# Protocol Title:
# Automated fetal cardiac function evaluation in pregnancy affected by congenital heart disease

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## Study Summary

**Study title:** Automated Fetal Cardiac Function Parameters in Congenital Heart Diseases

**Synopsis:** Congenital heart disease (CHD) is the most commonly diagnosed fetal malformation, affecting between 0.8 and 1.2% of livebirths worldwide (1). As with any other fetal cardiac injury, CHD can result in initial cardiac remodelling and dysfunction and later cardiac failure and hydrops (2).

A small number of studies have looked at fetal cardiac function parameters in fetuses affected by CHD (3-19). These studies suggest the complementary role of fetal cardiac function evaluation to the classical morphological assessment. Fetal cardiac parameters could potentially increase the predictive value of the cardiovascular profile score (CPS) to detect hydrops and cardiac failure (20,21). However, a few methodological limitations impede drawing any definitive conclusions on this matter (3-6, 8-14, 16,19).

In the last decades fetal functional cardiac parameters such as myocardial performance index (MPI) and tricuspid/mitral annular plane systolic excursion (TAPSE/MAPSE) have been applied in research settings (3-19). The lack of standardisation and the need for improvement of their repeatability and ease of measurement are crucial factors limiting their clinical application (22,23). Automation of these parameters could be the next step to overcome such limitations (22-24).

**Objectives:** This study will provide evidence as to whether or not, in pregnancy affected by CHD, a significant difference in terms of automated fetal cardiac function parameters could be detected when compared to healthy pregnancies.

- Primary objective: to compare automated Pulse Wave Doppler (PWD) MPI and Spatio-Temporal Image Correlation (STIC) tricuspid/mitral/septal annular plane excursion (TAPSE, MAPSE and SAPSE) in fetuses affected by CHD versus healthy fetuses.
- Secondary objectives: to estimate whether these automated functional cardiac parameters could improve the predictive value of the cardiovascular profile score in case of hydrops.

**Study design:** An international multicentre prospective observational study. An Australian lead study carried out in seven teaching hospitals across Europe and in Sydney.

**Planned sample size:** Total n = 495 (CHD Group: n = 165; Control Group: n = 330 healthy pregnancies)

**Selection criteria:** Singleton pregnancies diagnosed with CHD between 19+6 and 36+6 gestational weeks; and healthy singleton pregnancies at 19+6 and 27+6 gestational weeks.

**Study procedure:** Recruited pregnant women (CHD and healthy pregnancies) will be offered one or two fetal cardiac function evaluations, depending on gestational age at recruitment, to evaluate automated fetal functional parameters in fetuses affected by CHD. In a second phase a nested case-control study will be carried out evaluating hydrops fetuses matched to no hydrops.

Each fetal cardiac exploration will include the evaluation of automated pulsed wave doppler (PWD) myocardial performance index (MPI), spatio-temporal image correlation (STIC) tricuspid/mitral/septal annular plane excursion (TAPSE, MAPSE and SAPSE), alongside cardiac morphometric assessment and doppler evaluation of flow across the atrioventricular, aortic and pulmonary valves.

**Statistical considerations:** Comparisons between cases and controls in baseline characteristics will be performed using two-sample t-tests, Wilcoxon rank-sum tests or Pearson Chi-squared tests, as appropriate.

Analyses of changes in fetal function parameters over time, and the comparison of these changes between cases and controls will employ generalised linear mixed models, as appropriate for the parameter.

Logistic regression will be used to estimate the association between fetal cardiac parameters and the incidence of hydrops. Receiver-operating-characteristics-curve analysis will be carried out to assess functional cardiac parameters compared to the routinely used cardiovascular profile score to predict cardiac failure in fetuses with congenital heart disease.

**Duration of the Study:** 5 years

# Contents

# Background

## Disease Background

Congenital heart disease is the most frequently diagnosed fetal malformation afflicting approximately 0.8% to 1.2% of live births worldwide (1). Generally congenital heart disease is defined as a structural abnormality of the heart and (or) great vessels that is present at birth (25).

Recent studies have shown that in the initial stages of an insult, the fetal heart usually manages to adapt, undergoing subclinical changes in order to maintain optimal and efficient function (2).

These changes can include genome expression, molecular, cellular and interstitial variations, defined as cardiac remodelling and manifested clinically as changes in size, shape and function of the heart after cardiac injury (26).

Medical imaging investigations have defined normal and abnormal patterns of cardiac remodelling leading to a better understanding of how congenital cardiac malformations alter the hemodynamic transition to the extrauterine environment (27).

In most clinical situations, fetal cardiac dysfunction is subclinical. However, if the insult is severe enough or persists, impaired cardiac pumping and myocardial relaxation can occur, leading to clinical cardiac failure, which in utero essentially manifests as fetal hydrops (Figure 1) (2).

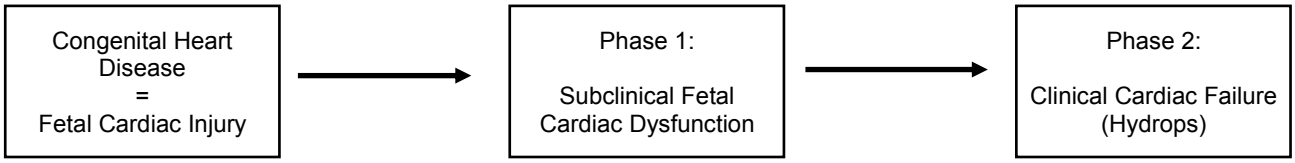


**Figure 1: Cardiac injury development**

## Rational for Performing the Study

Only a few studies have looked at the variation of cardiac functional parameters in fetuses affected by congenital heart diseases (3-19). These studies demonstrated that fetal cardiac function evaluation could be a valuable complement to morphological examination, since significant differences were identified between healthy and affected fetuses (3-6, 8-14, 16,19).

The limited number of studies and their methodological limitations (small sample size, retrospective design, heterogeneity of congenital heart diseases included etc.) impede the ability to draw definitive conclusions on this matter (3-19). This suggests that more evidence is needed to start employing routinely functional parameters to evaluate fetuses affected by congenital heart diseases.

During the last decades a heterogeneous group of cardiac function parameters and different ultrasound techniques have been used to evaluate fetal cardiac function. Previous studies on fetal cardiac function in congenital heart diseases evaluated mainly the value of morphometric measurements, the myocardial performance index (MPI) and/or tricuspid/mitral annular plane systolic excursion (TAPSE/MAPSE) carried out manually (3-19).

Myocardial performance index is a parameter capable of assessing overall cardiac function because it encompasses the systolic and diastolic components of the cardiac cycle and can be used in order to assess the two ventricles separately (28).

Atrioventricular (AV) plane displacement is often expressed as mitral annular plane systolic excursion (MAPSE), septal annular plane systolic excursion (SAPSE) and tricuspid annular plane systolic excursion (TAPSE) and it is a major contributor to cardiac pumping and a measure of longitudinal cardiac function.

Longitudinal cardiac function executed by longitudinal myocardial fibers is considered the first affected when hypoxia occurs (23).

