AUTOMATED ANALYSIS
OF FETAL CARDIAC FUNCTION

A New Approach Based on Tissue Doppler Imaging

Lotta Herling
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Automated Analysis of Fetal Cardiac Function
A New Approach Based on Tissue Doppler Imaging

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In loving memory of my grandmother Mary.
ABSTRACT

The heart is a central organ in fetal adaptation to various hemodynamic insults and it is possible that subtle changes caused by complications such as hypoxia and anemia could manifest themselves early as altered fetal cardiac function. Color tissue Doppler imaging (cTDI) is an ultrasound technique that evaluates myocardial motion by assessing myocardial velocities. cTDI has been attempted in fetuses, but its use has been hampered by a cumbersome and time-consuming process of image analysis. The main aim of this thesis was to lay the foundation for simplifying the process of image analysis, i.e. the analysis of myocardial velocity traces, using an automated algorithm allowing for quicker and easier assessment of fetal cardiac function during the second half of pregnancy.

In all studies, a four-chamber view of the fetal heart was acquired and cine-loops of consecutive cardiac cycles using cTDI were stored before off-line analysis was performed. Myocardial velocity traces describing longitudinal cardiac function in the left (LV) and right ventricular (RV) wall and interventricular septum (IVS) were obtained, and peak myocardial velocities, duration of cardiac cycle time intervals and displacement of the atrioventricular plane assessed.

In Study I the feasibility of a beta version of the automated algorithm was evaluated in 261 myocardial velocity traces obtained from 17 echocardiographic examinations in five women. The automated algorithm could analyze 203 out of 261 (78%) myocardial velocity traces. Furthermore, the effect of different sizes of regions of interest (ROIs) on the results at different gestational ages was assessed. With increasing ROI heights there was a loss of velocity information compared to a smaller reference ROI. However, the acceleration traces, used to define cardiac time intervals, were judged to be more well-defined, with increasing ROI heights, in later gestation.

In Study II, manual and automated analysis of cTDI velocity traces were compared and the feasibility of using the automated algorithm was assessed in 107 women ≥ 41 weeks of gestation. All myocardial velocity traces (n = 321) were possible to analyze with the manual method of analysis. Myocardial velocities and cardiac cycle time intervals could be measured in 96% of all traces using the automated method of analysis. There were significant positive correlations between all automatically and manually assessed myocardial velocity variables except one. However, only a few of the cardiac cycle time intervals measured by the two techniques correlated significantly, the agreement between methods sometimes showed considerable bias and precision was poor for some cTDI variables.

In Study III, gestational age specific reference ranges for normal pregnancies between 18 and 42 weeks of gestation were constructed using the automated analysis of cTDI velocity traces. This was a cross-sectional study with a final study population of 201 pregnant women examined between 18 and 42 weeks of gestation. This study demonstrated an increase of peak myocardial velocities with advancing gestation, whereas the time intervals remained more stable.
Study IV included 32 fetuses that underwent 70 intrauterine transfusions (IUTs). Twenty-seven fetuses underwent 63 IUTs because of fetal anemia due to maternal alloimmunization and five had other underlying diseases or the anemia was of unknown origin. There were significantly increased myocardial velocities during systole and diastole in the LV wall and IVS, whereas in the RV wall only the systolic velocity was increased before IUT. After IUT, there were significant decreases in the same velocities. When analyzing only first IUTs, there was a significant negative correlation between hemoglobin and myocardial velocity during rapid ventricular filling, i.e. LV Em (rho = -0.61, p = 0.036), as well as the ratio between myocardial velocity during rapid ventricular filling and atrial contraction, i.e. LV Em/Am (rho = -0.82, p = 0.001).

This thesis shows that it is possible to use an automated algorithm to analyze cTDI velocity traces to assess fetal cardiac function during the second half of pregnancy. It is proposed that the ROI size should be adjusted according to gestational age as results indicate that this could improve the function of the algorithm. Furthermore, the automated method of analysis was demonstrated feasible at ≥ 41 weeks of gestation. However, sometimes the agreement between the automated and manual methods of analysis of myocardial velocity traces was poor. While none of these methods are considered a gold standard today, the potential benefit of an automated assessment that repeatedly gives the exact same results is an important pre-requisite for the future application and use of cTDI in research and clinical practice. Gestational age specific reference ranges in the second half of normal pregnancy were constructed, which is also a pre-requisite to discriminate normal from abnormal cardiac function. Finally, the automated analysis was evaluated in fetuses undergoing IUT due to suspected fetal anemia, and illustrated increased myocardial velocities before transfusion that decreased with reversal and cessation of the hyperdynamic state after transfusion in accordance with previous findings in fetal blood flow velocities.

To conclude, cTDI with automated analysis shows promise as a feasible method to study fetal cardiac function and to detect cardiac dysfunction and adaptation to pathological situations. The use of an automated analysis could also facilitate its application in research and clinical practice.
LIST OF SCIENTIFIC PAPERS

This thesis is based on the following articles:

I. Automated analysis of color tissue Doppler velocity recordings of the fetal myocardium using a new algorithm

   Herling L, Johnson J, Ferm-Widlund K, Lindgren P, Acharya G and Westgren M.
   Cardiovasc Ultrasound. 2015;13:39

II. Automated analysis of fetal cardiac function using color tissue Doppler imaging


III. Automated analysis of fetal cardiac function using color tissue Doppler imaging in the second half of normal pregnancy

   Ultrasound Obstet Gynecol. Accepted.

IV. Fetal cardiac function before and after intrauterine transfusion assessed by automated analysis of color tissue Doppler recordings

   Manuscript.
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<th>Description</th>
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<tbody>
<tr>
<td>ALARA</td>
<td>As low as reasonably achievable</td>
</tr>
<tr>
<td>Am</td>
<td>Peak myocardial velocity during atrial contraction</td>
</tr>
<tr>
<td>AV</td>
<td>Atrioventricular</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>CCO</td>
<td>Combined cardiac output</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>cMPI</td>
<td>Myocardial performance index obtained by cTDI</td>
</tr>
<tr>
<td>cTDI</td>
<td>Color tissue Doppler imaging</td>
</tr>
<tr>
<td>C/T ratio</td>
<td>Cardiothoracic ratio</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>DA</td>
<td>Ductus arteriosus</td>
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<tr>
<td>DAPP</td>
<td>Dynamic adaptive piston pump</td>
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<tr>
<td>DV</td>
<td>Ductus venosus</td>
</tr>
<tr>
<td>Em</td>
<td>Peak myocardial velocity during rapid ventricular filling</td>
</tr>
<tr>
<td>EF</td>
<td>Ejection fraction</td>
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<tr>
<td>ET</td>
<td>Ejection time</td>
</tr>
<tr>
<td>GA</td>
<td>Gestational age</td>
</tr>
<tr>
<td>ICT</td>
<td>Isovolumic contraction time</td>
</tr>
<tr>
<td>IRT</td>
<td>Isovolumic relaxation time</td>
</tr>
<tr>
<td>IUGR</td>
<td>Intrauterine growth restriction</td>
</tr>
<tr>
<td>IUT</td>
<td>Intrauterine (intravascular) transfusion</td>
</tr>
<tr>
<td>IVS</td>
<td>Interventricular septum</td>
</tr>
<tr>
<td>LV</td>
<td>Left ventricle/ventricular</td>
</tr>
<tr>
<td>MCA</td>
<td>Middle cerebral artery</td>
</tr>
<tr>
<td>MI</td>
<td>Mechanical index</td>
</tr>
<tr>
<td>MoM</td>
<td>Multiple of median</td>
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<tr>
<td>MPI</td>
<td>Myocardial performance index</td>
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<tr>
<td>PI</td>
<td>Pulsatility index</td>
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<tr>
<td>PIV</td>
<td>Pulsatility index for veins</td>
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<tr>
<td>PSV</td>
<td>Peak systolic velocity</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>RV</td>
<td>Right ventricle/ventricular</td>
</tr>
<tr>
<td>SF</td>
<td>Shortening fraction</td>
</tr>
<tr>
<td>SGA</td>
<td>Small for gestational age</td>
</tr>
<tr>
<td>Sm</td>
<td>Peak myocardial velocity during ventricular ejection</td>
</tr>
<tr>
<td>sTDI</td>
<td>Spectral tissue Doppler imaging</td>
</tr>
<tr>
<td>TDI</td>
<td>Tissue Doppler imaging</td>
</tr>
<tr>
<td>TI</td>
<td>Thermal index</td>
</tr>
<tr>
<td>TTTS</td>
<td>Twin-to-twin transfusion syndrome</td>
</tr>
<tr>
<td>UA</td>
<td>Umbilical artery</td>
</tr>
<tr>
<td>UV</td>
<td>Umbilical vein</td>
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<tr>
<td>VTI</td>
<td>Velocity time integral</td>
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1 INTRODUCTION

There are few methods for monitoring fetuses before birth. Most are based on ultrasound and include evaluation of fetal size, behavior, heart rate and heart rate variability, as well as evaluation of blood flow in different fetal vessels to assess signs of compromise, such as redistribution of circulation and increased impedance in placental or maternal vessels. In clinical practice, we often experience a lack of adequate and reliable information about fetal well-being and have difficulties correctly distinguishing between normal and pathological conditions before late in the course of fetal disease.

The heart is a central organ in fetal adaptation to various insults, and it is possible that subtle changes caused by different complications, such as hypoxia, anemia and infection, could manifest themselves early as altered cardiac function. There are a variety of ultrasound techniques used to assess different aspects of fetal cardiac function, most of which are based on variables derived from evaluation of blood flow. Surprisingly little is known about the heart itself and the functioning of the myocardium in utero. Recent developments in imaging techniques have increased the possibility to investigate details of cardiac function describing myocardial tissue velocities, timing of myocardial events with measurement of cycle time intervals and displacement of the myocardium. One of these techniques is tissue Doppler imaging (TDI). In adults, it facilitates the detection of subclinical cardiac dysfunction and helps predict adverse cardiovascular outcomes\cite{1,2}. TDI was first demonstrated feasible in fetuses in 1999\cite{3}. Signs of possible subclinical cardiac dysfunction with a decrease in myocardial velocities have been shown in fetuses with intrauterine growth restriction (IUGR)\cite{4}. Moreover, there are indications that TDI might be more sensitive in detecting cardiac dysfunction than conventional blood flow evaluation\cite{5,6}. Consequently, TDI is an ultrasound technique that shows promise for increasing knowledge about fetal well-being and cardiac function that, hypothetically, could improve monitoring and fine-tune timing of delivery of compromised fetuses.

The focus of this thesis is on the use of color TDI (cTDI). As with all other techniques of monitoring attempted on the fetus, cTDI has its limitations. These relate primarily to fetal and maternal movements, the small size of the fetal heart, high fetal heart rate, and angle-dependency in the assessment of myocardial velocities. Furthermore, in the developing fetus the process of gestational age-related maturation influences fetal cardiovascular function as well as the performance of imaging techniques. This aspect must be taken into consideration when evaluating fetal heart function.

Clinical application of cTDI in the routine assessment of fetal cardiac function has been hampered by the need for specialized equipment and highly trained operators, as well as a cumbersome and time-consuming process of image analysis. There is still a lack of knowledge about fetal cardiac function/dysfunction and adaptation to various pregnancy complications. Consequently, there is a need to further develop and improve methods such as cTDI, to evaluate whether additional information on fetal disease states can be obtained to
improve the screening, diagnosis and monitoring of fetuses at risk of compromise. To facilitate the use of cTDI, and to adequately evaluate its applicability and role in the clinical assessment of different fetal conditions, the development of automation might be considered a pre-requisite.

The main objective of this thesis was to lay the foundation for simplifying the process of analyzing cTDI velocity traces by the use of an automated algorithm allowing for quicker and easier assessment of fetal cardiac function in research and clinical practice.
2 THE FETAL CARDIOVASCULAR SYSTEM

2.1 DEVELOPMENT OF THE FETAL HEART

The heart is the first organ to obtain its definitive function in the embryo\textsuperscript{7}. Formation is complex and sensitive to perturbations, as evidenced by an estimated 10\% incidence of severe cardiac malformations in early miscarriages\textsuperscript{8}. Furthermore, the heart needs to support the growing embryo before establishing its own final structure with four-chambers and it does so by growing and maturing while constantly adapting to the changing demands\textsuperscript{8}.

The structural development of the heart has been traditionally described in three major stages, i.e. formation of a primitive heart tube, looping of the heart tube and, finally, septation of the atria, ventricles and outflow tracts creating four chambers and separate outflows. However, recent knowledge suggests that the primary heart tube only serves as a scaffold for various surrounding cells that complete development. These different groups of cells give rise to different parts of the heart, such as the atria, inflow tracts and conducting system\textsuperscript{9-11}. Consequently, the development of the fetal heart is the result of complex, and fine-tuned, temporal and spatial development resulting in the final fetal anatomy.

Structural development is crucial, but equally important are the functional changes in cardiac contraction and relaxation that accompany structural development of the fetal heart. Much less is known about these processes in the human heart, and a lot of the knowledge is derived from animal studies and extrapolated to human physiology.

The main function of cardiac muscle cells (cardiomyocytes) is to maintain a cycle of contraction and relaxation. This is a complex process involving transport of ions and delivery of calcium to and from contractile proteins. Myocytes contain myofibrils that are formed from chains of sarcomeres, which are the primary contractile units of heart muscle. Within each sarcomere there are muscle filaments containing contractile proteins that create cross-bridges that create contraction. In short, the mature/adult heart achieves contraction-relaxation by creating a negative membrane potential at rest using sodium-potassium pumps located in the cell membrane. Contraction is initiated by depolarization of the membrane through opening of sodium channels, which leads to opening of voltage dependent calcium channels. This small influx of calcium into the cell causes the release of large amounts of calcium from the sarcoplasmic reticulum. This substantial increase of calcium inside the cell results in activation of the contractile proteins and cell shortening (contraction). The subsequent relaxation is an energy dependent process achieved by reuptake of calcium into the sarcoplasmic reticulum through calcium pumps. The resting membrane potential is then reestablished and a new contraction-relaxation cycle can be initiated\textsuperscript{8,11-13}.

By approximately day 21 to 22 post-conception (5\textsuperscript{th} gestational week), the elements required for rhythmic contraction and relaxation in the human embryonic heart are functional, and pulsations can be seen in the straight heart tube. In the beginning the heart beats are irregular and the heart does not pump blood. Eventually, the tubular heart becomes a suction pump...
where contraction causes complete obstruction of the lumen and generates 100% ejection fractions, which cannot be achieved at any later stage in development\textsuperscript{11}.

There are several differences between fetal and mature hearts that influence the contraction-relaxation cycle and, thus, function of the fetal heart. This results in significant age-related differences in the mechanisms of cardiac contraction, relaxation and regulation of contractile function. For instance, myocytes change considerably in size, shape and overall appearance through maturation. As demonstrated in studies on fetal sheep, myocytes can proliferate throughout most of gestation but this seems to stop around the time of birth suggesting that the maximal numbers of myocytes are then set\textsuperscript{14,15}. Continued growth of the heart is mainly caused by enlargement, i.e. hypertrophy, of existing myocytes. This switch from hyperplastic to hypertrophic growth is incompletely understood\textsuperscript{8}.

Another example is the sarcoplasmic reticulum, which in mature hearts stores, releases and re-accumulates most of the calcium involved in contraction and relaxation. In the fetus, the sarcoplasmic reticulum is underdeveloped and its position within the cell is different compared to that in adults. Details about its functional maturation are also largely unknown. Consequently, it is suggested that it has a less prominent role in the fetal cardiac contraction-relaxation cycle. The T-tubules, which are structures that mediate and facilitate contact between the cell membrane and inner structures, such as the sarcoplasmic reticulum, are also poorly developed or absent. Sarcomeres are seen already in early fetal life. However, they are relatively disorganized and myofibrils are initially irregular and scattered. With maturation, there is an increase in cellular content of myofilaments and myofibrils are eventually positioned along the long axis of the cell, improving myocardial function\textsuperscript{8}.

There are also other important age-related changes in regulatory processes, such as changes in enzyme activities, and protein isoforms, as well as changes in proteins involved in the interaction between calcium and contractile proteins. Troponins compose the protein complex to which calcium binds to trigger contraction. Changes in the expression of cardiac troponins occur during development and influence myocardial sensitivity to calcium and influence cardiac force development\textsuperscript{8,16}.

