Title: “Cardiac Alterations in Fetuses Exposed to Rheumatic Disease – A Fetal Speckle Tracking Echocardiography Study”

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1. ABSTRACT:

Understanding the influence of maternal rheumatological conditions on fetal cardiac function is crucial for early identification of potential cardiac abnormalities during gestation. This thesis contributes to the existing knowledge on the cardiac effects of maternal rheumatological conditions, aiding in the development of targeted interventions and improved management strategies for affected pregnancies. Inflammatory burdens associated with maternal rheumatological conditions can adversely affect both maternal and fetal outcomes. Furthermore, the inflammatory status of the mother can influence the formation and structure of the placenta, leading to vascular abnormalities, placental insufficiency, and alterations in placental structure. These changes can disrupt the exchange of nutrients and oxygen between the mother and fetus, resulting in fetal growth restriction, particularly in pregnancies affected by conditions such as systemic lupus erythematosus. Additionally, maternal autoantibodies, including anti-Ro and anti-La, have the ability to cross the placenta and trigger an inflammatory response, potentially damaging the cardiac tissues of the fetus. Offspring exposed to maternal Ro/La autoantibodies are at risk of developing cardiac conduction abnormalities, notably congenital heart block (CHB), which can progress to cardiomyopathy, typically of a dilated phenotype. The study presented within this thesis aims to investigate the impact of maternal rheumatological conditions on fetal cardiac function at 22-24 weeks of gestational age using speckle tracking echocardiography (STE), with a focus on the fetal apical four chamber view. STE enables the assessment of myocardial deformation by tracking speckles in ultrasound images throughout the cardiac cycle, while strain, derived from STE, provides quantitative information on myocardial deformation.

The study is retrospective and compares fetuses exposed to pregnancy complicated by a concomitant rheumatological conditions to those from pregnant individuals without such conditions. Retrospective recruitment of women with a rheumatological disease who underwent fetal echocardiography between 2013 to 2021 was conducted at a single center (McGill University Health Centre). Pregnancies were stratified by anti-Ro/La antibody status and compared to non-exposed pregnancies, matched by gestational age (22 to 24 weeks). Cardiac functional alterations were observed in fetuses exposed to maternal rheumatological conditions, regardless of the presence of CHB. Increased left ventricular ejection fraction and strain suggest early adaptations to an adverse in-utero environment, possibly influenced by elevated afterload resulting from unfavorable vascular resistance. These findings emphasize the importance of future studies to investigate later gestational and immediate postnatal cardiac adaptations in infants exposed to maternal rheumatological diseases.

2. RÉSUMÉ:

La compréhension de l'influence des pathologies rhumatologiques maternelles sur la fonction cardiaque du fœtus est cruciale pour l'identification précoce des anomalies cardiaques potentielles pendant la gestation. Cette thèse contribue aux connaissances actuelles sur les effets cardiaques des atteintes rhumatologiques maternelles, en aidant au développement d'interventions ciblées et de stratégies de gestion améliorées pour les grossesses affectées. Les charges inflammatoires associées aux pathologies rhumatologiques maternelles peuvent avoir des conséquences négatives sur les résultats maternels et fœtaux. En outre, l'état inflammatoire de la mère peut influencer la formation et la structure du placenta, entraînant des anomalies vasculaires, une insuffisance placentaire et des altérations de la structure du placenta. Ces changements peuvent perturber les échanges de nutriments et d'oxygène entre la mère et le fœtus, entraînant un retard de croissance du fœtus, en particulier dans les grossesses affectées par des maladies telles que le lupus érythémateux disséminé. En outre, les auto-anticorps maternels, y compris les anti-Ro et les anti-La, ont la capacité de traverser le placenta et de déclencher une réaction inflammatoire, ce qui peut endommager les tissus cardiaques du fœtus. Les fœtus exposés aux auto-anticorps maternels Ro/La risquent de développer des anomalies de la conduction cardiaque, notamment un bloc cardiaque congénital (BCC), qui peut évoluer vers une cardiomyopathie, typiquement de phénotype dilaté. L'étude décrite dans le contexte de cette thèse vise à étudier l'impact des conditions rhumatologiques maternelles sur la fonction cardiaque fœtale à 22-24 semaines d'âge gestationnel en utilisant l'échocardiographie par suivi de pixels, en se concentrant sur la vue apicale des quatre cavités du fœtus. L'échocardiographie par suivi de pixels permet d'évaluer la déformation du cœur en suivant les pixels composant les images de l'ultrason tout au long du cycle cardiaque, tandis que la déformation fournit des informations quantitatives sur la déformation du myocarde.

L'étude rétrospective présentée dans cette Thèse compare les fœtus exposés à des femmes enceintes souffrant de maladies rhumatologiques à ceux de femmes enceintes ne souffrant pas de telles maladies. Le recrutement rétrospectif des femmes atteintes d'une maladie rhumatologique ayant subi une échocardiographie fœtale entre 2013 et 2021 a été effectué dans un seul centre (Centre universitaire de santé McGill). Les grossesses ont été stratifiées selon la présence d'anticorps anti-Ro/La et comparées à des grossesses non exposées, appariées selon l'âge gestationnel (22 à 24 semaines). Des altérations fonctionnelles cardiaques ont été observées chez les fœtus exposés à des conditions rhumatologiques maternelles, indépendamment de la présence d'un BCC. L'augmentation de la fraction d'éjection et de la déformation du ventricule gauche suggère des adaptations précoces à un environnement in-utéro défavorable, possiblement influencées par une postcharge élevée résultant d'une résistance vasculaire augmentée. Ces résultats soulignent l'importance d'études futures pour évaluer les adaptations cardiaques plus tardives pendant la gestation et immédiatement après la naissance chez les nourrissons exposés à des maladies rhumatologiques maternelles.

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5. PREFACE & CONTRIBUTIONS OF AUTHORS:

As per the guidelines for manuscript-based thesis, this thesis represents my substantial contribution as the first author. I conducted the literature review, designed the methods, collected the data, analyzed, and interpreted the data, and wrote the manuscript with the aim of submitting it to a peer-reviewed journal.

Drs. Altit and Vinet contributed to the study design, supervised data collection, statistical analyses, interpretation of data, and provided critical revision and editing of the thesis and manuscript for important intellectual content. Dr. Wilk, Mayssa Moukarzel, Daniella Villegas Martinez, and Jessica Simoneau played a role in the collection of data, development, and interpretation of the results, and provided critical review of the study. Drs. Mendel, Malhamé, Mawad and Rudski provided valuable revisions to the manuscript. All authors will approve the final manuscript before submission to a journal and will agree to be accountable for all aspects of the work before final publication in the scientific literature.

6. LIST OF ABBREVIATIONS:

ACR (American College of Rheumatology)
AD (Autoimmune diseases)
AGA (Appropriate growth for gestational age)
aPLS (Antiphospholipid antibodies)
APS (Antiphospholipid syndrome)
ASp (ankylosing spondylitis)
AV (Atrioventricular)
AVC (Atrioventricular block)
CHB (Congenital heart block),
CI (Confidence interval)
CV (Cardiovascular)
DA (Ductus arteriosus)
DICOM (Digital Imaging and Communications in Medicine)
DM (Dermayomyositis)
ECG (Electrocardiogram)
ED (End-diastolic)
EDA (End-diastolic area)
EDSR (Early-diastolic strain rate)
EDV (End-diastolic volume)
EF (Ejection fraction)
ES (End-systolic)
ESV (End-systolic volume)
EULAR (European League Against Rheumatism)
FAC (Fractional area change)
fCTIs (Fetal cardiac time intervals)
fps (Frame per second)
GA (Gestational age)
GCS (Global circumferential strain)
GLS (Global longitudinal strain)
GLSR (Global longitudinal strain rate)
GRS (Global radial strain)
HELLP (hemolysis - elevated liver enzymes - low platelets)
HR (Heart Rate)
IgG (Immunoglobulin G)
IQR (Interquartile range)
IUGR (Intrauterine growth restriction)
IVC (Inferior vena cava)
LA (Left atrium)
LV (Left ventricle)
MAS (Meconium aspiration syndrome)
MCG (Magnetocardiography)

MUHC (McGill University Health Center)
NLE (Neonatal lupus erythematosus)
PACS (Picture Archiving and Communications System)
PH (Pulmonary hypertension)
pLS (Peak longitudinal strain)
pLSR (Peak longitudinal strain rate)
PM (Polymyositis)
RA (Right atrium)
RRNL (Research registry for neonatal lupus)
RV (Right ventricle)
SA (Sinoatrial)
SD (Standard deviation)
SGA (Small for gestational age)
SLE (Systemic lupus erythematosus)
SS (Sjögren syndrome)
SSc (Systemic sclerosis)
STE (Speckle-tracking echocardiography)
SVC (Superior vena cava)
TGF-β (Transforming growth factor beta)
TTP (Time to peak)
1C-DYS (One-chamber dyssynchrony)
2C-DYS (Two-chamber dyssynchrony)
2-D (Two-dimensional)
3-D (Three-dimensional)
4-CV (Four-chamber view)

7. INTRODUCTION:

Rheumatological conditions during pregnancy have been associated with increased risks of maternal, fetal, and neonatal complications, often conferring a high-risk status.[1] Impaired placental changes due to these conditions may result in compromised oxygen exchange and nutrient absorption, leading to chronic fetal hypoxia and malnutrition.[2] The fetal heart may undergo myocardial remodeling in response to loading variations induced by inflammation, chronic hypoxia, and elevated placental resistance.[3] Fetuses exposed to placental insufficiency may adapt their ventricles to enhance contractility.[4] Additionally, maternal autoimmune diseases, especially those with circulating autoantibodies anti-Ro/La, have been linked to myocardial inflammation, impaired atrioventricular valve function, and damaged conductive system in fetuses, potentially leading to congenital heart block or pre- and post-natal cardiomyopathy.[5,6] Speckle-tracking echocardiography (STE) is a non-invasive technique that can assess myocardial deformation, even in utero, to evaluate cardiac performance.

Current knowledge gaps in the field include understanding the effects of maternal rheumatological conditions on fetal cardiac performance through STE, elucidating the role of autoantibodies in influencing fetal cardiac adaptations and their impact on cardiac function using STE, and investigating the effects of congenital heart block (CHB) within pregnancies exposed to autoantibodies (anti-Ro/La) on fetal heart function. Hence, this thesis points to identify early markers of adverse cardiac involvement in fetuses exposed to pregnancies complicated by maternal rheumatological conditions.

This thesis aims to investigate the impact of maternal rheumatological conditions on fetal cardiac performance using STE. It hypothesizes that fetuses exposed to maternal rheumatological conditions would display distinct cardiac adaptation in utero, compared to an unexposed group. This thesis also seeks to assess the effect of circulating maternal autoantibodies (anti-Ro/La) and fetal CHB on fetal cardiac function, with a hypothesis that autoantibodies would further impact fetal cardiac adaptation and that the presence of CHB would have a more profound effect on fetal cardiac performance. This research will contribute to understanding the fetal cardiac health in pregnancies complicated by rheumatological conditions and may have clinical and future research implications regarding the monitoring and management of such pregnancies.

8. BACKGROUND AND RATIONALE:

8.1. Maternal Rheumatological Conditions in Pregnancy

Rheumatological conditions in pregnancy (such as systemic lupus erythematosus (SLE), Sjogren syndrome (SS), antiphospholipid syndrome (APS), mixed connective tissue disease, etc.) have been associated with an increase in maternal, fetal and/or neonatal complications, conferring a status of high-risk pregnancy (**Table A**). Maternal risks include: disease flares, gestational diabetes, pregnancy-induced hypertension, pre-eclampsia, eclampsia and thromboembolic events.[1,7] Additionally, several variables, including immunological alterations, maternal disease activity, degree of organ damage, circulating and burden of antibodies, as well as pharmacological treatment, might influence fetal prognosis in mothers with an autoimmune illness.[8] Maternal risk factors that affect the general population, such as advanced maternal age, anatomic malformations, chromosomal abnormalities, endocrine dysfunction, non-autoimmune immunological disorders, body weight, diet, smoking, and infections can also occur in autoimmune expectant mothers, thereby affecting fetal development.[8]

8.1.1. **Table A.** Rheumatological Conditions Associated with Maternal and Fetal/Neonatal Risks.

Condition	Definition	Common Symptoms	Maternal Risks	Fetal/Neonatal Risks
SLE[9]	Complex autoimmune disease characterized by widespread inflammation that can affect multiple organs and systems in the body with the generation of numerous autoantibodies.	Fatigue, joint pain, skin rashes, fever, kidney problems, cardiovascular issues.	Disease flares, gestational diabetes, PIH, pre-eclampsia, eclampsia, thromboembolic events.	Fetal death, prematurity, placental insufficiency, neonatal lupus, CHB.
Sjögren Syndrome[10,11]	Autoimmune disease primarily affecting	Dryness of mucosal surfaces,	Pulmonary hypertension.	Neonatal lupus, CHB, stillbirth, fetal

	exocrine glands, presenting as either primary or secondary to other connective tissue diseases.	extraglandular manifestations.		loss, IUGR, premature deliveries, spontaneous abortions.
Rheumatoid Arthritis[12]	Systemic autoimmune disorder marked by inflammatory synovitis leading to tissue damage.	Joint pain, swelling, morning stiffness, fatigue.	Pre-eclampsia, increased risk for caesarean section.	Low birth weight, premature birth, pre-eclampsia, small for gestational age, neonatal and perinatal death.
Mixed connective tissue disease[13,14]	Systemic autoimmune disorder characterized by features of multiple connective tissue diseases.	Raynaud phenomenon, hand edema, synovitis, histologically proven myositis, acrosclerosis, and muscle/joint pain.	Disease flares, pre-eclampsia, thromboembolic event, caesarean section, and maternal death.	Prematurity, IUGR, neonatal lupus, and perinatal death.
Undifferentiated connective tissue disease[15]	Connective tissue disease symptoms that do not fit specific criteria for other diseases.	Arthralgia, skin lesions, Raynaud phenomenon, mucocutaneous symptoms, arthritis, fever, non-specific interstitial pneumonia and thyroid disfunction	Disease flares, pre-eclampsia, thromboembolic event.	Prematurity, IUGR, neonatal lupus, miscarriage.
Antiphospholipid syndrome[16-18]	Presence of antiphospholipid antibodies in the presence of vascular thrombosis or pregnancy complications	Blood clots, rash, chronic headaches, dementia, and seizures.	Pre-eclampsia, miscarriage, infertility, repeated early pregnancy losses, increased risk for maternal thrombosis.	Pregnancy loss, fetal distress, premature birth, IUGR, placental insufficiency, abruptio placentae.
Idiopathic inflammatory myositis[19]	Autoimmune diseases causing muscle inflammation and weakness.	Muscle weakness, pain, fatigue, skin rashes	PIH, pre-eclampsia, caesarean section, antepartum hemorrhage	Low birth weight, premature birth, small for gestational age.
Ankylosis Spondylitis[20]	Inflammatory arthritis primarily affecting the spine and sacroiliac joints.	Back pain, stiffness, reduced spine flexibility	Pre-eclampsia, caesarean section	Premature birth, small for gestational age.
Legend: CHB: Congenital Heart Block; IUGR: Intrauterine Growth Restriction; PIH: Pregnancy Induced Hypertension; STE: Speckle Tracking Echocardiography.				

8.1.2. Systemic Lupus Erythematosus (SLE):

Women with SLE are at a higher risk of developing medical complications during pregnancy, including thrombosis, due to hormonal changes that increase the risk of thrombosis two to three times.[7,18] Pre-eclampsia, which is characterized by pregnancy-induced hypertension, proteinuria, and edema, is a common complication in pregnancy with SLE, with about 25% developing it.[7,18] Higher disease activity, lupus nephritis, antiphospholipid antibodies and pre-existing hypertension have all been related to an increased risk of pre-eclampsia in SLE-affected pregnancies.[1,7,18] Angiogenic factors and the complement system have been postulated to have a role in the pathophysiology of pre-eclampsia, which is found more commonly in SLE pregnancies.[1]

A significant percentage (up to 30%) of women diagnosed with SLE have detectable antiphospholipid antibodies (aPLs) including lupus anticoagulant, anticardiolipin antibodies, or anti-β2-glycoprotein-I antibodies.[8,21] The presence of these antibodies can lead to increased morbidity, including complications such as thromboembolic events, organ damage, and cognitive impairment.[21] aPLs also contributes to pregnancy complications, such as fetal death, and prematurity associated to pre-eclampsia or placental insufficiency.[8]

8.1.3. Sjögren Syndrome (SS):

Women who are pregnant and have been diagnosed with SS are more likely to face additional difficulties that are beyond the normal range of issues typically associated with pregnancy when compared to those who do not have the condition.[10] Although there has been limited research on the impact of SS on pregnancy outcomes, several studies have shown a heightened occurrence of fetal loss among women with the syndrome, along with intrauterine

growth restriction (IUGR) and premature deliveries.[10] Prominent fetal consequences observed in pregnancies complicated by SS include neonatal lupus and CHB, which are believed to be caused by the detrimental effects of anti-Ro or anti-La antibodies, or both, on the atrioventricular node.[10] Further discussion on these autoantibodies will take place in section *8.1.4.* The prevalence of SS varies between 0.29% and 0.77% and is considerably more common in women than in men, with a female-to-male sex ratio of 9:1.[22,23]

8.1.4. Placenta-Mediated Complications:

Maternal rheumatological conditions are also known to be associated with placenta-mediated pregnancy complications due to impaired placentation. This defective placentation is often associated with maternal malperfusion, which refers to a condition where there is inadequate blood flow between the mother and the developing fetus during pregnancy and can lead to anomalies in placental vascularity and coagulation, such as extensive infarction, decidual vasculopathy, placental abruption, chronic inflammation, vasculitis, and decreased placental weight.[2,24] Placental failure is complicated by the maintenance of high vascular resistances due to insufficient trophoblast cell invasion into the uterine arteries, which are responsible for supplying blood to the placenta.[2] This results in compromised oxygen exchange and nutrient absorption, initiating chronic fetal hypoxia and malnutrition.[2] For this reason, fetal cardiac development may be influenced by the prenatal environment and may adapt in structure and shape, with its function possibly being impacted by the adverse vascular resistances, growth/development and oxygenation.[3]

8.1.5. Anti-Ro/La Antibodies:

The presence of maternal anti-Ro (SSA) and, to a lesser extent, anti-La (SSB) autoantibodies might result in neonatal lupus syndrome. Transplacental passage of maternal anti-Ro and anti-La antibodies may lead to myocardial inflammation (leading to cardiac failure, hydrops, valvular regurgitation and endofibroelastosis) and injury to the fetal conducting system (leading to CHB). Maternal autoantibodies begin to cross the placenta at 12 weeks of gestation, with only a small proportion (5%-10%) of the mother's immunoglobulin G (IgG) antibodies being transferred through the placenta between weeks 17 and 22 of gestation.[8,25] However, the levels of transferred antibodies increase significantly as pregnancy progresses, reaching higher levels by the end of the third trimester, with up to 50% of maternal IgG antibodies being transferred by week 32 of gestation.[8,25] The SS-A antigen is a soluble nuclear antigen, while the Ro antigen is a soluble cytoplasmic antigen, and has been confirmed to be immunologically identical to the SS-A antigen using the double-immunodiffusion method.[26] Ro/SSA antigens refer to two cellular proteins, Ro52 and Ro60, which are targeted by autoantibodies in some autoimmune diseases (AD). Ro60 binds to small RNAs and may have a role in preventing autoimmune disease while Ro52 is an E3 ubiquitin ligase that interacts with a range of substrates and has important roles in intracellular antibody immunity and the regulation of inflammation.[27] Both Ro52 and Ro60 may also play a role in regulating mRNA stability.[28] Additionally, the structure of Ro52 and Ro60 protein epitopes affect how they are recognized. Anti-Ro52 antibodies recognize linear epitopes in the denatured protein, which are typically found in the leucine zipper region and not exposed on the surface of the native protein.[29] Anti-Ro60 antibodies recognize highly conformational epitopes, and these antibodies lose their ability to bind to the denatured protein.[29] Anti-Ro antibodies are present in

70-100% of SS patients and 40-90% of SLE patients, while anti-La antibodies can be found in 35-70% of SS patients and 45% of SLE patients. In addition, anti-Ro antibodies are detected in 3-11% of patients with systemic sclerosis (SSc), 5-15% of patients with polymyositis (PM) and dermatomyositis (DM), and 3-15% of patients with rheumatoid arthritis.[29]

