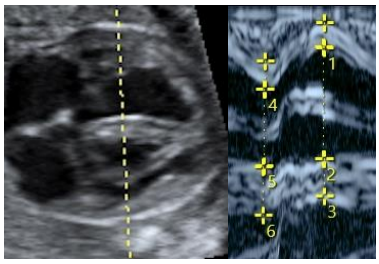
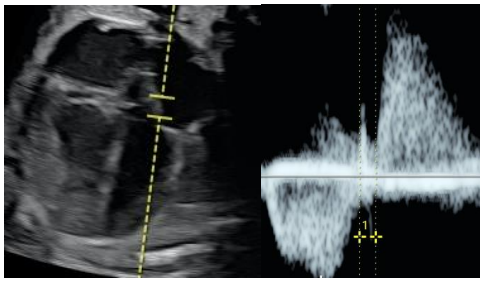
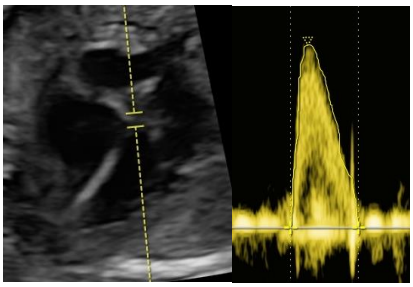
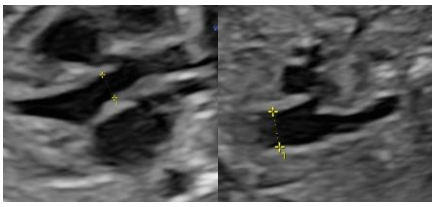
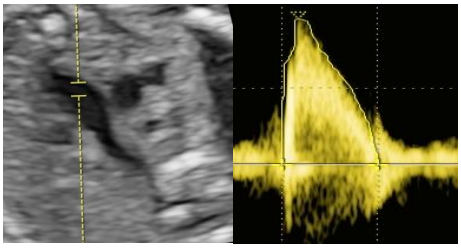
SUPPLEMENTARY TABLES

TABLE S1

Fetal echocardiographic measurements

Variables	Methodology	Example
Cardiac morphometry		
Cardiac and thoracic area	Cardiac area was measured in 2D images at maximal distension from an apical or basal 4-chamber view at end-diastole. End-diastole was defined as the frame at which the atrioventricular valves closed and, thus, when the ventricles reached their largest size. Thoracic area was measured in the same image as the cardiac area.	
Atrial areas	Atrial areas were measured in 2D images at maximum atrial distension from a 4-chamber view at end-systole (defined by the frame preceding the atrioventricular valves opening) and, thus, when the atria reached their largest size. The atrial measurements did not include the pulmonary veins/arteries or the atrioventricular valve annulus.	
Ventricular areas and sphericities	Ventricular dimensions and areas were measured in 2D images from an apical or basal 4-chamber view at end-diastole. The ventricular basal and longitudinal dimensions were measured at the level of the atrioventricular valves and from the atrioventricular valves (including the atrioventricular valves annulus) to the inner myocardium apex, respectively. Both ventricular areas were measured by manual	

	tracing along the true border of the inner myocardium, including the endocardium, the muscular trabeculations, and the moderator band.	
Myocardial wall thicknesses (mm)	Myocardial wall thicknesses were measured in 2D images from a transverse 4-chamber view at end-diastole below the atrioventricular valve leaflets.	
Relative wall thicknesses (M-Mode)	Myocardial wall thicknesses were measured using M mode images from a transverse 4-chamber view at end-diastole below the atrioventricular valve leaflets.	
Systolic function		
TAPSE, MAPSE	An apical or basal four-chamber view was used, applying M-mode at the tricuspid (TAPSE) or mitral (MAPSE) valve annulus to measure the maximum excursion from end-diastole to end-systole.	
End-systolic septal thickness (used for calculating septal thickening)	Septal thickness was measured in 2D images from a transverse 4-chamber view at end-systole.	
End-systolic right ventricular area (used for calculating	End systolic right ventricular area was measured in 2D images from an apical or basal 4-chamber view at end-systole by manual tracing along the true border of the inner myocardium, including the	

the right fractional change area (FAC))	endocardium, the muscular trabeculations, and the moderator band.	
Mid diameter of ventricular cavity (used for calculating left ventricular shortening fraction)	Left mid-ventricular cavity was measured at end-systole and end-diastole using M mode images from a transverse 4-chamber view.	
Left isovolumic contraction time (ICT)	Placing the Doppler sample volume in a four-chamber view on the medial wall of the ascending aorta and displaying the mitral biphasic inflow and the aortic outflow in the same spectral image. ICT includes the time interval from the closure of the mitral valve to the opening of the aortic valve.	
Left ejection time (ET)	Placing the Doppler sample volume in a four-chamber view on the medial wall of the ascending aorta and displaying in the same spectral image the mitral biphasic inflow and the aortic outflow. Ejection time includes the time interval from the opening to closure of the aortic valve.	
Aortic and pulmonary diameters (mm)	Aortic and pulmonary diameters were obtained in frozen real-time images during early to mid-systole by the leading-edge-to-edge method.	
Aortic and pulmonary velocity-time integrals (used for calculating left	Velocity-time integrals were calculated by manual trace of the spectral Doppler area and can be multiplied by the area of the outflow wave.	