Furthermore, in adult hearts long-chain free fatty acids are the main energy substrate, whereas in immature hearts the energy substrates are primarily lactate and glucose. This is logical as these are the most readily available substrates in the fetus\textsuperscript{7}. Another influencing factor might be that the enzyme transporting activated free fatty acids into the mitochondria, where they are metabolized, has decreased activity in immature hearts. Furthermore, the mitochondria are irregularly scattered throughout the cell and gradually become positioned along the myofilaments to more efficiently meet the high energy demands\textsuperscript{8}.

To conclude, control of contractile function in the immature heart is substantially different compared to the fully mature heart. The fetal myocardium contains a greater proportion of non-contractile elements (i.e. nuclei, mitochondria, etc.) that are more disorganized in architecture and the sarcoplasmic reticulum has a less prominent role in fetal cardiac
Despite these differences, myocardial velocity traces obtained by cTDI from human adults and fetuses in the second half of pregnancy are surprisingly similar.

2.2 THE FETAL CIRCULATION

The fetal circulatory system is flexible and adaptive compared to adult and neonatal circulatory systems due to the existence of specific particularities, including shunts and watershed areas. This allows for distributional changes that are not possible in later life and that influence fetal cardiac function and adaptation.

In the fetal heart, the two ventricles work in parallel to the systemic circulation\textsuperscript{20}, as opposed to children and adults where they work in series. The right ventricle is the dominant ventricle in the fetal heart as it ejects more blood than the left ventricle, but at the same filling and systemic pressures as the left ventricle\textsuperscript{21-23}. The estimation of combined cardiac output (CCO) differs depending on measurement techniques. According to some authors, the CCO at 20 weeks is approximately 210 mL/min and increases to 1900 mL/min at 38 weeks\textsuperscript{24}, whereas others report lower numbers with a CCO of approximately 140 mL/min at 20 weeks and 1470 mL/min at 40 weeks\textsuperscript{21}. The right ventricular cardiac output is slightly larger throughout pregnancy, reaching 60% of CCO at 38 weeks\textsuperscript{24}.

There are three main shunts allowing for redistribution of blood flow\textsuperscript{25} (Figure 2-1):

- The ductus venosus (DV) – connecting the intra-abdominal portion of the umbilical vein, via the portal vein, to the inferior vena cava at its inlet to the right atrium. The watershed area being the portal sinus, where the venous return from the placenta (umbilical vein) and portal circulation are connected.
- The foramen ovale – connecting the right and left atria.
- The ductus arteriosus (DA) – connecting the main pulmonary artery to the descending aorta just below the left subclavian artery. The watershed area being the aortic isthmus, where the blood streams from left and right ventricles are connected.

Well oxygenated blood, rich in nutrients and oxygen returning from the placenta through the umbilical vein, will pass either through the liver or be shunted through the DV directly to the heart. The blood passing through the DV is accelerated due to the portocaval pressure gradient and the trumpet-like construction of the vessel. This, as well as the position and structure of the foramen ovale, results in saturated blood from the DV reaching the left atrium and ventricle preferentially. This “preferential streaming” results in well-oxygenated and nutrient-rich blood reaching mainly the left ventricle and, thus, the myocardium, brain and upper extremities\textsuperscript{26,27}. The oxygen saturation in the ascending aorta is, however, only 65%, illustrating the relatively hypoxemic environment of the fetus. According to Kiserud \textit{et al.}, 20-30% of the umbilical venous return is shunted through the DV during the second half of pregnancy\textsuperscript{28}. The degree of shunting decreases as pregnancy advances and DV pulsatility declines\textsuperscript{29}. The degree of shunting is regulated by the diameter of the DV. This mechanism is
Figure 2-1. Schematic illustration of the fetal heart and circulation. Orange colors represent vessels towards the heart, blue colors vessels going out of the right ventricle (RV) and red colors vessels going out of the left ventricle (LV). The three shunts are illustrated, i.e. ductus venosus (DV), foramen ovale (FO) and ductus arteriosus (DA). SVC, superior vena cava; IVC, inferior vena cava; RPA, right pulmonary artery; LPA, left pulmonary artery; RPV, one of the two right pulmonary veins; LPV, one of the two left pulmonary veins; IVS, interventricular septum; TV, tricuspid valve; PV, pulmonary valve; MV, mitral valve; AV, aortic valve.

not fully defined, but there are indications that the diameter is under adrenergic control\textsuperscript{30}. A reduction in umbilical venous pressure and increase in hematocrit also favors shunting through the DV\textsuperscript{31}.

In animal studies, shunting through the DV has been shown to increase during hypoxia to maintain adequate coronary and cerebral circulation\textsuperscript{28, 32-34}. The proportion of umbilical venous blood flow passing through the DV has been shown to increase in pathology, for instance IUGR\textsuperscript{35}. Nevertheless, the difference in oxygen saturation between the right and left ventricle is not more than 10\%, but increases to 12\% during hypoxemia\textsuperscript{25}.

According to Mielke et al., approximately 33\% of the CCO passes through the foramen ovale and contributes to 76\% of the left cardiac output\textsuperscript{21}. Various investigators have indicated different proportions, and some also indicate that foramen ovale shunting diminishes in later gestation, constituting less of the left cardiac output as the pulmonary blood flow gradually increases until reaching a maximum at 28-32 weeks\textsuperscript{24, 26}. However, only a small part of the fetal blood flow, i.e. 11-25\% of the CCO, passes through the lungs, and returns to the left atrium through the pulmonary veins\textsuperscript{21, 24}. 
The poorly oxygenated blood returning to the right atrium from the venous circulation through the inferior and superior vena cava will mainly pass through the right ventricle, the main pulmonary artery and go through the ductus arteriosus to reach the lower body and the placenta. During the second and third trimesters, 32-46% of the CCO is directed through the DA\textsuperscript{21,24}.

The direction of diastolic blood flow in the aortic isthmus is determined by the difference in impedance between the brachiocephalic and placental (and lower body) circulations. In normal fetuses, the net blood flow in the aortic isthmus is forward. Due to a decrease in impedance in the cerebral circulation with advancing gestation, there is a brief reversal of flow in the aortic isthmus in early diastole in the third trimester\textsuperscript{26,36}.

Furthermore, distribution of circulation between the lower body of the fetus and the placenta can be influenced by altered resistance in the vascular beds. Normally, there is a progressive decline in placental vascular resistance that serves to maintain adequate perfusion of the placenta\textsuperscript{26}, ensuring gas exchange and nutrient transfer between the mother and the fetus.

Consequently, distributional changes unique to the fetal circulation need to be considered when interpreting findings describing cardiac function and adaptation.
3 FETAL CARDIAC FUNCTION AND DYSFUNCTION

3.1 BACKGROUND AND GENERAL PRINCIPLES

The main function of the heart is to eject blood to maintain adequate perfusion of the organs and tissues. The main factors that influence myocardial performance, i.e. stroke volume and cardiac output, are loading conditions (preload and afterload), contractility and heart rate\textsuperscript{12}.

**Preload**

In a normal adult heart, the amount of blood pumped is largely determined by the amount of blood returning to the heart, the venous return. When the venous return and the pressure are increased, there is increased stretching of the cardiac muscle during diastole and the heart contracts with greater force to pump the increased amount of blood. Preload is the ventricular load present in the end of diastole, preferably measured as the end-diastolic volume, but frequently substituted by end-diastolic pressure, which stretches the myocardium prior to the contraction. In the normal heart, increased end-diastolic volume leads to increased stroke volume. An increased stroke volume in response to increased preload is called the Frank-Starling mechanism\textsuperscript{12}. The Frank-Starling mechanism is operational in the fetus\textsuperscript{37-40}, although the fetal heart has a limited ability to increase stroke volume by increasing diastolic filling\textsuperscript{20}.

The fetal heart has a decreased ability for relaxation in early pregnancy, and this gradually improves with advancing gestation. Data from Doppler echocardiography suggests that fetal ventricular relaxation improves more than compliance during the course of pregnancy as, early diastolic filling (E-wave) increases relatively more than filling during atrial contraction (A-wave).

**Afterload**

Afterload is the load that the cardiac ventricles must overcome to eject blood. It is most easily thought of as arterial blood pressure, even though the terms are not synonymous\textsuperscript{12}. When afterload increases, wall stress, which is the tension on the individual myocardial fibers\textsuperscript{41}, increases and the heart will react with diminished contraction and less blood will be ejected, thus, decreasing stroke volume\textsuperscript{42}. Fetuses are sensitive to even small increases in afterload, resulting in diminished stroke volume as indicated by observations in human fetuses and newborns as well as in studies on fetal sheep and muscle preparations\textsuperscript{19, 43-46}. Importantly however, preload and afterload are interlinked, i.e. increased ventricular volume due to filling leads to increased systolic performance, which will increase systolic blood pressure and, consequently, increase afterload\textsuperscript{12}. In the fetal heart, the afterload of the right ventricle is largely determined by resistance in placental vessels, and that of the left ventricle by resistance in the circulation of the brain and upper body\textsuperscript{23}.

Importantly, the development of the ventricles is influenced by afterload and preload, as well as subsequent distributional changes, with consequences different to the adult situation. For instance, increased afterload due to an aortic stenosis will lead to a shift in blood volume to
the right ventricle through the foramen ovale, decreasing the filling/preload of the left ventricle, with left ventricular hypoplasia as a possible result. The right ventricle, on the other hand, will need to handle an increased blood volume and preload, which will result in compensatory growth of the ventricle\textsuperscript{20}. Another example of fetal adaptation to loading conditions is the recipient in twin-to-twin transfusion syndrome (TTTS). Suffering from both increased preload and afterload, due to hypervolemia and increased systemic vasoconstriction, they will develop right ventricular outflow obstruction due to excessive hypertrophy. This illustrates the adaptability of fetal cardiovascular development, and the close relationship between cardiac form and function\textsuperscript{47, 48}.

**Contractility**

Contractility is the innate ability of the heart muscle to contract independently of changes in preload, afterload or heart rate\textsuperscript{12}. This can be altered by the sympathetic nervous system releasing noradrenaline to increase fiber contractility\textsuperscript{47}. These changes seem to be mediated by alterations in calcium levels within the cell. The fetal heart has reduced inherent contractility as it has less contractile elements, the sarcoplasmic reticulum is immature and sympathetic innervation is functionally immature\textsuperscript{8, 19, 42}.

**Heart rate**

The normal fetal heart rate lies between 110-160 beats/minute. Within the normal range, a relatively constant cardiac output is maintained, as the stroke volume compensates for changes in heart rate. At very high fetal heart rates there could be negative consequences, as diastole shortens more than the systole/time for ejection, which leaves less time for ventricular filling\textsuperscript{42}.

**The cardiac cycle**

For the heart to function optimally, i.e. to achieve contraction, relaxation and filling, the temporal sequence of events of the cardiac cycle needs to be well synchronized. The cardiac cycle is often displayed as a Wiggers diagram (Figure 3-1). The phases are described as\textsuperscript{12}:

- Isovolumic contraction
- Ventricular ejection
- Isovolumic relaxation
- Rapid/early ventricular filling
- Slow ventricular filling/diastasis
- Atrial contraction

The Wiggers diagram describes the cycle where ventricular pressure builds up as calcium triggers the contractile proteins/contraction. When the pressure in the ventricle exceeds that in the atrium, the atrioventricular valves close. Isovolumic contraction is the period where both inflow and outflow valves are closed. There is normally no change in volume but still a continued development of pressure. When the intraventricular pressure exceeds that in the
aorta/pulmonary trunk, the semilunar valves open and *ventricular ejection* starts. During the early part of ventricular ejection, there is rapid expulsion of blood, whereas in the later part of ejection the cardiomyocyte calcium concentration starts to decline, and ventricular ejection gradually diminishes but is maintained by arterial (aortic) compliance and recoil, a phenomenon called the Windkessel effect. When pressure in the aorta and pulmonary artery exceeds intraventricular pressure, the semilunar valves close and *isovolumic relaxation* starts. During isovolumic relaxation, there is normally no change in ventricular volume, but relaxation continues and pressure diminishes. When ventricular pressure drops below the pressure in the atrium, the atrioventricular valves open and ventricular filling begins. The first phase is *rapid/early ventricular filling*, followed by *slow ventricular filling/diastasis* and, finally, *atrial contraction*\(^{12}\). In the adult heart, the rapid filling accounts for most of the ventricular filling, in contrast to the fetus where the atrial contraction is the most important component, even though a gradual increase of the rapid ventricular filling component is seen with advancing gestation. Slow ventricular filling is often very short or non-existent in the fetus, especially in early gestation with higher fetal heart rates.

![Figure 3-1. A simplified Wiggers diagram as observed in the adult. IVC, isovolumic contraction; IVR, isovolumic relaxation.](image)

The cardiac cycle is often divided into systole and diastole, and this could be achieved in two different ways. Clinical *cardiologic systole* starts with the first heart sound, i.e. the closure of the mitral and tricuspid valves, and ends with the second heart sound, i.e. the closure of the aortic and pulmonary valves. *Physiologic systole* starts with isovolumic contraction and ends at the peak of ejection, where intraventricular pressure starts to fall. Consequently, *physiologic diastole* starts when relaxation dominates over contraction, and calcium in the
myocyte are taken up by the sarcoplasmic reticulum\textsuperscript{12}, or in the fetus more likely cleared by passage through the cell membrane\textsuperscript{8}. In this thesis, systole is considered to include the isovolumic contraction time (ICT) and ejection time (ET), whereas diastole includes the isovolumic relaxation time (IRT) and all phases of ventricular filling.

### 3.2 CARDIAC WALL MECHANICS, MOTION AND DEFORMATION

There are some key concepts that are important to understand when discussing movement of the ventricular myocardial walls and how this could potentially be assessed by different imaging technologies.

The fibers in the different cardiac walls, i.e. the left ventricular (LV) and right ventricular (RV) walls and interventricular septum (IVS), are organized and structured differently. Although the exact structure is sometimes debated, most studies describe the myocardial structure in the LV as composed of obliquely arranged fibers in superficial layers, longitudinal (apex to base) fibers in the subendocardium and predominantly circular fibers in the middle part. In the RV wall, longitudinal and circumferential fibers pre-dominate\textsuperscript{49}. The IVS contains the subendocardial fibers from both ventricles and circular fibers from the LV wall\textsuperscript{50-52}. The orientation of fibers affects the movement of the myocardial wall and, consequently, to what degree different imaging technologies can assess this.

If all points within an object (such as a myocardial region) move with the same velocities, the object is described as having motion. If different points within the same object move with different velocities, the object alters its shape, i.e. it undergoes deformation. Myocardial motion is defined as the distance one specific point in the myocardium moves during a certain period of time and is defined by velocity and displacement, i.e. the distance covered. Myocardial deformation describes how two points move in relation to each other and is described as the change in length/thickness in percent of a segment (strain) and the velocity of change (strain rate)\textsuperscript{53, 54}. Deformation can be expressed as global or regional. There are two ways of describing the deformation, i.e. as Lagrangian or natural (Eulerian) strain. In Lagrangian strain deformation is expressed relative to the original length, whereas natural (Eulerian) strain uses the instantaneous length, and is expressed relative to the length at a previous instant\textsuperscript{55}. For example, considering deformation (Lagrangian strain) in a one-dimensional object such as a bar, a shortening to half its original length would mean a strain of -50% and a lengthening to double its length a strain of +100%\textsuperscript{56}. The deformation of a myocardial segment is complex, as the heart can perform longitudinal, radial and circumferential motion with lengthening/shortening or thickening/thinning possible in all these directions. Longitudinal motion is achieved by longitudinal fibers that run from base to apex. These are mainly located in the subendocardium and are the longest distance away from the epicardial blood supply and, consequently, the most susceptible to milder degrees of hypoxia\textsuperscript{57}. Radial motion is perpendicular to the epicardium and achieved by fibers in the middle of the wall, which can be affected in later stages of compromise. Circumferential
motion is perpendicular to both the other directions and takes part in rotation and twisting of the myocardial wall\textsuperscript{54}.

Cardiomyocyte contraction, and thus shortening, is the most important factor influencing deformation. However, other “external” factors also influence regional deformation. These include cavity pressure with different loading conditions, tissue elasticity and interaction between segments, i.e. the influence by other contracting segments of the wall\textsuperscript{53}.