8.2. Rheumatological Conditions and Cardiovascular Function

8.2.1. Impact of SLE on Maternal Cardiovascular Function during Pregnancy

Individuals with SLE are more likely to experience cardiovascular (CV) events, however it is unclear whether standard CV risk factors and SLE factors are related.[30] Recent studies have started to investigate the link between pregnancy and CV issues in SLE women.[30] The main cause of morbidity and death in SLE patients is early CV disease, which has a five-fold increased risk.[30] There are several risk factors that are common between SLE and CV disease, which could explain the association between pregnancy complications and CV disease in mothers with SLE. As outlined, SLE increases the risk of pregnancy complications including pre-eclampsia, low birth weight, and premature delivery, which share multiple risk factors with CV disease, such as obesity, smoking, hypertension, diabetes, and lipid abnormalities.[31] Taken together, these shared risk factors between SLE and CV disease may contribute to the increased risk of pregnancy complications in women with SLE, which could in turn contribute to the association between pregnancy complications and CV disease in SLE mothers. Additionally, placenta-mediated complications have been linked to endothelial dysfunction increasing the risk of later developing CV.[31] Significant CV illnesses, such as myocardial infarction, can develop rapidly and prematurely in women whose pregnancies were disrupted by placenta-mediated complications or adverse pregnancy outcomes.[30] Additionally, given that pre-eclampsia alters the inflammatory response in

comparison to healthy pregnancies and that inflammation plays a role in both SLE and atherosclerosis, this indicates a possibly harmful combination for the long-term risk of CV disease in SLE mothers.[31] Other complications affecting the fetus, such as pre-term birth and IUGR have also been associated with inflammation.[31] Furthermore, pre-term delivery at less than 34 weeks with a history of impaired placental changes has been associated with an increase in CV events by two-fold in these mothers, compared to those with uncomplicated pregnancies.[30]

8.3. Cardiac Mechanics

Cardiac function involves the entire process from ventricular filling (preload) to ejection in the outflow tracts and is influenced by various factors. Firstly, it depends on the architecture of each ventricle. The right ventricle (RV) and left ventricle (LV) exhibit noticeable variations in their shape and structure.[32] The RV has a crescent shape with thinner walls, designed to pump blood into the pulmonary circulation, which operates at lower pressures (secondary to the low resistances in the pulmonary vascular bed).[32] Conversely, the LV features a conical shape with thicker walls to effectively pump oxygenated blood into the high-pressure (high-resistance) systemic circulation to supply the body's tissues and organs.[32] These discrepancies in shape and wall thickness are reflective of the distinct functions and pressure requirements that each ventricle fulfills.[32] Cardiac function also depends on the contractility of each ventricle, which is the inotropic state of the heart muscle and is determined by factors such as the structure of the muscle fibers, energy substrates such as glucose, as well as the acid-base and electrolytic homeostasis including sodium, potassium, calcium and magnesium. Cardiac contractility is significantly influenced by the concentration of calcium within the cardiac cells.[33] Elevated levels of intracellular calcium promote a more forceful contraction, leading to increased contractility of the heart.[33] Additionally, the interaction between

the two ventricles is crucial since they share common myocardial fibres, as well as a common wall known as the septum. These fibers have very precise orientations that are geometrically different within the wall of the left and of the right ventricles. The RV free wall primarily consists of transverse muscle fibers, with a smaller proportion of subendocardial longitudinal fibers.[32] On the other hand, the LV is characterized by a combination of endocardial and epicardial fibers that form a helical structure, along with midwall circumferential fibers.[32] Consequently, the RV has fewer muscle fibers and is considerably thinner compared to the LV, accounting for approximately one-third of its thickness.[32] This discrepancy in fiber arrangement plays a significant role in determining distinct patterns of ventricular contraction.[32] Other elements affecting cardiac performance include the preload, the afterload and the myocardial activation (as well as dispersion of electrical influx). The afterload, which refers to the resistance the ventricles need to overcome during contraction, also affects underlying function. For instance, the LV is responsible for pumping blood throughout the vast systemic vascular network, achieving elevated pressures compared to the right ventricle.[33] As such, various cardiac chambers face various workload and mechanical resistance. Therefore, the LV which faces a higher workload has a significantly thicker free wall when reaching pediatric and adult age, in contrast to the RV.[33] As outlined, the electrical system plays a vital role in activating the heart and conferring a uniform and time-sensitive myocardial activation, as well as repolarization. The cardiac cycle, which represents a single heartbeat, is commonly categorized into two stages: systole, characterized by the active contraction of the heart, and diastole, marked by relaxation and the process of filling.[34] The electrophysiological process of the heart begins when an action potential is, typically, generated at the sinoatrial (SA) node.[33] This electrical signal spreads throughout the atria, causing them to

contract, and then proceeds to the atrioventricular (AV) node.[33] At the AV node, there is a brief delay of approximately 100 milliseconds (various timing dependent on age and heart rate) before the impulse is transmitted to the atrioventricular bundle of His.[33] From there, the electrical impulse travels down the right and left bundle branches, eventually reaching the Purkinje fibers.[33] Once the action potential reaches the ventricular contractile fibers, it triggers the mechanism known as excitation-contraction coupling.[33] This mechanism leads to the influx of calcium ions, which results in a synchronized contraction that starts at the apex of the heart and progresses upward.[33] As such, myocardial inflammation, ischemia and/or fibrosis will affect the dispersion of electricity, potentially leading to dyssynchrony. Abnormal synchronization of cardiac contraction and relaxation entails that certain portion of the cardiac structure may be contracting, while other portions are in a relax state. This may hinder performance. Further, injured myocardial cells may not achieve their intrinsic pacing functions (such as SA node inflammatory changes), as well as alter the capacity to transmit electrical impulse (such as when the AV node is affected, leading to heart block). Preload refers to the filling of a cardiac cavity. The right ventricle is dependent on right atrial filling, which is dependent on systemic venous return. Systemic venous return is dependent on the left ventricular cardiac output, lymphatic function, organic irrigation, and venous competency. The left ventricle is dependent on the left atrial filling, which is dependent on the pulmonary venous return, which is in turn dependent on appropriate pulmonary perfusion. Preload may also be influence by intra-cardiac or extra-cardiac shunts, volume status and presence of vascular steal (i.e.: bleeding, extravasation, inflammatory status leading to third-spacing). Preload of the ventricles is also dependent on the capacity of the myocardium to relax, which may be impaired in situation of increased stiffness (i.e.: fibrosis, abnormal depolarization, hypertrophy,

ischemia) or restriction (ex: pericardial effusion leading to tamponade; high intra-thoracic pressure with a pneumothorax). Preload affects ventricular function mainly by impacting its diastolic phase. It also impacts cardiac performance through the Frank-Starling relationship, which describes how an increase in preload, or the volume of blood filling the heart during diastole, leads to a more forceful contraction of the heart during systole. This results in an increased stroke volume and cardiac output.

The LV systolic function is achieved through various contraction patterns, including longitudinal contraction (vertical axis), circumferential contraction (towards the inside), radial contraction (wall thickening), and torsion (wringing motion). Indeed, the LV epicardial and endocardial layers follow distinct rotational patterns, at the apex and at the base of the heart.[35,36] This differential rotation between the two layers and two poles of the LV create a twisting motion known as LV torsion. In contrast, the RV primarily contracts longitudinally in a peristaltic motion to propel blood from the tricuspid valve to the outflow tract.[32] There is also a component of the free wall of the RV coming towards the septum, and the septum bulging towards the free wall during the contraction, which participates to the RV performance.

8.4. Fetal Circulation Physiology

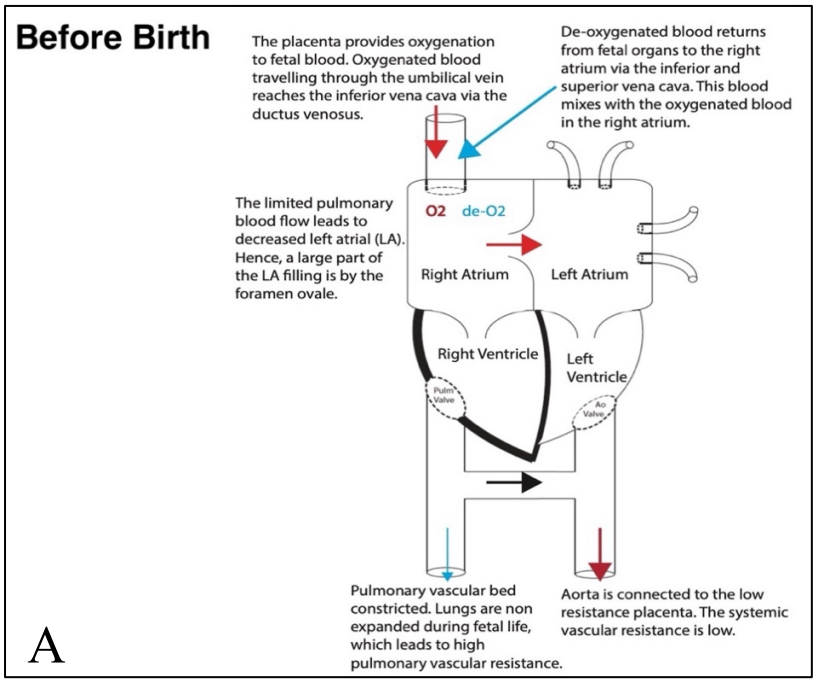
Fetal circulation (**Figure A)** differs significantly from the postnatal circulation (**Figure B)**. The placenta acts as the interface between the maternal and fetal circulatory systems, supplying oxygen and nutrients to the growing fetus, while removing metabolic waste and carbon dioxide.[37] The placenta performs a multitude of functions encompassing endocrinological, immunological, respiratory, nutritional, detoxification, hematological, and other processes. Oxygen from the maternal system gets extracted by the fetal blood at the placental interface, a process which is

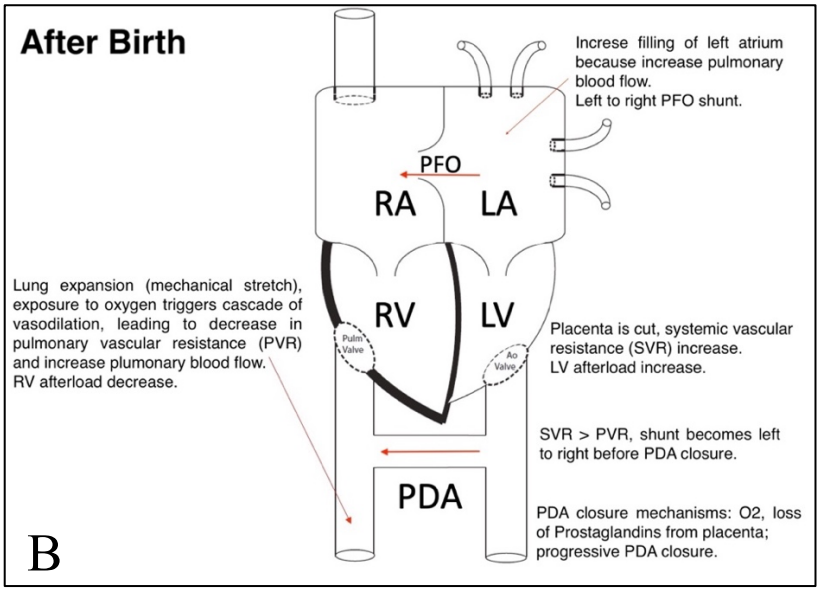
favored by the higher affinity of the fetal hemoglobin. Oxygenated blood flows the umbilical vein, filling the right atrium (RA) by the ductus venosus. The fetal RA is being filled by the systemic venous return of the fetus (superior vena [SVC] and inferior vena cava [IVC]), as well. The RA filing has two pathways for unloading during fetal life: the foramen ovale (a natural connection at the atrial level between the left atrium (LA) and RA), as well as through the tricuspid valve into the RV. In fetal circulation, the RA typically exhibits higher filling pressure than the LA, due to combination of placental and systemic venous return in comparison to the limited pulmonary blood flow underfilling the LA by its natural pulmonary venous return.[37] As a result, the oxygenated blood is preferentially shunted from the RA to the LA via the foramen ovale.[37] Streaming of oxygenated blood from the umbilical venous return has been shown to preferentially enter the LA via the foramen ovale, conferring the majority of the oxygen to the fetal cerebral circulation. The left atrium unloads into the LV through the mitral valve, and ejects into the coronary arteries and aorta, eventually returning to the placenta via the two umbilical arteries.[37] The aorta branches out to supply highly oxygenated blood to the brain, and eventually, to the lower body. The blood from the lower body is typically less oxygenated due to the right to left shunting at the ductal level, bringing some of the less oxygenated blood from the RV into the descending systemic circulation.[37] Indeed, a mixture of oxygenated blood from the umbilical venous return and of deoxygenated blood from the IVC and SVC enters the RV via the tricuspid route.[37] Once in the RV, it enters the right ventricular outflow tract, and into the pulmonary artery. Here, it encounters two potential routes for unloading: the pulmonary vasculature or, in more significant volume, travels through the ductus arteriosus (DA), which serves as a connection between the pulmonary artery and the aorta. The preference for blood volume to pass through the DA is due to the high

resistance in the pulmonary vascular bed compared to the low resistance in the systemic vascular bed, which is connected to the low-resistance vasculature of the placenta.[37] Due to the limited pulmonary blood flow in fetal life, the LA receives limited filling through the lungs, resulting in a preferential right-to-left shunt at the atrial level. As such, in fetal life, approximately 10-15% of the RV output goes to the fetal lungs and its majority is shunted away from the lungs through the DA. One notable difference between fetal and postnatal life is that, although there is a connection between both great vessels (aorta and pulmonary artery) through the DA, the resistances they face are not equal. While the pressures experienced by both ventricles theoretically equilibrate, the resistances differ significantly. Indeed, according to Ohm's Law, the pressure (P) in a system is directly proportional to the flow (Q) and the resistance (R), mathematically represented as P = Q * R. In fetal circulation, the pulmonary vascular bed exhibits high resistance, leading to low flow, whereas the systemic vasculature displays low resistance, resulting in higher flow to the body.[37] Consequently, the pressure between the pulmonary artery and the descending aorta, connected by the DA, equalizes between the two systems. During the process of birth, significant physiological changes occur to facilitate the transition from fetal circulation to infant circulation. These changes are triggered by the initiation of respiration and the cessation of placental blood flow. As a result, pulmonary resistance decreases due to the mechanical stretch of the lungs, exposure to oxygen (a potent pulmonary vasodilator) and the triggering of the release of various vasoactive substances (such as endogenous expression of nitric oxide by the endothelial nitric oxide synthase). This enhances pulmonary blood flow, which increases left atrial filling via the increase in pulmonary venous return. Following birth, there is a natural closure of the shunts mentioned.[37] The DA will close via exposure to oxygen, and the loss of prostaglandin-expression via the placenta and,

eventually, the lining of the ductal structure. The foramen ovale will eventually close via a flap that blocks its shunting, through the progressive reversal of the right to left towards a left to right shunting. This reversal occurs due to the increase in LA filling and to the increase in LV end-diastolic pressure due to the significant rise in LV afterload after the loss of the low-resistance vasculature from the placenta. The umbilical arteries undergo occlusion and transform into the medial umbilical ligaments, while the umbilical vein obliterates and forms the ligamentum hepatis teres.[38] The ductus venosus collapses and becomes the ligamentum venosus. The DA fibroses into the ligamentum arteriosum.[38]

8.4.1. **Figure A & B.** Fetal Physiology Circulation Before and After Birth.





Legend – Representation of fetal-placenta physiology (A). Post-natal cardiac physiology (B). Constructed by supervisor Dr. Gabriel Altit.

8.5. Impact of Rheumatological Conditions on Fetus

The management of rheumatological pregnancies requires careful consideration of potential fetal risks, as maternal disease activity, medication exposure, and other factors can impact fetal outcomes, necessitating close monitoring and appropriate interventions. (**TABLE B**). Potential fetal risks involve miscarriage, intrauterine fetal demise, preterm rupture of membranes, preterm birth, IUGR, neonatal hydrops and neonatal lupus.[1] Another important fetal risks includes pregnancy loss, which can occur as a result of the presence of lupus anticoagulant during the first trimester, which is a aPLs subtype linked to pregnancy loss and adverse pregnancy outcomes and has been proven to be a risk factor.[1] aPLs can lead to inflammation and activate complement, which can affect placental function and result in poor pregnancy outcomes.[7] Antiphospholipid Syndrome (APS), SLE and vasculitis carry a higher risk of poor fetal outcomes (stillbirth, miscarriage, preterm birth, IUGR, intrauterine fetal death) as opposed to rheumatoid arthritis and

spondyloarthropathies.[8] Fetal death during the second and third trimesters is more prevalent in pregnancies affected by APS compared to the general population, where most pregnancy losses typically occur in the first trimester. Additionally, 30% of APS pregnancies can be affected by IUGR, 18-48% by pre-eclampsia, and 50% by placental insufficiency, while prematurity occurs in around one-third of all APS pregnancies.[8]

8.5.1. **Table B.** Existing Studies on Rheumatological Conditions and Fetal/Neonatal Cardiac Function.

Authors	Pubmed ID	Study Type	Population	Key Findings
Abadir *et al*., 2015 J Am Heart Assoc.[39]	26675254	Retrospective	31 neonates with isolated congenital atrioventricular block.	Maternal SLE is associated with changes in left atrial conduction and inexcitability.
Duan *et al.,* 2021 Clin Rehumatol.[6]	33813619	Prospective	52 fetuses: 18 from mothers with autoimmune antibodies and 34 control.	Fetuses of mothers with autoimmune antibodies displayed longer one-chamber dyssynchrony (i.e., left ventricular systolic mechanical dyssynchrony).
Dörner *et al*., 1992 Clin Investig.[40]	1392417	Observational	42 babies with congenital cardiac conduction defects.	Anti-Ro antibodies associated with atrioventricular conduction blocks.
Friedman *et al*., 2010 Arthritis Rheum.[41]	20391423	Randomized Control Trial	20 mothers with anti-SSA/Ro antibodies and previous child with congenital heart block/neonatal lupus rash.	Intravenous immunoglobulin has been determined to be safe, yet when administered at lower doses as a replacement therapy, it does not hinder the recurrence of congenital heart block or lower maternal antibody levels.
Iruretagoyena *et al*., 2014 Am J Obstet Gynecol.[42]	24440565	Observational	18 fetuses: 9 with severe early onset of intrauterine growth restriction and 9 control.	Intrauterine growth restriction is linked to alterations in the contractile machinery of cardiomyocytes, leading to modifications in elements such as sarcomere length, which becomes shorter.
Izmiraly *et al*., 2020 J Am Coll Cardiol.[43]	32674792	Randomized Control Trial	54 anti-SSA/Ro-positive mothers with a previous pregnancy with a fetus with congenital heart block.	Hydroxychloroquine greatly reduced occurrence of fetal congenital heart block.
Izmiraly *et al.*, 2012 Circulation.[44]	22626746	Retrospective	257 mothers of anti-SSA/Ro-positive mothers: 40 exposed	Hydroxychloroquine use is associated with decreased risk of cardiac neonatal lupus.

			and 217 unexposed to hydroxychloroquine.	
Jaeggi *et al.*, 2011 J Am Coll Cardiol.[45]	21435519	Prospective	165 fetuses of anti-Ro/La antibody-positive women.	Fetal atrioventricular prolongation did not predict progressive heart block to birth.
Jaeggi *et al*., 2010 J Am Coll Cardiol.[46]	20538173	Prospective	186 fetuses/neonates: 146 with normal pregnancy outcomes and 40 with heart block and/or endocardial fibroelastosis.	The level of anti-Ro antibodies, rather than their presence, shows a correlation with the likelihood of experiencing cardiac complications (heart block, myocardial dysfunction, pericardial effusion, and endocardial fibroelastosis).
Madhusudan *et al*., 2016 J Obstet Gynaecol India.[47]	27651588	Prospective	13 fetuses of mothers with congenital heart block.	Fetal congenital heart block is linked to maternal presence of anti-Ro/SSA, anti-La/SSB and antinuclear antibodies, in cases of systemic lupus erythematosus. Steroid treatment has the potential to improve outcomes.
Mawad *et al*., 2022 J Am Heart Assoc.[48]	35001672	Retrospective	130 mothers diagnosed with cardiac neonatal lupus erythematosus.	Low risk of perinatal mortality and postnatal cardio myopathy in fetuses that received transplacental dexamethasone.
Meisgen *et al*., 2021 Annals of the Rheumatic Disease.[49]	35470161	Genome-wide association observational study	92 patients with congenital heart block and their families.	Fetal cardiomyocytes with a deficiency in auxilin exhibit irregular connectivity and calcium homeostasis, along with reduced expression of calcium channel on their cell surfaces. This results in compromised cardiomyocyte function, contributing to signs of congenital heart block such as ectopic beats, arrhythmias, and prolonged atrioventricular time.
Nield *et al*., 2002 Circulation.[50]	11854125	Retrospective	13 fetuses and children: 6 with prenatal and 7 postnatal endocardial fibroelastosis.	Endocardial fibroelastosis develops in cases of autoantibody-associated congenital heart block even when ventricular pacing is appropriately managed.
Silvetti *et al*., 2020 Pacing Clin Electrophysiol.[51]	32233121	Longitudinal	20 neonates and infants with congenital complete atrioventricular block who underwent	Left ventricular (LV) pacing conserved both LV systolic function and synchrony in neonates with congenital complete atrioventricular block, and this preservation

			pacemaker implantation.	was sustained over a 5-year period. Patients experienced an enhancement in left ventricular ejection fraction. Neither the pacing mode nor the presence of autoantibodies displayed any influence on LV function and synchrony.
Sonesson *et al*., 2019 Ultrasound Obstet Gyncecol.[52]	30620419	Prospective	212 anti-Ro52 antibody exposed pregnancies (18-24 weeks of gestational age) at risk of fetal atrioventricular block.	Fetal atrioventricular interval is a limited predictor of congenital heart block progression; however, vigilant monitoring facilitates the early identification of atrioventricular block II or III.
Trucco *et al*., 2011 J Am Coll Cardiol.[53]	21292131	Retrospective	20 mothers treated with intravenous immunoglobulin and corticosteroids.	The improvement of maternal autoantibody-induced fetal cardiomyopathy and endocardial fibroelastosis, particularly in terms of ventricular function, was observed with the administration of intravenous immunoglobulin and corticosteroids.