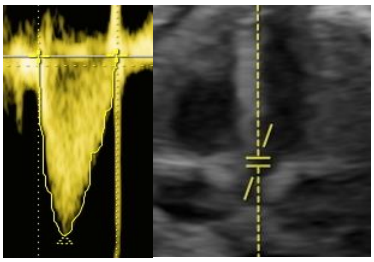
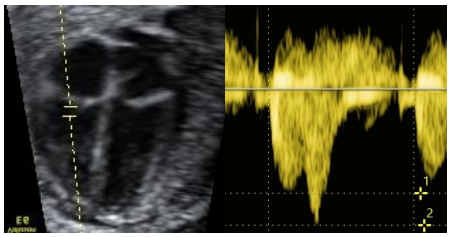
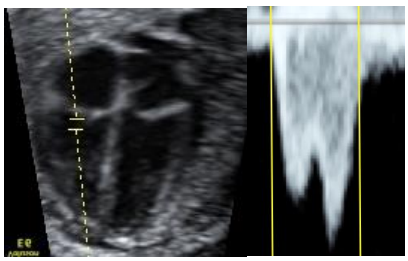
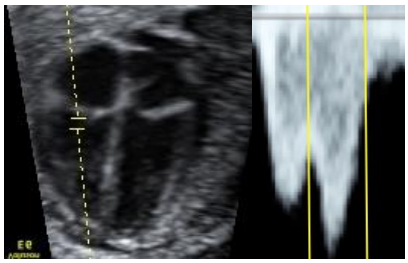
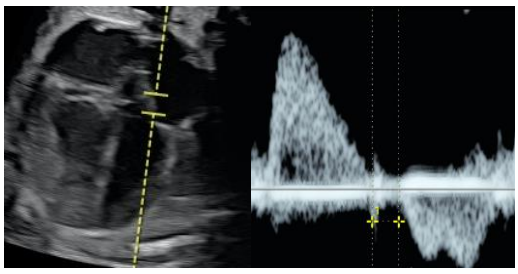
and right stroke volume, respectively)		
Diastolic function		
Tricuspid and mitral E/A ratios	Evaluated by the spectral Doppler sample volume below the atrioventricular valves, displaying a biphasic wave. The ratio is obtained by the division of the peak velocities of the E over the A waveform.	
Tricuspid and mitral inflow time.	Inflow times were evaluated by the spectral Doppler sample volume below the atrioventricular valves, displaying a biphasic wave. Inflow time includes the time interval from the opening to closure of the atrioventricular valves.	
Tricuspid and mitral A waves duration	A wave duration was evaluated by the spectral Doppler sample volume below the atrioventricular valves, displaying a biphasic wave. A duration includes the time interval of the A wave (second wave).	
Left isovolumic relaxation time (IRT)	Placing the Doppler sample volume in a four-chamber view on the medial wall of the ascending aorta and displaying the mitral biphasic inflow and the aortic outflow in the same spectral image. IRT includes the time interval from the closure of the aortic valve to the opening of the mitral valve.	

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TABLE S2

Microorganisms isolated in positive amniotic fluid cultures.

Gestational age at amniocentesis (weeks+days)	Microorganism isolated in amniotic fluid
23+3	*Ureaplasma urealyticum*
30+9	*Peptoniphilus indoliticus*
26+1	*Capnocytophaga sputigena* and *fusobacterium* spp.
30+9	*Ureaplasma urealyticum*
32+4	*Ureaplasma urealyticum*
33+4	*Ureaplasma urealyticum*
26+1	*Fusobacterium nucleatum*
27+6	*Peptoniphilus harei*
25+4	*Fusobacterium nucleatum*
29+0	*Ureaplasma urealyticum*
23+9	*Ureaplasma urealyticum*
30+1	*Ureaplasma urealyticum* and *streptococcus mitis*
27+0	Extended spectrum beta-lactamase producing *Escherichia coli*
30+4	*Ureaplasma urealyticum*
27+4	*Ureaplasma urealyticum*
25+7	*Ureaplasma urealyticum*
26+9	*Escherichia coli* and *Bacteroides vulgatus*
29+9	*Peptoniphilus indolicus*
26+1	*Ureaplasma urealyticum*

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TABLE S3

Amniocentesis in amniotic fluid samples from the Biobank.

Gestational age at amniocentesis (weeks+days)	Indication for amniocentesis
26+3	Congenital talipes equinovarus
28+0	Hyperechogenic fetal bowel
28+3	Moderate renal pyelectasis
28+5	Mild polyhydramnios + hyperechogenic fetal bowel
28+6	Mild renal pyelectasis
29+3	Hyperechogenic fetal bowel
29+6	Hyperechogenic fetal bowel
31+4	Hypospadias
36+1	Long bones at -1 to -2 standard deviations.
36+3	Long bones at -2 to -3 standard deviations.
27+4	8-9mm right and left brain atrial diameters
27+5	9-10mm right and left brain atrial diameters
28+2	Unilateral mild ventriculomegaly (10mm)
29+2	9mm right brain atrial diameter
29+6	8mm right brain atrial diameter
31,2	Mild delay in cortical maturation
31+3	9 mm left brain atrial diameter
32+6	9 mm right brain atrial diameter
35+2	8 mm left brain atrial diameter
37+4	3 mm arachnoid cyst

All the amniocenteses were performed for extra-cardiac reasons. Pregnancies did not present risk factors for cardiac remodeling, normal results were confirmed in the amniotic fluid analysis and healthy offspring were delivered in all cases.