The translational potential of fetal cardiac functional parameters is constrained by the lack of standardised methodology for demarcation of the time periods used in their calculation and the need for improvement of its repeatability and ease of measurement (22,23). Machine learning application to fetal cardiology and automation of the measurement may be considered as the next step towards such improvement leading to increase the applicability of fetal cardiac function parameters in standard clinical practice (22-24).

The term 'automation' refers to the act of taking measurements from Doppler waveforms. In conventional imaging, this is undertaken manually by the sonographer or sonologist, introducing a number of potential points for human error where cursor positions are chosen. In contrast, automation refers to the technique of using engineering techniques to select the correct point(s) for measurement of Doppler indices. This has been previously shown by our group to b technically possible and to result in perfectly repeatable interpretation of waveforms (ICC 1.0).

Furthermore, fetal cardiac parameters could potentially be of great value at identifying fetuses at risk of developing hydrops and cardiac failure (20,21). A cardiovascular profile score has been recognised as a valuable support to select fetuses at risk of developing hydrops (29-31).

As far as we are aware, the predictive value of automated cardiac parameters and their additional specificity and sensitivity at estimating the risk of hydrops and cardiac failure has not been previously explored. This could be of great value in improving the cardiovascular score profile predicting value of fetal cardiac failure.

# Study Objectives

The aim of the study is to determine the value of automated functional cardiac parameters in the evaluation of fetal congenital heart diseases.

In previous studies published by our group automated PWD-Mod MPI and STIC MAPSE/TAPSE were validated in healthy pregnancies (32,33). Automated PWD-MPI and STIC MAPSE and TAPSE have been shown to be the most useful indicators and most reliably achievable automated cardiac functional parameters in a normal fetal population by previous work by our research group (personal communication).

We now intend to apply these parameters to both healthy pregnancies and those affected by congenital heart diseases. We also wish to progress application of our offline engineering techniques to automation of measurement of other Doppler indices.

## Primary Objective

Our primary objective is to evaluate automated PWD-MPI and STIC TAPSE SAPSE and MAPSE comparing fetuses affected by congenital heart disease (CHD) to reference values across the fetal healthy population. More specifically the aim is to estimate whether there is a significant difference in each parameter between fetuses with CHD overall compared to healthy fetuses and then by subgroups of different CHDs. Furthermore, the analysis will be looking at the variation of these parameters over time comparing healthy fetuses to CHD overall and by subgroups.

## Secondary Objective

Our secondary objective is to estimate whether automated functional cardiac parameters could improve the predictive value of cardiovascular profile score in case of hydrops; facilitating earlier or optimised intervention in the form of elective delivery.

### Expected outcomes of the study

The expected outcome of the study would be to demonstrate a significant difference in terms of fetal cardiac dysfunction between the two groups. It is anticipated that the standardisation provided by automation of measurement will make this difference easier to detect.

If this is shown to be correct, the automated parameters could be used routinely in fetal cardiac evaluation of fetuses affected by congenital heart disease. This could inform and support further research to evaluate the predictive value of these parameters to help identify fetuses at higher risk of developing incipient hydrops/heart failure at need of immediate further interventions.

## Study design

### Design

An international multicentre prospective observational study will be carried out in seven Teaching Hospitals in Europe and Australia.

### Study Groups

A cohort of pregnant women whose fetus is detected and diagnosed with an isolated cardiac anomaly will be approached and recruited during an anomaly scan carried out up to 36+6 weeks gestation (***CHD Group***). A corresponding cohort of pregnant women without any congenital heart diseases will be recruited during the second trimester anomaly scan and up to 27+6 weeks of gestation (***Control Group***). Patients will be offered to participate in the study after inclusion/exclusion criteria verification. Informed consent will be discussed and signed with the researcher in each centre.

### Number of Participants

CHD Group: n = 165. Control Group: n = 330 healthy pregnancies.

### Number of Centres

This will be an Australian SESLHD/UNSW lead study with international centre participants assisting in data collection. Each study site will perform the same procedures as internationally published literature (34-57) to ensure uniformity. The following is the list of centres and expected number of participants:

1. Royal Hospital for Women, Randwick, NSW (Lead Centre); CHD Group: 20; Control Group: 40
2. Centre Hospitalier de Mayotte, Mayotte, France; CHD Group: 25; Control Group: 50
3. Department of Obstetrics and Gynaecology, Buzzi Children's Hospital, University Department of Clinical Sciences, University of Milan (Milan, Italy); CHD Group: 35; Control Group: 70
4. Department of Life, Health and Environmental Sciences, University of L'Aquila, Obstetrics & Gynaecology Unit, San Salvatore Hospital (Aquila, Italy); CHD Group: 10; Control Group: 20
5. Maternal and Child Health Institute - IRCCS Burlo Garofolo, Department of Medicine, Surgery and Health Science, University of Trieste (Trieste, Italy); CHD Group: 20; Control Group: 40
6. Sheba Medical Center (Tel Aviv, Israel); CHD Group: 20; Control Group: 40
7. Fetal Cardiology Unit, Medical Center, Ujastek, Cracow, Department of Pediatric Cardiology, Institute of Paediatrics, Faculty of Medicine, Jagiellonian University Medical College (Cracow, Poland); CHD Group: 35; Control Group: 70.

Ethics approval for each international site will be applied for separately in collaboration with the lead researcher from each participating centre.

### Duration

We estimate the duration to be a total of five years. Considering the sample size calculation and the incidence of cases in the different participating centres, we estimate the recruitment phase of the project will be 2-3 years. With data collection, analysis, reporting and follow up to an additional two years.

## Participant Selection

### Recruitment and Screening

Pregnant women will be recruited from each participating centre during their second or third trimester morphology scan.

They will be approached by a member of the research team (Dr Erenbourg) and not by their treating doctor.

The Control Group will be mostly recruited at the time of the second trimester morphology scan; the CHD Group during a second/third trimester anomaly scan with collaboration and referral from the hospital fetal cardiologist.

Healthy patients will be approached in the waiting room after their second trimester ultrasound scan, offered to participate and given a copy of a patient information sheet and consent form. CHD patients will be approached and offered to participate after consultation with perinatal cardiologists. A week after the first contact, we will phone patients to verify their intention to take part and to organize the fetal cardiac function follow up. Flyers will be placed at the hospitals and further pregnant women interested in participating in the study can contact the Research Investigators for further information.

After checking for exclusion and inclusion criteria, patients will be invited to participate accordingly. If women wish to take part, the consent form will be read, discussed and signed with the local researcher.

### Inclusion Criteria

***Inclusion criteria for the CHD Group are as follows:*** singleton pregnancies; gestational age between 19+6 and 36+6 weeks gestation, determined by the last menstrual period and confirmed by first trimester ultrasound; isolated congenital cardiac anomaly diagnosed.

***Inclusion criteria for the Control Group are as follows:*** singleton pregnancies; gestational age between 19+6 and 27+6 weeks gestation, determined by the last menstrual period and confirmed by first trimester ultrasound; no congenital cardiac anomaly diagnosed.