8.5.2. Intrauterine Growth Restriction

IUGR occurs when a fetus does not grow in accordance with its genetic potential, and therefore does not achieve in its entirety, its intrinsic growth potential at any given gestational age (GA)[54]. In pregnancies complicated by SLE, IUGR is a well-known and frequent complication, particularly in individuals with renal involvement or active lupus. IUGR has been associated with various other conditions such as pre-eclampsia, eclampsia, hemolysis - elevated liver enzymes - low platelets (HELLP) syndrome, chronic hypertension, diabetes, chronic renal insufficiency, sickle cell disease, infections, hematologic immune-mediated disorders, malignancies, neonatal/maternal heart conditions, and thyroid and AD.[1,8] IUGR is known to be caused by maternal conditions related with fetal hypoxia, such as pulmonary insufficiency, anemia, pre-eclampsia, and smoking[55]. Hypoxia induced IUGR within animal models is associated with

changes in the cardiac structure and function, as well as with increased susceptibility to myocardial ischemia and reperfusion injury[55,56]. Smoking, maternal malnutrition, APS, teratogen exposure, infections, genetic and structural diseases, and most importantly placental insufficiency, as shown by Doppler abnormalities throughout fetal life, have all been associated with increased risk of developing IUGR[57]. The most common etiology of fetal IUGR is uteroplacental vascular insufficiency, where the placenta is unable to provide an adequate amount of nutrients and oxygen to the fetus[58]. Prolonged oxygen and nutrient restriction frequently lead to circulatory alterations in growth restricted fetuses, which are in part alternative mechanisms to compensate for the unfavorable intrauterine environment[58]. These prenatal adaptations, for which the fetuses' cardiac output is redistributed, are intended to keep essential organs including the brain, heart, and adrenals supplied with oxygen and nutrients[58]. Although these adaptive circulatory shifts are favorable during intrauterine life, they are not beneficial after birth and are thought to affect the cardiovascular system's development of these fetuses, increasing their risk for lifelong cardiovascular morbidities and diseases in adulthood[58,59].

8.5.3. Congenital Heart Block

Fetuses of women with AD exposed to maternal autoantibodies have higher risk of developing damaged conductive system and cardiac function, as well as developing CHB and heart failure.[6] The association between maternal autoantibodies and CHB has been shown to be higher in offspring of mothers containing anti-Ro-52kD and anti-La-48kD.[46] Autoantibodies SSA/Ro and SSB/La are transported across the placenta into the circulation of the fetus. Ro autoantigen includes both isoforms (52kD and 60kD), in which Ro52 is found more commonly than Ro60 and is accountable for cardiac damage.[60] The conduction tissues can be damaged throughout the fetal

development of the heart, due to placental transference of maternal Ro/La autoantibodies, potentially inducing inflammation of the AV node and the myocardium, which in turn will be replaced with calcification and fibrosis, leading to potential heart block, myocardial dysfunction, and endocardial fibroelastosis.[5,46] Cardiac complications related to autoantibodies occurred mostly when offspring were exposed to levels ≥50 U/ml of anti-Ro and can lead to a reduction in fetal heart rate (HR), which can be as low as 50-70 beats per minute.[46,60] Interestingly, fetuses exposed to high anti-La levels, concomitant with anti-Ro levels, showed to be affected to a lesser extent compared to those exposed only to high anti-Ro levels (38%).[46]

Irreversible complete atrioventricular block is the foremost cardiac manifestation of CHB, although other severe cardiac complications can develop in fetuses/newborns, such as endocardial fibroelastosis, valvular insufficiency and cardiomyopathies with significantly reduced cardiac function.[5] Congenital atrioventricular block (AVB) can affect 2%-5% of fetuses emerging from mothers with an immunological disease, such as SLE or SS, with the presence of autoantibodies.[61]

Immune-mediated cardiac disease, such as autoimmune CHB, can be incorporated among factors that contribute to the development of neonatal lupus erythematosus (NLE).[5] NLE manifestations involve cutaneous rash, thrombocytopenia, leukopenia, anemia, and liver dysfunction, for which unlike complete AVB, all get resolved within a few months after birth, in addition to the disappearance of maternal antibodies from the fetus' circulation.[46] Maternal anti-SSA/Ro and anti-SSB/La have been observed to be implicated in the pathogenesis of these complications and can be transferred passively to the fetus as early as 11 weeks.[60] The correlation between cardiac NLE and anti-Ro levels have been supported, thereby indicating that the decrease in these autoantibodies may ward off those complications.[46] It is important to take into

consideration that CHB and cardiac NLE cases were more common in asymptomatic mothers.[46,60] According to the Research Registry for Neonatal Lupus (RRNL), mothers affected by SLE, SS, undifferentiated autoimmune syndrome or asymptomatic can still have babies with CHB, meaning that the presence of active autoimmune disease in mothers is not a prerequisite for the occurrence of CHB.[60] Occasionally, asymptomatic pregnant women with anti-Ro/La autoantibodies can have offspring with CHB, and bradycardia is often discovered during standard prenatal screening, though third-degree CHB may not be reversed by medication once established.[6]

CHB can range in severity from first-, second- and third-degree block, which are all strongly associated with maternal SSA/Ro autoantibodies. CHB can affect 2% of fetuses from pregnancies affected by anti-Ro antibody, with a recurrent rate of 15-20% in subsequent pregnancies and a mortality rate of 16-30% in utero or during the first three months of life in those affected by CHB.[8] It has been observed that anti-Ro/SSA is associated with CHB but does not negatively influence pregnancy outcomes.[8] Although the most common form of CHB is AV block, other manifestations have been noticed, such as sinus node dysfunction, ventricular and junctional tachycardia, long QT interval, atrial flutter, valvular disease, and dilated cardiomyopathy.[60]

8.5.4. Fetal Cardiac Adaptations and Remodeling

The thickening of arterial vessel walls is one indication of vascular adaptation to IUGR conditions, and it has been shown that children and adolescents born at a small-for-gestational-age (SGA) have increased arterial thickness.[62] Being born IUGR has been associated with early-life hypertension and with increased atherosclerosis risk. Barker's hypothesis is the epidemiologic concept where fetal development may affect health across the lifespan. As such, there are increasing data outlining that IUGR fetuses are at high risk for later cardiovascular disease.[62] IUGR

has also been associated with pulmonary vascular remodeling to varying extents, which causes pulmonary hypertension (PH)[54]. Additionally, growth restricted fetuses are also exposed to perinatal and neonatal complications, such as meconium aspiration syndrome (MAS), birth asphyxia and respiratory distress syndrome[54]. Hypoxia can increase the pulmonary vascular resistance, leading to PH in IUGR asphyxiated neonates[54,63].

In fetuses affected by IUGR, various patterns of cardiovascular remodeling have been observed depending on the severity of the disease (**Table C**). Placental insufficiency can directly impact the structure of fetal heart cells, while increased resistance to blood flow in the placenta leads to increased cardiac afterload. As a result, the fetal heart undergoes remodeling, becoming less efficient while adopting a more spherical or globular shape to better handle the increased afterload and to lessen wall tension[57]. Ongoing and worsening placental insufficiency may be associated to hypertrophic cardiac response, in an attempt to enhance contractility and reduce local wall stress[57]. The cardiomegaly and moderate pericardial effusion in some of these severe IUGR newborns may be attributed to volume overload and, unavoidably, changes in heart function are related to this significant remodeling[57,62]. Although ejection fraction (EF) is often maintained, this occurs in conjunction with a decrease in stroke volume and a concurrent compensatory rise in heart rate in order to maintain cardiac output and the proper perfusion to organs[57,62]. IUGR is also associated with profound alterations of the fetal heart structure, as well as at the cellular and organelle levels[57]. In regard to a large range of cardiac diseases, the disruption of normal sarcomere structure is implicated in cardiac dysfunction and remodelling[42]. The heart's contractility in IUGR fetuses seemed to be altered with shorter sarcomere, as well as a decrease in sarcomeric protein levels, such as tyrosine and myosin[57]. Seeing that sarcomeres is an important component of cardiac

contractility, and that cardiac energy consumption is proportional to the number of recruited cross bridges, shorter sarcomeres will feature fewer actin and myosin cross bridges, lower contractile force, and shorter distances of contraction[42,58]. It has been observed that IUGR is not only associated with cardiac dysfunction, but as well with shorter sarcomere length and an increase of troponin-I concentration[42]. Iruretagoyena *et al*. conducted a study and compared nine severe growth-restricted fetuses to nine with an appropriate growth for gestational age (AGA) fetuses. They found that the sarcomere length of IUGR fetuses was considerably shorter than that of AGA fetuses and that this can result in systolic dysfunction, decrease in longitudinal motion and impaired relaxation of the heart, suggesting that this may be an adaptive mechanism to prolonged oxygen and nutrient restriction[42,58,62].

In tissue remodelling, programmed cell death and cellular removal play a significant role. Failure of cardiocytes to consume apoptotic cardiocytes during embryonic cardiac development might be regarded as a sign of CHB linked to maternal antibodies.[60] Antigens SSA/Ro and SSB/La are translocated onto cardiomyocytes, allowing autoantibodies to bind them in the circulation and initiate an immunological response.[60] However, immunological complexes on phagocytic cardiocytes may hinder their clearance by nearby healthy cardiocytes, obstructing an important function in the proper development of the fetal heart.[60] The accumulation of these apoptotic cardiocytes lead to their infiltration by macrophages and triggers pro-inflammatory and fibrotic cytokines, resulting in tissue damage and the fibrosis characteristic of CHB.[60] Additionally, it has been observed that healthy normal hearts show no apoptotic cells, whereas fetal hearts affected by CHB showed an inflated amount of apoptotic cells.[60]

*8.5.4.1.***Table C.** IUGR Cardiac Clinical Manifestations and Mechanism of Fetal Heart Cardiac Adaptation.

IUGR Cardiac Clinical Manifestations	Mechanisms and Impact
Cardiac Dysfunction	Altered contractility, shorter distances of contraction, sarcomere length shortening, and poor oxygenation contribute to impaired cardiac function in IUGR fetuses.
Cardiac Remodeling and Shape Alteration	Placental insufficiency leads to a less efficient heart with a spherical shape to handle increased afterload. Accumulation of apoptotic cardiocytes triggers inflammation, fibrotic cytokines, and tissue damage characteristic of congenital heart block.
Pulmonary Hypertension (PH)	Pulmonary vascular remodeling due to IUGR leads to pulmonary hypertension. Hypoxia and increased pulmonary vascular resistance contribute to PH in IUGR asphyxiated neonates.
Systemic Hypertension	IUGR conditions can result in increased arterial thickness, potentially leading to systemic hypertension in children and adolescents born with small-for-gestational-age.

8.6. Fetal Echocardiography, Speckle Tracking Echocardiography and Myocardial Deformation

8.6.1. Concept of Echocardiography

8.6.1.1.Conventional Imaging Techniques

Fetal echocardiography is a non-invasive imaging ultrasound imaging technique that is widely used to evaluate cardiac function during pregnancy. It enables a detailed assessment of various aspects of fetal cardiac health, offering valuable information for prenatal care. One of the primary advantages of fetal echocardiography is its ability to assess multiple parameters of cardiac function and rhythm using a non-radiation modality. This includes evaluating the cardiac structure, heart rhythm, anatomic abnormalities, ventricular function, and valvular function.[6] This comprehensive evaluation helps to identify any potential concerns or abnormalities in the developing fetal heart, providing important diagnostic information for management and treatment

planning. Fetal heart monitoring has progressed with time to the extent of being able to detect morphological anomalies with the use of high-resolution echocardiography.[64] In addition to fetal echocardiography, conventional M-mode (motion-mode) echocardiography and pulsed-wave Doppler are also utilized to assess AVB. M-mode echocardiography provides a real-time, one-dimensional view of the fetal heart, allowing for precise measurements of cardiac dimensions and function.[65] Pulsed-wave Doppler, on the other hand, measures blood flow velocities in specific regions of the fetal heart (precise areas of interrogation), providing information about blood flow patterns and potential abnormalities, including AVB.[64-66]

8.6.1.2. Two-Dimensional Speckle Tracking Echocardiography in Cardiac Function

Two-dimensional speckle tracking echocardiography (2D-STE), a relatively new technique, can evaluate the myocardial deformation by tracking speckles (pixels) that form the ultrasound-derived images of the heart. The myocardium is tracked throughout the cardiac cycle, thus providing measures of cardiac function and time to peak of contraction of myocardial segments.[6,67]. The STE algorithm tracks the entire myocardial layer and can be assessed in multiple views, such that it can inform on the deformation in the longitudinal, circumferential, and radial plane. The percentage of deformation between systole and diastole is described as the "strain value", while the speed at which it occurs is described as the "strain rate". An assessment taking into account all the segments of the respective ventricles provide a global strain or global strain rate value. Segmental analysis is also feasible in order to detect areas of the ventricles that may be not functioning or functioning at suboptimal performance.[67] Indeed, tethering effect is a known limitation of conventional echocardiography metrics of functional assessment, whereby areas of the myocardium not participating to the contraction or relaxation are dragged by other surrounding

areas contracting and relaxing. Segmental analysis is taken into account within the global deformational analysis, such that lower values inform on global or segmental sub-performance of the myocardium. Further, STE allows to further assess the location and severity of the myocardial injury, by measuring regional changes and depicting values via its polar standardized projection, also known as the "bull's-eye" map. A high resolution clip of the 4CV, with visualization of the valve insertion and optimal contrast between the cavity and endocardium are essential to ensure an accurate tracking of the LV and the RV, as well as to avoid the underestimation of peak global longitudinal strain (GLS) and strain rate (GLSR).[4] This technology can be used on fetuses, children, and adults, whether it is in normal conditions or specific to a disease, and has been shown to be independent of the fetal position, compared to conventional imaging, making it a promising tool for assessing fetal heart function.[4,6,68]

8.6.2. Myocardial Strain and Strain Rate in Healthy Fetuses Using STE

Given the important role of the fetal heart in adapting to changes in hemodynamics, evaluating fetal cardiac function can be valuable in investigating non-cardiac disease, but it can also be helpful in diagnosing and monitoring congenital heart defects such as aortic coarctation and hypoplastic left-heart syndrome.[68] However, assessing fetal cardiac function is challenging due to factors such as the small size and motion of the fetal heart.[68] It has been implied that 2D-STE can be useful in early detection of myocardial remodeling or anomalies, through GLS and GLSR values, compared to Doppler.[4,68] GLSR is the velocity of the myocardial deformation and therefore reflect cardiac function taking into account underlying heart rate.[4] GLS represents the change in length of a myocardial segment compared to its original length, for which in most cases remains stable, although it can be impacted by preload and afterload alterations.[4] GLS and GLSR

are expressed as negative values upon a reduction in dimensions (such as during contraction in the longitudinal plane of the LV), and higher negative values indicate increased shortening.[67] Fetal cardiac function is likely to undergo changes throughout pregnancy due to the normal growth and development of the heart, as well as alterations in loading conditions as gestation progresses.[68] This suggest that GLS can be dependent on GA, seeing that the fetal hemodynamic system changes significantly through prenatal life.[4,68] In a longitudinal cohort study of 124 healthy pregnancies with normally growing fetuses, it was observed that values for GLS and GLSR increased significantly for both the LV and RV, from the second trimester until delivery.[68] Reference values for LV-GLS, LV-GLSR, RV-GLS, and RV-GLSR, measured using 2D-STE between 18 and 41 weeks' gestation, were determined in this study. It was found that all four parameters increased significantly (became less negative), indicating decreasing myocardial shortening with advancing gestation.[68] However, RV-GLS values were consistently higher than LV-GLS values throughout pregnancy.[68] Fetal ventricles are initially stiff with limited heart rate adaptation due to reduced adrenergic stimulation response, but as the heart grows during gestation, these limitations reduce, resulting in increased contraction capacity.[68] Additionally, GLSR values tend to increase with decreasing fetal HR as GA advances.[68]

The RV plays an important role in the fetal circulation by being accountable for 60% of the cardiac output, which is the amount of blood the heart pushes out with each contraction, thereby making it the dominant ventricle in fetal life.[4,69] The RV gets filled mostly from the upper body's systemic venous return via the superior vena cava, the lower body's venous return via the inferior vena cava and from the umbilical venous flow.[4, 68] LV gets filled mainly, during fetal life, by the blood coming from the right atrium and crossing the foramen ovale to fill the left atrium.

Additionally, a small amount of blood flows through the lungs and returns via the pulmonary veins, contributing to a lesser amount to the LV filling. The fetal LV ejects into the ascending aorta the blood which has the highest concentration in oxygen, which is destined to the cerebral circulation. Portion of the blood feeding the ascending aorta mixes with the flow from the DA coming from the pulmonary artery. This blood supplies the descending aorta of the fetus and, part of it, returns to the placenta via the umbilical arteries. As such, the fetal aorta is connected to the low resistance placental vascular network. In the fetal context, the pulmonary vascular bed is constricted, resulting in high pulmonary vascular resistances. As such, the DA, which connects the fetal aorta to the fetal pulmonary artery, is fed in blood by the pulmonary artery, which originated from the RV. Overall, alterations in both ventricular preload and afterload can be manifested in changes in GLS and GLSR values. A study revealed that RV-GLS values consistently exceeded LV-GLS values during pregnancy, which is in line with previous research indicating a similar trend with advancing GA.[68] Discrepancies in ventricular myocardial architecture may account for the variation in GLS values observed between the LV and the RV.[68] The LV has circumferential and longitudinal fibers, allowing for deformation in multiple directions including longitudinal, circumferential and radial planes, while the RV has predominantly longitudinal fibers, leading to longitudinal shortening.[68]

8.6.3. Echocardiography Concepts Applied to Rheumatological Populations

8.6.3.1. Detection of Atrioventricular Block in Fetuses Exposed to Maternal Rheumatological Conditions

These conventional imaging techniques are crucial in detecting and monitoring AVB. The four-chamber view (4CV) is a critical and commonly used imaging technique for evaluating the

fetal heart. [64] It is not only easy to obtain but also considered the most important view for assessing the fetal heart, as approximately 60% of congenital heart disease cases can be detected using this view.[66] In addition to the 4CV, evaluating the connections of the great arteries can further detect about 90% of serious congenital heart diseases.[66] Fetal echocardiography using Doppler also allows for the evaluation of the mitral-aortic time and the superior-vena cava to aorta time, which are estimates of the mechanical PR interval. M-mode assessment also allows for evaluation of wall motions with a high temporal resolution. As such, line of interrogation may be placed crossing the ventricular and atrial wall, in order to appreciate timing of atrial and ventricular contractions, as well as their relationship. Finally, pulse-wave Doppler in the fetal vessels (aorta or pulmonary artery) inform on heart rate, while Doppler in the umbilical, ductus venosus and fetal cerebral vessels inform on ultrasonographic signs of placental insufficiency.

Additionally, fetal cardiac time intervals (fCTIs) can be assessed through magnetocardiography (MCG), which is another non-invasive method that records magnetic fields generated by the electric currents of the heart and has shown to be effective in exploring fetal arrythmias.[61] PQ segment, which reflects pure AV conduction time and unaffected by atrial depolarization time (PR interval), has been demonstrated to be significantly longer in fetuses, thus suggesting early antibody-mediated tissue damage caused by maternal SSA/Ro and SSB/La antibodies.[61] This finding is consistent with a pathological model of AV node involvement in less advanced degrees of AVB, as demonstrated in a previously published study comparing exposed fetuses to anti-Ro/La antibodies at risk to a gestational age-matched unexposed group.[61]

Prolonged PR interval secondary to inflammation of the fetal cardiac conduction system may cause cardiac dyssynchrony, which can be categorized into two types: electrical dyssynchrony and

mechanical dyssynchrony. Electrical dyssynchrony is characterized by prolonged conduction time in the ventricles, leading to an extended QRS duration and is a result from abnormal electrical activation and spreading patterns (such as left bundle branch block), or structural remodeling, and can lead to disruptions in the normal timing of the heart's contraction and relaxation.[70] On the other hand, mechanical dyssynchrony refers to the lack of coordination in the mechanical contraction of the LV, often resulting in simultaneous contraction and stretch in different segments of the LV, as well as delays in the time to peak contraction between segments.[70] Moreover, dyssynchrony encompasses three main components, namely atrioventricular, interventricular, and intraventricular dyssynchrony.[70] This can manifest as regional differences in timing, abnormal shortening patterns, and variations in the amount of external work performed by the heart.[71] Yet, during the initial phases of the condition, changes in fetal cardiac function and dyssynchrony may be subtle in fetuses with normal heart rhythm, making it challenging to quantify them using conventional imaging methods.[6]

8.6.3.2. Myocardial Strain and STE in Fetal Applications Related to Rheumatological Conditions

A study conducted by Duan *et al.*, with the aim of comparing cardiac function and systolic dyssynchrony in fetuses of mothers with AD and those of healthy mothers using 2D-STE, demonstrated that although the LV and RV systolic and diastolic function remained unchanged in fetuses from mothers affected by AD, the LV systolic dyssynchrony was prolonged.[6] Additionally, the longitudinal strain of the LV free wall, interventricular septum and RV free wall had shown no significant difference compared to controls.[6] Ventricular dyssynchrony refers to an irregular or uncoordinated contraction of one or more of the lower chambers of the heart. Even a slight delay

in the timing of contractions between different parts of the ventricles or between the LV and the RV can result in dyssynchrony. Therefore, ventricular systolic dyssynchrony, which can influence ventricular function and structure, has shown to be significantly more prolonged in a one-chamber dyssynchrony (1C-DYS) than a two-chamber dyssynchrony (2C-DYS) in fetuses of pregnant individuals with AD and positive antibodies.[6] These results suggest that the LV conduction system in fetuses may have been adversely affected, possibly due to the presence of autoantibodies SSA/Ro or/and anti-SSB/La that could damage the conduction tissues of the fetal heart.