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TABLE S4a

Z-scores of fetal echocardiographic results different among groups

Variables	Group 1: PTL/PPROM IAI (n= 41)	Group 2: PTL/PPROM no-IAI (n=54)	Group 3: Control (n=48)	p1 *(1 vs. 2)*	p2 *(1 vs. 3)*	p3 *(2 vs. 3)*	p4 *(linearity trend among the three groups)*
Fetal-placental parameters							
Cardiac morphometry							
Cardiac sphericity index[1]	ZS: -0.53 (-1.2 - -0.05)	ZS: -0.33 (-1.05 – 0.03)	ZS: 0.14 (-0.43 – 0.83)	0.873	**0.005**	0.149	**0.001**
Right ventricular area	-0.06 (-0.7-0.6)	0.3 (-0.2-0.9)	-0.2 (-0.5-0.2)	0.440	0.438	**0.001**	0.124
Right ventricular sphericity index[1]	-0.2 (-0.8-0.3)	-0.3(-0.6-0.3)	ZS: 0.2 (-0.3-0.7)	0.533	**0.004**	**0.009**	0.528
Right ventricle free wall thickness (mm)	1.9 (1.1-2.7)	0.9 (-0.4-1.5)	0.4 (-0.5-1.3)	**0.017**	<**0.001**	0.056	**<0.001**
Right relative wall thickness[2]	1.8 (1.3-2.7)	1.4 (0.69-2.20)	0.9 (0.0-1.9)	**0.028**	**<0.001**	**0.013**	**0.001**
Left ventricular area	-0.1 (-0.6-0.7)	0 (-0.4-0.4)	-0.5 (-0.9-0.1)	0.825	0.054	**0.001**	**0.045**
Left ventricle free wall thickness (mm)	1.5 (0.4-2.3)	1.1 (-0.6-2.2)	0.2 (-0.4-1.2)	0.349	**<0.001**	**0.008**	**<0.001**
Left relative wall thickness[2]	1.8 (0.7-2.9)	1.7 (0.85-2.4)	1.0 (0.0-2.0)	0.839	**0.007**	**0.034**	**0.012**
Septal wall thickness (mm)	4 (2.4-4.8)	1.9 (1.0-3.7)	1.5 (0.8-2.4)	**0.010**	**<0.001**	**0.031**	**<0.001**
Systolic function							
TAPSE	-0.8 (-1.2- -0.3)	-0.6(-1.3- -0.1)	-0.9 (-1.3- -0.6)	0.628	0.028	**0.007**	0.426

Variables	*Group 1: PTL/PPROM IAI (n= 41)*	*Group 2: PTL/PPROM no-IAI (n=54)*	*Group 3: Control (n=48)*	*p1 (1 vs. 2)*	*p2 (1 vs. 3)*	*p3 (2 vs. 3)*	*p4 (linearity trend among the three groups)*
Left Isovolumic contraction time (ICT)	0.9(-0.1-1.4)	0.6 (0-1.4)	0.6(-0.1-1.2)	0.504	0.333	0.698	0.193
Left ejection time	-0.8(-2- -0.2)	-0.8 (-1.8-0)	-0.5(-1.2-0.4)	0.450	**0.016**	**0.042**	**0.032**
Diastolic function							
Left isovolumic relaxation time (IRT)	1.7(0.9-2.4)	1.1(0.5-1.6)	1.1(0.4-1.9)	0.373	0.111	0.677	0.362

Data are presented as number (percentage) for qualitative variables or median (25th centile-75th centile) for quantitative variables. ***p1*** *was adjusted for estimated fetal weight <10th centile and PPROM at admission.* ***p2 and p3*** *were adjusted for estimated fetal weight <10th centile.*

IAI, intra-amniotic infection or inflammation; GA, gestational age; EFW, estimated fetal weight; PI, pulsatility index; MPI: Myocardial performance index.

[1] **sphericities**: cardiac, auricular or ventricular longitudinal diameter (mm)/ transverse diameter (mm); [2] **Relative wall thickness (by M-Mode)**: (septal wall thickness (mm) + right or left free wall thickness (mm))/ right or left cavity diameter (mm)

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TABLE S4b

Percentage of patients below 5th or above 95th percentiles of fetal echocardiographic results different among groups

Variables	Group 1: PTL/PPROM IAI (n= 41)	Group 2: PTL/PPROM no-IAI (n=54)	Group 3: Control (n=48)	p1 *(1 vs. 2)*	p2 *(1 vs. 3)*	p3 *(2 vs. 3)*
Fetal-placental parameters						
Cardiac morphometry						
Cardiac sphericity index[1]	3/40 (7.5)	4/50 (8.0)	0	1.00	0.097	0.118
Right ventricular area[1]	1/38 (2.63)	0	0	0.447	0.452	NA
Right ventricular sphericity index[1]	1/39 (2.56)	0	0	0.448	0.459	NA
Right ventricle free wall thickness (mm)[2]	16/35 (45.71)	8/44 (18.18)	4/47 (8.51)	**0.008**	**<0.001**	0.222
Right relative wall thickness[2]	32/36 (88.89)	14/49 (28.57)	10/46 (21.74)	**<0.001**	**<0.001**	0.444
Left ventricular area[1]	1/38 (2.63)	0	2/47 (4.35)	0.447	1.00	0.242
Left ventricle free wall thickness (mm)[2]	12/35 (34.29)	12/44 (27.27)	5/48 (10.42)	0.501	**0.012**	**0.037**
Left relative wall thickness[2]	22/36 (61.11)	20/51 (39.22)	12/46 (26.09)	**0.044**	**0.001**	0.170
Septal wall thickness (mm)[2]	28/35 (80.0)	21/44 (47.73)	18/48 (37.5)	**0.033**	**<0.001**	0.321
Systolic function						
TAPSE[1]	1/39 (2.56)	1/52 (1.92)	4/38 (9.52)	1.00	0.361	0.169