3.3 THE FETAL HEART AS A PUMP

The mechanical functioning of the heart has previously been described mainly as a squeezing motion, but in recent years also as a mechanical pump controlled by its inflow according to the dynamic adaptive piston pump (DAPP) hypothesis proposed by Lundbäck in 1986\textsuperscript{58}. According to the DAPP hypothesis, the mechanical functioning of the heart is initiated by the movement of the AV-piston, which corresponds to the atrioventricular (AV) plane that creates an inflow controlled hydraulic return\textsuperscript{59-61}. Furthermore, it states that the IVS regulates the LV and RV stroke volumes and that the heart works with a rather constant outer volume, which has been demonstrated through MRI studies in adults\textsuperscript{62}. The DAPP hypothesis is the background to the method of analysis and automated algorithm investigated in this thesis.

3.4 FETAL HEART DYSFUNCTION AND FAILURE

Heart failure is defined as “a complex clinical syndrome that results from any structural or functional impairment of ventricular filling or ejection of blood”\textsuperscript{63}. Thus, it is a situation where the heart is unable to supply the tissues with sufficient blood to meet their needs. Overt fetal heart failure is, generally, a late event that has a typical appearance with increased cardiac size, increased systemic venous pressure, as displayed by changes in the flow pattern in precordial veins, atrioventricular valve insufficiency and abnormal movement of the myocardial walls. The end-stage of fetal cardiac failure manifests itself as hydrops with ascites, subcutaneous edema, pleural and pericardial effusions. This condition carries a high risk of mortality and morbidity\textsuperscript{64}. Severe degrees of cardiac failure are easily recognizable as opposed to the subtle signs of cardiac dysfunction that might precede them.

Cardiac dysfunction can be classified as either systolic or diastolic depending on what part of the cardiac cycle is affected. More discrete signs of dysfunction are likely to affect diastolic function first, such as prolongation of the isovolumic relaxation time (IRT). Another factor that might be detected at an early stage is impaired longitudinal function of the heart that might affect longitudinal motion, i.e. velocities and displacement. Moreover, early signs of fetal cardiac dysfunction are often visualized in right heart dynamics, such as regurgitation of the tricuspid valve and increased size of the right atrium as seen in arteriovenous fistulas due to increased volume load\textsuperscript{64, 65}.
During the subclinical period of cardiac dysfunction, when the fetal heart is trying to adapt, a process described as cardiac/ventricular remodeling could occur. Remodeling is the concept where structural changes occur in response to chronically altered loading conditions\textsuperscript{66}, e.g. the more globular hearts described in babies suffering from IUGR \textit{in utero}\textsuperscript{54}.

### 4 FETAL ECHOCARDIOGRAPHY

Fetal echocardiography is the main tool available for assessing the fetal heart \textit{in utero}. Since the advent of fetal echocardiography in the 1980s, the main focus has been on detecting structural defects by two-dimensional (2D) B-mode ultrasound using different examining planes\textsuperscript{67}. Initially mainly the four-chamber view was evaluated, but now additional ultrasound planes in a “five-transverse view” protocol describing the abdominal situs, the outflow tracts, the three-vessel and trachea view have been incorporated in the screening. Gradually other modalities, such as color and spectral Doppler imaging of blood flow and 3D/4D ultrasound, as well as recently developed new electronic probes, were introduced, and have the potential to improve and complement assessment\textsuperscript{68}. Today, many major congenital heart defects, e.g. hypoplastic left heart syndrome, are detected prenatally, contributing to improved perinatal outcomes, whereas some defects, such as coarctation of the aorta, often remain undetected prenatally\textsuperscript{69, 70}. There is an increasing interest in extending fetal echocardiography to functional assessment of the fetal heart, where new emerging imaging techniques, such as TDI and speckle tracking, show a potential to contribute with additional information.

### 5 ASSESSMENT OF FETAL CARDIAC FUNCTION

According to a Scientific Statement from the American Heart Association in 2014, assessment of fetal cardiac function is an important part of the fetal echocardiogram, even though there is no clear consensus regarding to what extent it should be performed and whether it should be qualitative or quantitative\textsuperscript{71}.

Different ultrasound modalities, and recently also magnetic resonance imaging, have been proposed to assess fetal cardiac function. Different parameters have been attempted to quantitatively evaluate different aspects of function. Traditionally, they involved evaluating cardiac dimensions by M-mode and 2D ultrasound, venous blood flow, timing of events of the cardiac cycle by measuring duration of time intervals, estimation of stroke volume and cardiac output using conventional Doppler, as well as shortening fraction (SF) and AV-plane displacement by M-mode. Recent developments have also enabled assessment of myocardial motion and deformation, through techniques such as TDI and speckle tracking that allow for measurement of strain and strain rate\textsuperscript{47, 72}.

However, few novel techniques have progressed from the research setting into routine clinical practice, and the main parameters and techniques used today are still the assessment of
venous flow, AV inflow and cardiac time intervals to calculate myocardial performance index (MPI) using conventional Doppler and AV-plane displacement measured by M-mode\textsuperscript{54}.

Most parameters used for the assessment of fetal cardiac function were originally intended for use in children and adults, and have been adapted for use in fetuses\textsuperscript{47}. Many of them have not been validated in fetuses. Moreover, in contrast to structural defects detected before birth, functional alterations may be difficult to ascertain after birth as the transition from fetal to neonatal circulation changes cardiac demands quite dramatically. There are also limitations to these techniques due to small size of the fetal heart, high heart rate, fetal position and movements, as well as restricted access via the maternal abdomen. All measurements need to be related to fetal size and gestational age, as cardiac size and maturation varies throughout gestation. Myocardial maturation and differing preload and afterload, due to distribution in the fetal circulation, might alter response to insults compared to adults and mask differences in pressure, as well as symptoms and signs that would be obvious postnatally. All these factors need to be considered when evaluating results of functional assessment of the fetal heart\textsuperscript{54, 72}.

Some important ultrasound techniques, parameters and examples of their use in fetuses will be described below with a focus on the use of TDI. Parameters and techniques are summarized in Table 5-1.

\subsection*{5.1 M-MODE}

M-mode, or motion-mode, ultrasound describes motion along one single scanning line with high temporal resolution. The scanning line that is represented by the cursor is usually positioned using 2D ultrasound but is sometimes used in combination with spatiotemporal imaging (STIC)\textsuperscript{73}. M-mode can also be used with color Doppler\textsuperscript{74}. M-mode can be performed on-line (real-time M-mode) or off-line (anatomical M-mode)\textsuperscript{72}, where the latter results in substantially lower resolution. A variant of M-mode with adjustable angle also exists\textsuperscript{76}.

A \textit{lateral four-chamber view} of the fetal heart with the cursor placed perpendicular to the septum at the tip of the mitral and tricuspid valves can be used to identify end-systole and end-diastole. This allows for measurement and calculation of\textsuperscript{77, 78}:

- Cardiac dimensions.
- Shortening fraction (SF), i.e. the change of ventricular inner diameter between the end of diastole and the end of systole expressed as a ratio.
- Ejection fraction (EF), i.e. the percentage of total blood volume in the ventricle ejected during one heartbeat.
An apical or basal four-chamber view, where the position of the cursor is placed in the annulus at right angle to the AV-plane can be used to assess AV-plane displacement and the following measurements can be obtained:

- Mitral annular plane systolic excursion (MAPSE).
- Tricuspid annular plane systolic excursion (TAPSE).
- Septum annular plane systolic excursion (SAPSE).

An apical or basal four-chamber view, where the position of the M-mode cursor is adjusted to pass through the atrial and ventricular walls to record their motion simultaneously can be used to assess:

- Fetal arrhythmias by evaluating atrial and ventricular rate and rhythm\textsuperscript{73}.

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### Table 5-1. Parameters and techniques commonly used to assess fetal cardiac function

<table>
<thead>
<tr>
<th>Parameters</th>
<th>B-mode/2D</th>
<th>M-mode</th>
<th>Conventional spectral Doppler</th>
<th>3D/4D</th>
<th>Speckle tracking</th>
<th>Spectral TDI</th>
<th>Color TDI</th>
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</thead>
<tbody>
<tr>
<td><strong>Systolic function</strong></td>
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<tr>
<td>Stroke volume (SV)/cardiac output (CO)</td>
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<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Ejection fraction (EF)</td>
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<td>X</td>
<td>X</td>
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<tr>
<td>Shortening fraction (SF)</td>
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<tr>
<td>Peak myocardial systolic velocity (S'/Sm)</td>
<td>X</td>
<td>X</td>
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<td>Strain (SI)</td>
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<td>Strain rate (SR)</td>
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<td><strong>Diastolic function</strong></td>
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<tr>
<td>E/A ratio</td>
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<td>X</td>
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<tr>
<td>E/E' ratio</td>
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<td>X</td>
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<tr>
<td>IRT/ post-ejection time</td>
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<td>X</td>
<td>X</td>
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<tr>
<td>Peak myocardial diastolic velocity (E', Em, A', Am)</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Ductus venosus PIV</td>
<td>X</td>
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<td><strong>Global function</strong></td>
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<tr>
<td>Myocardial performance index (MPI, MPI' or cMPI)</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>AV-plane displacement (AVPD)</td>
<td>X</td>
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<td>X</td>
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</tbody>
</table>

AV-plane, atrioventricular plane; TDI, tissue Doppler imaging; IRT, isovolumic relaxation time; PIV, pulsatility index for veins.

\textsuperscript{73} Fetal arrhythmias by evaluating atrial and ventricular rate and rhythm.
Normal values describing *cardiac dimensions* including chamber size, thickness of the ventricular walls and the septum have been presented in fetuses showing increasing dimensions throughout gestation\(^79,\ 80\), with RV end-diastolic being larger than LV end-diastolic dimensions\(^81\). According to Huhta *et al.*, there is often an increase in diastolic dimensions with impaired cardiac function\(^82\).

**Shortening fraction (SF)** is measured as the end-diastolic dimension minus end-systolic dimension divided by end-diastolic dimension, i.e. how much the ventricles shorten their inner-diameters in systole compared to diastole\(^64,\ 82\). SF is considered a surrogate of ventricular function\(^73,\ 83\). It is reported to be constant throughout gestation\(^79\), but a decrease in RV SF with advancing gestation has also been noted\(^84\). LV SF has been observed to increase in donor twins in TTTS\(^85\). An increase has also been noted in the RV SF in late gestation in fetuses of diabetic mothers\(^86\). A decrease in SF in both ventricles after intrauterine transfusion\(^87\) suggests decreased ventricular performance.

The *ejection fraction* (EF) is the percentage of the total amount of blood in the ventricle at the end of diastole that is being pumped out of the heart during one cardiac cycle. To determine EF, ventricular volumes are estimated from dimensions obtained by M-mode ultrasound, and the EF calculated through a formula. The drawbacks are that inaccuracies in M-mode measurements are increased by extrapolating ventricular volumes through a formula\(^77\). EF is considered an important parameter in classification of adult patients with heart disease. It is considered a measure of systolic function, but adults can have heart failure with or without reduced EF\(^63\). In fetuses, this variable is not often used and generally not recommended\(^71\).

Both *mitral* and *tricuspid annular plane excursion* (MAPSE and TAPSE) measure annular displacement, which is considered to reflect longitudinal function\(^37,\ 76,\ 88\). These variables are measured from end-diastole to end-systole describing movement of the AV-plane throughout the cardiac cycle\(^4,\ 49\). MAPSE and TAPSE have been used to evaluate ventricular function in various situations in both pediatric and adult cardiology\(^89,\ 90\). A reduced MAPSE reflects impaired longitudinal function and could be used as a sensitive early marker of LV systolic dysfunction in adult patients with hypertension and normal EF. It is also associated with poor prognosis in patients after myocardial infarction or with heart failure\(^91\). In adults, decreased TAPSE, as a sign of RV dysfunction, is an independent risk factor for mortality in patients with heart failure, independent of LV function\(^92\). Measurement of MAPSE and TAPSE is slightly different in adults, as an ECG can be used to more clearly delineate systole. In fetuses, a linear increase has been observed during gestation with higher values for TAPSE, followed by MAPSE and SAPSE\(^37,\ 75\). A decrease in both MAPSE and TAPSE has been observed in both donor and recipient twins in TTTS, suggesting decreased longitudinal motion\(^93\). Cruz-Lemini *et al.* also demonstrated significantly decreased values at all locations compared to controls in a group of fetuses with IUGR\(^4\).
5.2 B-MODE/2D

B-mode/2D ultrasound is used in functional assessment to measure cardiac dimensions and assess adverse signs of fetal compromise, such as the presence of hydrops. Commonly used measurements are:

- Cardiothoracic ratio (C/T ratio).
- Dimensions of various cardiac structures such as ventricular chambers, atria, myocardial walls and valves.

_Cardiothoracic ratio (C/T ratio)_ is performed in a transverse section of the fetal thorax at the level of the four-chamber view. Measurement is performed in diastole and the circumference or area of the heart and the thorax is expressed as a ratio. Increased C/T ratio is a sign of cardiomegaly considered to be an important sign of altered cardiac function and has been observed in fetuses with hydrops and various cardiac malformations\textsuperscript{64, 71, 94}.

_Dimensions of various cardiac structures_ can be measured to assess length, width, circumference and volumes as well as calculate cardiac output and ejection fraction. However, these are not regularly used in clinical practice\textsuperscript{77, 78}. Measurement of the diameter of the aortic and pulmonary valves can be measured during systole\textsuperscript{95} and the cross-sectional area calculated assuming a circular orifice. These measurements can be used in the estimation of stroke volume and cardiac output\textsuperscript{77} that will be further discussed below.

5.3 CONVENTIONAL COLOR DOPPLER ULTRASOUND

Color Doppler ultrasound superimposes blood flow information on the 2D image. Color Doppler can be used to evaluate the presence of blood flow and abnormalities, such as valve regurgitation, but also the direction of blood flow in the heart and in different fetal vessels\textsuperscript{71, 96}. It is usually not used for velocity measurements but is mainly used to selectively visualize vessels and to identify places (e.g. cardiac valves) with increased blood flow velocities, to allow for further investigation and measurements with spectral Doppler.

5.4 CONVENTIONAL SPECTRAL DOPPLER ULTRASOUND

Spectral Doppler ultrasound translates changes in the frequency of emitted and reflected ultrasound beams into velocities displayed as waveforms over time. This allows qualitative assessment of the waveform and quantification of blood flow dynamics by calculating velocities and different indices. Conventional spectral Doppler ultrasound is used to assess velocities across valves to verify and characterize valvular regurgitation and stenosis, to calculate the ratio between ventricular inflow during early and late diastole, to describe waveforms, measure velocities and calculate indices in different fetal blood vessels\textsuperscript{71}.
Some commonly used parameters to describe cardiac function are:

- E/A ratio, i.e. the ratio between ventricular inflow during early and late diastole.
- Pulsatility index and waveform analysis in veins.
- Myocardial performance index (MPI).
- Duration of individual cardiac time intervals.
- Stroke volume and cardiac output.

The **E/A ratio** represents the ratio between ventricular inflow during early and late diastole. The spectral Doppler trace describing the E- and A-waves is obtained in a four-chamber view by placing the Doppler gate just below the AV-valves. The first part is the E-wave that represents the early, diastolic filling that is largely dependent on ventricular relaxation. The second part is the A-wave, which corresponds to the active part of ventricular filling that is often referred to as “atrial contraction/atrial kick”. This is when the AV-plane moves a bit further towards the base finalizing the ventricular filling before closure of the AV-valves. Inflow to the ventricles through the AV-valves is normally characterized as biphasic, and in a healthy fetus the A-wave is normally greater than the E-wave\(^9\) (Figure 5-1). The E-wave increases relatively more than the A-wave throughout pregnancy, which is considered to be the result mainly of improved ventricular relaxation. Consequently, the E/A ratio is low in the beginning and increases gradually throughout pregnancy, but usually do not exceed one in normal fetuses\(^8\).