### Exclusion Criteria

***Exclusion criteria for both groups are as follows:*** fetuses whose mothers have comorbidities that have been proven to potentially affect cardiac function including intrahepatic cholestastis (34), pre-gestational and gestational diabetes (58-61) and preeclampsia (62-64); growth restricted fetuses defined as estimated fetal weight or abdominal circumference <3rd percentile for GA (63, 65-69); fetuses with other structural extracardiac anomalies at ultrasound examination; fetuses affected by any diagnosed genetic abnormalities.

In a second phase a nested case-control study will be carried out evaluating hydropic fetuses matched to no hydrops.

## Study Outline

### Investigation Plan

Participating patients will be offered a fetal cardiac function echocardiography between 27+6 and 29+6 weeks gestation and another between 34+6 and 36+6 gestational weeks (Figure 2). If a patient from the

congenital heart disease cohort is recruited after 29+6 weeks gestation, a fetal cardiac function echocardiography evaluation between 34+6 and 36+6 weeks gestation will be offered.

At the stage patients will come in to carry out fetal cardiac function echocardiography they will already know whether the baby is affected by congenital heart disease since malformation screening is carried out between 18 and 20 gestational weeks and will have already been undertaken. In the remote case of detecting an undiagnosed fetal malformation during one of the research scans, patients will be reassured, and appointments arranged at MFM clinic for further consultation along with notification of to their own treating doctor. Dr Erenbourg is a fetal medicine practitioner and Professor Welsh is Professor of Maternal-Fetal Medicine so in a number of cases immediate counselling will be available.

Every participant will be assigned a study number following recruiting order of participation. De-identification will be undertaken at image acquisition by the RHW doctors prior to image review by the engineers. Researchers will access an online patients' form system, collect the assigned patients' number and add the necessary outcomes information. Ultrasound images will be saved locally in the ultrasound machine by the same assigned patient's study number and uploaded to a specific onedrive folder created in Sydney.

The research ultrasound scans will be performed by a trained sonographer using either a clinical ultrasound system or a research ultrasound system (Verasonics Vantage 256 Ultrasound System: VVS) that is TGA approved for research imaging. The machine used clinically is made by one of the common commercial manufacturers (in this case General Electric E10) and is an identical model to that used in the Department of Matenal-Fetal Medicine. The machine used for research use that we intend to use is manufactured by a company called Verasonics and the machine is Vantage 256. It uses the same fundamental electronic circuitry and transducer design as conventional commercial machines, and in fact uses the same transducers as the commercial machines. However, the way that the ultrasound is delivered differs. Instead of transmitting beams as used in conventional ultrasound machines, Verasonics scans the area of interest by unfocused waves. The latest allows high quality images with a limited number of compounded plane-waves, and reduced acquisition time (70,71). There is no difference in the common indices used to monitor machine output (the Thermal and Mechanical Indices) from commercial machines. The nature of the Verasonics machine means that we have secured TGA approval to use this as a research tool and not one to be used for clinical diagnosis. This is the source of the definition of 'machine approved for research use'. Should any concerns arise from a diagnostic perspective during an ultrasound scan, then any images to be used for management purposes will be acquired using the clinical (GE E10) machine. Any information generated from the VVS will only be used for research and never for diagnosis or clinical tests. If any abnormalities are found while scanning with the VVS, a confirmation scan will be done using our clinical ultrasound machine.

Women will be placed in a semi-recumbent position, as standard for pregnancy ultrasounds. After routine biometry, the research fetal cardiac function ultrasound will be carried out.

Each fetal cardiac function examination will include the following parameters:

1. Fetal biometric parameters (biparietal diameter, head circumference, abdominal circumference, femur diaphysis length)
2. Standard fetal Doppler parameters (umbilical artery, medial cerebral artery, ductus venosus)
3. Fetal cardiac heart rate
4. Presence of pericardial effusion or hydrops
5. Cardiac morphometry - all measurements carried out at the end of diastole, with the exception of atrial dimensions measured in systole (at their maximum extension)
    - Heart/thorax area measurement
    - 4 heart chambers measurements (apical/basal 4-chambers view in 2D)

- Atrial and ventricular areas (apical/basal 4-chambers view in 2D)
- Ventricular and atrial sphericity calculation
- Inter-ventricular septum and myocardial walls thickness measurement (transversal 4-chambers view in 2D or M-mode)

6. Cardiac contractility
   - STIC M-Mode stroke volume, ejection fraction and shortening fraction
   - Automated STIC MAPSE, TAPSE, SAPSE
   - Automated PWD Left and Right Mod-MPI
7. AV valves function evaluation
   - Cine-loop evaluation of correct opening and closing
   - Anterograde Colour Doppler without regurgitation
   - Pulsed Doppler evaluation of flow velocity (monophasic or biphasic)
   - Left and right E/A ratio calculation
   - If any regurgitation: peak velocity and duration quantified
8. Aorta outflow evaluation
   - Aorta artery measurement (at the level of valvular ring in systole)
   - Aortic flow evaluation (Colour Doppler evaluation of systolic peak velocity)
9. Pulmonary outflow evaluation
   - Pulmonary artery measurement (at the level of valvular ring in systole)
   - Pulmonary flow evaluation (Colour Doppler evaluation of systolic peak velocity)
10. V-sign evaluation
    - Confirmation of anterograde flow in the entire length of the arteries
    - Pulsatility index of aortic isthmus and ductus arteriosus

All fetal morphometric and functional cardiac parameters will be normalised to Z-score by gestational age where possible. Fetal cardiac volumes and 2D images with inadequate quality due to fetal movements, presence of acoustic shadows of fetal ribs or spine, and maternal breathing will be excluded.

If hydrops develops, cardiovascular profile score will be added to the routine cardiac function exploration as shown in Table 1.

The study population will be followed up until delivery and discharge of both mother and neonate.

Patients' information will be collected anonymously. Each patient's history will be evaluated and information about previous pregnancies (maternal or fetal diseases during pregnancy) and outcomes (type of delivery, maternal and neonatal conditions at birth, long-term outcome of the pregnancy) will be collected. Furthermore, we plan to collect information about the current pregnancy (maternal and fetal observations during pregnancy) and outcomes (type of delivery, maternal and neonatal conditions at birth and up to hospital discharge of both).

### Study Procedure Risks

There is no increased risk related to participating in this study. The study uses conventional ultrasound machinery as used in routine fetal evaluation, with no alteration in power output (as defined by Thermal Index or Mechanical Index). The VVS research ultrasound system is also comparable to conventional ultrasounds in terms of risks.

We anticipate approximately 20-30 minutes scan duration for acquisition of the necessary research data which is in keeping with standard ultrasound examinations, and the ALARA (As Low As Reasonably Achievable) principle conventionally applied in fetal imaging (73).