Variables	*Group 1: PTL/PPROM IAI (n= 41)*	*Group 2: PTL/PPROM no-IAI (n=54)*	*Group 3: Control (n=48)*	*p1 (1 vs. 2)*	*p2 (1 vs. 3)*	*p3 (2 vs. 3)*
Left Isovolumic contraction time (ICT)[1]	1/38 (2.63)	0	0	0.427	0.442	NA
Left ejection time[1]	11/37 (29.73)	11/50 (22.0)	5/47 (10.64)	0.461	**0.027**	0.132
Diastolic function						
Left isovolumic relaxation time (IRT)[2]	14/37 (37.84)	10/50 (20.0)	10/47 (21.28)	0.066	0.095	0.877

Data are presented as number (percentage). ***p1*** *was adjusted for estimated fetal weight <10th centile and PPROM at admission.* ***p2 and p3*** *were adjusted for estimated fetal weight <10th centile.*

IAI, intra-amniotic infection or inflammation; GA, gestational age; EFW, estimated fetal weight; PI, pulsatility index; MPI: Myocardial performance index.

[1] Percentage of cases below 5th percentile

[2] Percentage of cases above 95th percentile

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TABLE S5

Correlation between amniotic fluid IL-6 and echocardiographic and biomarkers findings

Echocardiographic findings	Pearson's correlation coefficient	p
Cardiac sphericity index[1]	-0.04	0.731
Right atrial area to heart ratio[2]	-0.11	0.323
Right ventricular sphericity index[1]	0.03	0.778
Right ventricle free wall thickness (mm)	0.03	0.774
Right relative wall thickness[3]	0.31	**0.004**
Left ventricular area to heart ratio[2]		
Left ventricle free wall thickness (mm)	-0.01	0.929
Left relative wall thickness[3]	0.12	0.289
Septal wall thickness (mm)	0.05	0.639
TAPSE	-0.289	**0.006**
Left ventricular shortening fraction (%)[4]	0.04	0.729
Left Isovolumic contraction time (ICT, %)[5]	0.05	0.649
Left ejection time (ET, %)[5]	0.03	0.770
Tricuspid E/A ratio	0.10	0.362
Tricuspid inflow time fraction[6]	0.05	0.626
Tricuspid A duration fraction[6]	0.02	0.865
Left isovolumic relaxation time (IRT, %)[5]	0.06	0.600
Left myocardial performance index[7]	0.04	0.692
Amniotic fluid Troponin I concentrations (pg/ml)	0.29	**0.002**
Amniotic fluid NT-proBNP (pg/ml) in positive NT-proBNP samples	0.29	**0.001**

[1]**Sphericities**: cardiac, auricular or ventricular longitudinal diameter (mm)/ transverse diameter (mm); [2]**Areas to heart ratio**: right or left auricular or ventricular areas (cm^2) / cardiac area (cm^2); [3] **Relative wall thickness (by M-Mode)**: (septal wall thickness (mm) + right or left free wall

thickness (mm))/ right or left cavity diameter (mm); [4] **Left ventricular shortening fraction** (by M-Mode): ((End-diastolic ventricular diameter (mm) - End-systolic ventricular diameter (mm)) / End-diastolic ventricular diameter (mm))*100; [5] **IRT, ICT, ET**: (IRT, ICT or ET (ms) / total cycle duration (ms))*100; [6]**Diastolic times fraction**: tricuspid or mitral inflow or A duration (ms) / total cycle duration (ms); [7]**Myocardial performance index**: (ICT (ms) + IRT (ms)) / ET (ms)

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TABLE S6

Baseline, fetal and perinatal characteristics of the PTL/PPROM-IAI group sub-analysis

Variables	Intra-amniotic infection and inflammation (n= 19)	Sterile intra-amnotic inflammation (n= 22)	p
Maternal and fetal characteristics			
Maternal age (years)	33.8 (30.5-37.0)	33.7 (28.2-37.6)	0.389
BMI (Kg/m^2)	22.7 (20.0-28.3)	23.0 (21.1-25.8)	0.971
Race			
White, n (%)	12/17 (70.1)	17/21 (81)	
Maghrebi, n (%)	2/17 (11.8)	1/21 (4.8)	
Hispanic, n(%)	1/17 (5.9)	3/21 (14.3)	0.561
Asian, n(%)	1/17 (5.9)	0	
Other, n(%)	1/17 (5.9)	0	
Maternal smoking, n(%)	0	2/39 (5.1)	0.492
Primiparity, n(%)	6 (33.3)	13 (59.1)	0.105
ART technique, n(%)	1/15 (6.7)	2/19 (10.5)	1.000
GA at inclusion (weeks)	27.6(26.0-30.4)	28.9 (26.8-31.7)	0.524
Time from inclusion to delivery (days)	9 (5-16)	13 (6-23)	0.207
Perinatal data			
Neonatal male sex, n(%)	11 (61.1)	15 (68.2)	0.641
GA at delivery (weeks)	30.3 (27.4-32.1)	31.4 (28.3-33.0)	0.188
Birthweight (g)	1300 (1040-1600)	1748 (1282-2240)	**0.030**
Birthweight centile	11.5 (3-21)	29.5 (6-51)	0.333
Clinical chorioamnionitis at delivery	4 (21.1)	3 (13.6)	0.685