![Figure 5-1. Spectral Doppler recording of ventricular inflow, demonstrating inflow during early diastole (E) and atrial contraction (A).](image)

In adults, a decreased E/A ratio is considered a sign of diastolic dysfunction\(^4\). In fetuses, a decreased E/A ratio has been observed in the recipient twin in TTTS\(^9\). An increase has also been observed in certain complications, such as IUGR\(^2\). In some situations, with more severe myocardial hypertrophy and disturbed diastolic filling, a monophasic inflow is observed and this can be associated with severe cardiac compromise or disease, such as in aortic stenosis and TTTS. However, it is important to distinguish this from monophasic inflow due to the fusion of the E- and A- waves sometimes seen with high heart rate\(^4\).
Pulsatility index and waveform analysis in veins is often evaluated in vessels connected to the right atrium as their blood flow reflects the atrial or central venous pressure. Low atrial pressures favor forward flow and higher atrial pressures, such as during atrial contraction, and result in decreased forward flow, and sometimes absent or reversed flow. The most commonly used vessel is the ductus venosus (DV), where the pulsatile flow seen in spectral Doppler recordings reflects changes in atrial pressure during the cardiac cycle. The DV blood flow velocity waveform is usually assessed by calculating the pulsatility index for veins (PIV), and by visually analyzing and describing the waveform during atrial contraction (the a-wave) as present, absent or reversed. The DV normally displays a forward flow throughout the entire cardiac cycle at any gestational age, and the PIV gradually declines. The PIV and the a-wave are used to monitor and time delivery in severely ill, often preterm fetuses. An increased PIV and, eventually, an absent or a reversed flow in the a-wave, may indicate hypoxemia, acidosis and/or cardiac dysfunction. At the cardiac level, a DV velocity profile with abnormal flow during atrial contraction indicates that atrial and ventricular filling pressure are higher than normal. Different diseases might primarily affect cardiac preload, afterload, contractility or diastolic relaxation, finally resulting in decreased diastolic function and increased venous pressure.

The umbilical vein (UV) could also be used in the assessment of venous flow. After the first trimester it is normally protected from changes in atrial pressure and has a constant, non-pulsatile blood flow. A pulsatile UV blood flow with double venous pulsations is an ominous sign that is related to increased risk of mortality in high risk pregnancies.

Myocardial performance index (MPI) is an index based on the duration of cardiac cycle time intervals. MPI is obtained by simultaneously recording inflow and outflow blood flow velocity waveforms at the level of the four-chamber view, where the Doppler gate is placed to include both the aortic and the mitral valve. Development of measurement techniques have led to a focus on recording valve clicks, with the aim of clear visualization. MPI is calculated as the sum of the isovolumic contraction (ICT) and relaxation time (IRT) divided by ejection time (ET), and considered to reflect global cardiac function. MPI obtained from spectral Doppler recordings in fetuses, is predominantly used for the LV, as simultaneous recording of inflow and outflow velocities from the same cardiac cycle is not possible on the right side due to the anatomical configuration.

The myocardial performance index was first introduced in adults to assess cardiac function in patients with cardiomyopathy and called the Tei index. In fetuses, it was first reported in 1999. Most investigators report it to be relatively constant throughout pregnancy, whereas others report a decrease or an increase with gestation. Recently, an automated analysis to reduce variation has been proposed and is being evaluated. In fetuses, MPI has been validated by demonstrating that it correlates well with EF. An increased MPI has been shown in recipient twins in TTTS, fetuses with α-thalassemia, in hydrops and large for gestational age fetuses of diabetic mothers. Prolongation of MPI can be attributed to prolongation of IRT, which is considered a sign of diastolic dysfunction. A shortening of
MPI, due to shortening of ICT and prolongation of ET, has also been shown in anemic fetuses, suggesting enhanced systolic myocardial performance\textsuperscript{113}.

Assessment of the duration of individual cardiac time intervals can be performed using spectral Doppler ultrasound where the most common ones are the ICT, ET and IRT included in the MPI. However, time intervals could also be evaluated individually\textsuperscript{114} where prolonged IRT in the LV has been suggested as a sign of diastolic dysfunction in fetuses with cardiomyopathies\textsuperscript{115}.

The ventricular filling during diastole, i.e. the time from the opening of the mitral/tricuspid valve to its closure, has also been suggested for assessing diastolic function in accordance with a monophasic inflow indicating dysfunction. Inflow duration as a percentage of cycle length on the right side has been observed to be shortened, and to precede the development of overt cardiomyopathy, in recipient twins in TTTS\textsuperscript{114, 116, 117}.

Stroke volume and cardiac output can be calculated by combining the evaluation of ventricular outflow, fetal heart rate assessed by spectral Doppler, and the dimension of the outflow valves measured by 2D ultrasound. Cardiac output is calculated as stroke volume $\times$ heart rate. Stroke volume is defined as the amount of blood that flows out of the heart during each heartbeat. Measurement of ventricular volume is cumbersome in the fetus. Therefore, stroke volume is usually estimated and calculated by multiplying the velocity time integral (VTI) of the blood flow across the semilunar valves and the cross-sectional area of the valves. VTI is measured from the spectral Doppler recording by tracing the outflow velocity profile and integrating the area underneath as an estimation of the blood flow across the valve. The

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure5-2.png}
\caption{The upper trace demonstrates a spectral Doppler recording for the measurement of myocardial performance index (MPI) with focus on valve clicks. The lower trace defines the delineating valve clicks and time intervals. 1, mitral valve opening; 2, mitral valve closure; 3, aortic valve opening; 4, aortic valve closure; ICT, isovolumic contraction time; ET, ejection time; IRT, isovolumic relaxation time. Modified from Cruz-Martinez et al. 2012, with permission from S. Karger AG, Basel.}
\end{figure}
cross-sectional area of the valve is calculated using the diameter measurement, obtained by 2D ultrasound, as: \( \pi \left( \frac{\text{diameter}}{2} \right)^2 \). It is important to bear in mind that there is a substantial risk of error when calculating the valve area from the diameter as the measurement is squared\(^47, 77\). Cardiac output is often expressed as the sum of the LV and RV cardiac output, i.e. combined cardiac output, that increases during gestation as mentioned earlier\(^118\).

5.5 3D/4D ULTRASOUND

The most basic form of 3D/4D ultrasound is an acquisition of a static volume by an automated two-dimensional sweep of the fetal heart. 3D/4D spatiotemporal imaging correlation (STIC), however, adds temporal and spatial information, which results in the creation of a reconstructed cardiac cycle with high resolution. This may also be combined with other imaging modalities, such as color or power Doppler\(^71\). The technique is dependent on optimal fetal position and a minimum of fetal movements to produce good results. It could be used to complement anatomical assessment and has been attempted for calculations of volumes to calculate EF and CO\(^47, 77\).

5.6 SPECKLE TRACKING

Speckle tracking is an approach used to assess myocardial motion and deformation. This technique identifies acoustic markers, i.e. speckles, within the 2D image that are tracked between image frames. When the frame rate is known, the change in speckle position allows for calculation of velocity and displacement data, as well as deformation (strain and strain rate)\(^53-55\).

In fetuses, a four-chamber view is obtained. As the full thickness of the LV and RV walls and the IVS should be visualized, it is preferable to obtain a slightly angled four-chamber view\(^9\). Consecutive cardiac cycles are recorded and transferred to separate software. Different systems and manufacturers use different algorithms for tracking the speckles\(^71, 119, 120\). The user then draws a tracking line that defines the endocardial border and, in the absence of an ECG, the cardiac cycle is determined, for instance, through mechanical movement using valve movement or anatomical M-mode\(^120\). Speckle tracking does not use Doppler and is relatively angle independent, but is still dependent on an adequate frame rate, which is even more important in the fetus due to high heart rate\(^119\). The method can give information on both global and segmental strain. Mainly longitudinal and circumferential motion and deformation is assessed\(^53\).

True intrinsic contractility cannot be measured \textit{in vivo}, but strain and strain rate could be considered as surrogates in clinical practice\(^121, 122\). In adults, they have been demonstrated to be sensitive markers of cardiac dysfunction\(^1, 53, 123\). Assessment of strain using speckle tracking (2D strain) has been attempted in fetuses, but disagreement exists regarding
gestational age-related changes in myocardial strain where increased, decreased and constant values have been reported\textsuperscript{71, 120, 124}. Lower RV strain values in TTTS recipients\textsuperscript{125} and decreased global strain in fetuses with hypoplastic left heart syndrome compared to controls have been observed\textsuperscript{126}.

5.7 TISSUE DOPPLER IMAGING (TDI)

TDI is an ultrasound technique that gives quantitative, non-invasive assessment of myocardial motion and mechanics by measuring tissue velocities. Tissue velocities are lower but have higher amplitude compared to blood flow velocities\textsuperscript{127, 128}.

There are two main echocardiographic TDI modalities, i.e. spectral TDI (sTDI) and color TDI (cTDI). In both techniques, the location most commonly used for measurement in fetuses is in the myocardium at the level of the AV-plane in a four-chamber view to assess longitudinal motion, which might be affected in early stages of hypoxia and dysfunction. The apex is normally stationary within the thorax, and with contraction the base of the heart moves towards the apex. As a result, the velocities obtained from the level of the AV-plane are considered to reflect the movement of the entire wall and, thus, a measurement of global longitudinal cardiac function. Declining velocities are seen approaching the apex. The regions of interest (ROIs) or sample volumes can be placed in the IVS, the LV and RV walls. As the measurement of velocities with Doppler techniques is angle dependent, it is very important that the angle of insonation is as small as possible. With both sTDI and cTDI, it is possible to measure myocardial velocities and cardiac time intervals\textsuperscript{54, 128}. The common velocities measured are:

- Peak myocardial velocity during atrial contraction ($A'/Am$).
- Peak myocardial velocity during ventricular ejection/systole ($S'/Sm$).
- Peak myocardial velocity during rapid ventricular filling/early diastole ($E'/Em$).

There are several ways of denoting myocardial velocities in the literature. In this thesis, myocardial velocities obtained by sTDI are noted as $A'$, $S'$ and $E'$, whereas velocities obtained with cTDI are noted $Am$, $Sm$ and $Em$. In adult echocardiography, lower case letters ($a'$, $s'$ and $e'$) are often used\textsuperscript{129}. In addition, myocardial velocities are sometimes measured during the isovolumic phases, i.e. isovolumic contraction velocity (IVCV) and isovolumic relaxation velocity (IVRV)\textsuperscript{130, 131}. Examples of sTDI and cTDI are demonstrated in Figure 5-3. The $Em/Am$ ratio and the ratio between the E-wave assessed using blood flow velocity and E-wave assessed by TDI ($E/E'$ or $E/Em$) can also be calculated.

The cardiac time intervals evaluated by TDI are the same as those measured using blood flow velocity waveforms, but they are based on myocardial mechanical events and may not exactly correspond to hemodynamic events. In this thesis, the isovolumic phases when using cTDI with automated analysis are called pre- and post-ejection phases. MPI derived from TDI (MPI' or cMPI) can be calculated from the same cardiac cycle in the IVS, LV or RV wall.
Figure 5-3. sTDI and cTDI in the fetus. **A.** Demonstration of sTDI in the right ventricular wall with the sample volume at the atrioventricular plane and myocardial velocity trace obtained on-line. A narrow sector width is used for better resolution. **B.** Demonstration of cTDI with the ROIs positioned off-line in the interventricular septum and the right and left ventricular walls at the level of the atrioventricular plane. Myocardial velocity trace from the left ventricular wall is demonstrated. E' and Em, peak myocardial velocity during rapid ventricular filling/early diastole; A' and Am, peak myocardial velocity during atrial contraction; S' and Sm, peak myocardial velocity during ventricular ejection/systole.
The assessment of myocardial velocities is affected by tethering, i.e. the passive motion produced by adjacent cardiac segments, and translational movement that relates to other types of movement that can be superimposed on the measurement, such as maternal or fetal body movements\textsuperscript{1, 56}. Further limitations are that high frame rates are required to detect temporal events in the cardiac cycle of short duration, such as velocity changes during the isovolumic or pre- and post-ejection phases\textsuperscript{54, 128, 132}.

In adults, TDI has been used both in the assessment of systolic and diastolic cardiac function. It facilitates the detection of subclinical dysfunction in various conditions, such as diabetes, hypertrophic cardiomyopathy and valvular disease. Furthermore, it helps in predicting the prognosis of major cardiac diseases, such as acute myocardial infarction\textsuperscript{1, 133-135}. The E' and E/E' ratio have been shown to relate to abnormal relaxation (diastolic dysfunction) and filling pressures in the LV\textsuperscript{136, 137}, and the latter is a predictor of long-term cardiovascular outcome, heart failure and mortality\textsuperscript{1}. TDI has been proven feasible in human fetuses\textsuperscript{3, 128, 138}, and it has been tried for evaluation of fetal cardiac function in various pregnancy complications, such as IUGR\textsuperscript{5, 139}, TTTS\textsuperscript{114} and gestational diabetes\textsuperscript{140}. Further details about the separate modalities are provided below.

\subsection{Spectral TDI (sTDI)}

sTDI instantaneously registers the frequency shifts between the emitted and returning ultrasound signal at a chosen location in the myocardium. A mathematical process called Fast Fourier transformation is used to analyze the reflected ultrasound and displays the distribution of myocardial velocities as a function of time. sTDI is generally considered to display peak velocities, but actually a spectrum is obtained, and its width is influenced by the gain settings used\textsuperscript{141}. sTDI is an on-line method, where only one location in the myocardium can be assessed at a time and there are no post-processing possibilities.

In fetuses, studies have demonstrated increasing myocardial velocities with advancing gestational age from late second trimester to term in normal pregnancies\textsuperscript{3, 37, 127, 142, 143}. MPI' has also been evaluated, and an increase in the LV MPI' throughout gestation has been reported\textsuperscript{143}. sTDI seems feasible and reproducible in the fetus\textsuperscript{128}.

In IUGR fetuses, significantly lower systolic and diastolic myocardial velocities have been demonstrated, as well as increased MPI' values, suggestive of cardiac dysfunction\textsuperscript{4, 5}. Increased values of E'/A' ratios have also been seen in the LV wall and IVS in IUGR fetuses compared to normal. No or few differences were found in the corresponding blood flow variables, indicating that TDI might be more sensitive for detection of fetal cardiac dysfunction\textsuperscript{5, 144}. The use of MPI' has also been attempted in monochorionic twins, where LV MPI' was found to be significantly higher in twins that subsequently developed into TTTS recipients\textsuperscript{110}, also indicating the detection of subclinical dysfunction. sTDI has also been attempted to assess fetuses before and after fetoscopic laser ablation of placental vessels in TTTS\textsuperscript{145}. Furthermore, differences in systolic and diastolic function between fetuses of diabetic mothers and normal controls have been visualized using sTDI\textsuperscript{146, 147}. 

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5.7.2 Color TDI (cTDI)

cTDI detects phase shifts and uses a complex method called autocorrelation to process the signals from the myocardial wall\textsuperscript{148}. Myocardial velocity information is obtained from several points along several scanning lines, as opposed to sTDI where one sample point is chosen. The velocity can then be displayed in real-time and, consequently, allows for post-processing off-line\textsuperscript{128, 141}.

Practically, cTDI is performed by acquiring an apical or basal four-chamber view of the fetal heart to assess longitudinal function. Cine-loops of consecutive cardiac cycles are recorded, and the data are transported to software for off-line analysis. There, ROIs are positioned, and myocardial velocity traces obtained for further analysis.

In addition to the evaluation of velocities and cardiac time intervals, cTDI also allows for calculation of displacement, which is the distance covered by the AV-plane. As multiple areas within the myocardium can be assessed simultaneously, parameters describing deformation, such as strain and strain rate, can also be obtained. Displacement, strain and strain rate are calculated by post-processing the velocity data off-line in separate software. It is important to understand that strain measurements obtained by cTDI and speckle tracking only show weak correlations, and that they are not interchangeable\textsuperscript{123}. As strain and strain rate involve measuring two/several points within the myocardium, it is not influenced by tethering and translational movement in the same way as velocity measurements. It is also important to understand that cTDI displays lower velocities than sTDI, as it registers the average (modal) velocities within the ROI\textsuperscript{56, 129} rather than maximum velocities.

There are fewer studies describing cTDI than sTDI throughout gestation in normal pregnancies. There is an increase in myocardial velocities throughout the second and third trimesters, as shown by cTDI, although absolute values are lower compared to those measured by sTDI\textsuperscript{131, 138, 149, 150}. Few studies have described cardiac cycle time intervals and MPI\textsuperscript{131, 151}. Decreased myocardial velocities in IUGR fetuses indicate the presence of systolic and diastolic dysfunction\textsuperscript{139, 152}. In a study by Larsen et al., a LV Sm < 1.6 cm/s seemed to predict perinatal mortality in IUGR fetuses with cerebroplacental redistribution\textsuperscript{139}. cTDI has also been attempted in fetuses with hypoplastic left heart syndrome, showing altered RV function with changes in myocardial velocities, cMPI and IRT already in utero\textsuperscript{153}. Furthermore, cTDI has been attempted in fetal arrhythmia\textsuperscript{154} and impaired RV diastolic and systolic function evaluated by myocardial velocities are suggested in fetuses with intra-amniotic infection\textsuperscript{155}. The role of measurements of myocardial velocities and time intervals obtained by cTDI in detecting subclinical cardiac dysfunction in various fetal conditions needs to be further investigated.