**Table 1: The cardiovascular profile score (72)**

| Category | 2 points | 1 point | 0 point |
|---|---|---|---|
| **Hydrops** | None | Ascites or pericardial or pleural effusion | Skin oedema |
| **Heart size (HA/CA)** | > 0.2 and ≤ 0.35 | 0.35 - 0.5 | < 0.2 or > 0.5 |
| **Cardiac function** | Normal MV and TV, biphasic diastolic filling, LV or RV SFs > 0.28 | Holosystolic TR or LV or RV SFs < 0.28 | Holosystolic MR or TR dP/dt < 400, monophasic diastolic filling |
| **Arterial Umbilical Doppler** | Normal | AEDV | REDV |
| **Venous Doppler** | Normal | DV atrial reversal | UV pulsations |

AEDV, absent end-diastolic velocity; DV, ductus venosus; HA/CA, heart to chest area ratio; LV, left ventricle; MR, mitral valve; REDV, reversed end-diastolic velocity; RV, right ventricle; SF, ventricular shortening fraction; TR dP/dt, change in pressure over time of TR jet; TR, tricuspid valve regurgitation; TV, tricuspid valve; UV, umbilical vein

## Sample Size Calculation

The primary outcome of the study is the difference in the mean of automated fetal functional cardiac parameters between CHD cases and controls. This will be analysed using a two-sample t-test.

To estimate our sample size, we used the most commonly applied fetal functional parameter, specifically the left ventricle MPI (LV-MPI) as a proxy of all the automated fetal cardiac parameters.

Due to the rarity of congenital heart disease, we performed the sample size calculation based on recruiting two controls for each case. Pooled across cases with isolated pulmonary valve stenosis (n = 16) and controls (n = 48), Guirado et al (2018) observed a standard deviation of 0.098 in LV-MPI measurements (4). Using this observed pooled SD, we require a total sample of 381 pregnancies (127 CHD + 254 controls) with completed measurements to achieve at least 80% power to detect a difference of 0.03 in mean LV-MPI (4), with a two-sided type I error rate of 5%.

We acknowledge that some pilot data may be required to evaluate the limited number of pathological cases and therefore some approximations are necessary e.g. for standard deviation within the population. For this reason, we have aimed to recruit a larger number of participants (approximately 30%), 165 CHD and 330 Controls, allowing also for some patient exclusions due to patient drop out, difficulties in scanning due to fetal movements etc, and incomplete data sets.

The aim would be to recruit sufficient cases to be able to estimate if there is significant difference in terms of fetal cardiac function parameters between affected and not affected fetuses to inform further research.

## Data Analysis Plan

Raw (radio-frequency) ultrasound data generated using the VVS will allow the researchers to analyse the signal/image processing that takes place prior to display, enabling refinement of this imaging technique.

Image analysis will be carried out first manually through optical evaluation and then through the use of mathematical algorithms which will recognise and analyse only high-quality images. This could be a limitation because automatically only high-quality images will be included (which is not representative of real clinical work) but also guarantees that parameters are collected only from almost perfect research material (showing true differences if they exist).

Ultrasound images will be analysed and cardiac function parameters interpreted by SESLHD/UNSW researchers based at the Royal Hospital for Women, Randwick. Images will be analysed by a team of fetal medicine doctors at Royal Hospital for Women and UNSW engineers (led by Professor Tracie Barber) to assure that algorithms are correctly applied to calculate fetal cardiac function parameters.

Comparisons of interest between cases and controls in baseline characteristics will be performed using two-sample t-tests, Wilcoxon rank-sum tests or Pearson Chi-squared tests, as appropriate.

Secondary outcomes comparing cases and controls at a single time point will be analysed in a similar way to the primary outcome, subject to checks of assumptions. Analyses of changes in fetal function parameters over time (i.e. between the 27+6-29+6 and 34+6-36+6 week scans), and the comparison of these changes between cases and controls will employ generalised linear mixed models, as appropriate for the parameter.

Logistic regression will be used to estimate the association between fetal cardiac parameters and the incidence of hydrops. Receiver-operating-characteristics-curve analysis will be carried out to assess functional cardiac parameters compared to the routinely used cardiovascular profile score to predict cardiac failure in fetuses with congenital heart disease.

Statistical analysis will be performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA).

For those cases without complete data acquisition (i.e. intending but not undertaking a second scan), analysis will take place for only the isolated value and not for any temporal change. Their single gestational data set of ultrasound measurements will still be included in analysis but excluded from any analysis of sequential change.

# Safety

## Adverse Event Reporting

Any potential breach of privacy will be guarded against by de-identification of data following study completion and storage of utilised data in either password-protected electronic databases and/or locked filing cabinet within the Perinatal Imaging Research Group office at RHW.

Should there be any concerns or unexpected findings during the research scanning, the patient's usual antenatal carer will be contacted and appropriate follow-up care will be organised by them.

## Serious adverse event reporting

No serious adverse events are anticipated but should they occur they will be reported through the primary investigator, Professor Welsh and the obstetrical team based at the Royal Hospital for Women.

## Data Safety and Monitoring Board

To assure high quality data collection, images will be collected at each participating centre by experienced fetal medicine doctors who have practiced fetal medicine for over 10 years. At RHW this will be Dr Anna Erenbourg. Each image will be stored securely. Data will be anonymised and monitored by researchers at SESLHD/UNSW. Researchers at UNSW will also review revise and complete data collection in case of missing data.

Professor Alec Welsh as principal investigator will supervise and guarantee the quality of the data. Dr Anna Erenbourg will work both as international coordinator and as site researcher at RHW with this work being undertaken for her PhD thesis through UNSW.

Each participating centre will have a site researcher, a fetal cardiologist who will discuss and sign consent form, collect ultrasound images and relevant patients' data. The data collected will be uploaded and securely stored onto the UNSW platform, Dr Anna Erenbourg will be responsible for images analysis, data analysis and publication drafting supervised by Professor Welsh and Professor Barber.

Anna Erenbourg will propose the study to patients, discuss and sign the consent form and collect data in Sydney. Once ethical approval for each overseas center will be granted, Anna Erenbourg (under the supervision of Alec Welsh and Tracie Barber) will download data from all centers, merge the information in a unique database and analyze them with the help of a statistician at UNSW. All the participants will then collaborate in drafting manuscripts for publication.

Outcome data will be accessed only by authorised researchers using an encrypted code for data protection. Final drafts for publication will be reviewed by all the authors from each research site.

### Dissemination of results and publication policy

Results of the study will be published in peer-reviewed scientific journals, presentations at conferences or other professional forums. In any publication, patient privacy will be protected and presented in a de-identified manner.

## Storage and Archiving of Study Documents

Patients' data and ultrasound images will be collected at each participating centre and safely secured using a UNSW Research Data Management Plan and the UNSW ResToolKit System for an online data management using UNSW servers. Patient privacy will be protected by deidentifying data once initial data abstraction has occurred, and through storage of data in password-protected computers and servers. Identified data will only be accessible to Professor Welsh and Dr Erenbourg. This data will be stored for a minimum of 5 years after publication, then the data record will be securely destroyed in accordance with the UNSW Records Disposal processes.