Variables	Intra-amniotic infection and inflammation (n= 19)	Sterile intra-amnotic inflammation (n= 22)	p
Histological funisitis or chorioamnionitis			
Acute chorioamnionitis without funisitis, n(%)	8 (42.1)	4 (18.2)	0.241
Acute chorioamnionitis + funisitis, n(%)	7 (36.8)	12 (54.6)	
Cesarean, n(%)	12 (63.2)	6 (27.3)	**0.021**
Induction of labor, n(%)	4 (21.1)	3 (13.6)	0.685
Non cephalic presentation, n(%)	9 (47.4)	5 (22.7)	0.097
APGAR <7 at 5 min, n(%)	1/18 (5.6)	2/19 (10.5)	1.000
pH umbilical artery	7.26 (7.08-7.35)	7.27 (7.18-7.34)	0.302
NICU admission n(%)	13/18 (72.2)	13/20 (65.0)	0.632
Major neonatal morbidity[1] or mortality, n(%)	6/18 (33.3)	8/20 (40.0)	0.671

Data are presented as number (percentage) for qualitative variables or median (25th centile; 75th centile) for quantitative variables.

BMI, body mass index; ART, artificial reproduction technique; GA, gestational age; NICU, neonatal intensive care unit

[1]Major complications defined by the presence of bronchopulmonary dysplasia, necrotizing enterocolitis, intraventricular hemorrhage, periventricular leukomalacia, retinopathy or early onset sepsis.

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TABLE S7

Admission characteristics of the PTL/PPROM-IAI group sub-analysis

Variables	Intra-amniotic infection and inflammation (n= 19)	Sterile intra-amniotic inflammation (n= 22)	p
PPROM at admission, n(%)	13 (68.4)	15 (68.2)	0.781
C-reactive protein (mg/L)	1.71 (1.22-3.1)	0.59 (0.23-1.01)	**<0.001**
White blood cell count (x10^9 / L)	13915 (8720-17950)	11250 (8660 – 13280)	0.370
Neutrophils (%)	83 (80-89)	77 (73-88)	0.137
Cervical length (mm)	20 (16-30)	21 (10-31)	0.777
AF glucose (mg/dL)	20.5 (3-32)	25.5 (10-38)	**<0.001**
AF interleukin-6 (pg/mL)	67994.45 (37593.2 – 143387.0)	17600.4 (4282.1-33581.8)	**<0.001**
Positive AF culture, n(%)	19 (100)	0	**<0.001**
Latency from admission to ultrasound (days)	1.5 (1-3)	2 (1-3)	0.064

Data are presented as number (percentage) for qualitative variables or median (25th centile; 75th centile) for quantitative variables.

PPROM, preterm premature rupture of membranes; AF, amniotic fluid

TABLE S8

Fetal echocardiographic results of the PTL/PPROM-IAI group sub-analysis

Variables	Intra-amniotic infection and inflammation (n= 19)	Sterile intra-amniotic inflammation (n= 22)	p
Fetal-placental parameters			
GA at ultrasound	27.6 (26.3 – 30.6)	29.2 (26.3- 31.7)	0.411
EFW at ultrasound (gr)	980 (857 – 1508)	1230 (966 – 1712)	0.372
EFW centile	20 (10-43)	26 (7-64)	0.832
Small for gestational age (EFW< 10th centile), n(%)	5 (26.3)	6 (27.3)	0.972
Umbilical artery PI	0.81 (0.71-0.98)	0.93 (0.73-1.16)	0.115
Middle cerebral artery PI	1.81 (1.68-2.31)	1.82 (1.54-2.05)	0.217
Ductus venosus PI	0.41 (0.31-0.59)	0.39 (0.31-0.53)	0.586
Uterine arteries PI average	0.85 (0.63-1.10)	0.74 (0.63-0.845)	0.886
Cardiac morphometry			
Cardiothoracic ratio[1] (%)	29.5 (25.9 - 33.3)	29.1 (25.6-33.0)	0.775
Cardiac sphericity index[2]	1.12 (1.07-1.19)	1.15 (1.08-1.22)	0.236
Right atrial area to heart ratio[3]	0.15 (0.14-0.20)	0.17 (0.14-0.21)	0.234
Right ventricular area to heart ratio[3]	0.20 (0.16-0.22)	0.21 (0.18-0.27)	0.088
Right ventricular sphericity index[2]	1.61 (1.38 - 1.84)	1.62 (1.34 - 1.77)	0.411
Right ventricle free wall thickness (mm)	3.4 (3.2 - 3.9)	3.3 (2.8 - 4.2)	0.881
Right relative wall thickness[4]	1.0 (0.92-1.12)	1.05 (0.85 – 1.23)	0.786
Left atrial area to heart ratio[3]	0.14 (0.13 - 0.15)	0.15 (0.14 - 0.20)	0.380
Left ventricular area to heart ratio[3]	0.21 (0.16 - 0.25)	0.23 (0.19 - 0.27)	0.412
Left ventricular sphericity index[2]	1.71 (1.59 - 1.79)	1.64 (1.47-1.95)	0.438
Left ventricle free wall thickness (mm)	3.3 (2.9 - 4.5)	3.2 (2.8 - 3.8)	0.610