There are certain limitations of cTDI, including angle-dependency, but also the fact that separate software is needed for off-line analysis. The total process of analysis is, therefore, cumbersome and time-consuming. However, the advantage is the possibility of
simultaneously obtaining information on displacement and deformation parameters, as well as the possibility of automation of the analysis of velocity traces.

6 THE EFFECT OF HYPOXEMIA/ACIDEMIA ON FETAL CARDIAC FUNCTION

In many pregnancy complications affecting fetal well-being, hypoxemia/hypoxia and/or acidemia plays an important role. Hypoxia refers to a situation where oxygen supply is insufficient, leading to a low oxygen content in tissue, whereas hypoxemia is an abnormally low oxygen content in the blood. Acidemia is an increase in hydrogen ions in the blood. The concept of ischemia commonly used in adults is a situation with a reduced blood supply, but blood with normal oxygen content, that causes not only a lack of oxygen but also a lack of nutrients, as well as inadequate removal of metabolic waste products. The fetus grows under conditions with a low oxygen concentration compared to adults. The umbilical vein, which carries the most highly oxygenated blood, only reaches an oxygen saturation of approximately 85%, and after blending with systemic and pulmonary venous blood returning to the heart, the oxygen saturation drops. Thus, oxygen saturation in the fetal ascending aorta is only 65%. Further oxygen deprivation could be caused by placental insufficiency, anemia or high altitude, but also contractions during labor. Most fetuses tolerate these hypoxic challenges to some degree, whereas some are more sensitive. The main aim of methods assessing and monitoring fetal well-being is to identify fetuses that are unable to tolerate hypoxic challenges at an early stage. As TDI shows potential to identify cardiac dysfunction and impaired longitudinal function at an early stage, it might be a method that could add valuable information in the assessment of fetal well-being in situations with reduced oxygen supply.

The main energy substrates in the heart are glucose and lactate. When there is a lack of oxygen, cells need to switch to anaerobic metabolism, which is less efficient and generates less energy. With anaerobic metabolism there is a gradual build-up of lactate, which could lead to metabolic acidosis. Fortunately, the fetal heart is less vulnerable to hypoxia due to an enhanced capacity for glycolysis and, thus, anaerobic energy production. Animal studies also indicate that glycogen levels in the immature heart are substantial, which might contribute to increased tolerance to oxygen insufficiency. However, hypoxemia may alter expression of several genes, such as hypoxia inducible factor 1 (HIF-1), that could trigger cardiovascular remodeling and influence cardiovascular health in later life.

In children subjected to chronic hypoxia due to high altitude, there is evidence of RV diastolic dysfunction with decreased E’ velocity and E’/A’ ratio as well as prolonged IRT. Furthermore, these differences were only seen using TDI and not conventional Doppler assessing blood flow. This indicates that early signs of cardiac dysfunction might first appear in the myocardial wall. In adults, acute exposure to hypoxia due to high altitude altered RV
and LV diastolic function with prolonged IRTs as assessed by sTDI and prolonged RV MPI assessed using conventional Doppler evaluating blood flow pattern\textsuperscript{159}. This is in line with cardiac findings evaluated by sTDI after hypoxic breathing that showed a prolongation of RV IRT, but also an increase in LV systolic myocardial velocity\textsuperscript{160}. In adults with chronic exposure to high altitudes, there were also more pronounced alterations in RV diastolic function and signs of systolic dysfunction\textsuperscript{159}.

Studies on sheep fetuses using conventional Doppler have indicated that acidemia is associated with prolonged ICT\textsuperscript{161}, and prolongation of the pre- and post-ejection phases corresponding to ICT and IRT was demonstrated using cTDI during acute hypoxia/acidemia\textsuperscript{162}. However, in this study hypoxia was caused by cord clamping and an effect of increased afterload and preload might have influenced the results. Another study using sTDI showed reduced isovolumic contraction and relaxation velocities and E', as well as increased ICT in sheep fetuses with metabolic acidemia\textsuperscript{130}. There are also indications that acute fetal hypoxemia could be associated with reduced LV longitudinal strain in the fetal sheep\textsuperscript{163}.

7 FETAL ANEMIA AND CARDIOVASCULAR FUNCTION BEFORE AND AFTER INTRAUTERINE TRANSFUSION (IUT)

Fetal anemia is a complication that could subject the fetus to a hypoxic challenge and the transfusion required could also add further strain on cardiac adaptive mechanisms. The physiologic changes and adaptive mechanisms occurring will be outlined below.

7.1 BACKGROUND

The main cause of fetal anemia is maternal red cell alloimmunization, where maternal antibodies against fetal erythrocyte antigens cause hemolysis of red blood cells. There are more than 50 red cell antibodies that could cause hemolytic disease of the fetus and newborn. The most common antibodies where intrauterine therapy is needed are anti-RhD, anti-Rhc and anti-Kell\textsuperscript{164}. In Sweden, the prevalence of RhD immunizations in RhD-negative women is approximately 1.5%\textsuperscript{165}. Other causes of fetal anemia exist, but are less frequent, and include infection with parvovirus B19 and homozygous α-thalassemia. Fetal anemia can result in the development of hydrops with ascites, subcutaneous edema and pleural and pericardial effusions. Before the advent of intrauterine therapy, fetal anemia often led to either perinatal death or substantial morbidity\textsuperscript{164}.

In 1963, Liley performed the first intrauterine intraperitoneal blood transfusion\textsuperscript{166}, and the first intravascular intrauterine transfusion (IUT) was performed in 1981 using a fetoscope\textsuperscript{167}. The technique used today involves direct intravascular transfusion through an ultrasound
guided procedure. The preferred sites of transfusion are either the intrahepatic part of the umbilical vein or the umbilical vein at the cord insertion in the placenta. After introduction of IUT, the survival rates in anemic fetuses with RhD immunizations have increased to live birth rates between 88.9-100%\textsuperscript{168}. The perinatal survival after treatment due to parvovirus B19 infection is considerably lower (67-85\%)\textsuperscript{169}.

The aim of surveillance is to monitor at-risk pregnancies and predict and treat anemia before the development of hydrops. Today this is achieved by assessing peak systolic velocity (PSV) in the middle cerebral artery (MCA), which increases in anemic fetuses. The MCA PSV is converted into multiple of the median (MoM), where 1.5 MoM is considered the threshold for umbilical vein blood sampling and transfusion if required. A sensitivity of 100\% for predicting fetal anemia with a false positive rate of 12\% has been demonstrated using this threshold\textsuperscript{170, 171}.

Short-term consequences are well described and procedure related complications, such as bleeding from the puncture site, cord hematoma, fetal bradycardia or preterm delivery due to complication, have decreased with increasing expertise and are now as low as 1.2\% per procedure\textsuperscript{172}. The knowledge about long-term consequences is less. The incidence of children with neurodevelopmental impairment after IUT due to fetal anemia was 4.8\% when assessed between 2-17 years\textsuperscript{173}.

Long-term cardiac consequences have also been demonstrated, as indicated by an association between a reduced left ventricular mass and left atrial area in childhood after fetal anemia due to red cell alloimmunization\textsuperscript{174}. Recently, further evidence has been provided by Wallace \textit{et al.} who investigated cardiovascular function in adults surviving intrauterine anemia and the first intraperitoneal transfusions. They demonstrate smaller left ventricular volumes, increased relative left ventricular wall thickness, decreased myocardial perfusion at rest and increased sympathetic tone compared to controls, suggesting altered cardiovascular development\textsuperscript{175}.

### 7.2 FETAL ANEMIA AND CARDIOVASCULAR FUNCTION

Anemia increases the demands on the fetal heart, which will react by increasing cardiac output\textsuperscript{176, 177} to maintain adequate tissue oxygenation. Briefly, anemia leads to decreased blood viscosity, decreased vascular resistance\textsuperscript{176} and a hyperdynamic circulation with increased blood flow velocities\textsuperscript{170, 178}, cardiac enlargement\textsuperscript{15, 179, 180} and, if untreated, hydrops with decreased chance of survival\textsuperscript{181}.

Data from experiments in fetal sheep indicate that \textit{acute} anemia, with its reduced blood oxygen concentration, increases blood flow to organs due to a decrease in vascular resistance in these organs as well as a decrease in blood viscosity. Various organs respond differently to anemia. The brain, heart and adrenal glands raise their blood flows to keep oxygen delivery stable even at very low hematocrits (12\%)\textsuperscript{182}. This function is crucial to the heart, which is
dependent on consistent oxygen delivery to maintain aerobic metabolism, as oxygen
extraction in the heart varies little with changes in blood oxygen concentration. In contrast,
other organs, such as the gastrointestinal tract, spleen and kidneys, show a more limited
capacity of vasodilation and demonstrate relatively little variation in blood flow. However,
in fetal sheep with chronic anemia, corresponding to the situation in human maternal red cell
alloimmunization, there is a more general decrease in vascular resistance resulting in
increased blood flow to all tissues except for the placenta. The rest of the information
below will concern chronic anemia unless stated otherwise.

In most cases of fetal anemia without hydrops, blood pH, pCO2 and pO2 remain normal, but
with increased severity of anemia tissue hypoxia occurs, as indicated by increased lactate
levels. Chronic anemia increases the demand on the heart, i.e. to deliver a higher cardiac output to
supply the fetal body with oxygen. Factors increasing cardiac output are an increase
in heart rate, decrease in afterload, increase in preload (which might be mediated by
ventricular remodeling) or increase in contractility. There is no clear evidence of an
increase in heart rate in chronic anemia, and the increase in cardiac output is probably
achieved by increased stroke volumes, due mainly to a decrease in afterload and an
increase in preload. This is supported by evidence suggesting that a reduction in afterload is
caused by decreases in viscosity and vascular resistance. Increased preload is probably
achieved largely by a cardiac enlargement, where end-diastolic chamber volumes are
increased without an increase in filling pressure. This is supported by data from animal
experiments in fetal sheep with chronic anemia, where central venous pressure and right atrial pressure did not change. Consequently, the ventricular chambers seem to improve
their filling without increased filling pressure or changes in wall stress by a gradual cardiac adaptation, i.e. remodeling. This is supported by findings that the ventricular-radius-to-wall-
thickness ratio is maintained in the RV, meaning that end-diastolic volume increases in
proportion to cardiac enlargement. Furthermore, an increase in short axis dimension and radius of curvature have been suggested to expand chamber volume in anemic fetal sheep.

The increase in cardiac output observed during anemia also seems to be equally shared
between the ventricles, maintaining RV dominance in the anemic fetus.

The increased filling of the right ventricle through an increase of venous return and preload is
indicated by increased blood flow velocities in the ductus venosus and inferior vena cava. The idea of increased preload/ventricular filling, and possibly improved diastolic relaxation, is also supported by findings that the blood flow E/A ratio might be increased in
anemic fetuses. An increase in umbilical venous circulation, necessary to increase filling, has been observed and is caused by both an increase in velocity and vessel diameter.
Furthermore, there is an increase in UV blood flow that is inversely correlated to cord hemoglobin at birth. There are also indications that the ventricular shortening fraction
might be increased in anemic fetuses before their first IUT, which might indicate increased
contractility but might also be explained by decreased afterload, or increased preload and remodeling.

As a result of increased cardiac output/stroke volumes due to the decrease in afterload, decrease in blood viscosity, decrease in vascular resistance and the increase in preload, the fetus also demonstrates increased arterial and left-sided intracardiac blood flow velocities, e.g. in the aorta\textsuperscript{191,192}, middle cerebral artery\textsuperscript{191} and across the mitral and aortic valves\textsuperscript{179}. In the clinical evaluation of the anemic fetus, the assessment of arterial blood flow velocities, especially in the middle cerebral artery, is an important tool to evaluate the degree of anemia and the need for fetal blood sampling and transfusion.

The assessment of cardiac function by evaluating cardiac time intervals and, thus, timing of events in the cardiac cycle have been less studied than the assessment of blood flow velocities. Cardiac dysfunction in anemic fetuses have been described with increased MPI seen in anemic fetuses with α-thalassemia\textsuperscript{197} with prolongation of ICT and ICT + IRT\textsuperscript{198}. Early changes at 12-14 weeks of gestation with a prolongation of ICT and MPI, indicating systolic dysfunction, have also been demonstrated in this group\textsuperscript{199}. However, there is evidence from fetuses with anemia due to maternal red cell alloimmunization that LV MPI actually shortens because of an increase in ET and reduction in IRT as anemia worsens. Consequently, the latter suggests that, initially, the fetus is able to enhance its cardiac performance to cope with the increased demands caused by the anemia\textsuperscript{113}.

Further evidence that cardiac function can be negatively influenced by anemia is that fetal cardiac troponin T values negatively correlated to hemoglobin z-scores and that elevated values before the first IUT were significantly associated to perinatal death in a small group of fetuses\textsuperscript{200}.

Most evidence in human anemic fetuses has been generated using 2D ultrasound, M-mode or conventional Doppler assessing blood flow parameters. Very little is known about the myocardium itself and its response to fetal anemia, except for the cardiac enlargement and the trend towards increased shortening fraction measured by M-mode\textsuperscript{87}. There are few studies trying to assess the effects of anemia on the myocardium itself. Michel \textit{et al.} used cTDI to assess systolic tissue velocities (Sm), displacement and systolic strain at different locations in the LV and RV walls. The analysis comprised a mixture of both longitudinal and lateral views of the fetal heart. They demonstrated that the LV Sm correlated negatively with the MCA pulsatility index (PI). Furthermore, both the Sm and systolic displacement in the LV correlated negatively with umbilical artery (UA) PI. Both the LV and RV strain were increased in anemic fetuses. RV systolic strain showed a negative correlation with UA PI and the LV Sm and displacement, both correlated positively with DV PI. However, no correlations were found between systolic cTDI variables and MCA PSV. The authors concluded that cTDI could be used to evaluate cardiac function in fetal anemia\textsuperscript{201,202}. 
7.3 CHANGES IN FETAL CARDIOVASCULAR FUNCTION AFTER IUT

In chronic fetal anemia, the heart is working with a high output against a low afterload. During and after IUT, the fetal cardiovascular system is further subjected to a relatively large volume load with high viscosity, which might quickly increase afterload and require a considerable fetal circulatory adaptation. Worsening fetal condition and cardiac dysfunction following IUT have been described and could be deleterious to the fetus\textsuperscript{203-205}. This is a challenging hemodynamic adaptation that might also have implications for long-term outcomes.

After IUT, there is an immediate cessation of the hyperdynamic circulation, with a decrease in blood flow velocities in the aorta and carotid artery\textsuperscript{194}, thought to be caused by a decrease in cardiac output\textsuperscript{187}. The reason for this decrease in cardiac output might be due to an increase in afterload\textsuperscript{187} because of an increase in hematocrit, increase in blood viscosity\textsuperscript{203, 206} and a possible decrease in cardiac performance after transfusion\textsuperscript{87}. The cessation of the hyperdynamic situation might not only be due to the normalization of blood flow to organs that were hyperperfused during anemia, but also due to an additional decrease in cardiac performance associated with an abnormal increase in systemic vascular resistance.

Some of the changes initiated might be temporary, as the time interval between IUT and the cardiac assessment have an impact on results, and acute alterations might be transient. For instance, two separate investigators showed a decrease in LV and RV cardiac output with assessment within two hours after transfusion\textsuperscript{187, 193}, whereas Copel \textit{et al.}\textsuperscript{207} could not demonstrate any differences 24 hours after transfusion. In animal studies, similar alterations have been described. Kilby \textit{et al.} demonstrated that LV afterload increased after transfusion but returned to baseline levels after one hour, whereas viscosity increased and remained elevated. Furthermore, they showed that end-diastolic pressure (EDP) and end-diastolic volume (EDV) increased but EDP returned to baseline after one hour, whereas EDV did not. However, heart rate, LV stroke volume and contractility did not change significantly in this study\textsuperscript{208}. On the other hand, the shortening fraction in the LV and RV has been shown to decrease significantly immediately after IUT in human fetuses, supporting the suggestion that decreased cardiac contractility or performance contributes to reversal of the hyperdynamic circulation\textsuperscript{87}. Rizzo \textit{et al.} also demonstrated increased E/A ratios after IUT, which returned to normal within two hours after transfusion\textsuperscript{193}.