## Study Flow Chart

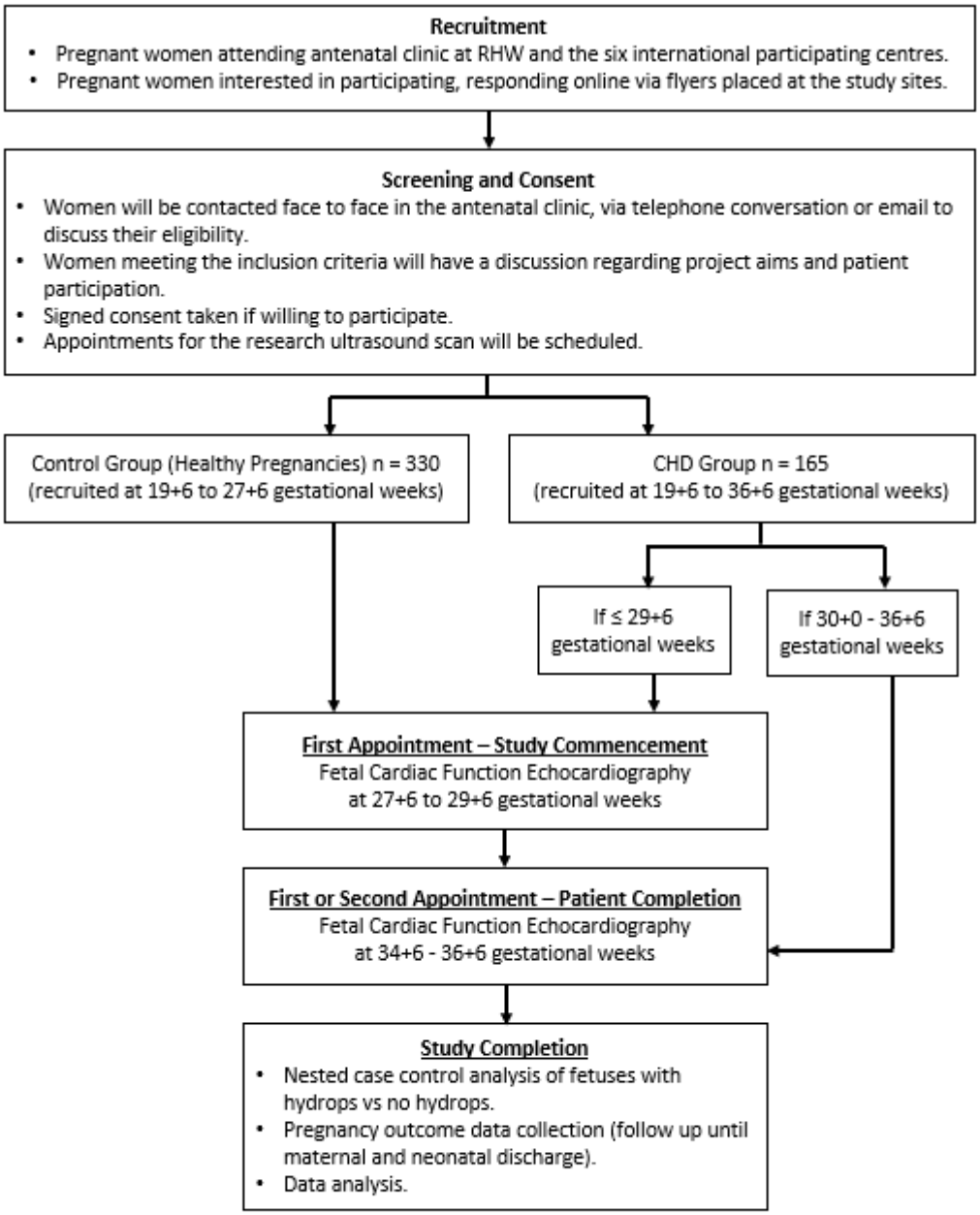


**Figure 2: Patient Recruitment and Study Flow Chart**

# References


1. Wu W, He J, Shao X. Incidence and mortality trend of congenital heart disease at the global, regional, and national level, 1990–2017. Medicine 2020;99:23 (e20593).
2. Crispi F, Sepúlveda-Martínez Á, Crovetto F, Gómez O, Bijnens B, Gratacós E. Main Patterns of Fetal Cardiac Remodeling. Fetal Diagn Ther. 2020;47(5):337-344. doi: 10.1159/000506047. Epub 2020 Mar 26. PMID: 32213773.
3. Walter C, Soveral I, Bartrons J, Escobar MC, Carretero JM, Quirado L, Gómez O, Sánchez-de-Toledo J. Comprehensive Functional Echocardiographic Assessment of Transposition of the Great Arteries: From Fetus to Newborn. Pediatr Cardiol. 2020 Apr;41(4):687-694. doi: 10.1007/s00246-019-02279-w. Epub 2020 Jan 10. PMID: 31919591.
4. Guirado L, Crispi F, Masoller N, Bennasar M, Marimon E, Carretero J, Gratacós E, Martínez JM, Friedberg MK, Gómez O. Biventricular impact of mild to moderate fetal pulmonary valve stenosis. Ultrasound Obstet Gynecol. 2018 Mar;51(3):349-356. doi: 10.1002/uog.17456. PMID: 28295792.
5. Inamura N, Taketazu M, Smallhorn JF, Hornberger LK. Left ventricular myocardial performance in the fetus with severe tricuspid valve disease and tricuspid insufficiency. Am J Perinatol. 2005 Feb;22(2):91-7. doi: 10.1055/s-2005-837739. PMID: 15731988.
6. Lasa JJ, Tian ZY, Guo R, Rychik J. Perinatal course of Ebstein's anomaly and tricuspid valve dysplasia in the fetus. Prenat Diagn. 2012 Mar;32(3):245-51. doi: 10.1002/pd.2939. PMID: 22430722.
7. Wohlmuth C, Wertaschnigg D, Wieser I, Arzt W, Tulzer G. Tissue Doppler imaging in fetuses with aortic stenosis and evolving hypoplastic left heart syndrome before and after fetal aortic valvuloplasty. Ultrasound Obstet Gynecol. 2016 May;47(5):608-15. doi: 10.1002/uog.14885. Epub 2016 Apr 17. PMID: 25914144.
8. Chen J, Xie L, Dai L, Yu L, Liu L, Zhou Y, Wu G, Qin F, Liu H. Right Heart Function of Fetuses and Infants with Large Ventricular Septal Defect: A Longitudinal Case-Control Study. Pediatr Cardiol. 2016 Dec;37(8):1488-1497. doi: 10.1007/s00246-016-1462-z. Epub 2016 Aug 25. PMID: 27562129.
9. Natarajan S, Szwast A, Tian Z, McCann M, Soffer D, Rychik J. Right ventricular mechanics in the fetus with hypoplastic left heart syndrome. J Am Soc Echocardiogr. 2013 May;26(5):515-20. doi: 10.1016/j.echo.2013.02.001. Epub 2013 Mar 6. PMID: 23473605.
10. Nawaytou HM, Peyvandi S, Brook MM, Silverman N, Moon-Grady AJ. Right Ventricular Systolic-to-Diastolic Time Index: Hypoplastic Left Heart Fetuses Differ Significantly from Normal Fetuses. J Am Soc Echocardiogr. 2016 Feb;29(2):143-9. doi: 10.1016/j.echo.2015.08.014. Epub 2015 Sep 26. PMID: 26394829.
11. Chen Y, Lv G, Li B, Wang Z. Cerebral vascular resistance and left ventricular myocardial performance in fetuses with Ebstein's anomaly. Am J Perinatol. 2009 Apr;26(4):253-8. doi: 10.1055/s-0028-1103152. Epub 2008 Nov 20. PMID: 19023852.
12. Khandoker AH, Al-Angari HM, Marzbanrad F, Kimura Y. Investigating fetal myocardial function in heart anomalies by Doppler myocardial performance indices. Annu Int Conf IEEE Eng Med Biol Soc. 2017 Jul;2017:2197-2200. doi: 10.1109/EMBC.2017.8037290. PMID: 29060332.
13. Axt-Fliedner R, Graupner O, Kawecki A, Degenhardt J, Herrmann J, Tenzer A, Doelle A, Willruth A, Steinhard J, Gembruch U, Bahlmann F, Enzensberger C; Fetal Cardiac Imaging Research Group, Germany. Evaluation of right ventricular function in fetuses with hypoplastic left heart syndrome using tissue Doppler techniques. Ultrasound Obstet Gynecol. 2015 Jun;45(6):670-7. doi: 10.1002/uog.14736. Epub 2015 May 11. PMID: 25418127.
14. Graupner O, Enzensberger C, Wieg L, Willruth A, Steinhard J, Gembruch U, Doelle A, Bahlmann F, Kawecki A, Degenhardt J, Wolter A, Herrmann J, Axt-Fliedner R; Fetal Cardiac Imaging Research Group Germany. Evaluation of right ventricular function in fetal hypoplastic left heart syndrome by color tissue