Variables	Intra-amniotic infection and inflammation (n= 19)	Sterile intra-amniotic inflammation (n= 22)	p
Left relative wall thickness[4]	0.92 (0.76 – 1.11)	0.95 (0.69 – 1.20)	0.635
Septal wall thickness (mm)	4.1 (3.8 - 5.0)	4.1 (3.5 - 4.9)	0.932
Systolic function			
TAPSE	5.9 (5.3-6.4)	6.7 (5.4-7.1)	0.197
MAPSE	4.4 (3.6-4.8)	4.4 (3.6-5.2)	0.636
Septal thickening[5]	0.04 (-0.04-0.17)	0.07 (-0.03-0.14)	0.929
Right fractional change area (FAC)[6]	37.0 (24.2-43.4)	30.7 (22.8-38.1)	0.715
Left shortening[7]	0.51 (0.44-0.53)	0.50 (0.42-0.59)	0.448
Isovolumetric contraction time (ICT, %)[8]	8.6 (7.0-9.6)	8.9 (7.4-10.1)	0.972
Ejection time (ET, %)[8]	39.0(37.6-40.6)	39.0 (37.7-41.6)	0.808
Heart rate (bpm)	144 (139-153)	146 (136-153)	0.856
Pulmonary diameter (mm)	5.8 (5.4-6.8)	6 (4.8-6.5)	0.582
Right stroke volume (ml/contraction/kg)[9]	2.4 (2.0-2.7)	1.6 (1.3-1.8)	**<0.001**
Right cardiac output (ml/min/kg)[10]	363 (283-402)	232 (191-271)	**<0.001**
Aortic diameter (mm)	4.5 (4.0-5.2)	4.4 (3.7-5.1)	0.663
Left stroke volume (ml/contraction/kg)[9]	1.4 (1.0-1.8)	1.0 (0.9-1.3)	**0.016**
Left cardiac output (ml/min/kg)[10]	200 (148-263)	143 (113-183)	**0.014**
Cardiac index (ml/min/kg)[11]	564.7 (481.0-601.6)	372.8 (334.8-463.1)	**<0.001**
Diastolic function			
Tricuspid E/A ratio	0.72 (0.64-0.76)	0.70 (0.65-0.78)	0.587
Tricuspid inflow time fraction[12]	0.39 (0.36 – 0.41)	0.39 (0.37- 0.43)	0.490
Tricuspid A time fraction[12]	0.23 (0.22 – 0.24)	0.24 (0.20 – 0.25)	0.983
Mitral E/A ratio	0.70 (0.61-0.77)	0.72 (0.63-0.82)	0.466
Mitral inflow time fraction[12]	0.42 (0.39 – 0.46)	0.42 (0.40 – 0.47)	0.959
Mitral A time fraction[12]	0. 23 (0.22 – 0.25)	0.22 (0.21 – 0.25)	0.715

Left isovolumic relaxation time (IRT, %)[8]	12.9 (11.2-14.7)	13.1 (11.7-14.5)	0.950
Combined systolic and diastolic function			
MPI index[13]	0.54 (0.46-0.65)	0.58 (0.47-0.66)	0.869

Data are presented as number (percentage) for qualitative variables or median (25th centile; 75th centile) for quantitative variables.

GA, gestational age; EFW, estimated fetal weight; PI, pulsatility index; MPI: Myocardial performance index.

[1] **Cardio-thoracic ratio**: (cardiac area(cm^2)/thoracic area (cm^2))*100; [2] **sphericities**: cardiac, auricular or ventricular longitudinal diameter (mm)/ transverse diameter (mm); [3] **Areas to heart ratio**: right or left auricular or ventricular areas (cm^2) / cardiac area (cm^2); [4] **Relative wall thickness (by M-Mode)**: (septal wall thickness (mm) + right or left free wall thickness (mm))/ right or left cavity diameter (mm); [5]**Septal Thickening**: (End-systolic septal thickness (mm) – end-diastolic septal thickness (mm))/ End-systolic septal thickness (mm); [6] **FAC**: ((End-diastolic right ventricular area (cm^2) – End-systolic right ventricular area (cm^2)) / End-diastolic right ventricular area (cm^2))*100; [7] **Left ventricular shortening fraction** (by M-Mode): ((End-diastolic ventricular diameter (mm) - End-systolic ventricular diameter (mm)) / End-diastolic ventricular diameter (mm))*100; [8] **ICT, ET, IRT**: (ICT, ET or IRT (ms) / total cycle duration (ms))*100; [9] **Stroke volumes**: (Pulmonary or aortic area (cm^2)* pulmonary or aortic VTI (cm/contraction))/EFW (Kg); [10] **Cardiac outputs index:** (Pulmonary or aortic stroke volume (ml/contraction) * heart rate (bpm)) / EFW (Kg); [11] **Cardiac index:** (Right cardiac output (ml/min)+ left cardiac output (ml/min)) / EFW (Kg); [12]**Diastolic times fraction**: tricuspid or mitral inflow or A duration (ms) / total cycle duration (ms); [13] **MPI index**: (ICT (ms) + IRT (ms)) / ET (ms)

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TABLE S9

Amniotic fluid biomarkers.