8.6.3.3.Myocardial Strain and Rheumatological Conditions Using STE

Transthoracic STE screening is a non-invasive and practical tool that can assess the systolic and diastolic functions of the heart, as well as identify myocardial impairments and prevent early mortality.[72,73] Conventional EF measurement using Biplane Simpson's disc method is often used for the assessment of LV systolic function. However, some data suggests that it may fail to detect subtle anomalies detectable by GLS or GLSR, which have also been associated to mortality outcomes in SLE patients.[67,73] It has been established that prior to the development of a reduced EF in SLE patients, LV-GLS measurements can identify cardiac disease.[73] Many studies have observed the impact of rheumatologic conditions on the CV function. For RA, studies have reported some cardiac contractile performance impairment, such as a significant decrease in GLS, global circumferential strain (GCS), global radial strain (GRS), and GLSR due to the inflammatory status, compared to healthy controls.[67,74-77] STE analysis was also evaluated in patients with ankylosing spondylitis (ASp) patients by several studies. It has been shown that GLS was significantly lower in ASp subjects compared to controls, as well as GCS and GRS.[67,78,79] Additionally, one of the studies also reported an important reduction in LV diastolic and systolic

strain and strain rate values in the context of ASp.[67,80] In regard to SLE patients, numerous studies have described a decrease in GLS, GCS and GRS, when compared to healthy subjects.[67,73,81] Additionally, left atrial peak systolic strain and global strain were revealed to be reduced in all segments, while the expected positive left atrial strain on diastole in healthy patients was also found to be decreased in SLE patients, along with a reduction in systolic strain rate and early diastolic strain rate.[67,82] SS is also known to be associated to elevated CV risks. GLS in the apical four-chamber view and GRS in the short axis view were discovered to be significantly lower in SS patients, as opposed to controls.[67,83] Overall, many other rheumatological disease such as psoriatic arthritis, systemic sclerosis, Churg-Strauss syndrome, Behcet's disease, Kawasaki vasculitis and others, demonstrated similar correlations in terms of CV impairments.[67] Thus, STE can be an interesting method in identifying myocardial functional alterations, possibly enhancing patient's assessment and risk management.

8.6.4. Rationale

The rationale for our research is based on the established association between maternal rheumatological conditions and adverse pregnancy outcomes, including fetal cardiac dysfunction. However, limited studies have utilized STE to investigate this relationship in the fetus. Autoantibodies, specifically anti-Ro/La, have been implicated in CHB and fetal heart dysfunction. Despite existing evidence, there are knowledge gaps in understanding the impact of maternal rheumatological conditions on fetal cardiac adaptations, the role of autoantibodies, and the potential of STE in evaluating fetal cardiac function. Therefore, our retrospective study aims to address these gaps by utilizing STE to compare fetal cardiac function in pregnancies with and without exposure to maternal rheumatological conditions.

*8.6.4.1.***Table D.** Knowledge Gaps and Study Aims.

Knowledge Gaps	Aims of Study
Fetal cardiac function in fetuses exposed to maternal rheumatological conditions.	Utilize STE to assess fetal cardiac function and explore the influence of maternal rheumatological conditions on it. Compare these findings with fetuses not exposed to maternal rheumatological conditions
Impact of circulating autoantibodies (Anti-Ro/La) on fetal cardiac function.	Investigate the effect of detectable circulating autoantibodies (Anti-Ro/La) on fetal cardiac function.
Influence of CHB presence on fetal heart function in fetuses exposed to positive Anti-Ro/La autoantibodies.	Examine the impact of CHB presence on fetal cardiac function using STE among fetuses exposed to positive Anti-Ro/La autoantibodies. Compare these findings to fetuses without CHB.
Legend: CHB: Congenital Heart Block; STE: Speckle Tracking Echocardiography.	

OBJECTIVES AND HYPOTHESIS:

9.1. Primary objective:

Among a cohort of fetuses exposed to rheumatological conditions closest to 23 weeks of GA, we aimed to assess the fetal heart's function through STE and examine how maternal rheumatological conditions affect it, comparing them to unexposed fetuses.

Hypothesis: We hypothesized that fetuses whose mothers had a rheumatological condition would display distinct cardiac function in utero, as determined by STE, in comparison to an unexposed group of fetuses.

9.2. Secondary objectives

i. To assess how detectable circulating autoantibodies affect the underlying fetal cardiac function.

Hypothesis: We hypothesized that the function of the cardiac system would be further affected by exposure to autoantibodies.

ii. To assess the impact of CHB presence or absence on fetal heart function using STE among those who were exposed to positive autoantibodies.

Hypothesis: We hypothesized that fetal cardiac function would be significantly more affected by the presence of CHB.

10. PREFACE TO MANUSCRIPT:

The complex interplay between maternal health and fetal development has been well-documented, and evidence suggests that rheumatological conditions can significantly influence the outcome of pregnancies, with implications for maternal, fetal, and neonatal health.

The link between rheumatological conditions and adverse pregnancy outcomes, such as maternal malperfusion, placental anomalies, and compromised fetal oxygen exchange and nutrient absorption, has been established in previous research. Moreover, the transplacental passage of autoantibodies, particularly anti-Ro and anti-La antibodies, has been associated with various degrees of CHB and fetal heart dysfunction. These findings highlight the need for further investigation into the impact of maternal rheumatological conditions on fetal cardiac adaptation and performance.

To address these gaps in knowledge, our research aims to compare fetal ventricular and deformation parameters using STE in pregnancies with positive and negative anti-Ro/La autoantibodies to those who did not have exposure to these autoantibodies. We hypothesize that fetuses exposed to maternal rheumatological conditions would exhibit different cardiac function in utero compared to unexposed fetuses, and that the presence of autoantibodies would further impact its function. Additionally, we hypothesize that the occurrence of CHB would have a more profound effect on fetal cardiac performance.

We anticipate that our research will contribute to a novel approach for early risk stratification of women with rheumatological conditions and their offspring at high risk of adverse cardiovascular outcomes. By determining the association between maternal autoantibodies and

subclinical cardiac function in offspring, our findings may offer insights into implementing preventive strategies and optimal management. Moreover, our methodology could potentially be applicable to the assessment of subclinical cardiac involvement in other rheumatic diseases and may guide future clinical trials in this area.

11. MANUSCRIPT:

Cardiac Alterations in Fetuses Exposed to Rheumatic Disease – A Fetal Speckle Tracking Echocardiography Study

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Conflicts of interest: None to declare.

What are the novel findings of this work?

Using speckle-tracking analysis on fetal echocardiography scans performed at around 22-23 weeks of gestational age, we identified alterations on fetal left ventricular (LV) function in those exposed to maternal rheumatological conditions. LV ejection fraction and longitudinal strain were increased when compared to unexposed fetuses, regardless of antibody presence or fetal heart block.

What are the clinical implications of this work?

Our findings indicate that the fetal heart is initially able to compensate for unfavorable intrauterine conditions, which may not necessarily be interpreted as a positive indicator. Pregnancies complicated by rheumatological conditions should be monitored for fetal cardiac functional status.

Abbreviations: ACR (American College of Rheumatology), CHB (congenital heart block), CI (confidence interval), DICOM (Digital Imaging and Communications in Medicine), ED (end-diastolic), EDA (end-diastolic area), EDSR (early-diastolic strain rate), EDV (end-diastolic volume), EF (ejection fraction), ES (end-systolic), ESV (end-systolic volume), EULAR (European League Against Rheumatism), FAC (fractional area change), fps (frame per second), GA (gestational age), HB (heart block), IQR (interquartile range), IUGR (intrauterine growth restriction), LV (left ventricle), MUHC (McGill University Health Center), PACS (Picture Archiving and Communications System), pLS (peak longitudinal strain), pLSR (peak longitudinal strain rate), RV (right ventricle), SD (standard deviation), SLE (systemic lupus erythematosus), STE (speckle-tracking echocardiography), TTP (time to peak), 2-D (two-dimensional).

Keywords: anti-Ro/La antibodies, congenital heart block, fetal echocardiography, rheumatological pregnancies, speckle tracking echocardiography, systemic lupus erythematosus.

Data sharing: Derived data generated will be shared on reasonable request to the corresponding author.

Disclosure of prior presentation: None.

11.1 Contributors Statement Page:

AO conceptualized and designed the study, collected the data, analyzed the data, drafted the manuscript, and adjusted the manuscript according to the comments of the co-authors.

NW, MM, **DVM**, **JS** collected the data, critically appraised the analysis and revised the manuscript.

AM, **IM**, **WM**, **LR** critically appraised the analysis of the data and reviewed and revised the manuscript.

GA and **EV** conceptualized and designed the study, supervised data collection, critically appraised the analysis of the data, wrote, and critically reviewed the manuscript for important intellectual content.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

11.2 **Abstract:**

OBJECTIVES: We sought to valuate the fetal cardiac performance in pregnancies complicated by a maternal rheumatological condition using speckle-tracking echocardiography (STE), compared to a cohort of unexposed fetuses. In addition, we aimed to characterize the effect of circulating maternal autoantibodies and fetal atrio-ventricular block (AVB) on fetal cardiac function.

METHODS: Single-center, retrospective cross-sectional study of pregnancies in women with a rheumatological disease who underwent fetal echocardiography between 2013 to 2021. Rheumatic disease pregnancies were stratified by anti-Ro/La antibody status and were compared to pregnancies occurring in women without a rheumatic disease and/or anti-Ro/La antibodies, matched on gestational age (22 to 24 weeks).

RESULTS: We identified 60 pregnancies exposed to a rheumatological condition. Of those, 46 had positive anti-Ro/La antibodies including 3 twin pregnancies, resulting in 49 positive anti-Ro/La antibodies fetuses. Most pregnancies occurred in the context of systemic lupus erythematosus (65%) and 83% were exposed to hydroxychloroquine (Table 1). Fetuses exposed to a rheumatological pregnancy were compared to the fetal echocardiography performed on 61 fetal non-exposed fetuses. Left ventricular (LV) ejection fraction (EF) was higher in fetuses exposed to rheumatological conditions, regardless of anti-Ro/La antibodies status, compared to unexposed fetuses (Table 2). Positive anti-Ro/La antibodies fetuses also showed increased LV deformation (global and LV free wall) compared to unexposed fetuses. Right ventricular (RV) parameters were similar between groups. Within the positive anti-Ro/La antibodies fetuses, 5 (11%) had a third-degree AVB (Table 3). AVB fetuses demonstrated a delayed LV and RV activation by the time-to-peak deformation using STE. Positive anti-Ro/La antibodies fetuses without AVB still demonstrated an increased LV-EF when compared to unexposed fetuses (LV-EF 56.4% [SD 9.4] vs 51.9% [SD 7.7]; p=0.01); 95% CI: [53, 59]% vs [50, 54]%) and LV anterolateral peak longitudinal strain (-24.2 [SD 6.3] vs -21.8 [SD 5.1]%; p=0.03; 95%CI: [-26.0, -22.4]% vs [-23.1,-20.5]%). Point estimate of difference being -4.7% (95% CI [-7.8, -1.3]%) for the LV-EF and for 2.4% (95% CI [0.2, 4.5]%) LV anterolateral peak longitudinal strain.

CONCLUSION: Fetuses exposed to maternal rheumatological conditions show early cardiac functional alterations by STE, regardless of AVB status. The increase in LV-EF and strain may represent changes due to an adverse in-utero environment. Indeed, these findings may reflect an early adaptation to a higher afterload secondary to adverse vascular resistance. Therefore, these subtle differences suggest a hyperdynamic heart response to the placental reaction. However, it remains uncertain whether these early changes will have a later clinical impact. Future studies are needed to evaluate the long-term effects of this phenomenon and should outline the later

gestational and immediate post-natal cardiac adaptation of infants exposed to maternal rheumatological diseases.

11.3 Introduction

Rheumatological conditions affecting pregnancies have been associated with an increase in maternal, fetal and/or neonatal complications, conferring a high-risk status.[1] Maternal rheumatological conditions are known to be associated with placenta-mediated pregnancy complications due to im-paired placental implantation. These changes are often indicators of maternal malperfusion and include anomalies in placental vascularity and coagulation.[2,3] This may result in compromised oxygen exchange and nutrient absorption, initiating chronic fetal hypoxia and malnutrition.[2] As such, the fetal heart's development may be influenced by the prenatal environment and may adapt in structure and shape, with its function possibly being impacted by the adverse vascular resistances and oxy-genation.[4] Indeed, the fetal heart may undergo myocardial remodeling due to loading variations induced by the ongoing inflammation of the mother's rheumatological state, the chronic hypoxia and the elevated placental resistance. Fetuses exposed to placental insufficiency have been described to adapt their left ventricles to shift from an elliptical to a globular/hypertrophic form, in attempt to enhance contractility.[5] Additionally, fetuses of women with autoimmune diseases, especially those with circulating anti-Ro and anti-La antibodies, have higher risk of developing myocardial inflammation, impaired atrio-ventricular valve function and damaged conductive system, with some potentially developing various degree of congenital heart block (CHB) or post-natal cardiomyopathy.[6]

Speckle-tracking echocardiography (STE) is a technique that tracks pixels from a frame-by-frame basis to quantify the degree of myocardial deformation, even in utero, in order to assess the under-lying cardiac performance.[7-10] Therefore, we sought to evaluate the fetal cardiac performance, using STE, to understand the impact of maternal rheumatological conditions on the fetal heart. In

addition, we aimed to characterize the effect of circulating maternal anti-Ro/La antibodies and fetal atrio-ventricular block (AVB) on fetal cardiac function. We hypothesized that fetuses exposed to a ma-ternal rheumatological condition would have a different cardiac adaptation in utero, as measured by STE, when compared to a group of unexposed fetuses. As well, we hypothesized that exposure to anti-Ro/La antibodies would further impact this adaptation and that the occurrence of AVB would have a more pronounced effect on the fetal cardiac performance.

11.4 Methods

This is a single-center, retrospective, cross-sectional study of pregnancies between 22 and 24 weeks of gestational age (GA), undergoing fetal echocardiography from 2013 to 2021 at the McGill University Health Centre (MUHC). At the MUHC, pregnancies are referred to our fetal cardiology service when a suspicion arises based on prenatal risk factors (family history of con-genital heart defect, concern for genetic condition or syndrome, fetal malformation, maternal condition, documentation of maternal anti-Ro/La antibodies, or other concerns on obstetrical ultrasound). The fetal cardiologist emits a report that is digitalized, which includes the suspected diagnosis and reason for referral. Data from the echocardiography is stored in the format of Digital Imaging and Communications in Medicine [DICOM] files on the Picture Archiving and Communications System (PACS) (SyngoPACS, Siemens Medical Solutions, United States). SyngoPACS is searchable. As such, we identified pregnancies with the following keywords: “congenital heart block”, “anti-Ro”, “anti-La”, “Lupus”, “SLE”, “Sjogren”, or “mixed connective tissue disease”. At our center, rheumatic diseases are usually clinically diagnosed relying on the classification criteria established by the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) for systemic lupus erythematosus (SLE),

Sjogren's syndrome, rheumatoid arthritis, antiphospholipid syndrome, idiopathic inflammatory myositis and mixed connective tissue disease, respectively.[11-14] Inclusion criteria consisted in pregnancies complicated by a maternal rheumatological condition with or without positive anti-Ro and/or La antibodies, as ascertained by medical chart review. Exclusion criteria included fetuses with major cardiac defects and pregnancies with insufficient documentation in the chart when followed at an outside institution. Pregnancies diagnosed with maternal rheumatological conditions after diagnosis of fetal AVB based on incidental findings of fetal bradycardia at a routine obstetrical visit were included. Ethics approval was obtained for this study from the MUHC - Research Ethics Board.

11.4.1 Clinical information

For all the subjects included in the study, maternal age at fetal echocardiography was obtained, as well as other clinical information such as subtype of maternal rheumatological condition, maternal co-morbidities, pregnancy complications (i.e CHB, gestational diabetes, gestational hypertension, hemolysis elevated liver Enzymes and low platelet (HELLP), induced abortion, fetal growth restriction and fetal death), presence of a multiple pregnancy, and current medications. Gestational age at fetal echocardiography was obtained from the best obstetrical assessment, which involved the combining information from fetal ultrasound and the date of the last menstrual period recorded at the first trimester obstetrical visit or on their first trimester ultrasound. Additionally, regarding the maternal characteristics, pregnant women were accounted for only once when there was an occurrence of a multiple pregnancy, while if pregnant women had more than one pregnancy over the study period, we included each of these pregnancies. Available placental reports describing placental weight (classified by expected weight for gestational age at birth) and histopathology

were retrospectively reviewed, and data was collected as per the Amsterdam consensus classification criteria.[15]

11.4.2 Selection of the Unexposed Group

The unexposed group was selected based on a convenience sample established from the fetal echo-cardiography database. These pregnancies were evaluated at the fetal echocardiography laboratory of our institution between November 2013 to August 2021. Only pregnancies followed by the cardiology department at our institution were included, in order to obtain information from the mother, the fetus and the newborn. They were selected if adequate fetal echocardiography images were available. We included fetuses that were described as having a normal fetal echocardiography by our expert fetal cardiologist. We excluded fetuses that were evaluated for the presence of a congenital malformation or if there were significant maternal or fetal indications necessitating a fetal echo-cardiography (such as severe maternal diabetes requiring insulin). The unexposed group in our cohort study primarily consisted of pregnancies referred for mild gestational diabetes (diet-controlled) or screening due to a family history of congenital heart disease. These pregnancies were referred for fetal echocardiography at 22 to 24 weeks of GA.

11.4.3 Fetal echocardiography

Fetal echocardiography images were acquired using a GE Vivid E9 or E95 (Wisconsin, USA) by trained medical imaging technologists with expertise in fetal echocardiography or by fetal cardiologists. Echocardiography were performed according to the American Institute of Ultrasound in Medicine and the American Society of Echocardiography Guidelines.[16,17] Data were extracted from the images stored in the DICOM files. Images were stored at a frame rate of 25 frame per second (fps). Our local fetal cardiology guidelines warrant evaluation of pregnant

women with a positive antibody status starting 16 to 18 weeks of GA. These mothers are then followed every two weeks. However, for each fetus exposed to a maternal rheumatological condition, data from their fetal echocardiogram closest to 23 weeks of GA was extracted in order to match it to the GA at which the fetal echocardiography was performed in our unexposed group of fetuses. The rheumatological exposure status during the analysis of echocardiograms was unconcealed to the data extractor, although within the rheumatological group, the autoantibody status was blinded.

11.4.4 Fetal echocardiography data extraction

STE was used to evaluate various deformation parameters, such as strain (the magnitude of deformation in %), strain rate (the velocity of the deformation in 1/s), as well as the time to the peak strain of each segment of the cardiac wall (in milliseconds). STE was performed on the software TomTEC Arena (Munich, Germany). Measurements were obtained by two-dimensional (2-D) manual tracking of the endocardial border for the left (LV) and the right ventricle (RV), from a fetal apical four-chamber view at the end of systole and diastole, as previously described.[18] A minimum of two consequent cardiac cycles were used for the analysis of the myocardial deformation by STE. Cardiac cycles were identified through the M-mode tracing obtained through the corresponding atrio-ventricular annulus. As such, based on the mitral (LV) or tricuspid (RV) valve motion, we outlined the end-diastolic (ED) and the end-systolic (ES) periods of the cardiac cycle and we selected the period corresponding to two cardiac cycles. This method is a surrogate for the R wave detection, seeing that there was an absence of electrocardiogram-gating. The endocardial border was firstly traced, point-by-point, at the peak of systole followed by the peak of diastole, for which both were manually adjusted if needed upon tracking revision. The software

then provides an estimate of the ejection fraction (EF), the end-diastolic volume (EDV), and the end-systolic volume (ESV) for the LV, as well as the fractional area change (FAC) and the end-diastolic area (EDA) for the RV. The analysis also provides segmental deformational measurements. The early-diastolic strain rate (EDSR), the peak longitudinal strain (pLS), the peak longitudinal strain rate (pLSR), and the time to peak strain (TTP) were extracted for both the RV and LV (values accounted the free wall and septum for each ventricle). Additionally, segmental values for the free wall and for the septum were extracted for each ventricle. The corresponding ventricular heart rate was also obtained for each ventricular analysis, based on the M-Mode of each ventricle. The aortic heart rate was extracted from the Pulse-Wave Doppler of the fetal aorta at the time of fetal echocardiography.