Doppler imaging. Ultrasound Obstet Gynecol. 2016 Jun;47(6):732-8. doi: 10.1002/uog.14940. PMID: 26138790.
15. Pedra SR, Hornberger LK, Leal SM, Taylor GP, Smallhorn JF. Cardiac function assessment in patients with family history of nonhypertrophic cardiomyopathy: a prenatal and postnatal study. Pediatr Cardiol. 2005 Sep-Oct;26(5):543-52. doi: 10.1007/s00246-004-0688-3. PMID: 16132314.
16. Clur SB, Vink AS, Etheridge SP, Robles de Medina PG, Rydberg A, Ackerman MJ, Wilde AA, Blom NA, Benson DW, Herberg U, Donofrio MT, Cuneo BF. Left Ventricular Isovolumetric Relaxation Time Is Prolonged in Fetal Long-QT Syndrome. Circ Arrhythm Electrophysiol. 2018 Apr;11(4):e005797. doi: 10.1161/CIRCEP.117.005797. PMID: 29654130.
17. Yozgat Y, Kilic A, Ozdemir R, Karadeniz C, Kucuk M, Karaarslan U, Mese T, Unal N. Modified myocardial performance index is not affected in fetuses with an isolated echogenic focus in the left ventricle. J Matern Fetal Neonatal Med. 2015 Feb;28(3):333-7. doi: 10.3109/14767058.2014.916679. Epub 2014 May 22. PMID: 24749803.
18. Acharya G, Pavlovic M, Ewing L, Nollmann D, Leshko J, Huhta JC. Comparison between pulsed-wave Doppler- and tissue Doppler-derived Tei indices in fetuses with and without congenital heart disease. Ultrasound Obstet Gynecol. 2008 Apr;31(4):406-11. doi: 10.1002/uog.5292. PMID: 18340627.
19. Patey O, Carvalho JS, Thilaganathan B. Urgent neonatal balloon atrial septostomy in simple transposition of the great arteries: predictive value of fetal cardiac parameters. Ultrasound Obstet Gynecol. 2021 May;57(5):756-768. doi: 10.1002/uog.22164. PMID: 32730671.
20. Crispi F, Valenzuela-Alcaraz B, Cruz-Lemini M, Gratacós E. Ultrasound assessment of fetal cardiac function. Australas J Ultrasound Med. 2013 Nov;16(4):158-167. doi: 10.1002/j.2205-0140.2013.tb00242.x. Epub 2015 Dec 31. PMID: 28191192; PMCID: PMC5030052.
21. Acharya G, Archer N, Huhta JC. Functional assessment of the evolution of congenital heart disease in utero. Curr Opin Pediatr. 2007 Oct;19(5):533-7. doi: 10.1097/MOP.0b013e3282efd2a2. PMID: 17885470.
22. Maheshwari P, Henry A, Welsh AW. The Fetal Modified Myocardial Performance Index: Is Automation the Future? Biomed Res Int. 2015;2015:215910. doi: 10.1155/2015/215910. Epub 2015 Jun 22. PMID: 26185751; PMCID: PMC4491561.
23. Herling L, Johnson J, Ferm-Widlund K, Zamprakou A, Westgren M, Acharya G. Automated quantitative evaluation of fetal atrioventricular annular plane systolic excursion. Ultrasound Obstet Gynecol. 2021 Dec;58(6):853-863. doi: 10.1002/uog.23703. PMID: 34096674.
24. Garcia-Canadilla P, Sanchez-Martinez S, Crispi F, Bijnens B. Machine Learning in Fetal Cardiology: What to Expect. Fetal Diagn Ther. 2020;47(5):363-372. doi: 10.1159/000505021. Epub 2020 Jan 7. PMID: 31910421.
25. Liu Y, Chen S, Zuhlke L, et al. Global birth prevalence of congenital heart defects 1970-2017: updated systematic review and meta-analysis of 260 studies. Int J Epidemiol 2019;48:455–63.
26. Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling--concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an International Forum on Cardiac Remodeling. J Am Coll Cardiol. 2000 Mar 1;35(3):569-82. doi: 10.1016/s0735-1097(99)00630-0. PMID: 10716457.
27. Tan CMJ, Lewandowski AJ. The Transitional Heart: From Early Embryonic and Fetal Development to Neonatal Life. Fetal Diagn Ther. 2020;47(5):373-386. doi: 10.1159/000501906. Epub 2019 Sep 18. PMID: 31533099; PMCID: PMC7265763.
28. Peixoto AB, Bravo-Valenzuela NJ, Rocha LA, Araujo Júnior E. Spectral Doppler, tissue Doppler, and speckle-tracking echocardiography for the evaluation of fetal cardiac function: an update. Radiol Bras. 2021 Mar-Apr;54(2):99-106. doi: 10.1590/0100-3984.2020.0052. PMID: 33854264; PMCID:

PMC8029930.Araujo Junior E, Bravo-Valenzuela NJ, Peixoto AB. Perinatal Cardiology Part 1&2. Bentham Science Publishers. Singapore 2020.
29. Donofrio MT, Moon-Grady AJ, Hornberger LK, Copel JA, Sklansky MS, Abuhamad A, Cuneo BF, Huhta JC, Jonas RA, Krishnan A, Lacey S, Lee W, Michelfelder EC Sr, Rempel GR, Silverman NH, Spray TL, Strasburger JF, Tworetzky W, Rychik J; American Heart Association Adults With Congenital Heart Disease Joint Committee of the Council on Cardiovascular Disease in the Young and Council on Clinical Cardiology, Council on Cardiovascular Surgery and Anesthesia, and Council on Cardiovascular and Stroke Nursing. Diagnosis and treatment of fetal cardiac disease: a scientific statement from the American Heart Association. Circulation. 2014 May 27;129(21):2183-242. doi: 10.1161/01.cir.0000437597.44550.5d. Epub 2014 Apr 24. Erratum in: Circulation. 2014 May 27;129(21):e512. PMID: 24763516.
30. Wieczorek A, Hernandez-Robles J, Ewing L, Leshko J, Luther S, Huhta J. Prediction of outcome of fetal congenital heart disease using a cardiovascular profile score. Ultrasound Obstet Gynecol 2008; 31 (3): 284–88.
31. Huhta JC. Diagnosis and treatment of foetal heart failure: foetal echocardiography and foetal hydrops. Cardiol Young. 2015 Aug;25 Suppl 2:100-6. doi: 10.1017/S104795111500089X. PMID: 26377716.
32. Welsh AW, Maheshwari P, Wang J, Henry A, Chang D, Crispi F, Gardiner HM, Hernandez-Andrade E, Meriki N, Redmond S, Yagel S. Evaluation of an automated fetal myocardial performance index. Ultrasound Obstet Gynecol. 2016 Oct;48(4):496-503. doi: 10.1002/uog.15770. Epub 2016 Sep 13. PMID: 26423314.
33. Philip, M.E., Sowmya, A., Avnet, H., Ferreira, A., Stevenson, G.N., & Welsh, A.W. (2019). Convolutional Neural Networks for Automated Fetal Cardiac Assessment using 4D B-Mode Ultrasound. 2019 IEEE 16th International Symposium on Biomedical Imaging (ISBI 2019), 824-828.
34. Salomon LJ, Alfirevic Z, Berghella V, Bilardo C, Hernandez-Andrade E, Johnsen SL, Kalache K, Leung KY, Malinger G, Munoz H, Prefumo F, Toi A, Lee W; ISUOG Clinical Standards Committee. Practice guidelines for performance of the routine mid-trimester fetal ultrasound scan. Ultrasound Obstet Gynecol. 2011 Jan;37(1):116-26. doi: 10.1002/uog.8831. PMID: 20842655.
35. Bhide A, Acharya G, Baschat A, Bilardo CM, Brezinka C, Cafici D, Ebbing C, Hernandez-Andrade E, Kalache K, Kingdom J, Kiserud T, Kumar S, Lee W, Lees C, Leung KY, Malinger G, Mari G, Prefumo F, Sepulveda W, Trudinger B. ISUOG Practice Guidelines (updated): use of Doppler velocimetry in obstetrics. Ultrasound Obstet Gynecol. 2021 Aug;58(2):331-339. doi: 10.1002/uog.23698. Epub 2021 Jul 19. PMID: 34278615.
36. García-Otero L, Soveral I, Sepúlveda-Martínez Á, Rodriguez-López M, Torres X, Guirado L, Nogué L, Valenzuela-Alcaraz B, Martínez JM, Gratacós E, Gómez O, Crispi F. Reference ranges for fetal cardiac, ventricular and atrial relative size, sphericity, ventricular dominance, wall asymmetry and relative wall thickness from 18 to 41 gestational weeks. Ultrasound Obstet Gynecol. 2021 Sep;58(3):388-397. doi: 10.1002/uog.23127. Erratum in: Ultrasound Obstet Gynecol. 2021 Nov;58(5):788. PMID: 32959925.
37. Bravo-Valenzuela NJ, Peixoto AB, Carrilho MC, Siqueira Pontes AL, Chagas CC, Simioni C, Araujo Júnior E. Fetal cardiac function by three-dimensional ultrasound using 4D-STIC and VOCAL - an update. J Ultrason. 2019 Dec;19(79):287-294. doi: 10.15557/JoU.2019.0043. Epub 2019 Dec 31. PMID: 32021711; PMCID: PMC6988455.
38. Gonçalves LF, Lee W, Espinoza J, Romero R. Examination of the fetal heart by four-dimensional (4D) ultrasound with spatio-temporal image correlation (STIC). Ultrasound Obstet Gynecol. 2006 Mar;27(3):336-48. doi: 10.1002/uog.2724. PMID: 16482611.
39. Tongsong T, Wanapirak C, Piyamongkol W, Sirichotiyakul S, Tongprasert F, Srisupundit K, Luewan S. Fetal ventricular shortening fraction in hydrops fetalis. Obstet Gynecol. 2011 Jan;117(1):84-91. doi: 10.1097/AOG.0b013e3181fc3887. PMID: 21173648.

40. Hernandez-Andrade E, López-Tenorio J, Figueroa-Diesel H, et al. A modified myocardial performance (Tei) index based on the use of valve clicks improves reproducibility of fetal left cardiac function assessment. Ultrasound Obstet Gynecol. 2005; 26: 227–32.
41. Hernandez-Andrade E, Figueroa-Diesel H, Kottman C, Illanes S, Arraztoa J, Acosta-Rojas R, Gratacos E: Gestational age adjusted reference values for the modified myocardial performance index for evaluation of fetal left cardiac function. Ultrasound Obstet Gynecol 2007; 29: 321–325.
42. Hernandez-Andrade E, Benavides-Serralde JA, Cruz-Martinez R, Welsh A, Mancilla-Ramirez J: Evaluation of conventional Doppler fetal cardiac function parameters: E/A ratios, outflow tracts, and myocardial performance index. Fetal Diagn Ther 2012; 32: 22–29.
43. Mahajan A, Henry A, Meriki N, Hernandez-Andrade E, Crispi F, Wu L, Welsh AW. The (Pulsed-Wave) Doppler Fetal Myocardial Performance Index: Technical Challenges, Clinical Applications and Future Research. Fetal Diagn Ther. 2015;38(1):1-13. doi: 10.1159/000363181. Epub 2015 Apr 28. PMID: 25926030.
44. Meriki N, Izurieta A, Welsh A: Reproducibility of constituent time intervals of right and left fetal modified myocardial performance indices on pulsed Doppler echocardiography: a short report. Ultrasound Obstet Gynecol 2012; 39: 654–658.
45. Meriki N, Welsh AW: Technical considerations for measurement of the fetal left modified myocardial performance index. Fetal Diagn Ther 2012; 31: 76–80.
46. Meriki N, Welsh AW: Development of Australian reference ranges for the left fetal modified myocardial performance index and the influence of caliper location on time interval measurement. Fetal Diagn Ther 2012; 32: 87–95.
47. Meriki N, Izurieta A, Welsh AW: Fetal left modified myocardial performance index: technical refinements in obtaining pulsed- Doppler waveforms. Ultrasound Obstet Gynecol 2012; 39: 421–429.
48. Denes M, Farkas K, Erdei T, Lengyel M. Comparison of tissue Doppler velocities obtained by different types of echocardiography systems: are they compatible? Echocardiography 2010; 27: 230–235.
49. Tei C. New non-invasive index for combined systolic and diastolic ventricular function. J Cardiol 1995;26:135–136.
50. Van Mieghem T, Gucciardo L, Lewi P, Lewi L, Van Schoubroeck D, Devlieger R, De Catte L, Verhaeghe J, Deprest J: Validation of the fetal myocardial performance index in the second and third trimesters of gestation. Ul- trasound Obstet Gynecol 2009;33:58–63.
51. Bravo-Valenzuela NJ, Peixoto AB, Mattar R, Arujio Junior E. Fetal cardiac function by mitral and tricuspid annular plane systolic excursion using spatio-temporal image correlation M-mode and left cardiac output in fetuses of pregestational diabetic mothers. Obstet Gynecol Sci 2021;64(3):257-265.
52. Tedesco GD, Bezerra M, Bello Barros F, Martins WP, Marcondes Machado Nardozza L, Mattar R, Fernandes Moron A, Rolo CL, Araujo Júnior E. Fetal Heart Function by Tricuspid Annular Plane Systolic Excursion and Ventricular Shortening Fraction Using STIC M-Mode: Reference Ranges and Validation. Am J Perinatol 2017;34:1354–1361.
53. Paladini D. Standardization of on-screen fetal heart orientation prior to storage of spatio-temporal image correlation (STIC) volume datasets. Ultrasound Obstet Gynecol 2007;29(06): 605–611.
54. Peixoto AB, Bravo-Valenzuela NJ, Martins WP, Tonni G, Mattar R, Moron AF, Pares DB, Araujo Júnior E. Reference ranges for the fetal mitral, tricuspid, and interventricular septum annular plane systolic excursions (mitral annular plane systolic excursion, tricuspid annular plane systolic excursion, and septum annular plane systolic excursion) between 20 and 36 + 6 weeks of gestation. J Perinat Med. 2020 Jul 28;48(6):601-608. doi: 10.1515/jpm-2020-0002. PMID: 32609650.
55. Kiserud T, Ebbing C, Kessler J, Rasmussen S. Fetal cardiac output, distribution to the placenta and impact of placental compromise. Ultrasound Obstet Gynecol. 2006 Aug;28(2):126-36. doi: 10.1002/uog.2832. PMID: 16826560.