Variables	Intra-amniotic infection and inflammation (n= 19)	Sterile intra-amniotic inflammation (n= 22)	p
AF positive NT-proBNP , n (%)	7/18 (38.9)	7 (31.8)	0.641
AF NT-proBNP (pg/ml) in positive NT-proBNP samples	7450 (1565.5-10570)	3086 (648.6-10300)	0.737
AF Troponin-I (pg/ml)	1438 (1058.4-2421.3)	1173.8 (927.3-2234.6)	0.945

Data are presented as number (percentage) for qualitative variables or median (25^{th} centile; 75^{th} centile) for quantitative variables.

FIGURES

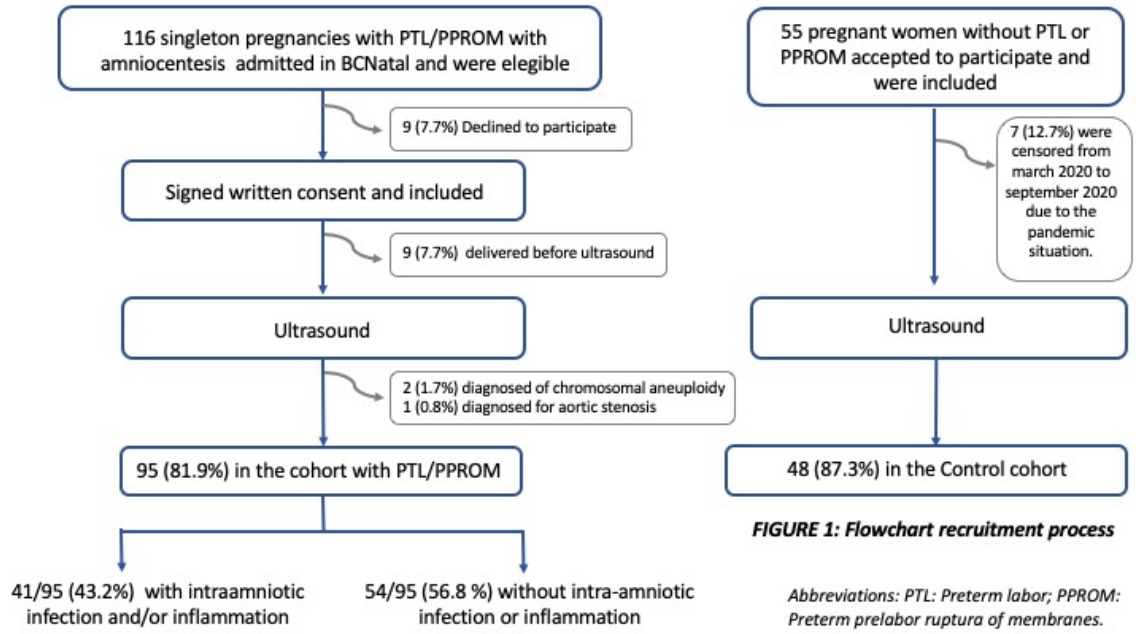


FIGURE 1: Flowchart recruitment process

Abbreviations: PTL: Preterm labor; PPROM: Preterm prelabor ruptura of membranes.

Figure 1

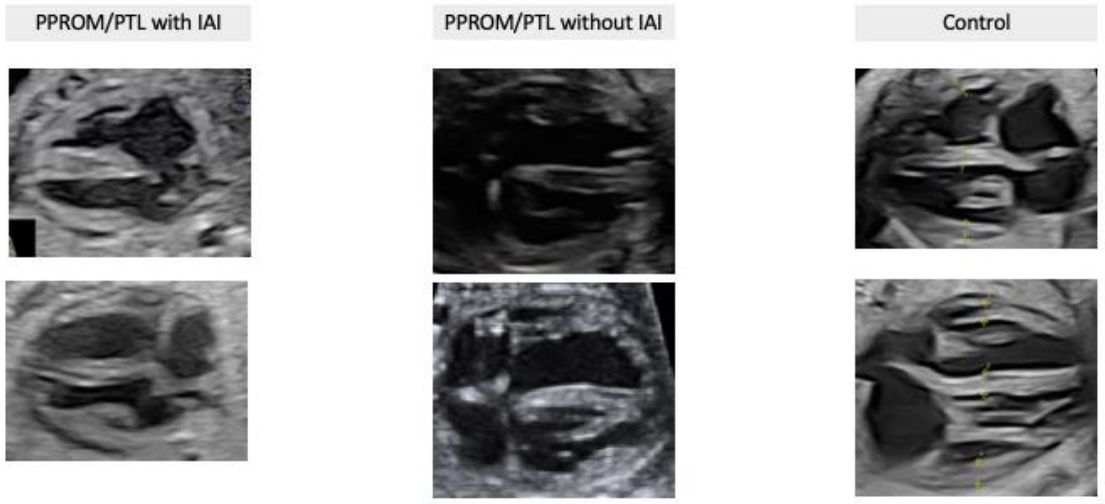


FIGURE 2: Heart morphology according study groups

Abbreviations: PTL: Preterm labor; PPROM: Preterm prelabor rupture of membranes; IAI: Intraamniotic infection and/or inflammation.
Group with PTL/PPROM with IAI: concentric cardiac hypertrophy (higher myocardium thickness but normal cardiac size).
Group with PTL/PPROM without IAI: Intermediate group with some degree of concentric cardiac hypertrophy.
Control group: Normal heart.