11.4.5 Statistical analysis

Descriptive statistics were used, including mean with standard deviation (SD) and 95% confidence interval when continuous variables were parametric and median with interquartile range (IQR) for when continuous variables were non-parametric. Additionally, counts with proportion were used for categorical variables. Shapiro-Wilk test was used to evaluate for the normality of the distribution of the continuous variables. The Student t-test (or analysis of variance [ANOVA]) and the Wilcoxon-Mann-Whitney test were used for continuous variables with a parametric and with a non-parametric distribution, respectively. To assess the relationship between exposure to a maternal rheumatological condition and the fetal LV-EF outcome, we conducted a multiple linear regression analysis. To ensure comparability, we initially matched the GA at fetal echocardiography between the groups. Subsequently, we further adjusted with GA at fetal echocardiography in the regression model to account for its potential influence on the association

between exposure and outcome of interest (LV-EF). To further investigate the impact of anti-Ro/La antibodies, a second multiple linear regression analysis adjusting for GA at fetal echocardiography was conducted including 3 exposure groups: unexposed fetuses, fetuses exposed to circulating anti-Ro/La antibodies, as well as fetuses exposed to a maternal rheumatological condition without anti-Ro/La antibodies. Lastly, a third model was introduced within the subgroup of fetuses exposed to positive anti-Ro/La antibodies, for which the exposure was stratified into three levels once again: unexposed fetuses, fetuses exposed to circulating anti-Ro/La antibodies with CHB and fetuses exposed to circulating anti-Ro/La antibodies without CHB. The analysis assessed the association for LV-EF as the outcome and adjusted for GA at fetal echocardiography. The regression (β) coefficients were reported with their corresponding 95% confidence intervals. The level of significance was set at 0.05. Statistical analyses were done with StataSE 14.2 (Texas, USA). In addition, a post-hoc power calculation with an alpha of 0.05 was performed, with LV-EF as the primary outcome within the entire cohort, using https://clincalc.com/stats/Power.aspx.

11.5 Results

We identified 61 fetuses unexposed to maternal autoantibodies from our fetal echocardiography database. Regarding pregnancies with a rheumatological condition, we assessed 105 pregnancies for eligibility (**Figure 1**), from which 45 were excluded for one or more of the following reasons: insufficient documentation or followed at outside of institution (n=44), congenital heart defect detected in the fetus (n=1). We included 60 pregnancies with adequate fetal echocardiography images available and followed at our institution. Among these, 46 pregnancies had confirmed maternal anti-Ro/La antibodies (3 twin pregnancies, therefore 49 fetuses) and 14 were

serologically negative. Within the 49 fetuses exposed to positive anti-Ro/La antibodies, fetal AVB occurred in 5 fetuses.

Demographic and clinical characteristics are exposed in **Table 1**. There was no difference between the maternal age at fetal echocardiography between groups (unexposed pregnancies: 32.6 (4.9) years, positive anti-Ro/La antibodies: 33.8 (3.9) years; and negative of anti-Ro/La antibodies: 31.3 (6.0) years, p=0.16 by ANOVA). Regarding maternal rheumatological conditions, most pregnancies occurred in the context of SLE (30/46, 65%). Medications for rheumatological conditions were administered in most pregnancies (56/60; 93%), such as hydroxychloroquine (83% of positive anti-Ro/La pregnancies and 64% of negative anti-Ro/La pregnancies). Concerning the Ro/La autoantibody status, within the groups with positive circulating antibodies, 98% were anti-Ro, 39% were anti-La, and 37% had both autoantibodies. Maternal co-morbidities are presented in **Table 1**. Pregnancy complications including gestational diabetes and gestational hypertension were observed in all groups. Gestational diabetes was observed in 23% of the unexposed group, 9% in the positive anti-Ro/La antibodies group and 14% in the negative anti-Ro/La antibodies group; while gestational hypertension was present in 3% of the unexposed group, 9% in the positive anti-Ro/La group and 14% in the negative anti-Ro/La group.

Over the study period, CHB was detected in 5/46 (11%) pregnancies, all on their first fetal echo-cardiography and were treated throughout their pregnancy with dexamethasone. Other pregnancy complications were observed, such as fetal growth restriction in positive (8/46, 17%) and negative (3/14, 21%) anti-Ro/La antibodies fetuses. Placental changes were described in 46% (29/63) of placentas exposed to a rheumatological condition (**Table 1**).

11.5.1 Deformation Analysis in Fetuses Exposed to Rheumatological Conditions Compared to Unexposed Fetuses

Gestational age at the fetal echocardiography used for analysis was similar between the unexposed group and the rheumatological groups, respectively: 23.1 (22.4 – 24.0) vs 23.0 (21.7 – 24.3) weeks (p=0.85) (**Table 2**). Further, there was no difference in GA at fetal echocardiography when stratified between the unexposed (23.4 [2.1]), the positive anti-Ro/La antibodies (23.6 [2.7]) or the negative anti-Ro/La antibodies groups (23.2 [2.1]), p=0.79 by ANOVA. LV-EF was higher in fetuses exposed to a maternal rheumatological condition (56.5% [9.4]) compared to unexposed fetus-es (51.9% [7.7]; p=0.003; 95% CI [54.2, 58.9]% vs [49.9, 53.8]%). There was no difference between the two groups for the remaining measurements of the LV and the RV. Additionally, no differences in LV-EF were observed upon assessing the 29 fetuses exposed to maternal rheumatological conditions with placental anomalies (EF: 56.4% [9.9]) compared to the 6 unexposed fetuses with placental pathology (53.2% [9.4]). It is important to note, however, that the cohort with placental anomalies consisted of only 35 fetuses, limiting significantly power for this analysis.

11.5.2 Deformation Analysis in Fetuses Exposed to Rheumatological Conditions by Anti-Ro/La Autoantibody Status

There was no difference between the LV-EF in those exposed to positive anti-Ro/La antibodies compared to those with negative anti-Ro/La antibodies within pregnancies complicated by a rheumatological condition (56.4% [9.5] vs 56.9% [9.3]; p=0.88; 95%CI: [53.7, 59.1]% vs [51.5, 62.2]%). Similarly, EDV (0.74 mL [0.52 – 1.03] vs 0.90 mL [0.66 – 1.13]; p=0.34), global LV-

pLS (-22.4% [5.5] vs -20.7% [5.7]; p=0.32; 95%CI: [-23.9, -20.8]% vs [-23.9, -17.4]%), global RV-pLS (-19.6% [-23.0 – -15.4] vs -22.8 [-24.2 – -17.1]; p=0.17), LV-pLSR (-1.2% [-1.6 – -1.0] vs -1.2% [1.4 – -0.8]; p=0.29) and RV-pLSR (-1.1% [-1.2 – -0.9] vs -1.2% [-1.3 – -1.0]; p=0.38) were not different (**Table 3**). Remaining parameters for the LV and the RV were similar between both groups.

11.5.3 Deformation Analysis in Fetuses Exposed to Rheumatological Conditions with Negative Anti-Ro/La Autoantibodies Compared to Unexposed Fetuses

LV-EF was higher in negative anti-Ro/La antibodies fetuses compared to fetal unexposed fetuses (56.9% [9.3] vs 51.9% [7.7]; p=0.04; 95%CI [51.5, 62.2]% vs [49.9, 53.8]%) (**Table 4**). The remaining measurements of the LV and RV in both groups did not show any statistically significant differences.

11.5.4 Deformation Analysis in Fetuses Exposed to Rheumatological Conditions with Positive Circulating Anti-Ro/La Autoantibodies Compared to Unexposed Fetuses

LV-EF was higher in positive anti-Ro/La antibodies fetuses compared to unexposed fetuses (56.4% [9.4] vs 51.9% [7.7]; p=0.01; 95%CI [53.7, 59.1]% vs [49.9, 53.8]%). Further, LV strain was increased in fetuses exposed to positive anti-Ro/La antibodies for the global LV strain (LV free wall and LV septum) and for the isolated LV free wall (global pLS: -22.4% [5.5] vs -20.5% [4.3]; p= 0.04; 95%CI [-23.9, -20.8]% vs [-21.6, -19.4]%; free wall: -24.2% [6.3] vs -21.8% [5.1]; p=0.03; 95%CI [-26.0, -22.4]% vs [-23.1, -20.5]%) (**Table 4**). RV deformational parameters were similar between groups.

11.5.5 Deformation Analysis between Unexposed Fetuses and Positive Anti-Ro/La Autoantibodies Fetuses with CHB

Within the fetuses exposed to positive anti-Ro/La autoantibodies, 5 (11%) had a third-degree fetal HB (**Table 5**). Compared to unexposed fetuses, theses fetuses demonstrated a delayed activation of the myocardium by the time from activation to the peak strain for the LV (280ms [236 – 804] vs 200ms [161 – 201]; p=0.01) and for the RV (501ms [260 – 684] vs 201ms [167 – 240]; p=0.01]. Additionally, RV septal-pLS was higher in CHB fetuses compared to unexposed fetuses (-22.7% [6.8] vs -17.1% [5.7]; p=0.04; 95%CI: [-31.2, -14.3]% vs [-18.6, -15.7]%). Remaining measurements for both groups showed no differences for the LV and the RV.

11.5.6 Deformation Analysis between Unexposed Fetuses and Positive Anti-Ro/La Autoantibodies Fetuses without CHB

Positive anti-Ro/La autoantibodies fetuses without CHB demonstrated an increased LV-EF compared to unexposed fetuses (LV-EF 56.3% [9.6] vs 51.9% [7.7]; p=0.01; 95%CI: [53, 59]% vs [50, 54]%), as well as an increase in the LV free wall pLS (-24.13% [6.5] vs -21.83% [5.1]; p=0.04; 95%CI: [-26.1, -22.2]% vs [-23.1, -20.5]%) (**Table 5**). Remaining measurements for both groups were similar for the LV and the RV. Point estimate of difference being -4.7% (95% CI [-7.8, -1.3]%) for the LV-EF and 2.4% (95% CI [0.2, 4.5]%) for LV anterolateral peak longitudinal strain.

11.5.7 Multiple Regression Analysis for the LV-EF Outcome

LV-EF measured by STE remained associated with the exposure to a maternal rheumatological condition before and after adjusting for GA at echocardiography (β = 4.6%, 95% CI [1.6, 7.6]%) (**Table 6**). Additionally, when stratifying the rheumatological status into presence and absence of

anti-Ro/La autoantibodies, we observed an association in the unadjusted model in the presence of Ro/La autoantibodies compared to the unexposed group, but not with the absence of Ro/La autoantibodies (presence of Ro/La antibodies: β = 4.6%, 95% CI [1.3, 7.7]%; absence of Ro/La anti-bodies: β = 5.0%, 95% CI [-0.1, 10.1]%). After adjustment for GA, LV-EF was associated with both status when compared to the unexposed group (presence of Ro/La antibodies: β = 4.4%, 95% CI [1.2, 7.7]%; absence of Ro/La antibodies: β = 5.1%, 95% CI [0.1, 10.1]%). Lastly, when stratifying within the positive Ro/La autoantibody group with CHB compared to the unexposed group there was no association with LV-EF before and after adjusting for GA (unadjusted: β = 5.3%, 95% CI [-2.6, 13.2]%; adjusted: β = 5.8%, 95% CI [-2.0, 13.6]%). However, for positive Ro/La autoantibody without CHB, we observed an association before and after the adjustment for GA (unadjusted: β = 4.5%, 95% CI [1.1, 7.8]%; adjusted: β = 4.2%, 95% CI [0.9, 7.6]%). Additionally, the post-hoc power calculation revealed a power of 77.2% with an alpha level set at 0.05.

11.6 Discussion

In this retrospective, cross-sectional study, we described alterations in fetal LV cardiac metrics by STE at 20-23 weeks in the context of exposure to a maternal rheumatological condition. Although RV parameters were similar across groups, we found that the LV-EF and the LV-longitudinal strain (especially the LV free wall strain) were higher in fetuses exposed to maternal rheumatological conditions, regardless of anti-Ro/La antibody status, compared to the unexposed group. These findings suggest early cardiac functional alterations and may reflect changes secondary to an ad-verse in-utero environment, such as an adaptation to a higher afterload due to altered placental vasculature.

11.6.1 Fetal Cardiac Parameters Impacted by Maternal Rheumatological Conditions

The maternal rheumatological condition may have a specific impact on the LV-EF in fetuses, but not necessarily on the overall development of the LV and RV, at least at around 20-23 weeks. Indeed, we did not detect any differences in fetal cardiac dimensions for the LV (end diastolic volume) and RV (end diastolic area) in the fetuses exposed to a maternal rheumatological condition. Additionally, the lack of differences in RV functional parameters suggests that maternal rheumatological condition may not significantly impact all aspects of fetal cardiac function. The RV is the dominant ventricle during fetal life, functioning as the "systemic ventricle" and providing two-third of the combined ventricular output secondary to the shunting via the ductus arteriosus and foramen ovale.[19] As described in our cohort, a majority of patients exposed to a maternal rheumatological conditions were found to have placental pathological anomalies. Previous studies have also demonstrated that pregnancies complicated by rheumatological conditions are at higher risk for placental insufficiency and placental histopathological anomalies, mainly through anomalies related to vascular placentation.[2,20,21] As such, a rise in fetal systemic vascular resistance exposure, secondary to placental vascular stiffness, may possibly to fetal cardiac reaction with isolated LV compensation. The left ventricle may have the capacity to adapt to increased vascular resistance, similarly to post-natal life, while the RV may not compensate similarly to stress in the fetal life.[4,22]

During gestation, maternal arterial stiffness and peripheral vascular resistance decreases, stimulating an increase in plasma volume, a an essential process for adequate placental circulation and fetal growth.[23,24] Increased maternal arterial stiffness, observed in those with pregestational or

gestation-al diabetes, was associated with altered echocardiographic measures of fetal cardiovascular function at 20 to 28 weeks of GA.[24] Indeed, these fetuses were found to have significantly higher velocity time integral at the level of the aortic valve, indicating increased fetal LV stroke volume.[24] The authors hypothesized that the increased LV stroke volume could be secondary to the unequal distribution of flow between the right and left hearts related to placental resistance, similarly to our findings in the rheumatological population.[24] Further, one cross-sectional study compared fetuses exposed to an increased placental vascular resistance, defined as an umbilical artery pulsatility index >95th percentile, to normal fetuses.[25] They were described to have lower ventricular volumes, stroke volumes, and cardiac output, but with a higher mean cardiac ejection fraction and, specifically, LV-ejection fraction compared to normal fetuses.[25] Additionally, RV volume, stroke volume, and cardiac output were higher than those of the LV.[25] These findings suggest that fetal hearts contraction against adverse placental vascular impedance may respond differently by increasing ventricular inotropy, which is characterized by a decrease in end-systolic volume and an increase in ejection fraction.[25] Our results showed similar findings at 22-24 weeks of GA within fetuses exposed to rheumatological conditions. Our findings concur with a left ventricular adaptation, which may reflect other subtle adverse fetal cardiac changes not detected by STE at that gestational age.

11.6.2 Cardiovascular impact after exposure to a rheumatological pregnancy

Increased LV-pLS was driven by a segmental increase in the anterolateral pLS (the free wall of the LV), rather than the septal pLS (shared with the RV). It is noteworthy to mention that the septal wall interrogated by STE is at the endocardial portion of the ventricles, outlining potential differences between the sheer movements of the septum on the LV or RV compartment, depending

on the endocardial portion being interrogated. While we evaluated fetuses around 23 weeks of GA, a prolonged increase in contractility, as an adaptive mechanism, may lead to eventual fetal cardiac remodeling leading to post-natal changes that may be long-lasting. Inflammation related to the rheumatological exposure may also lead to eventual cardiac alterations and fibrosis.[26] As such, monitoring of fetal cardiac parameters throughout the pregnancy, regardless of Ro/La autoantibody status, may be warranted. The subtle early signs of increased LV performance described in our cohort should not be interpreted as positive indicators. Indeed, Hamill et al. (2013) described that the fetal LV appears to be more receptive to inotropic stimulation (in the context of increased placental vascular resistance), leading to a proportionately greater decrease in end-systolic volume (-72%), compared to the right (-40%), and consequently a greater increase in LV-EF (17%), compared to the right (10%).[25] This may be an adaptive mechanism to preserve cerebral circulation, as previous animal and human studies have shown a "brain-sparing" effect with preferential blood flow to the brain in the presence of hypoxia.[25] The lower cardiac output measured in their fetal population indicates that the LV's ability to compensate can been exceeded, implying the possibility of subclinical fetal cardiac failure, which may occur through similar pathways in pregnancies complicated by rheumatological conditions.[25]

11.6.3 Impact of the Adverse Placental Environment

Intrauterine growth restriction (IUGR) is, on its own, associated with inflammation and frequently complicates rheumatological pregnancies.[2,3] Among our cohort, 11 fetuses (17%) exposed to a rheumatological condition (8 exposed to positive Ro/La autoantibodies and 3 exposed to negative Ro/La autoantibodies) were small for gestational age at birth (<10th percentile for birth weight as per Fenton growth charts). Growth restriction is often secondary to a deficiency in uteroplacental

blood supply, resulting in insufficient nutrients and oxygen reaching the fetus.[4,27] Growth-restricted fetuses may also undergo circulatory and vascular modifications to compensate for the unfavorable intrauterine conditions.[27,28] Although these alterations are compensatory during fetal development, they can negatively affect the long-term cardiovascular phenotype in the postnatal setting.[27,28] IUGR fetuses have shorter cardiac sarcomere length and increased troponin-I concentration.[29] Our findings may also be driven by altered cardiac metabolism and development, secondary to growth restriction in the context of the adverse placental environment. In our cohort, we did not detect any cardiac dimensions differences between groups, possibly secondary to technical limitations regarding the methodology or the insufficient power secondary to the relatively small sample. Future studies should evaluate whether improving fetal growth with the modulation of maternal inflammation or strategies to improve placental oxygenation may further impact cardiac performance within this population of high-risk fetuses.

11.6.4 Influence of heart block within the Positive Anti-Ro/La Antibodies Group

Our results suggest that the described cardiac functional alterations were independent of fetal HB, although only 5 fetuses developed HB in our cohort. As expected, these fetuses were found to have delayed myocardial activation, as evidenced by the increased time to peak contraction for both the LV and the RV. This is consistent with their lower baseline heart rate. Indeed, the abnormal myocardial activation leads to alterations in the timing and coordination of the myocardial contraction, resulting in delayed activation. These fetuses also presented increased RV-sided septal strain. Fetuses with HB are known to have myocardial inflammation and remodeling, and cardiac markers may deteriorate with time during fetal growth. The cardiac inflammatory is secondary to direct damage to the fetal myocardium and conducting system[30,31], which typically occurs between

16-24 weeks[32-34], and may not have been overtly detected in our cohort at 22-23 weeks. According to a study by Jaeggi et al., offspring exposed to high titers of anti-Ro and anti-La antibodies have a 5% risk of developing CHB.[35] The development of CHB is also more frequent in newborns exposed to high titers of anti-Ro-52kD and anti-La-48kD antibodies.[35] Therefore, the amount of fetal tissue damage caused by maternal antibodies is partly dose-dependent and type-dependent (anti-Ro to a more significant extent than anti-La).[35]

11.6.5 Clinical Significance

Based on the study findings, there is currently insufficient evidence to warrant changes in guidelines or approaches. However, the results do highlight the need for further research. Cardiac function changes can be detected as early as 22-23 weeks of gestation, prompting the investigation of long-term implications. Additional research is required both during the fetal and postnatal stages, as current guidelines do not recommend postnatal echocardiograms. The results indicate potential concerns and provide a basis for future studies to better understand postnatal follow-up. The clinical relevance lies in exploring whether these differences evolve and become clinically significant over time.