56. Comas M, Crispi F, Gómez O, Puerto B, Figueras F, Gratacós E. Gestational age- and estimated fetal weight-adjusted reference ranges for myocardial tissue Doppler indices at 24-41 weeks' gestation. Ultrasound Obstet Gynecol. 2011 Jan;37(1):57-64. doi: 10.1002/uog.8870. PMID: 21046540.
57. Schneider C, McCrindle BW, Carvalho JS, Hornberger LK, McCarthy KP, Daubeney PE. Development of Z-scores for fetal cardiac dimensions from echocardiography. Ultrasound Obstet Gynecol. 2005 Nov;26(6):599-605. doi: 10.1002/uog.2597. PMID: 16254878.
58. Henry A, Welsh AW. Monitoring intrahepatic cholestasis of pregnancy using the fetal myocardial performance index: a cohort study. Ultrasound Obstet Gynecol. 2015 Nov;46(5):571-8. doi: 10.1002/uog.14769. PMID: 25516144.
59. Russell NE, Foley M, Kinsley BT, Firth RG, Coffey M, McAuliffe FM: Effect of pregestational diabetes mellitus on fetal cardiac function and structure. Am J Obstet Gynecol 2008; 199: 312.e1–7.
60. Garcia-Flores J, Ja ez M, Gonzalez MC, Martinez N, Espada M, Gonzalez A: Fetal myocardial morphological and functional changes associated with well-controlled gestational diabetes. Eur J Obstet Gynecol Reprod Biol 2011; 154: 24–26.
61. Wong M, Wong W, Cheung Y: Fetal myocardial performance in pregnancies complicated by gestational impaired glucose tolerance. Ultrasound Obstet Gynecol 2007; 29: 395–400.
62. Depla AL, De Wit L, Steenhuis TJ, Slieker MG, Voormolen DN, Scheffer PG, De Heus R, Van Rijn BB, Bekker MN. Effect of maternal diabetes on fetal heart function on echocardiography: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2021 Apr;57(4):539-550. doi: 10.1002/uog.22163. PMID: 32730637; PMCID: PMC8048940.
63. Api O, Emeksiz MB, Api M, Uğurel V, Unal O: Modified myocardial performance index for evaluation of fetal cardiac function in preeclampsia. Ultrasound Obstet Gynecol 2009; 33: 51–57.
64. Youssef L, Miranda J, Paules C, Garcia-Otero L, Vellvé K, Kalapotharakos G, Sepulveda-Martinez A, Crovetto F, Gomez O, Gratacós E, Crispi F. Fetal cardiac remodeling and dysfunction is associated with both preeclampsia and fetal growth restriction. Am J Obstet Gynecol. 2020 Jan;222(1):79.e1-79.e9. doi: 10.1016/j.ajog.2019.07.025. Epub 2019 Jul 20. PMID: 31336074.
65. Balli S, Kibar AE, Ece I, Oflaz MB, Yilmaz O. Assessment of fetal cardiac function in mild preeclampsia. Pediatr Cardiol. 2013 Oct;34(7):1674-9. doi: 10.1007/s00246-013-0702-8. Epub 2013 Apr 17. PMID: 23591803.
66. Gordijn SJ, Beune IM, Thilaganathan B, Papageorghiou A, Baschat AA, Baker PN, Silver RM, Wynia K, Ganzevoort W. Consensus definition of fetal growth restriction: a Delphi procedure. Ultrasound Obstet Gynecol. 2016 Sep;48(3):333-9. doi: 10.1002/uog.15884. PMID: 26909664.
67. Cruz-Martinez R, Figueras F, Benavides-Serralde A, Crispi F, Hernandez-Andrade E, Gratacos E: Sequence of changes in myocardial performance index in relation to aortic isthmus and ductus venosus Doppler in fetuses with early-onset intrauterine growth restriction. Ultrasound Obstet Gynecol 2011; 179–184.
68. Hernandez-Andrade E, Crispi F, Benavides-Serralde JA, Plasencia W, Diesel HF, Eixarch E, Acosta-Rojas R, Figueras F, Nicolaides K, Gratacos E: Contribution of the myocardial performance index and aortic isthmus blood flow index to predicting mortality in preterm growth-restricted fetuses. Ultrasound Obstet Gynecol 2009; 34: 430–436.
69. Crispi F, Hernandez-Andrade E, Pelsers MMLC, Plasencia W, Benavides-Serralde JA, Eixarch E, Le Noble F, Ahmed A, Glatz JFC, Nicolaides KH: Cardiac dysfunction and cell damage across clinical stages of severity in growth-restricted fetuses. Am J Obstet Gynecol 2008; 199: 254.e1–8.
70. Tanter M et al. Ultrafast imaging in biomedical ultrasound. IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control 2014.
71. Joos P et al. High-Frame-Rate Speckle tracking echocardiography. IEEE TRANSACTIONS ON ULTRASONICS, FERROELECTRICS, AND FREQUENCY CONTROL 2018.

72. Wieczorek A, Hernandez-Robles J, Ewing L, Leshko J, Luther S, Huhta J. Prediction of outcome of fetal congenital heart disease using a cardiovascular profile score. Ultrasound Obstet Gynecol. 2008 Mar;31(3):284-8. doi: 10.1002/uog.5177. PMID: 18253925.
73. Rapid Response Group. Executive Board of the International Society of Ultrasound in Obstetrics and Gynecology.. Safety statement, 2000. International Society of Ultrasound in Obstetrics and Gynecology (ISUOG). Ultrasound Obstet Gynecol. 2000 Nov;16(6):594-6. doi: 10.1046/j.1469-0705.2000.00296.x. PMID: 11169361.