Less is known about the effects of IUT on fetal cardiac time intervals. LV MPI obtained from blood flow measurements increased significantly after transfusion according to Assunçao \textit{et al.}, and greater changes are associated with an earlier gestational age (GA) at transfusion, lower MPI z-score before transfusion and smaller feto-placental volume expansion\textsuperscript{209}. The higher MPI values could indicate ventricular dysfunction just after transfusion.
In the assessment of the myocardium using cTDI, systolic strain has been observed to decrease in both ventricles after IUT\textsuperscript{201}.

In summary, the anemic fetus undergoing IUTs is subjected to a hemodynamic challenge that requires a substantial cardiovascular adaptation, which might have both short- and long-term consequences. Knowledge about fetal cardiac adaptation is largely based on animal studies and assessment of fetal blood flow parameters, whereas less is known about myocardial function itself.

8 PROLONGED PREGNANCIES

Hypoxic challenges to the fetus can occur during labor and, traditionally, prolonged and post-term pregnancies have been considered as high-risk groups for intrapartum complications.

A pregnancy that reaches to or beyond 42 weeks of gestation (294 days) is defined as post-term\textsuperscript{210}. In Sweden, 7.2% of singleton pregnancies were delivered post-term in 2016\textsuperscript{211}. Several studies have demonstrated increased fetal, maternal and neonatal complications with increased risk of perinatal morbidity and mortality after 41 weeks of gestation\textsuperscript{212}. There is, however, no conclusive evidence that it is the actual prolongation of pregnancy that increases the risk, as other associated specific risk factors, such as IUGR and fetal malformations, have also been identified\textsuperscript{210, 213}.

Two options for the management of pregnancies $\geq 41 + 0$ have been proposed, i.e. routine induction or expectant management with intermittent fetal monitoring. There is no conclusive evidence that routine induction of labor improves the outcome. According to the American College of Obstetricians and Gynecologists, induction of labor can be considered between 41 + 0 and 42 + 0 and is recommended after 42 + 0. The Society of Obstetricians and Gynecologists of Canada recommends that women are offered an induction of labor at 41 + 0 to 42 + 0 weeks of gestation\textsuperscript{212, 214}.

There is no clear consensus on what tests to use and how often to monitor these fetuses. Commonly used tests are: non-stress test with cardiotocography (CTG), assessment of amniotic fluid volume by ultrasound, fetal biophysical profile, estimation of fetal weight and Doppler assessment of umbilical artery and other fetal vessels, such as the middle cerebral artery. There has been interest in the use of fetal Doppler in the assessment of blood flow to evaluate these pregnancies, but the results are conflicting\textsuperscript{215-219}.

The pathophysiological basis for the increased risk is unclear, even though placental ageing is often suggested\textsuperscript{210}. If fetal hypoxia due to placental ageing/impairment is an underlying cause, then, hypothetically, performing cTDI to detect subtle changes in cardiac function, such as impaired longitudinal function, might be a way of improving risk assessment in these pregnancies.
Except when there are clear signs of fetal compromise evidenced by reduced fetal heart rate variability or unprovoked decelerations, little is known about other cardiac function parameters associated with adverse outcome in prolonged pregnancies or during labor. Weiner et al. demonstrated that changes in aortic peak blood flow velocity and cardiac output correlated with changes in amniotic fluid index. A notching of the aortic flow velocity waveform of unclear significance has been noted as a common finding in post-term pregnancies. There are also indications that normally grown, term fetuses that develop intrapartum compromise and need emergency delivery have lower LV and higher RV cardiac outputs than those who do not need emergency delivery. The situation in prolonged pregnancies is not known.

9 DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE/FETAL PROGRAMMING

There is a link between the intrauterine environment and cardiovascular health in later life. Therefore, assessing fetal cardiovascular function before birth could be useful and important. The concept of the developmental origins of health and disease was first proposed by Barker in the 1980s. He observed that the geographical distribution of infant mortality closely mirrored death from ischemic heart disease 60 years later, where the most deprived regions displayed the greatest mortality. He proposed that cardiovascular disease might have its origins in fetal life, where the fetus adapts to survive in utero, but that this adaptation causes permanent structural, metabolic and physiological changes. These changes would then “program” the individual to have increased risk of cardiovascular disease in later life. The inverse relationship between birth weight with blood pressure and coronary heart disease have since then been demonstrated in numerous studies.

There is now evidence that signs of fetal cardiovascular adaptation can already be demonstrated in utero. Fetuses with IUGR show signs of both systolic and diastolic cardiac dysfunction, with increased E/A ratios and MPI as well as decreased myocardial velocities, when assessed by TDI. As previously mentioned, there are also indications that TDI might be a more sensitive tool than conventional Doppler to detect cardiac dysfunction. In both IUGR and small for gestational age (SGA) fetuses, there are cardiovascular changes persisting into childhood, e.g. more globular hearts, reduced longitudinal motion and impaired relaxation with decreased S’ and E’ velocities. The children also showed increased blood pressure and carotid intima thickness. Recently, similar findings were identified in preadolescent children (8-12 years of age), further illustrating that fetal cardiovascular adaptation identified in utero can be followed through adolescence. Shorter sarcomere length in deceased IUGR fetuses has been suggested as an explanation for the observed cardiac dysfunction.

Fetal anemia has also been proposed as a programming agent of cardiac function and disease. In animal studies, fetal anemia has been shown to result in cardiac enlargement due to larger,
increased numbers and more mature cardiomyocytes compared to controls. There is also a
change in the coronary vascular tree with an increase in coronary blood flow and conductance
that might persist into adulthood, even if the anemia is corrected\cite{15, 229, 230}.

Long-term cardiac consequences in humans have also been demonstrated by studies on
children that had fetal anemia due to red cell alloimmunization\cite{174}. Recently, further evidence
has been provided by Wallace et al. investigating cardiovascular function in adults surviving
intrauterine anemia and the first intraperitoneal transfusions. They demonstrate smaller left
ventricular volumes, increased relative left ventricular wall thickness, decreased myocardial
perfusion at rest and increased sympathetic tone compared to controls, suggesting altered
cardiovascular development\cite{175}.

Complications such as IUGR/SGA and intrauterine anemia, demonstrate the importance of
correct and thorough assessment of fetal cardiac function to define high-risk situations and
groups to optimize monitoring, timing of delivery, postnatal follow-up and, hopefully in the
future, also allow for the development of preventive strategies.

10 SAFETY ISSUES USING ULTRASOUND IN PREGNANCY

When applying any method to the fetus, safety needs to be considered. The use of ultrasound
during pregnancy is, generally, considered safe. However, the lack of harmful effects does
not prove its safety. As diagnostic ultrasound involves exposure to energy, there is a potential
to induce biological effects. Epidemiological studies have not demonstrated harmful effects,
such as fetal growth delay, occurrence of congenital malformations, impaired postnatal
neurological development, poor school performance, impaired motor development, disturbed
attention/perception or deficient hearing or vision. However, a possible increase in non-right
handedness has been suggested, and an experimental study on mice indicated that prolonged
exposure might influence neuronal migration\cite{231, 232}. Whether the association between non-
right handedness and ultrasound screening is causal is not known\cite{233}.

Different ultrasound modalities involve possible exposure to different levels of energy. B-
mode is probably the safest, with the lowest acoustic outputs. In M-mode and 3D ultrasound,
the risks are probably not higher than for B-mode. However, both spectral and color Doppler
have the potential to generate exposure to higher levels of energy\cite{234}. Therefore, the ALARA
(as low as reasonably achievable) principle, should always be applied. This means that at
every examination benefits should be weighed against potential risks.

Furthermore, the time of exposure should be minimized and ultrasound modalities emitting
higher energy should be handled with knowledge and care. Special attention should be given
to the use of fetal Doppler ultrasound in the first trimester, where it should not be used
routinely\cite{235}. There is also a consensus that ultrasound for the sole purpose of providing
“souvenir images” should not be performed\cite{231}.
The user is responsible for evaluating risks and benefits of the examination. Output display standards require that indices indicating risks of exposure (thermal indices and mechanical index) are continuously displayed on the ultrasound screen during examination. The thermal index (TI) indicates the risk of temperature rise and mechanical index (MI) the risk of mechanically induced damage, such as cavitation\textsuperscript{234}.

In the examinations performed in this thesis, care was taken to follow the published safety guidelines and to minimize exposure when using color and spectral Doppler ultrasound.
\textbf{11 AIMS}

The overall aim of this thesis was to evaluate the feasibility and value of assessing fetal cardiac function using automated analysis of color tissue Doppler imaging (cTDI) during the second half of pregnancy.

The specific aims were:

- To assess the feasibility of a newly developed automated algorithm for the analysis of myocardial velocity traces obtained by cTDI, and to evaluate the effect of different sizes of regions of interest (ROIs) on the results at different gestational ages. (Study I)

- To evaluate the feasibility of using the automated method of analysis of cTDI velocity traces in fetuses close to labor, at $\geq 41$ weeks of gestation, to compare manual and automated methods of analysis and explore the association between fetal cTDI variables and adverse perinatal outcomes. (Study II)

- To construct reference ranges for variables describing fetal cardiac function obtained by automated analysis of cTDI velocity recordings during the second half of normal pregnancy. (Study III)

- To evaluate fetal cardiac function before and after intrauterine transfusion using automated analysis of cTDI velocity traces. (Study IV)
12 MATERIAL AND METHODS

12.1 STUDY POPULATION AND METHODS

Study I was performed at the University Hospital of North Norway, Tromsø, Norway. Women in Studies II-IV were recruited from the Center for Fetal Medicine at Karolinska University Hospital, which has been the National Center for Fetal Therapy in Sweden since 2013. All intrauterine transfusions (IUTs) in Sweden have been centralized to this center since 2013.

12.1.1 Study I – feasibility of automated analysis

In this study, we analyzed 261 myocardial velocity traces obtained from 17 fetal echocardiographic examinations performed on five healthy pregnant women at different gestational ages. These women were randomly selected from a larger longitudinal study evaluating fetal cardiac function in women with uncomplicated pregnancies, and were examined serially in approximately four weekly intervals from 18 weeks of gestation until term.

The material in Study I was divided into three different groups according to gestational age (GA) at examination, i.e. GA I, week 18-24; GA II, week 25-32 and GA III, week 33-41. In these GA groups, different ROI sizes were tested and the sizes were increased with increasing GA. The ROI length/height covered different proportions of the ventricular length. Therefore, ROI height was divided by the length of the ventricular septum measured from the atrio-ventricular (AV) plane to the apex in end-diastole to estimate the proportion of septal, and thus, myocardial length occupied by the ROI. In all cases, a ROI of 2 x 2 mm was also used as a reference to enable comparison of velocity information between different ROI sizes. Difference in myocardial velocities and cardiac cycle time intervals were calculated as percentage change compared to the reference ROI.

All myocardial velocity traces were analyzed manually and with the automated algorithm. The results from the automated analysis were shown as percentage feasible traces, whereas the manually assessed velocity traces were used for the comparison between ROI sizes.

12.1.2 Study II – automated analysis at ≥ 41 weeks

This was a prospective, cross-sectional observational study that was conducted between 2013 and 2014. It included 107 women with uncomplicated singleton pregnancies examined between 41 + 0 and 41 + 5 weeks of gestation. Exclusion criteria were maternal complications such as pre-eclampsia, chronic hypertension, diabetes, or fetal chromosomal or major structural abnormalities. Adverse perinatal outcome was defined as the presence of at least one of the following: intrapartum fetal scalp blood lactate > 4.8 mmol/L, operative delivery for suspected fetal asphyxia, cord arterial pH < 7.15, Apgar < 7 at five minutes or Apgar score for muscle tone < 2 at five minutes.
Both automated and manual assessments of cTDI velocity traces were performed. Measurements were compared using Wilcoxon signed rank test and Spearman’s correlation coefficients (rho) were calculated. The agreement between automated and manual analysis of the peak myocardial velocities, cardiac cycle time intervals and myocardial performance index (cMPI) was assessed using Bland-Altman plots. We defined the precision as the 95% limits of agreement (± 1.96 standard deviations [SD]) and the bias as the mean of individual difference between values obtained with the two methods. The proportion of measurements that differed ≤ 20% between automated and manual assessment were calculated. Mann-Whitney U-test was used to compare normal and adverse perinatal outcome groups.

To assess intra- and inter-observer variability of the manual assessment, 10 randomly chosen scans, i.e. 30 myocardial velocity traces, 10 from each location, were analyzed manually by the same observer and a second observer. The pre-ejection, ventricular ejection and post-ejection phases were evaluated and coefficients of variation (CVs) calculated.

12.1.3 Study III – reference ranges in normal pregnancy

This was a prospective, cross-sectional study conducted between 2009 and 2011. The final study population included 201 women with uncomplicated singleton pregnancies examined between 18 and 42 weeks of gestation. Exclusion criteria were maternal complications such as pre-eclampsia, chronic hypertension or diabetes at inclusion or major structural abnormalities discovered during pregnancy or at a postnatal examination.

To construct reference ranges, the statistical method described by Royston and Wright was used. In summary, Box-Cox power transformation was used to decide on the appropriate type of transformation to achieve normal distribution of data. Linear polynomial regression was used to estimate the relationship between the studied variables and GA, expressed in exact gestational weeks. Normal distribution of absolute standardized residuals was checked with Shapiro-Wilk’s test. Mean and standard deviation (SD) curves were calculated as a function of GA. The polynomial regression equations were used to calculate the fitted mean (50th), 5th and 95th centiles for the corresponding GA.

Spearman’s correlation coefficient (rho) was used to analyze correlation between the variables describing cardiac function and GA. An analysis was performed to compare cardiac function between female and male fetuses using Mann-Whitney U-test.

To assess inter- and intra-observer variability, pregnant women between 19 and 41 weeks of gestation were examined. The inter-observer variability was assessed by two different operators examining 25 patients each. Intra-observer variability was evaluated by comparing two separate recordings in 22 patients obtained by the same operator 5-10 minutes apart. All ROIs were placed by one operator and myocardial velocity traces were analyzed by the automated algorithm. CVs were then calculated.
12.1.4 Study IV – cardiac function before and after IUT

This study included 32 women undergoing 70 IUTs between 2009 and 2016. Exclusion criteria were multiple pregnancy, major structural/chromosomal fetal abnormality or intraperitoneal transfusion.

cTDI was performed before and within 2.5 hours after IUT. All cTDI variables were converted to z-scores using the gestational age specific references ranges constructed in Study III. Z-scores were calculated as, (measurement-predicted mean)/predicted SD. Delta (Δ) z-scores were calculated as z-score after IUT minus z-score before IUT. Peak systolic velocity (PSV) in the middle cerebral artery (MCA) was recorded and adjusted to multiple of median (MoM) for each gestational age. The presence of fetal hydrops or ascites was noted. Before transfusion, a complete blood count was obtained, and the hemoglobin values were converted to multiple of median (Hb MoM). Fetal anemia was defined as mild (0.84-0.65 MoM), moderate (0.65-0.55 MoM) or severe (< 0.55 MoM).

All cTDI z-scores before IUT were expressed as means with 95% confidence intervals (CI). Spearman’s correlation (rho) was analyzed between the Hb MoM and MCA PSV MoM before IUT, and cTDI variables converted to z-scores. Changes in cTDI variables before and after transfusion were evaluated using Wilcoxon signed rank test for related samples for first and all IUTs.

12.2 ECHOCARDIOGRAPHIC EQUIPMENT

In Study I, a Vivid 7 Dimension ultrasound machine with a M4S sector transducer (1.5-4.3 MHz) was used (GE Vingmed Ultrasound AS, Horten, Norway). A Vivid S6 ultrasound imaging system with a M4S-RS (1.9-4.1 MHz) phased array transducer (GE CV Ultrasound, Haifa, Israel) was used in Study II, the inter- and intra-variability assessment in Study III and in most examinations in Study IV. A Vivid-i ultrasound imaging system with a 3S-RS (1.9-3.8 MHz) phased array transducer (GE Vingmed Ultrasound AS, Horten, Norway) was used in Study III and some examinations in Study IV.