11.6.6 Strengths and Limitations

This study is limited by its retrospective, unicentric design with the necessity to exclude a significant portion (43%) of subjects, mainly due to insufficient documentation because they're being followed at outside institution. Future research should aim to evaluate the generalizability of these findings in a larger sample size through a prospective multi-institutional study. Our study was constrained to a 1:1 case-to-control ratio, considering the difficulty to obtain suitable control subjects retrospectively. However, a higher ratio, such as a 1:2, could potentially have improved

precision and allowed for a more comprehensive assessment of the observed cardiac functional alterations in fetuses exposed to maternal rheumatological conditions. Subsequent research studies should contemplate utilizing an elevated case-to-control ratio. Despite these limitations, it represents one of the largest studies of fetuses exposed to maternal rheumatological conditions assessed for fetal cardiac performance. Echocardiography measures were not analyzed for intra-reader or inter-reader variability. The STE analysis was conducted by a single reader, and no assessment of inter-reader reliability was conducted. Previous reports have indicated a strong reproducibility of strain measurements, whether performed by the same reader or a different reader using different strain vendors and echocardiography machines.[36,37] However, measurements were performed by a trained data extractor from the raw images, and not extracted from the report. The data extractor was masked to the anti-Ro/La antibody status. The frame rate at which the analyzed echocardiography images were stored was 25 fps. However, a recent study described no difference between strain evaluations at lower (below 60 fps) and higher (≥ 60 fps) frame rates.[38] Although our echocardiography laboratory has a set protocolized approach for image acquisition, scans were acquired by multiple technologists and cardiologists, which may have introduced a variability. Regarding the unexposed group, it was selected from subjects referred for fetal echocardiography, which may limit the representativeness regarding the general population. The exposure and rheumatological status during the analysis of echocardiograms was unconcealed to the data extractor, which may have introduced bias in the data extraction process. Placental evaluations were retrospectively obtained from clinical reports (which may lead to variability in diagnosis and may not encompass the entire placenta in its evaluation) and were not available for 15 newborns within the unexposed group. Further, we were not able to evaluate the relationship between the

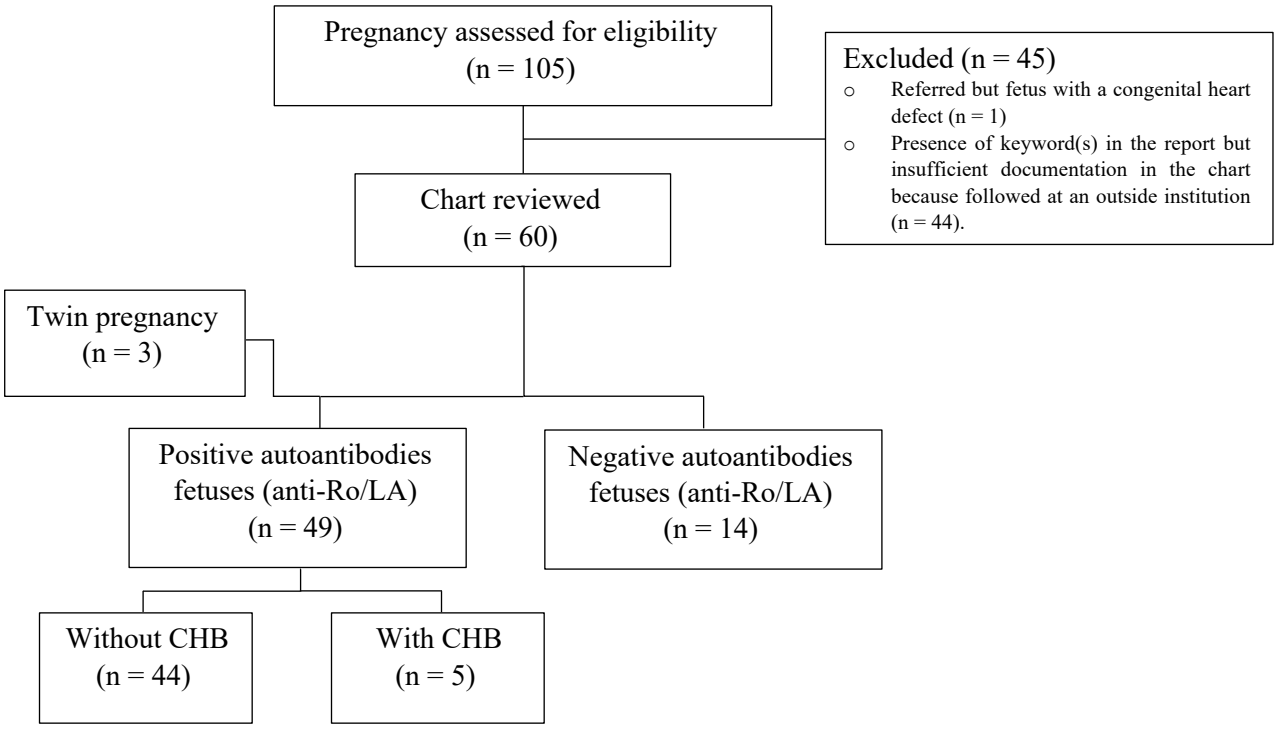
degree of placental anomalies and the cardiovascular findings. The severity of the maternal condition, anti-Ro/L antibodies titers and the impact of exposure to management strategies (such as the use of anti-inflammatory medications) were not explored in this study. Lastly, this study only collected data during a specific range of GA and did not assess long-term outcomes, which may limit the ability to draw conclusions about the long-term effects of maternal rheumatological conditions on fetal cardiac function.

11.6.7 Conclusion

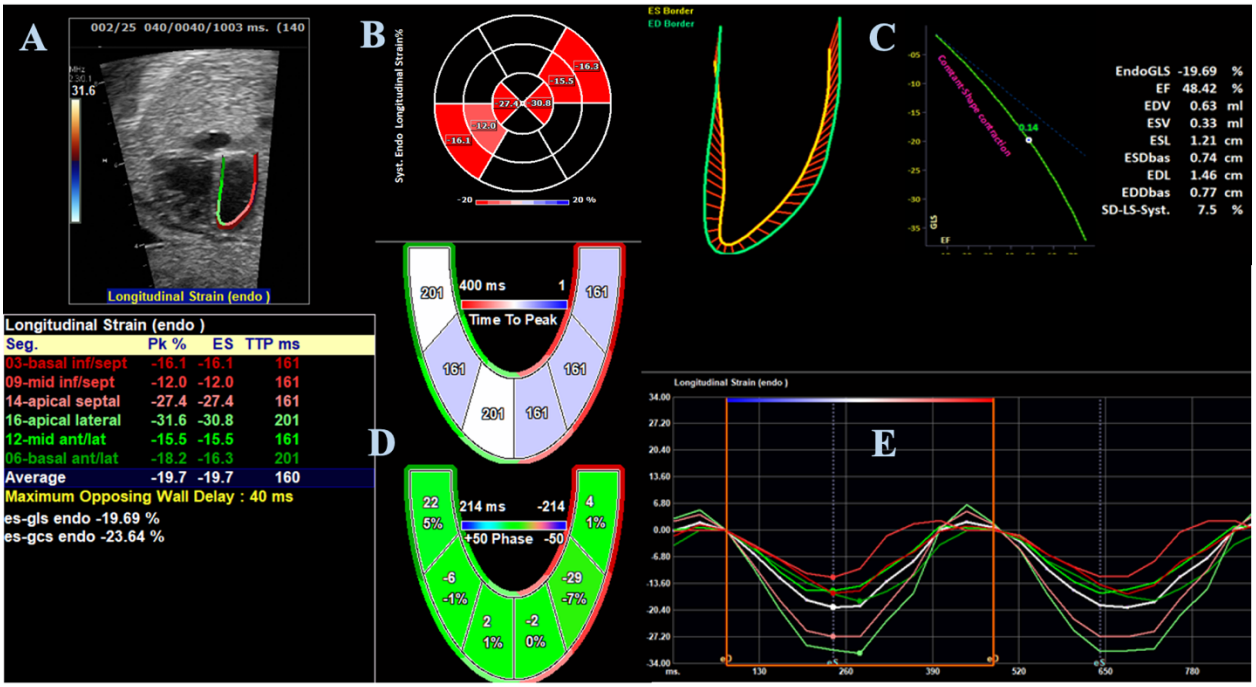
In conclusion, maternal rheumatological conditions may have a specific impact on fetal cardiac function, primarily on the LV, regardless of maternal anti-Ro/La presence or fetal AVB status. Fetuses exposed to maternal rheumatological conditions, with or without maternal anti-Ro/La antibodies, had echocardiographic signs of increased LV contractility at around 22-23 weeks, indicating early cardiac alterations. Our findings may indicate that the fetal heart is initially able to compensate for unfavourable intrauterine conditions, which may not necessarily be interpreted as a positive indicator. Future studies should investigate the later fetal and post-natal neonatal cardiac impact of maternal rheumatological conditions. In the meantime, our data suggests that only pregnancies complicated by such conditions should be monitored for fetal cardiac functional status.

11.7 Tables and Figures:

11.7.1 **Figure 1.** Patient Inclusion Flow Diagram for Fetuses Exposed to a Maternal Rheumatological Condition.



11.7.2 **Figure 2.** Speckle Tracking Echocardiography.



Legend – TomTEC analysis for the LV. Measurements are taken for the endocardium (A). The "Bull's eye" demonstrates various segments of the LV strain (B). The summary panel for two-dimension analysis of the longitudinal deformation of the LV (C). The summary table shows the peak of each segment as well as the average peak (D & E). These curves represent each segment in the A4C related to the longitudinal strain in % (E).

11.7.3 **Table 1.** Demographic and Maternal Characteristics.

	Unexposed	Positive (+) Anti-Ro/La	Negative (-) Anti-Ro/La
	N=61	N=46	N=14
Maternal age in years, mean (SD)	32.6 (4.9)	33.8 (3.9)	31.3 (6.0)
Maternal autoantibodies, n (%)			
Anti-Ro		45 (98)	0
Anti-La		18 (39)	0
Both		17 (37)	0
Maternal rheumatic diseases, n (%)			
SLE	0	30 (65)	9 (64)
Sjögren's syndrome	0	9 (20)	0
Rheumatoid Arthritis	0	1 (2)	2 (14)
Mixed connective tissue disease	0	1 (2)	0
Undifferentiated connective tissue disease	0	1 (2)	0
Antiphospholipid syndrome	0	2 (4)	2 (14)
Idiopathic inflammatory myositis	0	0	2 (14)
Ankylosis Spondylitis	0	0	1 (7)
Maternal co-morbidities, n (%)			
Pre-gestational diabetes	12 (20)	1 (2)	0
Pre-gestational hypertension	4 (7)	2 (4)	0
Pregnancy complications, n (%)			
Congenital heart block	0	5 (11)	0
Gestational diabetes	14 (23)	4 (9)	2 (14)
Gestational hypertension	2 (3)	4 (9)	2 (14)
HELLP	0	1 (2)	0
Induced abortion	0	3 (7)	0

Fetal growth restriction	0	8 (17)	3 (21)
Fetal death (IUFD)	0	2 (4)	0
Placental changes, n (%)			
Any placental anomaly	6 (10)	25 (51)	4 (29)
Placental abruption	0	1 (2)	0
Decidual vasculopathy	1 (2)	7 (15)	2 (14)
Decidual thrombi	1 (2)	2 (4)	1 (7)
Fetal thrombi	1 (2)	2 (4)	0
Chronic villitis	1 (2)	3 (7)	0
Decreased placental weight	0	10 (22)	2 (14)
Placental infarction	0	1 (2)	0
Villous infarction	0	4 (9)	1 (7)
Chorioamnionitis	3 (5)	6 (13)	0
Fetal vascular malperfusion	1 (2)	5 (11)	0
Multiple pregnancies, n (%)	0	3 (7)	0
Medications, n (%)			
Medications for rheumatological condition	0	43 (93)	13 (93)
Hydroxychloroquine during pregnancy	0	38 (83)	9 (64)
Dexamethasone during pregnancy	0	5 (11)	0

Legend: Categorical results are expressed as count **(%).** Continuous results are expressed as mean (SD). SD: standard deviation; SLE: Systemic Lupus Erythematosus; HELLP: Hemolysis, Elevated Liver Enzymes and Low Platelet; IUFD: Intrauterine Fetal Demise.

11.7.4 **Table 2.** Echocardiography Deformation Analysis by Speckle Tracking Echocardiography Between Unexposed and Rheumatological Fetuses.

	Unexposed	Rheumatological	*p*-values*
	N=61	N=63	
Gestational Age at ECHO in weeks	23.1 (22.4 - 24.0)	23.0 (21.7 - 24.3)	0.85
Left Ventricle			
Ejection Fraction (%)	51.9 (7.7)	56.5 (9.4)	**0.003***
End-Diastolic Volume (mL)	0.87 (0.69 - 1.28)	0.78 (0.54 - 1.13)	0.08
Septal pLS (%)	-19.3 (5.0)	-20.5 (5.8)	0.22
Septal pLS Rate (1/s)	-1.1 (-1.3 - -0.9)	-1.1 (-1.5 - -0.9)	0.44
Anterolateral pLS (%)	-21.8 (5.1)	-23.6 (6.6)	0.09
Anterolateral pLS Rate (1/s)	-1.2 (-1.5 - -1.0)	-1.3 (-1.6 - -1.0)	0.31
pGLS (%)	-20.5 (4.3)	-22.0 (5.5)	0.20
pGLS Rate (1/s)	-1.2 (-1.4 - -1.0)	-1.2 (-1.6 - -1.0)	0.25
End-Diastolic Strain Rate (1/s)	1.12 (0.93 - 1.36)	1.14 (0.92 - 1.5)	0.41
Time-to-peak Average (ms)	200 (161 – 201)	201 (164 – 236)	0.19
Right Ventricle			
Fractional Area Change (%)	37.2 (7.3)	36.2 (7.1)	0.43
End-Diastolic Area (cm^2)	1.05 (0.89 - 1.33)	1.17 (0.86 - 1.43)	0.49
Septal pLS (%)	-17.1 (5.7)	-17.4 (5.3)	0.79
Septal pLS Rate (1/s)	-0.9 (-1.2 - -0.7)	-0.9 (-1.1 - -0.7)	0.99
Lateral pLS (%)	-23.2 (-27.8 - -19.1)	-22.7 (-26.8 - -17.8)	0.67
Lateral pLS Rate (1/s)	1.3 (-1.6 - -1.1)	-1.3 (-1.5 - -1.0)	0.39
pGLS (%)	-20.2 (-22.9 - -17.8)	-20.1 (-23.5 - -15.9)	0.77
pGLS Rate (1/s)	-1.2 (-1.3 - -0.9)	-1.1 (-1.3 - -0.9)	0.47
End-Diastolic Strain Rate (1/s)	1.07 (0.91 - 1.26)	1.09 (0.86 - 1.40)	0.95
Time-to-peak Average (ms)	201 (167 - 240)	201 (200 – 240)	0.42

Legend: Continuous results are expressed as mean (Standard Deviation) or median (Interquartile Range). ECHO: echocardiography; pLS: peak longitudinal strain; pGLS: peak global longitudinal strain.
***p-values compared to unexposed. The student's t-test or Wilcoxon-Mann-Whitney test were used for continuous variables with a parametric or non-parametric distribution, respectively.**

11.7.5 **Table 3.** Echocardiography Deformation Analysis by Speckle Tracking Echocardiography Between Negative and Positive Anti-Ro/La Antibody Fetuses.

	Negative (-) Anti-Ro/La	Positive (+) Anti-Ro/La	*p*-values*
	N=14	N=49	
Gestational Age at ECHO in weeks	24.1 (21.7 - 24.7)	22.7 (21.7 – 24.1)	0.32
Left Ventricle			
Ejection Fraction (%)	56.9 (9.3)	56.4 (9.5)	0.88
End-Diastolic Volume (mL)	0.90 (0.66 - 1.13)	0.74 (0.52 - 1.03)	0.34
Septal pLS (%)	-20.1 (5.5)	-20.6 (5.9)	0.76
Septal pLS Rate (1/s)	-1.1 (-1.3 - -0.9)	-1.1 (-1.5 - -0.9)	0.53
Anterolateral pLS (%)	-21.7 (7.5)	-24.2 (6.3)	0.21
Anterolateral pLS Rate (1/s)	-1.2 (-1.5 - -0.8)	-1.3 (-1.6 - -1.1)	0.17
pGLS (%)	-20.7 (5.7)	-22.4 (5.5)	0.32
pGLS Rate (1/s)	-1.2 (-1.4 - -0.8)	-1.2 (-1.6 - -1.0)	0.29
End-Diastolic Strain Rate (1/s)	1.07 (0.81 – 1.28)	1.14 (0.93 – 1.52)	0.16
Time-to-peak Average (ms)	195 (160 - 201)	201 (167 - 240)	0.15
Right Ventricle			
Fractional Area Change (%)	36.5 (33.7 - 38.3)	34.5 (30.9 - 41.4)	0.43
End-Diastolic Area (cm^2)	1.39 (0.93 - 1.52)	1.15 (0.84 - 1.37)	0.39
Septal pLS (%)	-18.8 (-21.1 - -16.9)	-16.6 (-19.4 - -12.4)	0.17
Septal pLS Rate (1/s)	1.0 (-1.2 - -0.8)	-0.9 (-1.1 - -0.7)	0.53
Lateral pLS (%)	-26.8 (-25.4 - -21.7)	-26.1 (-21.9 - -17.7)	0.35
Lateral pLS Rate (1/s)	-1.4 (-1.5 - -1.2)	-1.3 (-1.5 - -1.0)	0.64
pGLS (%)	-22.8 (-24.2 - -17.1)	-19.6 (-23.0 - -15.4)	0.17
pGLS Rate (1/s)	-1.2 (-1.3 - -1.0)	-1.1 (-1.2 - -0.9)	0.38
End-Diastolic Strain Rate (1/s)	1.18 (0.95 - 1.40)	1.07 (0.84 - 1.41)	0.59
Time-to-peak Average (ms)	201 (161 - 240)	201 (200 - 240)	0.40

Legend: Continuous results are expressed as mean (Standard Deviation) or median (Interquartile Range). ECHO: echocardiography; pLS: peak longitudinal strain; pGLS: peak global longitudinal strain.
The student's t-test or Wilcoxon-Mann-Whitney test were used for continuous variables with a parametric or non-parametric distribution, respectively.

11.7.6 **Table 4.** Echocardiography Deformation Analysis by Speckle Tracking Echocardiography for Negative and Positive Anti-Ro/La Antibody Fetuses Compared to Unexposed.

	Unexposed	Negative (-) Anti-Ro/La	*p*-values*	Positive (+) Anti-Ro/La	*p*-values*
	N=61	N=14		N=49	
Gestational Age at ECHO in weeks	23.1 (22.4 - 24.0)	24.1 (21.7 - 24.7)	0.36	22.7 (21.7 - 24.1)	0.52
Left Ventricle					
Ejection Fraction (%)	51.9 (7.7)	56.9 (9.3)	**0.04***	56.4 (9.4)	**0.01***
End-Diastolic Volume (mL)	0.87 (0.69 - 1.28)	0.90 (0.66 - 1.13)	0.80	0.74 (0.52 – 1.03)	0.05
Septal pLS (%)	-19.3 (5.0)	-20.1 (5.5)	0.61	-20.6 (5.9)	0.21
Septal pLS Rate (1/s)	-1.1 (-1.3 - -0.9)	-1.1 (-1.3 - -0.9)	0.95	-1.1 (-1.5 - -0.9)	0.34
Anterolateral pLS (%)	-21.8 (5.1)	-21.7 (7.5)	0.94	-24.2 (6.3)	**0.03***
Anterolateral pLS Rate (1/s)	-1.2 (-1.5 - -1.0)	-1.15 (-1.5 - -0.8)	0.51	-1.3 (-1.6 - -1.1)	0.13
pGLS (%)	-20.5 (4.3)	-20.7 (5.7)	0.87	-22.4 (5.5)	**0.04***
pGLS Rate (1/s)	-1.2 (-1.4 - -1.0)	-1.2 (-1.4 - -0.8)	0.88	-1.2 (-1.6 - -1.0)	0.15
End-Diastolic Strain Rate (1/s)	1.12 (0.93 - 1.36)	1.07 (0.81 – 1.28)	0.43	1.14 (0.93 - 1.52)	0.18
Time-to-peak Average (ms)	200 (161 – 201)	195 (160 – 201)	0.67	201 (167 – 240)	0.08
Right Ventricle					
Fractional Area Change (%)	37.2 (7.3)	36.2 (3.1)	0.63	36.1 (7.9)	0.48
End-Diastolic Area (cm^2)	1.05 (0.89 - 1.33)	1.39 (0.93 - 1.52)	0.26	1.15 (0.84 - 1.37)	0.74
Septal pLS (%)	-17.1 (5.7)	-18.7 (4.5)	0.35	-17.0 (5.5)	0.93
Septal pLS Rate (1/s)	-0.9 (-1.2 - -0.7)	-1.0 (-1.2 - -0.8)	0.81	-0.9 (-1.1 - -0.7)	0.93
Lateral pLS (%)	-23.2 (-27.8 - -19.1)	-25.4 (-26.8 - -21.7)	0.64	-21.9 (-26.1 - -17.7)	0.48
Lateral pLS Rate (1/s)	1.3 (-1.6 - -1.1)	-1.4 (-1.5 - -1.2)	0.81	-1.3 (-1.5 - -1.0)	0.35
pGLS (%)	-20.2 (-22.9 - -17.8)	-22.8 (-24.2 - -17.1)	0.30	-19.6 (-23 - -15.4)	0.41
pGLS Rate (1/s)	-1.2 (-1.3 - -0.9)	-1.2 (-1.3 - -1.0)	0.88	-1.1 (-1.2 - -0.9)	0.35
End-Diastolic Strain Rate (1/s)	1.07 (0.91 - 1.26)	1.18 (0.95 - 1.4)	0.58	1.07 (0.84 - 1.41)	0.75
Time-to-peak Average (ms)	201 (167 - 240)	201 (161 – 240)	0.86	201 (200 – 240)	0.30

Legend: Continuous results are expressed as mean (Standard Deviation) or median (Interquartile Range). ECHO: echocardiography; pLS: peak longitudinal strain; pGLS: peak global longitudinal strain.
***p-values compared to unexposed. The student's t-test or Wilcoxon-Mann-Whitney test were used for continuous variables with a parametric or non-parametric distribution, respectively.**

11.7.7 **Table 5.** Echocardiography Deformation Analysis by Speckle Tracking Echocardiography for Positive Anti-Ro/La Antibody with CHB and without CHB Fetuses Compared to Unexposed.