Figure 2

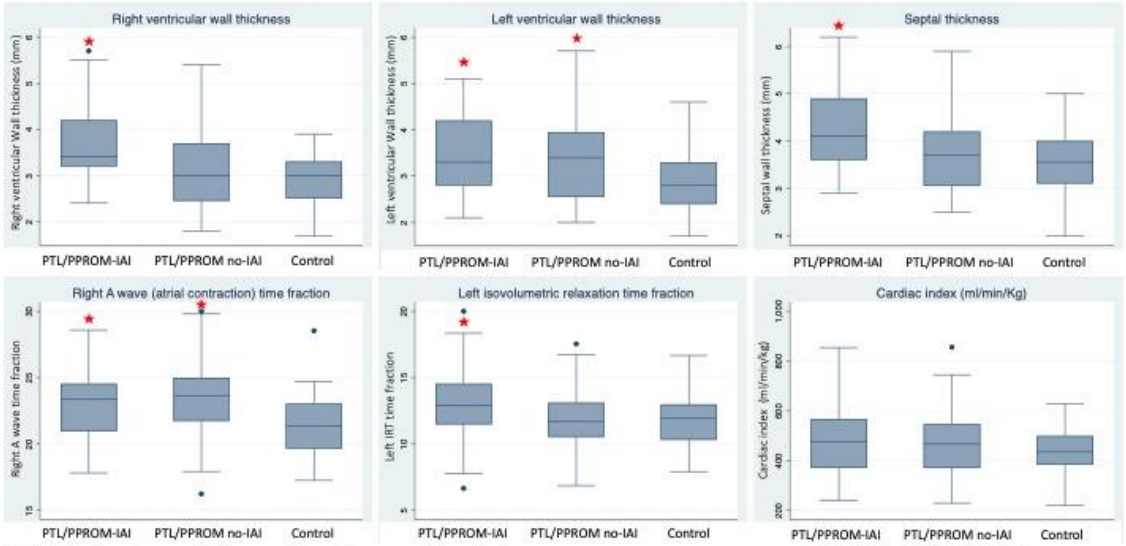
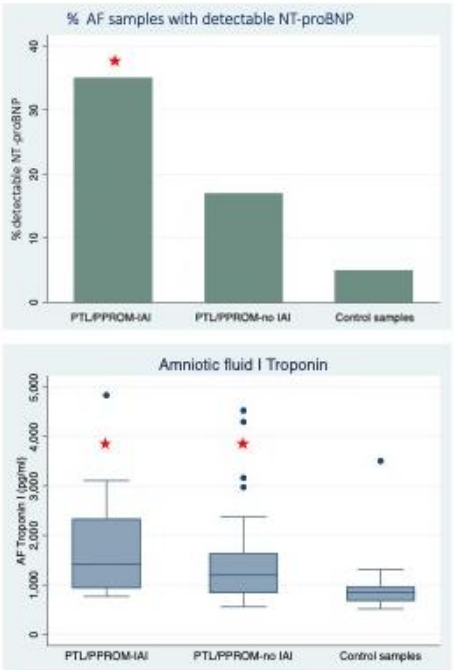


FIGURE 3: Heart morphology and function according study groups

Abbreviations: PTL: Preterm labor; PPROM: Preterm prelabor rupture of membranes; IAI: Intraamniotic infection and/or inflammation.
Right A time fraction: tricuspid A wave duration (ms) / total cycle duration (ms); **Left isovolumetric relaxation time (IRT)**: IRT (ms) / total cycle duration (ms))*100.
Cardiac index: (Right cardiac output (ml/min)+ left cardiac output (ml/min)) / Estimated fetal weight (Kg).

Figure 3



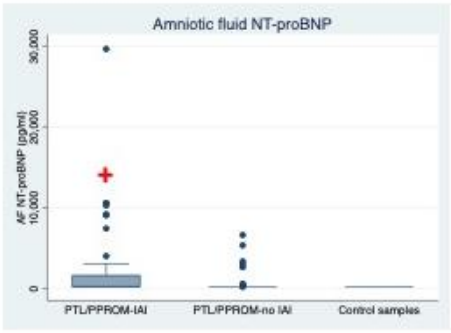


FIGURE 4: Amniotic fluid biomarkers

★ *p<0.05 compared to control samples*

✚ *p<0.05 compared to no-IAI group*

Abbreviations: AF: Amniotic fluid; PTL: Preterm labor; PPROM: Preterm prelabor rupture of membranes; IAI: Intraamniotic infection and/or inflammation.

Figure 4

GLOSSARY OF TERMS

AF: Amniotic fluid

EFW: Estimated fetal weight

IAI: Intra-amniotic infection and/or inflammation.

IL-6: Interleukin-6

NT-proBNP: N-terminal prohormone of brain natriuretic peptide

PPROM: Preterm prelabor rupture of membranes

PTL: Preterm labor

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CM, FC, TC, and MP contributed to the concept.

CM, OG, EG, FC, TC and MP contributed to the study design.

CM, CR, ML, DB, LG, JP, AH, SF and TC contributed to the inclusion of participants and data collection.

CM, VA, OG, EG, FC, TC, and MP contributed to data analysis and interpretation.

All authors contributed to the drafting and critical revision of the manuscript.