12.3 IMAGE ACQUISITION

cTDI was performed by acquiring an apical or basal four-chamber view of the fetal heart. Cine-loops of consecutive cardiac cycles were recorded. The insonation angle was kept as close to the long-axis of the heart as possible (always < 30°), and the image was adjusted to obtain as high a frame rate as possible.

All echocardiographic examinations were performed by experienced operators (Bodil Hvingel – Study I, Kjerstin Ferm-Widlund – Study II, III, IV and Lotta Herling – Study III and IV).
In Studies I, II and IV, other fetal measurements were also performed. An estimation of fetal weight was done by measuring the biparietal diameter of the head, the mean abdominal diameter/circumference and the femur length according to local protocols. Blood flow Doppler measurements were obtained from the umbilical artery (UA) and middle cerebral artery (MCA) according to guidelines for measurements of pulsatility index and peak systolic velocity\(^{239}\). The ALARA principle was always applied and care was taken to keep the mechanical index (MI) and the thermal index (TI) at recommended levels\(^{231}\).

### 12.4 OFF-LINE ANALYSIS

A flow chart of the entire process from image acquisition to manual and automated analysis is provided in Figure 12-1.

#### 12.4.1 ROI placement and size

Data from the cine-loops obtained with cTDI were transferred to EchoPAC version 201 (GE Vingmed Ultrasound AS, Horten, Norway). ROIs were placed at the level of the AV-plane in the interventricular septum (IVS) and left ventricular (LV) and right ventricular (RV) walls of the fetal heart to produce myocardial velocity traces describing longitudinal cardiac function. The aim was to place the ROI with the AV-plane reaching approximately one third into the ROI at end-systole. The placement of ROIs is demonstrated in Figure 12-2.

In Study I, various ROI sizes were used and compared (Figure 12-3). ROI widths were chosen to cover the entire width/thickness of the myocardium at that gestational age:

- GA I (18-24 weeks). ROI width 2 mm.
- GA II (25-32 weeks). ROI width 3 mm.
- GA III (33-41 weeks). ROI width 3-4 mm.

The ROI height was increased in steps to cover different proportions of the myocardial wall/ventricular length, with the maximum ROI heights being 4, 6 and 8 mm in GA I, II and III, respectively. The smallest ROI size, 2 x 2 mm, was analyzed for all recordings to enable comparison of myocardial velocities and cardiac cycle time intervals between different ROI sizes.

In Studies II-IV, a 2 x 2 (height x width) mm ROI was used at 18-24 weeks, a 4 x 3 mm ROI at 25-32 weeks and a 6 x 4 mm ROI at 33-41 weeks of gestation according to the results from Study I.
Figure 12-1. Flow chart describing the process from the ultrasound examination, i.e. the fetal echocardiography, to the automated and manual analysis in Studies I-IV.
Figure 12-2. A four-chamber view of the fetal heart demonstrating placement of regions of interest (ROIs) in the right ventricular wall (yellow), interventricular septum (blue) and left ventricular wall (red).

Figure 12-3. Different ROI sizes and corresponding myocardial velocity traces in a fetus in gestational age (GA) group III. Reprinted from Herling et al. 2015, with permission from BioMed Central.
12.4.2 Manual analysis of velocity traces

The myocardial velocity traces were subsequently transferred to GHLab software (Gripping Heart AB, Stockholm, Sweden). In Studies I and II, visual identification and manual definition of cardiac cycle time intervals were performed for one selected cardiac cycle. This was achieved by simultaneously evaluating the original myocardial velocity trace and a constructed acceleration trace according to a protocol where certain acceleration shifts define the beginning and end of the cardiac cycle time intervals (Figure 12-4).

In Study I, acceleration traces were also assessed by two operators and divided into three categories depending on the degree of well-defined acceleration shifts, as well-defined shifts are a pre-requisite for the algorithm to function. An acceleration score of three represented clear and well-defined shifts, a score of two represented less well-defined shifts, often biphasic, and a score of one represented indistinct shifts, often with a flat appearance. An average score was calculated for the IVS, LV and RV walls and the total number of traces, and an acceleration score of three for each ROI size was recorded as a subjective measure of quality.

Peak myocardial velocities and AV-plane displacement were automatically derived by the software after manual definition of the cardiac cycle time intervals. In Studies III and IV, manual assessment was not performed, and myocardial velocity traces were transferred for automated analysis.

Figure 12-4. Definition of cardiac cycle time intervals with manual analysis in one cardiac cycle. The thicker line demonstrates a velocity trace and the thinner line an acceleration trace. The time intervals identified are: 1, atrial contraction; 2, pre-ejection; 3, ventricular ejection; 4, post-ejection; 5, rapid ventricular filling/early diastolic filling.
12.4.3 Automated analysis of velocity traces

All myocardial velocity traces were transferred to MATLAB (R2010a/R2015a, Mathworks, MA, USA) for automated analysis. The algorithm for performing the automated analysis was constructed by Fredrik Bergholm and Jonas Johnson, in cooperation with KTH-The Royal Institute of Technology, Sweden. It was based on the dynamic adaptive piston pump (DAPP) hypothesis.58

The algorithm was constructed as a rule-based system that involves pattern recognition, several adaptive processes and some auxiliary algorithms, such as heart cycle determination documented in Bergholm (2016a). It identifies landmarks, such as maximum and minimum peaks and crossings through the zero line. The algorithm then uses these landmarks together with pre-defined rules to correctly determine the cardiac cycle time intervals. The beginning and the end of the cardiac cycles are estimated by an auxiliary algorithm and defined from the time intervals. The rule-based system contains 30-40 rules some of which are empirical constraints, some are logical rules, such as the order in which certain (detected) events can occur, and some describe strategies finding minima in the non-systolic part of signal (Em, Am, etc.). Moreover, the algorithm compares all identified heart beats and uses that information in identification of cardiac cycle time intervals. It can also compare different segments, i.e. the IVS, LV and RV walls, over time to more exactly define the time intervals.

This is a fully automated procedure and no manual marking of the traces is needed once the velocity traces are transferred to MATLAB. All available cardiac cycles can be analyzed. It takes approximately three milliseconds to analyze ten heart beats for all three segments using an Intel Core i7-6700 @3.4 GHz desktop computer with 16 GB RAM, 64 bits operating system. An example of the first step of automated analysis is shown in Figure 12-5. AV-plane displacement can also be analyzed by the automated algorithm, but this is not presented in these studies. In Study I, a beta version of the algorithm was used that was further developed and refined into the version used in Studies II-IV.

Figure 12-5. This is an example of the first step of automated analysis. The green line is the velocity trace and the red line the acceleration trace. The asterisks indicate identification of defined events in the cardiac cycle.
12.5 VARIABLES DESCRIBING MECHANICAL CARDIAC FUNCTION

All automated measurements were means of all available cardiac cycles that were acquired in a cine-loop of cTDI, whereas manual measurements were obtained from one selected cardiac cycle. All measurements were performed in the IVS, LV and RV walls separately. Measured variables are described below.

12.5.1 Myocardial velocities and Em/Am ratio

Am: Peak myocardial velocity during atrial contraction.

Sm: Peak myocardial velocity during ventricular ejection.

Em: Peak myocardial velocity during rapid ventricular filling/early diastole.

Myocardial velocities were measured in cm/s. Examples of velocity traces and the Am, Sm and Em waves are shown in Figures 5-3 and 12-3.

The Em/Am ratio was calculated from the above measurements.

12.5.2 Mechanical cardiac time intervals and cMPI

The time intervals obtained using cTDI are defined according to changes in the mechanical work of the heart and, therefore, we decided to name them mechanical cardiac cycle time intervals, to distinguish them from the time intervals measured using blood flow velocity waveforms, which are based on hemodynamic events (Figure 12-6).

Figure 12-6. The mechanical cardiac time intervals displayed in two different ways A. As a myocardial velocity trace B. In a circle as a cardiac state diagram, where the whole circle represents one cardiac cycle. Adapted from Herling et al. 2017, with permission from Wiley.
**Atrial contraction phase:** The time required for the lifting of the AV-plane towards the base of the heart with the aim of increasing the stroke length. It completes the filling of the ventricles in late diastole.

**Pre-ejection phase:** The time it takes for the ventricles to rearrange/restructure and contract before the actual expulsion of blood.

**Ventricular ejection phase:** The time it takes for the ventricles to contract and eject blood during systole. This involves a large movement of the AV-plane towards the apex of the heart.

**Post-ejection phase:** The time it takes for the ventricles to rearrange/restructure and start to relax.

**Rapid ventricular filling/early diastolic filling phase:** The time when the ventricles are filling rapidly with blood during diastole and the AV-plane moves towards the base of the heart.

**Slow ventricular filling phase/diastasis:** The time when the filling of the ventricles slows down. This phase is often very short in fetuses except during a period of low heart rate and, sometimes, in later gestation.

**Myocardial performance index (cMPI)** was calculated from the durations of (pre-ejection + post-ejection)/ventricular ejection.

The last two mechanical time intervals, i.e. rapid and slow ventricular filling were not evaluated when utilizing the automated algorithm at this stage in development.

**12.5.3 AV-plane displacement**

AV-plane displacement could also be analyzed both from the manual and automated analysis, but have not been the focus of this thesis. The definitions used in Study II are included below.

**Displacement during ventricular ejection (DispV):** Describes the movement (in mm) of the AV-plane towards the apex during ventricular ejection.

**Displacement during atrial contraction (DispA):** Describes the movement (in mm) of the AV-plane towards the base during atrial contraction.

**12.6 ETHICAL APPROVALS**

Informed consents were obtained from all participants. Ethical approvals were obtained from:

- The Stockholm Regional Ethics Committee DNr 2012/895-31/4 (Studies II and IV), DNr 2009/1617-31/2 and 2017/539-32 (Study III).
13 RESULTS

When the automated algorithm was run repeatedly, exactly the same results were obtained each time.

13.1 STUDY I – FEASIBILITY OF AUTOMATED ANALYSIS

Automated analysis of myocardial velocity traces obtained by cTDI was feasible as it was possible to analyze 203 out of 261 (78%) velocity traces with the automated algorithm. It was possible to analyze 93% (81/87) from the RV wall, 82% (71/87) from the LV wall and 59% (51/87) from the IVS. All traces could be analyzed with the manual method.

With the manual method of analysis there was a clear trend towards decreasing myocardial velocities and increasing interquartile ranges with increasing ROI heights (Table 13-1). The cardiac cycle time intervals showed minimal variation, with median differences from -3.2% to +2.6% and interquartile ranges of 2-13% compared to the reference ROI. The number of velocity traces with an acceleration score of three, used as a measure of quality, increased with ROI height in GA II and GA III but not in GA I. Details about ROI sizes, traces with acceleration scores of three and percentage of the ventricular/septal length occupied by each ROI are presented in Table 13-2.

| Table 13-1. Difference in peak myocardial velocity between different sizes of regions of interest according to gestational age group |
|-------------------------------|---------------|----------|----------|----------|----------|
|                              | ROI height (mm) |         |         |         |         |
| Δ Sm (%)                      | n              | 3        | 4        | 6        | 8        |
| GA I                          | 30             | -3.5 (14)| -9.3 (19)|         |          |
| GA II                         | 54             | -1.6 (7) | -1.0 (11)| -8.2 (16)|          |
| GA III                        | 126            | -2.9 (9) | -4.6 (17)| -9.2 (16)| -15.8 (16)|
| Δ Em (%)                      | n              | 3        | 4        | 6        | 8        |
| GA I                          | 30             | -1.2 (9) | -6.3 (10)|         |          |
| GA II                         | 54             | -0.1 (7) | -0.7 (11)| -1.9 (20)|          |
| GA III                        | 126            | -3.2 (14)| -6.0 (18)| -8.5 (27)| -13.5 (31)|
| Δ Am (%)                      | n              | 3        | 4        | 6        | 8        |
| GA I                          | 30             | -2.3 (6) | -8.6 (9) |         |          |
| GA II                         | 54             | -4.8 (8) | -8.7 (16)| -13.7 (16)|          |
| GA III                        | 126            | -2.1 (6) | -5.2 (6) | -7.7 (14)| -9.5 (20) |

Data are presented as median difference (Δ) in % (interquartile range) compared to the reference ROI of 2 x 2 mm. ROI, region of interest; GA, gestational age group; Sm, peak myocardial velocity during ventricular ejection; Em, peak myocardial velocity during rapid ventricular filling; Am, peak myocardial velocity during atrial contraction. Adapted from Herling et al. 2015, with permission from BioMed Central.
<table>
<thead>
<tr>
<th>Table 13-2. Details on gestational age groups and sizes of regions of interest</th>
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<tr>
<td><strong>ROI size (mm)</strong></td>
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<td><strong>GA I (n = 45)</strong></td>
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<td><strong>GA II (n = 72)</strong></td>
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<td>6 x 3</td>
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<td><strong>GA III (n = 144)</strong></td>
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ROI size is presented as height x width. ROI, region of interest; GA, gestational age group; n = total number of velocity traces.

*Adapted from Herling et al. 2015, with permission from BioMed Central.*
13.2 STUDY II – AUTOMATED ANALYSIS AT ≥ 41 WEEKS

Fetal echocardiographic examinations using cTDI were performed in 107 women between gestational age 41 + 0 and 41 + 5. It was possible to obtain a recording of the four-chamber view of the fetal heart in all fetuses. It was possible to analyze all myocardial velocity traces manually, and the inter- and intra-observer variability CV for the analysis of pre-ejection, ventricular ejection and post-ejection phases were 6.2% and 7.6%, respectively. Maternal characteristics and pregnancy outcomes are displayed in Table 13-3.

| Table 13-3. Maternal characteristics, ultrasound and pregnancy outcome data, Studies II-IV |
|---------------------------------|-----------------|-----------------|-----------------|
| Maternal data                  | Study II (n = 107) | Study III (n = 201) | Study IV (n = 32) |
| Age (years)                    | 31.0 ± 5.2       | 30.1 ± 5.2       | 31.2 ± 5.3       |
| BMI (kg/m²)                    | 24.9 ± 5.2       | 23.7 ± 3.9       | 24.1 ± 3.3       |
| Nulliparous                    | 45 (42.1)        | 81 (40.3)        | 4 (12.5)         |
| Ultrasound data                |                 |                 |                 |
| Frame rate (frames/s)          | 206.3 ± 15.5     | 208.3 ± 6.8      | 207.7 ± 13.1     |
| Pregnancy outcomes             |                 |                 |                 |
| GA at delivery (weeks)         | 41.7 ± 0.4       | 40.0 ± 1.4       | 35.7 ± 1.4       |
| Mode of delivery:              |                 |                 |                 |
| Normal vaginal delivery        | 85 (79.4)        | 147 (74.2)       | 11 (34.4)        |
| Vacuum extraction              | 9 (8.4)          | 22 (11.1)        | 0 (0.0)          |
| Cesarean section               | 13 (12.1)        | 29 (14.6)        | 21 (65.6)        |
| Birth weight (g)               | 3840 ± 423       | 3576 ± 501       | 2793 ± 443       |
| Female babies                  | 55 (51.4)        | 96 (48.5)        | 15 (46.9)        |
| Cord arterial pH               | 7.22 ± 0.09      | 7.25 ± 0.09      | 7.27 ± 0.10      |
| Five min Apgar score < 7       | 1 (0.9)          | 0 (0.0)          | 2 (6.3)          |
| Pre-term delivery < 37 weeks   | 0 (0.0)          | 4 (2.0)          | 28 (87.5)        |

Data are presented as mean ± SD or n (%). BMI, body mass index; GA, gestational age. Adapted from Herling et al. 2017, with permission from Wiley, and Studies III and IV.

13.2.1 Automated analysis

A total of 321 myocardial velocity traces, i.e. 107 velocity traces from each location of measurement, were analyzed with the automated and manual methods of analysis. With the automated method, an average of 8-9 cardiac cycles were evaluated. It was possible to analyze all myocardial velocity traces at all three locations, i.e. the IVS, LV and RV walls with the automated algorithm but complete data were not obtained for all variables. In the LV wall and IVS results were obtained for all peak myocardial velocities, whereas in the RV wall there were 10 missing values in the Em variable. In the IVS, LV and RV walls, information on all mechanical cardiac time intervals were obtained except for two LV post-ejection phases. Consequently, results were obtained on myocardial velocities and mechanical cardiac
time intervals in 96% of all traces and the exact same results were obtained when the algorithm was run repeatedly.