	Unexposed	Positive (+) Anti-Ro/La with CHB	*p*-values*	Positive (+) Anti-Ro/La without CHB	*p*-values*
	N=61	N=5		N=44	
Gestational Age at ECHO in weeks	23.1 (22.4 - 24.0)	22.7 (22.4 - 23.4)	0.87	22.9 (21.6 - 24.2)	0.52
Left Ventricle					
Ejection Fraction (%)	51.9 (7.7)	57.2 (9.8)	0.15	56.3 (9.6)	**0.01***
End-Diastolic Volume (mL)	0.87 (0.69 - 1.28)	0.80 (0.46 - 0.83)	0.37	0.74 (0.53 - 1.09)	0.06
Septal pLS (%)	-19.3 (5.0)	-22.6 (6.7)	0.17	-20.4 (5.9)	0.31
Septal pLS Rate (1/s)	-1.1 (-1.3 - -0.9)	-1.4 (-1.5 - -0.9)	0.49	-1.1 (-1.5 - -0.9)	0.40
Anterolateral pLS (%)	-21.8 (5.1)	-24.8 (5.4)	0.22	-24.1 (6.5)	**0.04***
Anterolateral pLS Rate (1/s)	-1.2 (-1.5 - -1.0)	-1.4 (-1.6 - -1.3)	0.50	-1.3 (-1.7 - -1.1)	0.15
pGLS (%)	-20.5 (4.3)	-23.7 (5.3)	0.12	-22.2 (5.5)	0.07
pGLS Rate (1/s)	-1.2 (-1.4 - -1.0)	-1.4 (-1.5 - -1.1)	0.40	-1.2 (-1.6 - -1.0)	0.18
End-Diastolic Strain Rate (1/s)	1.12 (0.93 - 1.36)	1.18 (0.72 – 1.19)	0.51	1.14 (0.94 - 1.57)	0.10
Time-to-peak Average (ms)	200 (161 – 201)	280 (236 - 804)	**0.01***	201 (166 - 203)	0.25
Right Ventricle					
Fractional Area Change (%)	37.1 (7.3)	42.7 (14.0)	0.14	35.4 (6.7)	0.21
End-Diastolic Area (cm^2)	1.05 (0.89 - 1.33)	0.96 (0.84 - 1.54)	0.88	1.16 (0.86 - 1.37)	0.69
Septal pLS (%)	-17.1 (5.7)	-22.7 (6.8)	**0.04***	-16.4 (5.1)	0.49
Septal pLS Rate (1/s)	-0.9 (-1.2 - -0.7)	-1.1 (-1.4 - -0.9)	0.25	-0.9 (-1.1 - -0.7)	0.69
Lateral pLS (%)	-23.2 (-27.8 - -19.1)	-28.0 (-33.8 - -20.3)	0.33	-21.9 (-26.1- -17.5)	0.30
Lateral pLS Rate (1/s)	1.3 (-1.6 - -1.1)	-1.5 (-1.6 - -1.0)	0.79	-1.3 (-1.5 - -1.0)	0.28
pGLS (%)	-20.2 (-22.9 - -17.8)	-24.9 (-33.6 - -18.0)	0.23	-19.4 (-22.6 - -15.4)	0.23
pGLS Rate (1/s)	-1.2 (-1.3 - -0.9)	-1.3 (-1.5 - -0.9)	0.47	-1.1 (-1.2 - -0.85)	0.23
End-Diastolic Strain Rate (1/s)	1.07 (0.91 - 1.26)	1.30 (0.89 - 1.41)	0.53	1.07 (0.83 - 1.38)	0.61
Time-to-peak Average (ms)	201 (167 - 240)	501 (280 - 684)	**0.01***	201 (200 - 240)	0.70

Legend: Continuous results are expressed as mean (Standard Deviation) or median (Interquartile Range). ECHO: echocardiography; pLS: peak longitudinal strain; pGLS: peak global longitudinal strain.
***p-values compared to unexposed. The student's t-test or Wilcoxon-Mann-Whitney test were used for continuous variables with a parametric or non-parametric distribution, respectively.**

11.7.8 **Table 6.** Multiple Linear Regression Analysis Evaluating Association Between Exposure and Outcome (LV Function by EF), Adjusting for Gestational Age at Echocardiography.

LV-EF	Unadjusted β	95% Confidence Interval	Adjusted β*	95% Confidence Interval
Model 1: Exposure = Rheumatological vs Unexposed; Outcome variable = LV-EF by STE				
Rheumatological vs Unexposed	4.6%	1.6 – 7.6%	4.6%	1.6 – 7.6%
Model 2: Exposure = Positive Anti-Ro/La Antibody vs Negative Anti-Ro/La Antibody vs Unexposed; Outcome variable = LV-EF by STE				
Positive (+) Anti-Ro/La vs Unexposed	4.6%	1.3 – 7.7%	4.4%	1.2 – 7.7%
Negative (-) Anti-Ro/La vs vs Unexposed	5.0%	-0.1 – 10.1%	5.1%	0.1 – 10.1%
Model 3: Exposure = Positive Anti-Ro/La Antibody with CHB vs Positive Anti-Ro/La Antibody without CHB vs Unexposed; Outcome variable = LV-EF by STE				
CHB vs Unexposed	5.3%	-2.6 – 13.2%	5.8%	-2.0 – 13.6%
Without CHB vs Unexposed	4.5%	1.1 – 7.8%	4.2%	0.9 – 7.6%
Legend: EF: ejection fraction; GA: gestational age; LV: left ventricle. *Adjusted for estimated gestational age in weeks at the exact time for the fetal echocardiography				

11.8 References

1. Petri M. Pregnancy and Systemic Lupus Erythematosus. *Best Pract Res Clin Obstet Gynaecol.* 2020;64:24-30.
2. Castellanos Gutierrez AS, Figueras F, Morales-Prieto DM, Schleußner E, Espinosa G, Baños N. Placental damage in pregnancies with systemic lupus erythematosus: A narrative review. *Front Immunol.* 2022;13:941586.
3. Levy R, Mendoza Pinto C, Domingues V, et al. Systemic autoimmune diseases and pregnancy. In: Anaya JM SY, Rojas-Villarraga A, Levy RA, Cervera R., ed. *Autoimmunity: From Bench to Bedside [Internet].* Bogota (Colombia): El Rosario University Press2013.
4. Crispi F, Sepúlveda-Martínez Á, Crovetto F, Gómez O, Bijnens B, Gratacós E. Main Patterns of Fetal Cardiac Remodeling. *Fetal Diagnosis and Therapy.* 2020;47(5):337-344.
5. van Oostrum NHM, de Vet CM, van der Woude DAA, Kemps HMC, Oei SG, van Laar J. Fetal strain and strain rate during pregnancy measured with speckle tracking echocardiography: A systematic review. *Eur J Obstet Gynecol Reprod Biol.* 2020;250:178-187.
6. Duan S, Ha S, Li S, et al. Evaluation of cardiac function and systolic dyssynchrony of fetuses exposed to maternal autoimmune diseases using speckle tracking echocardiography. *Clin Rheumatol.* 2021;40(9):3807-3815.
7. Altit G, Bhombal S, Van Meurs K, Tacy TA. Diminished Cardiac Performance and Left Ventricular Dimensions in Neonates with Congenital Diaphragmatic Hernia. *Pediatr Cardiol.* 2018;39(5):993-1000.
8. Altit G, Bhombal S, Van Meurs K, Tacy TA. Ventricular Performance is Associated with Need for Extracorporeal Membrane Oxygenation in Newborns with Congenital Diaphragmatic Hernia. *J Pediatr.* 2017;191:28-34.e21.
9. Altit G, Bonifacio SL, Guimaraes CV, et al. Cardiac Dysfunction in Neonatal HIE Is Associated with Increased Mortality and Brain Injury by MRI. *Am J Perinatol.* 2021.
10. de Carvalho Nunes G, Wutthigate P, Simoneau J, et al. The biventricular contribution to chronic pulmonary hypertension of the extremely premature infant. *J Perinatol.* 2022.
11. Aletaha D, Neogi T, Silman AJ, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum.* 2010;62(9):2569-2581.
12. Aringer M, Costenbader K, Daikh D, et al. 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for systemic lupus erythematosus. *Ann Rheum Dis.* 2019;78(9):1151-1159.
13. Lundberg IE, Tjärnlund A, Bottai M, et al. 2017 European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. *Ann Rheum Dis.* 2017;76(12):1955-1964.
14. Shiboski CH, Shiboski SC, Seror R, et al. 2016 American College of Rheumatology/European League Against Rheumatism Classification Criteria for Primary Sjögren's Syndrome: A Consensus and Data-Driven Methodology Involving Three International Patient Cohorts. *Arthritis Rheumatol.* 2017;69(1):35-45.

15. Khong TY, Mooney EE, Ariel I, et al. Sampling and Definitions of Placental Lesions: Amsterdam Placental Workshop Group Consensus Statement. *Arch Pathol Lab Med.* 2016;140(7):698-713.
16. AIUM Practice Parameter for the Performance of Fetal Echocardiography. *J Ultrasound Med.* 2020;39(1):E5-e16.
17. Rychik J, Ayres N, Cuneo B, et al. American Society of Echocardiography guidelines and standards for performance of the fetal echocardiogram. *J Am Soc Echocardiogr.* 2004;17(7):803-810.
18. DeVore GR, Satou G, Sklansky M, Cuneo B. Speckle Tracking Analysis in Fetuses with D-Transposition: Predicting the Need for Urgent Neonatal Balloon Atrial Septostomy. *Pediatr Cardiol.* 2023.
19. Noori S, Seri I. Chapter 1 - Principles of Developmental Cardiovascular Physiology and Pathophysiology. In: Seri I, Kluckow M, eds. *Hemodynamics and Cardiology (Third Edition).* Philadelphia: Elsevier; 2019:3-27.
20. Doss BJ, Jacques SM, Mayes MD, Qureshi F. Maternal scleroderma: placental findings and perinatal outcome. *Hum Pathol.* 1998;29(12):1524-1530.
21. Magid MS, Kaplan C, Sammaritano LR, Peterson M, Druzin ML, Lockshin MD. Placental pathology in systemic lupus erythematosus: a prospective study. *Am J Obstet Gynecol.* 1998;179(1):226-234.
22. Barbera A, Giraud GD, Reller MD, Maylie J, Morton MJ, Thornburg KL. Right ventricular systolic pressure load alters myocyte maturation in fetal sheep. *Am J Physiol Regul Integr Comp Physiol.* 2000;279(4):R1157-1164.
23. Camm EJ, Botting KJ, Sferruzzi-Perri AN. Near to One's Heart: The Intimate Relationship Between the Placenta and Fetal Heart. *Frontiers in Physiology.* 2018;9.
24. Moodley S, Arunamata A, Stauffer KJ, et al. Maternal arterial stiffness and fetal cardiovascular physiology in diabetic pregnancy. *Ultrasound Obstet Gynecol.* 2018;52(5):654-661.
25. Hamill N, Romero R, Hassan S, et al. The fetal cardiovascular response to increased placental vascular impedance to flow determined with 4-dimensional ultrasound using spatiotemporal image correlation and virtual organ computer-aided analysis. *Am J Obstet Gynecol.* 2013;208(2):153.e151-113.
26. Sandoval-Valdez D, Silveira LH, Rodríguez E. Cardiovascular risk in the postnatal life of children born to women with systemic lupus erythematous. *Arch Cardiol Mex.* 2022;92(4):522-529.
27. Cohen E, Wong FY, Horne RS, Yiallourou SR. Intrauterine growth restriction: impact on cardiovascular development and function throughout infancy. *Pediatr Res.* 2016;79(6):821-830.
28. Fouzas S, Karatza AA, Davlouros PA, et al. Neonatal cardiac dysfunction in intrauterine growth restriction. *Pediatr Res.* 2014;75(5):651-657.
29. Iruretagoyena JI, Gonzalez-Tendero A, Garcia-Canadilla P, et al. Cardiac dysfunction is associated with altered sarcomere ultrastructure in intrauterine growth restriction. *Am J Obstet Gynecol.* 2014;210(6):550.e551-557.
30. Brito-Zerón P, Izmirly PM, Ramos-Casals M, Buyon JP, Khamashta MA. Autoimmune congenital heart block: complex and unusual situations. *Lupus.* 2016;25(2):116-128.

31. Brito-Zerón P, Izmirly PM, Ramos-Casals M, Buyon JP, Khamashta MA. The clinical spectrum of autoimmune congenital heart block. *Nat Rev Rheumatol.* 2015;11(5):301-312.
32. Madhusudan D, Raju A, Vijaya N. Correlation of Maternal Autoantibodies with Fetal Congenital Heart Block. *J Obstet Gynaecol India.* 2016;66(Suppl 1):112-116.
33. Pruetz JD, Miller JC, Loeb GE, Silka MJ, Bar-Cohen Y, Chmait RH. Prenatal diagnosis and management of congenital complete heart block. *Birth Defects Res.* 2019;111(8):380-388.
34. Tunaoglu FS, Yildirim A, Vurali D. Isolated congenital heart block. *Tex Heart Inst J.* 2010;37(5):579-583.
35. Jaeggi E, Laskin C, Hamilton R, Kingdom J, Silverman E. The importance of the level of maternal anti-Ro/SSA antibodies as a prognostic marker of the development of cardiac neonatal lupus erythematosus a prospective study of 186 antibody-exposed fetuses and infants. *J Am Coll Cardiol.* 2010;55(24):2778-2784.
36. Costa SP, Beaver TA, Rollor JL, Vanichakarn P, Magnus PC, Palac RT. Quantification of the variability associated with repeat measurements of left ventricular two-dimensional global longitudinal strain in a real-world setting. *J Am Soc Echocardiogr.* 2014;27(1):50-54.
37. Risum N, Ali S, Olsen NT, et al. Variability of global left ventricular deformation analysis using vendor dependent and independent two-dimensional speckle-tracking software in adults. *J Am Soc Echocardiogr.* 2012;25(11):1195-1203.
38. Liu MY, Tacy T, Chin C, Obayashi DY, Punn R. Assessment of Speckle-Tracking Echocardiography-Derived Global Deformation Parameters During Supine Exercise in Children. *Pediatr Cardiol.* 2016;37(3):519-527.

12. DISCUSSION:

12.1 Summary

In this thesis, we investigated the influence of maternal rheumatological disorders on fetal heart function assessed through speckle-tracking echocardiography, as well as the effect of circulating maternal autoantibodies and fetal heart block on fetal cardiac function. Our retrospective study used data collected from cardiology databases of the McGill University Health Centre (MUHC) and included 124 fetuses. Their echocardiography retained for analysis was performed between the estimated gestational ages of 22 to 24 weeks. The population was comprised of fetuses exposed to maternal rheumatological conditions, sub-stratified by two groups: fetuses exposed to positive anti-Ro/La antibodies (n=49) and fetuses exposed to negative anti-Ro/La antibodies (n=14). They were compared to non-exposed fetuses (n=61). While there were no significant differences in RV parameters between the groups, our analysis revealed that fetuses exposed to maternal rheumatological conditions, regardless of their anti-Ro/La antibody status, displayed higher LV-EF and LV longitudinal strain, compared to the unexposed group. We also found that fetuses exposed to positive anti-Ro/La antibodies, after excluding those with fetal CHB, still exhibited early cardiac functional changes as observed through STE.

In our manuscript, we explored how our findings suggest the presence of early cardiac functional alterations that might be attributed to an adverse in-utero environment, potentially resulting from adaptations to increased afterload caused by modifications in placental vasculature. We also discussed a few possibilities as to why maternal anti-Ro/La autoantibodies and/or inflammatory changes may impact the cardiovascular function in this population of fetuses. We postulate that the presence of placental pathological anomalies may result in a rise in fetal systemic

vascular resistance exposure, leading to a LV compensation reaction due to the LV's ability to adapt to increased vascular resistance. Additionally, ongoing inflammatory responses associated with maternal rheumatological conditions can contribute to subsequent cardiac changes and, eventually fibrosis (especially when in presence of anti-Ro/La autoantibodies). However, in the earlier processes of pregnancy, our data suggests an initial LV reactional process, which may be a compensatory mechanism also related to the increased inflammatory environment. As CHB may dramatically impact cardiac performance, we evaluated our subgroup of fetuses exposed to circulating anti-Ro/La autoantibodies but free of CHB and found a similar effect. This outlines that the part of the observed changes in cardiac function possibly occur irrespective of the occurrence of CHB.

The results and the knowledge gained from this thesis, highlight the importance of furthering the research in this population of fetuses, especially regarding ongoing cardiovascular surveillance in the pre-natal setting, as well as gaining more insight on the post-natal evolution throughout the lifespan. Indeed, the differences described in our cohort regarding LV performance are subtle, but at a crucial moment of embryogenesis, organogenesis and fetal programming. Lifespan exposure to various cardiovascular hits may further aggravate these early alterations and long-term data regarding these cohorts of fetuses exposed to maternal rheumatological conditions are lacking.

Pregnant women with rheumatological conditions experience immunological and inflammatory alterations that intersect with the immune modulation processes occurring during pregnancy. The immune system undergoes modifications, including shifts in cytokine profiles, changes in T cell subsets, and adjustments in immune cell functions. These adaptations are crucial

for establishing immune tolerance towards the fetus. In the context of rheumatological pregnancies, cells present in the decidua and placenta play a significant role in coordinating tolerogenic mechanisms.[84] The immune system recognizes fetal antigens early on, initiating tolerance.[84] Factors like seminal fluid containing substances such as transforming growth factor beta (TGF-β) and prostaglandins contribute to a pro-inflammatory state, thereby promoting the induction of regulatory T cells that will suppress the immune responses against fetal antigens and prevent it from attacking the developing embryo, which are essential for successful implantation.[84] Therefore, to prevent rejection of the fetus and allow normal placentation and fetal growth, a rapid establishment of a tolerogenic environment at the fetal-maternal interface is necessary. The balance between pro-inflammatory and anti-inflammatory cytokines, as well as the modulation of immune cell populations, such as regulatory T cells and natural killer cells, can influence disease activity and management during pregnancy for individuals with rheumatological conditions. Inflammation related to autoimmune processes may impact the placentation, the vascular system of the pregnant individual, as well as trigger a transfer of cytokines and autoantibodies to the fetus during a critical stage of development. Adaptation in the fetus is nested within genetic susceptibility, variations in inflammatory response, exposure, and timing of exposure to variation in titers of autoantibodies and variations in repair mechanisms. Specifically, placental transfer of autoantibodies has been well documented to increase the risk of triggering multi-organ inflammation, mostly affecting the heart myocardium, and conducting system, as well as other organs such as the liver and the skin. In response to inflammation, the fetus potentially exhibits adaptive mechanisms by increasing its cardiac function through an increase in EF and contractility at the time of evaluation. This adaptation may occur directly due to the inflammatory changes, as

well as indirect mechanisms due to shifts in vascular resistances. Data is lacking regarding the paracrine alterations in these fetuses exposed to inflammation, such as through the renin-angiotensin-aldosterone system and its effects on the growing heart. In our cohort, infants were not systematically evaluated by echocardiography in the postnatal setting, except for those with a prenatal diagnosis of CHB. This prevented us from extending the analysis to the entire cohort in the postnatal setting in order to evaluate for the persistence of the described changes after undergoing neonatal transition.

In our cohort, we observed at 22-24 weeks GA, measures of increased LV contraction, exemplified by more profound negative values of the strain on the longitudinal axis of the heart in the apical 4 chamber view. We postulated that these changes may be secondary to factors such as a higher systemic vascular resistance mediated by the placental changes and, possibly, the vascular system of the fetus exposed to inflammation. This response is further possibly nested within a stage of development (22-24 weeks), where the cardiac tissue has the capacity to adapt. Indeed, cardiac fibrosis has been described in offspring exposed to autoantibodies and/or inflammation, but only starts to develop between 16 to 24 weeks of GA.[85] Nonetheless, with ongoing inflammation and altered loading conditions, fibrosis may set in and trigger adverse and irreversible cardiac alterations. As such, it is imperative to address this concern by conducting further investigations to better understand the implications in later stages of pregnancy and post-birth since it can potentially provide valuable insights into the developmental trajectory and shed light on potential long-term effects. Additionally, the lack of significant differences in the remaining LV metrics and RV metrics might be attributed to factors such as the restricted GA range, limitations inherent in the echocardiography methodology employed, and the possible

subtlety of fetal cardiac alterations that can evade immediate detection. Furthermore, our assessment was conducted on two specific planes of the heart, excluding the anterior and posterior walls. This could potentially result in the oversight of functional cardiac alterations occurring in those regions due to limitations in our echocardiography techniques. As emphasized, future research studies should be aimed at delving into the evolving nature of cardiac function and its potential consequences over time.

12.2 Thesis Limitations and Future Directions

The study's data collection was limited to a specific range of GA and did not include an assessment of long-term outcomes. This limitation restricts the ability to make conclusions about the prolonged effects of maternal rheumatological conditions on fetal cardiac function. Future research should focus on investigating the cardiac impact of maternal rheumatological conditions during later stages of fetal development and in the post-natal neonatal period, as well as long-term during the lifespan of these infants. STE offers a wide range of metrics for quantifying cardiac function, making it a valuable tool for quantifying changes in cardiac deformation of fetuses. It provides an angle-independent methodology, allowing measurements despite variation of orientation. In contrast to other echocardiography-based metrics that depend on manual measurements from a restricted number of frames, speckle tracking employs frame-to-frame analysis to track motion. This methodology offers the advantage of quantifying both global behavior of the myocardium in systole and diastole, as well as detecting alterations in segments of the heart (portion of a ventricular wall or atrium). However, strain analysis should ideally be done at a high frame rate, and our dataset was comprised of clinically acquired images encoded at 25 frame per second. Further, electrocardiogram (ECG)-gating allows for precise evaluation of the

timing of cardiac activation, which is not currently feasible in the context of a fetal echocardiography with commercial tools. Additionally, the use of an apical 4 chamber view by bidimensional echocardiography limited the assessment to a single plane of the heart (septum and free wall), disregarding its complex geometry. Multiple planes of imaging may have outlined other alterations, such as in the right ventricle. Also, inflammatory changes may impact differently various component of the heart, which may not have been picked up by the current methodology. While this view is commonly used and standardized for assessing fetal RV and LV function, it falls short in representing the complete three-dimensional (3-D) dynamics of the heart, potentially missing other regions such as the anterior and posterior wall. To overcome this limitation, acquiring comprehensive 3-D images of all cardiac walls would be ideal, but currently, obtaining such images with sufficient temporal resolution is challenging due to the need for ECG synchronization. Therefore, the study acknowledges the need to explore advanced techniques for capturing 3-D beating volumes of the RV and LV in fetal echocardiography or using new methods such as fetal magnetic resonance imaging.[86,87]

12.4 Conclusion

In conclusion, this thesis has shed light on the influence of maternal rheumatological conditions, maternal autoantibodies, and placental changes on fetal cardiac function. The findings support the presence of early cardiac alterations, possibly as a reactional process to the adverse fetal environment. Fetal exposure to maternal rheumatological conditions, with or without circulating anti-Ro/La autoantibodies, showed signs of increased LV contractility during echocardiographic assessment around 22-24 weeks, indicating early cardiac alterations. These results imply that the fetal heart may initially compensate for adverse intrauterine conditions. The

implications of these adaptations during fetal life and their long-term effects remain important questions for future studies. Future studies should investigate the lasting impact of these adaptations and how they may influence the individual's health and well-being over the lifespan. Future studies should also explore potential biomarkers to assess the involvement of cytokines in the pathophysiology of fetal adaptation and the development of CHB in rheumatological conditions.

13. REFERENCES:

1. Petri M. Pregnancy and Systemic Lupus Erythematosus. *Best Pract Res Clin Obstet Gynaecol.* 2020;64:24-30.
2. Castellanos Gutierrez AS, Figueras F, Morales-Prieto DM, Schleußner E, Espinosa G, Baños N. Placental damage in pregnancies with systemic lupus erythematosus: A narrative review. *Front Immunol.* 2022;13:941586.
3. Crispi F, Sepúlveda-Martínez Á, Crovetto F, Gómez O, Bijnens B, Gratacós E. Main Patterns of Fetal Cardiac Remodeling. *Fetal Diagnosis and Therapy.* 2020;47(5):337-344.
4. van Oostrum NHM, de Vet CM, van der Woude DAA, Kemps HMC, Oei SG, van Laar J. Fetal strain and strain rate during pregnancy measured with speckle tracking echocardiography: A systematic review. *Eur J Obstet Gynecol Reprod Biol.* 2020;250:178-187.
5. Brito-Zerón P, Izmirly PM, Ramos-Casals M, Buyon JP, Khamashta MA. Autoimmune congenital heart block: complex and unusual situations. *Lupus.* 2016;25(2):116-128.
6. Duan S, Ha S, Li S, et al. Evaluation of cardiac function and systolic dyssynchrony of fetuses exposed to maternal autoimmune diseases using speckle tracking echocardiography. *Clin Rheumatol.* 2021;40(9):3807-3815.
7. Jain V, Gordon C. Managing pregnancy in inflammatory rheumatological diseases. *Arthritis Res Ther.* 2011;13(1):206.
8. Carvalheiras G, Faria R, Braga J, Vasconcelos C. Fetal outcome in autoimmune diseases. *Autoimmun Rev.* 2012;11(6-7):A520-530.

9. He WR, Wei H. Maternal and fetal complications associated with systemic lupus erythematosus: An updated meta-analysis of the most recent studies (2017-2019). *Medicine (Baltimore).* 2020;99(16):e19797.

10. Gupta S, Gupta N. Sjögren Syndrome and Pregnancy: A Literature Review. *Perm J.* 2017;21:16-047.

11. Psianou K, Panagoulias I, Papanastasiou AD, et al. Clinical and immunological parameters of Sjögren's syndrome. *Autoimmun Rev.* 2018;17(10):1053-1064.

12. Tsai YC, Chang HC, Chiou MJ, Luo SF, Kuo CF. Fetal-neonatal and maternal pregnancy outcomes in women with rheumatoid arthritis: a population-based cohort study. *BMJ Open.* 2022;12(10):e059203.

13. Sapkota B, Al Khalili Y. Mixed Connective Tissue Disease. In: *StatPearls.* Treasure Island (FL): StatPearls Publishing

14. Tardif ML, Mahone M. Mixed connective tissue disease in pregnancy: A case series and systematic literature review. *Obstet Med.* 2019;12(1):31-37.

15. Serena C, Clemenza S, Simeone S, et al. Undifferentiated Connective Tissue Disease in Pregnancy: A Topic Yet to be Explored. *Front Pharmacol.* 2022;13:820760.

16. Bustamante JG, Goyal A, Singhal M. Antiphospholipid Syndrome. In: *StatPearls.* Treasure Island (FL): StatPearls Publishing

17. Chaturvedi S, McCrae KR. Diagnosis and management of the antiphospholipid syndrome. *Blood Rev.* 2017;31(6):406-417.

18. Mitchell K, Kaul M, Clowse ME. The management of rheumatic diseases in pregnancy. *Scand J Rheumatol.* 2010;39(2):99-108.

19. Che WI, Hellgren K, Stephansson O, Lundberg IE, Holmqvist M. Pregnancy outcomes in women with idiopathic inflammatory myopathy, before and after diagnosis-a population-based study. *Rheumatology (Oxford).* 2020;59(9):2572-2580.

20. Redeker I, Strangfeld A, Callhoff J, Marschall U, Zink A, Baraliakos X. Maternal and infant outcomes in pregnancies of women with axial spondyloarthritis compared with matched controls: results from nationwide health insurance data. *RMD Open.* 2022;8(2).

21. Ünlü O, Zuily S, Erkan D. The clinical significance of antiphospholipid antibodies in systemic lupus erythematosus. *Eur J Rheumatol.* 2016;3(2):75-84.

22. Geng B, Zhang K, Huang X, Chen Y. A meta-analysis of the effect of Sjögren's syndrome on adverse pregnancy outcomes. *Clinics (Sao Paulo).* 2022;77:100140.

23. Parisis D, Chivasso C, Perret J, Soyfoo MS, Delporte C. Current State of Knowledge on Primary Sjögren's Syndrome, an Autoimmune Exocrinopathy. *J Clin Med.* 2020;9(7).

24. Levy R, Mendoza Pinto C, Domingues V, et al. Systemic autoimmune diseases and pregnancy. In: Anaya JM SY, Rojas-Villarraga A, Levy RA, Cervera R., ed. *Autoimmunity: From Bench to Bedside [Internet].* Bogota (Colombia): El Rosario University Press2013.

25. Ciobanu AM, Dumitru AE, Gica N, Botezatu R, Peltecu G, Panaitescu AM. Benefits and Risks of IgG Transplacental Transfer. *Diagnostics (Basel).* 2020;10(8).

26. Reed BR, Lee LA, Harmon C, et al. Autoantibodies to SS-A/Ro in infants with congenital heart block. *J Pediatr.* 1983;103(6):889-891.

27. Boccitto M, Wolin SL. Ro60 and Y RNAs: structure, functions, and roles in autoimmunity. *Crit Rev Biochem Mol Biol.* 2019;54(2):133-152.

28. Verhagen AP, Pruijn GJ. Are the Ro RNP-associated Y RNAs concealing microRNAs? Y RNA-derived miRNAs may be involved in autoimmunity. *Bioessays.* 2011;33(9):674-682.

29. Yoshimi R, Ueda A, Ozato K, Ishigatsubo Y. Clinical and pathological roles of Ro/SSA autoantibody system. *Clin Dev Immunol.* 2012;2012:606195.

30. Soh MC, Dib F, Nelson-Piercy C, Westgren M, McCowan L, Pasupathy D. Maternal-placental syndrome and future risk of accelerated cardiovascular events in Parous Swedish women with systemic lupus erythematosus - a population-based retrospective cohort study with time-to-event analysis. *Rheumatology (Oxford).* 2016;55(7):1235-1242.

31. Lin P, Rhew E, Ness RB, et al. Adverse pregnancy outcomes and subsequent risk of cardiovascular disease in women with systemic lupus erythematosus. *Lupus Sci Med.* 2014;1(1):e000024.

32. Bernal-Ramirez J, Díaz-Vesga MC, Talamilla M, et al. Exploring Functional Differences between the Right and Left Ventricles to Better Understand Right Ventricular Dysfunction. *Oxid Med Cell Longev.* 2021;2021:9993060.

33. Berman MN, Tupper C, Bhardwaj A. Physiology, Left Ventricular Function. In: *StatPearls.* Treasure Island (FL): StatPearls Publishing

34. Voorhees AP, Han HC. Biomechanics of Cardiac Function. *Compr Physiol.* 2015;5(4):1623-1644.

35. Omar AM, Vallabhajosyula S, Sengupta PP. Left ventricular twist and torsion: research observations and clinical applications. *Circ Cardiovasc Imaging.* 2015;8(6).

36. Sengupta PP, Krishnamoorthy VK, Korinek J, et al. Left ventricular form and function revisited: applied translational science to cardiovascular ultrasound imaging. *J Am Soc Echocardiogr.* 2007;20(5):539-551.

37. Remien K, Majmundar SH. Physiology, Fetal Circulation. In: *StatPearls.* Treasure Island (FL): StatPearls Publishing

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38. Marty M, Kerndt CC, Lui F. Embryology, Fetal Circulation. In: *StatPearls.* Treasure Island (FL): StatPearls Publishing

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39. Abadir S, Fournier A, Vobecky SJ, Rohlicek CV, Romeo P, Khairy P. Left Atrial Inexcitability in Children With Congenital Lupus-Induced Complete Atrioventricular Block. *J Am Heart Assoc.* 2015;4(12).

40. Dörner T, Hiepe F, Göldner B, Apostoloff E. Investigations into Ro-specific antibody-associated congenital cardiac conduction defects. *Clin Investig.* 1992;70(6):492-496.

41. Friedman DM, Llanos C, Izmirly PM, et al. Evaluation of fetuses in a study of intravenous immunoglobulin as preventive therapy for congenital heart block: Results of a multicenter, prospective, open-label clinical trial. *Arthritis Rheum.* 2010;62(4):1138-1146.

42. Iruretagoyena JI, Gonzalez-Tendero A, Garcia-Canadilla P, et al. Cardiac dysfunction is associated with altered sarcomere ultrastructure in intrauterine growth restriction. *Am J Obstet Gynecol.* 2014;210(6):550.e551-557.

43. Izmirly P, Kim M, Friedman DM, et al. Hydroxychloroquine to Prevent Recurrent Congenital Heart Block in Fetuses of Anti-SSA/Ro-Positive Mothers. *J Am Coll Cardiol.* 2020;76(3):292-302.

44. Izmirly PM, Costedoat-Chalumeau N, Pisoni CN, et al. Maternal use of hydroxychloroquine is associated with a reduced risk of recurrent anti-SSA/Ro-antibody-associated cardiac manifestations of neonatal lupus. *Circulation.* 2012;126(1):76-82.

45. Jaeggi ET, Silverman ED, Laskin C, Kingdom J, Golding F, Weber R. Prolongation of the atrioventricular conduction in fetuses exposed to maternal anti-Ro/SSA and anti-La/SSB antibodies did not predict progressive heart block. A prospective observational study on the effects of maternal antibodies on 165 fetuses. *J Am Coll Cardiol.* 2011;57(13):1487-1492.

46. Jaeggi E, Laskin C, Hamilton R, Kingdom J, Silverman E. The importance of the level of maternal anti-Ro/SSA antibodies as a prognostic marker of the development of cardiac neonatal lupus erythematosus a prospective study of 186 antibody-exposed fetuses and infants. *J Am Coll Cardiol.* 2010;55(24):2778-2784.

47. Madhusudan D, Raju A, Vijaya N. Correlation of Maternal Autoantibodies with Fetal Congenital Heart Block. *J Obstet Gynaecol India.* 2016;66(Suppl 1):112-116.

48. Mawad W, Hornberger L, Cuneo B, et al. Outcome of Antibody-Mediated Fetal Heart Disease With Standardized Anti-Inflammatory Transplacental Treatment. *J Am Heart Assoc.* 2022;11(3):e023000.

49. Meisgen S, Hedlund M, Ambrosi A, et al. Auxilin is a novel susceptibility gene for congenital heart block which directly impacts fetal heart function. *Ann Rheum Dis.* 2022;81(8):1151-1161.

50. Nield LE, Silverman ED, Taylor GP, et al. Maternal anti-Ro and anti-La antibody-associated endocardial fibroelastosis. *Circulation.* 2002;105(7):843-848.

51. Silvetti MS, Muzi G, Unolt M, et al. Left ventricular (LV) pacing in newborns and infants: Echo assessment of LV systolic function and synchrony at 5-year follow-up. *Pacing Clin Electrophysiol.* 2020;43(6):535-541.

52. Sonesson SE, Ambrosi A, Wahren-Herlenius M. Benefits of fetal echocardiographic surveillance in pregnancies at risk of congenital heart block: single-center study of 212 anti-Ro52-positive pregnancies. *Ultrasound Obstet Gynecol.* 2019;54(1):87-95.

53. Trucco SM, Jaeggi E, Cuneo B, et al. Use of intravenous gamma globulin and corticosteroids in the treatment of maternal autoantibody-mediated cardiomyopathy. *J Am Coll Cardiol.* 2011;57(6):715-723.

54. Abbas G, Shah S, Hanif M, et al. The frequency of pulmonary hypertension in newborn with intrauterine growth restriction. *Sci Rep.* 2020;10(1):8064.

55. Rueda-Clausen CF, Morton JS, Lopaschuk GD, Davidge ST. Long-term effects of intrauterine growth restriction on cardiac metabolism and susceptibility to ischaemia/reperfusion. *Cardiovasc Res.* 2011;90(2):285-294.

56. Xu Y, Williams SJ, O'Brien D, Davidge ST. Hypoxia or nutrient restriction during pregnancy in rats leads to progressive cardiac remodeling and impairs postischemic recovery in adult male offspring. *Faseb j.* 2006;20(8):1251-1253.

57. Crispi F, Crovetto F, Gratacos E. Intrauterine growth restriction and later cardiovascular function. *Early Hum Dev.* 2018;126:23-27.

58. Cohen E, Wong FY, Horne RS, Yiallourou SR. Intrauterine growth restriction: impact on cardiovascular development and function throughout infancy. *Pediatr Res.* 2016;79(6):821-830.

59. Fouzas S, Karatza AA, Davlouros PA, et al. Neonatal cardiac dysfunction in intrauterine growth restriction. *Pediatr Res.* 2014;75(5):651-657.

60. Wainwright B, Bhan R, Trad C, et al. Autoimmune-mediated congenital heart block. *Best Pract Res Clin Obstet Gynaecol.* 2020;64:41-51.

61. Kiefer-Schmidt I, Lim M, Preissl H, et al. Fetal magnetocardiography (fMCG) to monitor cardiac time intervals in fetuses at risk for isoimmune AV block. *Lupus.* 2014;23(9):919-925.

62. Sehgal A, Skilton MR, Crispi F. Human fetal growth restriction: a cardiovascular journey through to adolescence. *J Dev Orig Health Dis.* 2016;7(6):626-635.

63. Lapointe A, Barrington KJ. Pulmonary hypertension and the asphyxiated newborn. *J Pediatr.* 2011;158(2 Suppl):e19-24.

64. Chaubal NG, Chaubal J. Fetal echocardiography. *Indian J Radiol Imaging.* 2009;19(1):60-68.

65. Ashley EA, Niebauer J. Cardiology explained. In: *Chapter 4, Understanding the echocardiogram.* . London: Remedica; 2004.

66. Lee MY, Won HS. Technique of fetal echocardiography. *Obstet Gynecol Sci.* 2013;56(4):217-226.

67. Lo Gullo A, Rodríguez-Carrio J, Gallizzi R, Imbalzano E, Squadrito G, Mandraffino G. Speckle tracking echocardiography as a new diagnostic tool for an assessment of cardiovascular disease in rheumatic patients. *Prog Cardiovasc Dis.* 2020;63(3):327-340.

68. van Oostrum NHM, de Vet CM, Clur SB, et al. Fetal myocardial deformation measured with two-dimensional speckle-tracking echocardiography: longitudinal prospective cohort study of 124 healthy fetuses. *Ultrasound Obstet Gynecol.* 2022;59(5):651-659.

69. van Oostrum NHM, van der Woude DAA, Clur SB, Oei SG, van Laar J. Right ventricular dysfunction identified by abnormal strain values precedes evident growth restriction in small for gestational age fetuses. *Prenat Diagn.* 2020;40(12):1525-1531.

70. Spartalis M, Tzatzaki E, Spartalis E, et al. Pathophysiology and Current Evidence for Detection of Dyssynchrony. *Cardiol Res.* 2017;8(5):179-183.

71. Nguyên UC, Verzaal NJ, van Nieuwenhoven FA, Vernooy K, Prinzen FW. Pathobiology of cardiac dyssynchrony and resynchronization therapy. *Europace.* 2018;20(12):1898-1909.

72. Guşetu G, Pop D, Pamfil C, et al. Subclinical myocardial impairment in SLE: insights from novel ultrasound techniques and clinical determinants. *Med Ultrason.* 2016;18(1):47-56.

73. Nikdoust F, Bolouri E, Tabatabaei SA, Goudarzvand M, Faezi ST. Early diagnosis of cardiac involvement in systemic lupus erythematosus via global longitudinal strain (GLS) by speckle tracking echocardiography. *J Cardiovasc Thorac Res.* 2018;10(4):231-235.

74. Ayyildiz YO, Vural MG, Efe TH, et al. Effect of Long-Term TNF-α Inhibition with Infliximab on Left Ventricular Torsion in Patients with Rheumatoid Arthritis. *Hellenic J Cardiol.* 2015;56(5):406-413.

75. Cioffi G, Viapiana O, Ognibeni F, et al. Prognostic Role of Subclinical Left Ventricular Systolic Dysfunction Evaluated by Speckle-Tracking Echocardiography in Rheumatoid Arthritis. *J Am Soc Echocardiogr.* 2017;30(6):602-611.

76. Ikonomidis I, Tzortzis S, Lekakis J, et al. Lowering interleukin-1 activity with anakinra improves myocardial deformation in rheumatoid arthritis. *Heart.* 2009;95(18):1502-1507.

77. Lo Gullo A, Rodríguez-Carrio J, Aragona CO, et al. Subclinical impairment of myocardial and endothelial functionality in very early psoriatic and rheumatoid arthritis patients: Association with vitamin D and inflammation. *Atherosclerosis.* 2018;271:214-222.

78. Chen Y, Chung HY, Zhao CT, et al. Left ventricular myocardial dysfunction and premature atherosclerosis in patients with axial spondyloarthritis. *Rheumatology (Oxford).* 2015;54(2):292-301.

79. Midtbø H, Semb AG, Matre K, Rollefstad S, Berg IJ, Gerdts E. Left Ventricular Systolic Myocardial Function in Ankylosing Spondylitis. *Arthritis Care Res (Hoboken).* 2019;71(9):1276-1283.

80. Ustun N, Kurt M, Nacar AB, Karateke HP, Guler H, Turhanoglu AD. Left ventricular systolic dysfunction in patients with ankylosing spondylitis without clinically overt cardiovascular disease by speckle tracking echocardiography. *Rheumatol Int.* 2015;35(4):607-611.

81. Huang BT, Yao HM, Huang H. Left ventricular remodeling and dysfunction in systemic lupus erythematosus: a three-dimensional speckle tracking study. *Echocardiography.* 2014;31(9):1085-1094.

82. Dai M, Li KL, Qian DJ, et al. Evaluation of left atrial function by speckle tracking echocardiography in patients with systemic lupus erythematosus. *Lupus.* 2016;25(5):496-504.

83. Atzeni F, Sarzi-Puttini P, Signorello MC, et al. New parameters for identifying subclinical atherosclerosis in patients with primary Sjögren's syndrome: a pilot study. *Clin Exp Rheumatol.* 2014;32(3):361-368.

84. Förger F, Villiger PM. Immunological adaptations in pregnancy that modulate rheumatoid arthritis disease activity. *Nat Rev Rheumatol.* 2020;16(2):113-122.

85. Saxena A, Izmirly PM, Mendez B, Buyon JP, Friedman DM. Prevention and treatment in utero of autoimmune-associated congenital heart block. *Cardiol Rev.* 2014;22(6):263-267.

86. Sun L, Marini D, Saini B, Schrauben E, Macgowan CK, Seed M. Understanding Fetal Hemodynamics Using Cardiovascular Magnetic Resonance Imaging. *Fetal Diagn Ther.* 2020;47(5):354-362.

87. Roy CW, van Amerom JFP, Marini D, Seed M, Macgowan CK. Fetal Cardiac MRI: A Review of Technical Advancements. *Top Magn Reson Imaging.* 2019;28(5):235-244.