At all three locations, the Am was highest followed by Em and Sm. All peak myocardial velocities were highest in the RV wall. Details about variables from the automated analysis are presented in Table 13-4.

<table>
<thead>
<tr>
<th>Table 13-4. Myocardial velocities, mechanical cardiac time intervals and myocardial performance index assessed by automated analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td><strong>Myocardial velocities</strong></td>
</tr>
<tr>
<td>Em (3.0-4.6)</td>
</tr>
<tr>
<td>Am (2.9-5.0)</td>
</tr>
<tr>
<td>Sm (2.4-4.0)</td>
</tr>
<tr>
<td>Em/Am (0.74-1.36)</td>
</tr>
<tr>
<td><strong>Cardiac time intervals</strong></td>
</tr>
<tr>
<td>Atrial contraction (51.2-69.2)</td>
</tr>
<tr>
<td>Pre-ejection (49.1-64.5)</td>
</tr>
<tr>
<td>Ventricular ejection (128.9-153.7)</td>
</tr>
<tr>
<td>Post-ejection (68.7-86.9)</td>
</tr>
<tr>
<td>cMPI (0.83-1.19)</td>
</tr>
</tbody>
</table>

Data are given as median (interquartile range). † No significant difference between automated and manual analyses assessed with Wilcoxon signed rank test, p > 0.05. LV, left ventricular wall; IVS, interventricular septum; RV, right ventricular wall; Em, peak myocardial velocity during rapid ventricular filling; Am, peak myocardial velocity during atrial contraction; Sm, peak myocardial velocity during ventricular ejection. Adapted from Herling et al. 2017, with permission from Wiley.

**13.2.2 Comparison between automated and manual analysis**

There were significant positive correlations between all myocardial velocity variables assessed automatically and manually, except septal Em/Am. The correlation was strongest for LV Em/Am (rho = 0.69) and LV Em (rho = 0.67). There were only a few weak significant positive correlations between automatically and manually assessed cardiac cycle time intervals, i.e. LV atrial contraction and post-ejection phases, and RV atrial contraction and pre-ejection phases (rho 0.28-0.34).
Large differences were observed between methods in atrial contraction, pre-ejection and ventricular ejection phases in the RV wall and IVS. Measurements were more similar in the post-ejection phases and LV ventricular ejection phase. Bland-Altman plots displaying the RV pre-ejection and post-ejection phases are given as examples of time intervals with differing bias and, thus, differing percentages in measurements with a difference ≤20% (Figure 13-1). Detailed information on precision, bias and proportion of velocity traces with a difference ≤20% is shown in Table 13-5.

![Bland-Altman plots](image)

**Figure 13-1.** Bland-Altman plots comparing the automated and manual methods of analysis of the pre- and post-ejection times in the right ventricular wall. The pre-ejection time demonstrates a large bias, whereas the post-ejection time demonstrates less bias.  
Adapted from Herling et.al 2017, with permission from Wiley.

<table>
<thead>
<tr>
<th></th>
<th>Precision</th>
<th>Bias</th>
<th>≤ 20%</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>LV</td>
<td>IVS</td>
<td>RV</td>
</tr>
<tr>
<td>Myocardial velocities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Em</td>
<td>2.2</td>
<td>2.4</td>
<td>3.1</td>
</tr>
<tr>
<td>Am</td>
<td>2.9</td>
<td>2.4</td>
<td>3.3</td>
</tr>
<tr>
<td>Sm</td>
<td>2.3</td>
<td>1.5</td>
<td>2.6</td>
</tr>
<tr>
<td>Cardiac time intervals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrial contraction</td>
<td>39.2</td>
<td>42.3</td>
<td>33.1</td>
</tr>
<tr>
<td>Pre-ejection</td>
<td>35.8</td>
<td>39.3</td>
<td>46.9</td>
</tr>
<tr>
<td>Ventricular ejection</td>
<td>39.7</td>
<td>51.5</td>
<td>55.3</td>
</tr>
<tr>
<td>Post-ejection</td>
<td>37.0</td>
<td>58.2</td>
<td>43.2</td>
</tr>
<tr>
<td>cMPI</td>
<td>0.70</td>
<td>0.71</td>
<td>0.63</td>
</tr>
</tbody>
</table>

The precision was defined by the 95% limits of agreement (± 1.96 SD), and the bias as the mean of individual difference between values obtained with the two methods. LV, left ventricular wall; IVS, interventricular septum; RV, right ventricular wall; Em, myocardial velocity during rapid ventricular filling; Am, myocardial velocity during atrial contraction; Sm, myocardial velocity during ventricular ejection; cMPI, myocardial performance index.  
Adapted from Herling et.al 2017, with permission from Wiley.
13.2.3 cTDI variables and perinatal outcome

Twenty-five babies (23%) were considered to have an adverse perinatal outcome according to the wide definition used. When using the automated method of analysis, there were significant differences between the normal and adverse outcome groups with regards to the RV post-ejection time, RV cMPI and septal pre-ejection time.

13.3 STUDY III – REFERENCE RANGES IN NORMAL PREGNANCY

A total of 202 women were initially included. One baby had muscular septal defects and a bicuspid aortic valve and was excluded from analysis, which resulted in a final study population of 201 women.

A four-chamber view of the fetal heart was possible to obtain in all pregnancies. A total of 603 myocardial velocity traces, i.e. 201 each from the IVS, LV and RV walls, were available for automated analysis. One velocity trace was omitted due to severe artifacts. It was possible to analyze all atrial contraction, pre-ejection, ventricular ejection and post-ejection phases and all peak myocardial velocities (except RV Em in 11 cases). Outliers where the algorithm did not manage a correct delineation of cardiac time intervals were discarded, resulting in a final number of observations per variable between 187-201. The best fitting model for all parameters was a first-degree linear polynomial after normalization, but different transformations were used to normalize data distribution.

All myocardial velocities, as well as LV and RV Em/Am, showed an increase with and positive correlations with GA. Graphs demonstrating the evolution of myocardial velocities in the LV wall in the second half of pregnancy are shown in Figure 13-2. The cardiac time intervals generally remained more stable throughout pregnancy. The time intervals that showed both increased duration and positive correlations with GA were septal and RV atrial contraction, LV pre-ejection time and septal ventricular ejection time, whereas septal post-ejection time and cMPI demonstrated a decrease and negative correlation with GA.

There was a difference between female and male fetuses in Sm at all three locations, septal and RV Am, septal and RV atrial contraction time, LV pre-ejection and septal ventricular ejection time.
The inter- and intra-variability, including the fetal echocardiography and placement of ROIs, sometimes showed considerable CVs. However, there were no major differences between the inter- and intra-variability groups. The lowest CV for both groups was observed in ventricular ejection time (8.3-13.7%), whereas pre-ejection was the cardiac time interval with the highest CV (28.0-35.2%). Peak myocardial velocities showed the lowest CVs in septal Sm intra-variability (11.8%), followed by inter-variability in RV Am (13.0%). The highest CVs were found in intra-variability LV Am and LV Sm (43.1 and 34.5%).

13.4 STUDY IV – CARDIAC FUNCTION BEFORE AND AFTER IUT

This study included 32 fetuses that altogether underwent 70 IUTs. In 66 of 70 transfusions, cTDI recordings obtained both before and after the procedure were available, and in four cases only before IUT. Details of the study population and pregnancy outcomes are summarized in Table 13-3. There were 27 fetuses, undergoing 63 IUTs, because of fetal anemia due to maternal alloimmunization. The most common maternal antibody was anti-D (n = 22). Five fetuses underwent IUTs due to other causes. One fetus had Diamond-Blackfan anemia, one a suspicion of CINCA (Chronic Infantile Neurological and Auricular) syndrome242 and in three babies the cause of the anemia was unknown. Fourteen of the IUTs were first transfusions, and in one the before examination hemoglobin value was missing.
Fetal hydrops was present at three (4.3%) IUTs in three fetuses and isolated ascites at eight (11.4%) IUTs in six fetuses. The transfusion data is as shown in Table 13-6.

<table>
<thead>
<tr>
<th>Table 13-6. Data on intrauterine transfusions IUTs (n = 70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of IUTs per fetus</td>
</tr>
<tr>
<td>Site of transfusion</td>
</tr>
<tr>
<td>Intrahepatic</td>
</tr>
<tr>
<td>Cord insertion in placenta</td>
</tr>
<tr>
<td>Mean GA at IUT (weeks)</td>
</tr>
<tr>
<td>Hemoglobin before IUT (g/L)</td>
</tr>
<tr>
<td>Hemoglobin after IUT (g/L)</td>
</tr>
<tr>
<td>Degree of anemia (n = 69)</td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>Mild</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>Severe</td>
</tr>
<tr>
<td>Infused volume (mL)</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD or n (%). GA, gestational age. Adapted from Study IV.

When extreme outliers in variables were identified, the individual cTDI recordings and velocity traces were visually inspected and measurements scrutinized. Values were omitted in a few cases where the identification of time intervals by the automated algorithm was incorrect or when the trace was of insufficient quality for the algorithm to function. This, together with missing Em values, resulted in a mean of 68 observations per variable before IUT (range, 64-70).

13.4.1 cTDI variables before IUT compared to normal values

In the LV wall and IVS, all myocardial velocities were significantly increased, whereas in the RV wall only the systolic velocity was increased before IUT compared to reference ranges constructed in Study III. Details about myocardial velocities and cardiac cycle time intervals before IUT are demonstrated in Table 13-7.
When analyzing first IUTs with available Hb values, there was a significant negative correlation between Hb and LV Em (rho = -0.61, p = 0.036) and LV Em/Am (rho = -0.82, p = 0.001) (Figure 13-3). In first IUTs, the median LV Em z-score was 1.03 (95% CI -0.12-1.95) and the median LV Am z-score was -0.13 (95% CI -0.58-0.34) indicating a possible trend towards increasing LV Em. When analyzing all IUTs, only weaker negative correlations were found between Hb and LV Em and Em/Am.

In first IUTs, there was a significant positive correlation between MCA PSV with LV Em (rho = 0.66, p = 0.03) and LV Em/Am (rho = 0.84, p = 0.001) (Figure 13-3). In all IUTs, only weaker positive correlations were found with LV Em and Em/Am.
13.4.3 Comparison of cTDI variables before and after IUT

In fetuses receiving their first IUT, there was a significant decrease in LV Am and Sm as well as septal Sm and Em following IUT with median Δ z-scores of -0.69 to -1.50; p < 0.05. There was also a significant increase in septal post-ejection time, septal cMPI and RV pre-ejection time with median Δ z-scores of +0.45 to +0.85; p < 0.05.

In the analysis of all IUTs, with available data, there was a significant decrease in heart rate (p = 0.001). All peak myocardial velocities, that were increased before transfusion, decreased significantly after IUT (median Δ z-scores of -0.34 to -0.97; p < 0.01). The LV Em/Am ratio also increased after transfusion (median Δ z-score of +0.58; p < 0.001). Several time intervals demonstrated a significant increase after IUT, i.e. LV ventricular ejection, septal post-ejection, RV pre-ejection and RV post-ejection (median Δ z-scores of +0.45 to +0.66; p < 0.05). Interestingly, also cMPI in the RV wall showed an increase with median Δ z-score of +0.48; p < 0.05.

Graphs illustrating the change in peak myocardial velocity during atrial contraction (Am) in the LV wall before and after IUT are shown in Figure 13-4.

Figure 13-3. Correlation between left ventricular (LV) Em/Am z-scores and hemoglobin (Hb) and middle cerebral artery peak systolic velocity (MCA PSV) multiples of median (MoM) in first IUTs.
Adapted from Study IV.
Figure 13-4. Graphs demonstrating the change in peak myocardial velocity (absolute values) during atrial contraction (Am) in the left ventricular wall before and after intrauterine transfusion. Red squares indicate fetuses with severe anemia. The dotted lines represent the 5th, 50th and 95th centiles according to reference ranges in Study III.
14 DISCUSSION

14.1 PRINCIPAL FINDINGS

The studies included in this thesis demonstrate that an automated analysis of cTDI velocity traces was feasible and could be used in the assessment of fetal cardiac function during the second half of pregnancy. It also showed the clinical value of the automated method, as it could be applied to construct reference ranges, that could be used to visualize differences in fetal cardiac adaptation to anemia and volume load.

*Study I* demonstrated that the analysis of myocardial velocity traces obtained by cTDI was feasible using an automated algorithm. Furthermore, we suggested that the ROI size should be adjusted according to gestational age due to the growing fetal heart. This conclusion was drawn by balancing the positive effects of a larger ROI on the acceleration traces, considered to improve the functioning of the automated algorithm, versus the loss of velocity information with increasing ROI heights.

*Study II* demonstrated that the automated analysis can be used in pregnancies at an advanced gestational age, which is important if this method is to be used for monitoring fetal well-being just before or during labor. Sometimes large differences in agreement between the automated and manual assessment of velocity traces were seen. While none of these methods is considered a gold standard today, the potential benefit of an automated assessment that repeatedly gives the same results was considered an important pre-requisite for future application and use of cTDI in research and clinical practice.

In *Study III*, gestational age specific reference ranges for normal pregnancies between 18 and 42 weeks of gestation were constructed using the automated analysis of cTDI velocity traces. The knowledge of normal values is a pre-requisite before evaluating pathological conditions. The study showed an increase in myocardial velocities with advancing gestation, whereas the mechanical cardiac time intervals remained more stable. The inter- and intra-variability assessment, including the whole procedure from the fetal echocardiography, the ROI placement and automated analysis, sometimes showed considerable variation, further emphasizing the need to reduce variability using the automated method.

*Study IV* demonstrated that cTDI with automated analysis of myocardial velocity traces can be used to evaluate fetal cardiac function before and after IUT, where altered cardiac function due to hemodynamic adaptation to anemia and volume load can be expected. This was illustrated by increased myocardial velocities before transfusion, which decreased with the reversal and cessation of the hyperdynamic state after transfusion in accordance with findings previously reported in fetal blood flow velocities. A significant negative correlation between the hemoglobin value before IUT and the LV Em and LV Em/Am ratio in first IUTs was also demonstrated.
14.2 INTERPRETATION OF RESULTS

14.2.1 Study I – feasibility of automated analysis

The automated analysis was judged to be feasible as results could be obtained from a majority of fetal myocardial velocity traces (78%). The impact of using different ROI sizes was evaluated. In most previous publications this topic is not discussed and the reason for choosing a certain ROI size is often unclear. Using the same ROI size throughout the second half of pregnancy is not logical, as the fetal heart increases its size substantially during this period. There was an increasing loss of velocity information with increasing ROI height. This is logical, as the ROI will cover a larger area of the myocardium and, consequently, will average lower velocities towards the apex. However, the acceleration traces appeared better defined with increasing ROI heights in more advanced gestations, which was considered to improve the algorithm function.

14.2.2 Study II – automated analysis at ≥ 41 weeks

As previously mentioned, there are indications from animal studies and from research on adult humans that some TDI variables might change in response to hypoxia/hypoxemia and acidemia. Both acute and chronic fetal hypoxia can occur at advanced gestations or during labor and, therefore, it is of utmost importance that this method can be used even at ≥ 41 weeks of gestation. cTDI with automated analysis was judged feasible at this gestational age as it was possible to acquire four-chamber views of the fetal heart and analyze cTDI velocity traces to obtain values in 96% of all traces.

In this study, automated and manual methods of analysis were also compared. There were significant positive correlations between all automatically and manually assessed myocardial velocity variables except one. However, only a few time intervals measured by the two techniques correlated significantly and the agreement between methods sometimes showed considerable bias, and precision was poor for some cTDI variables. These differences were partly due to the different definitions of the cardiac cycle time intervals using the automated and manual methods of assessment. This is illustrated by the pre-ejection time, where the definition of the delineating time events differs considerably between methods. Therefore, only 9% of LV pre-ejection time measurements showed a difference ≤ 20%. In the post-ejection phases, the definitions are more similar. Therefore, it resulted in 50-65% of measurements having a difference of ≤ 20%. Consequently, when analyzing differences, it is also important to bear in mind that none of these methods constitute a gold standard and that none is yet validated. Another important aspect is that the comparison is performed between one manually assessed cardiac cycle that was subjectively chosen and the average of all automatically analyzed cardiac cycles for each variable. In our opinion, the advantage of several automatically analyzed cycles, where the same results are obtained repeatedly, should be the method of choice and possibly a gold standard. This is supported by findings from a previous study by Maheshwari et al., who showed that with automated analysis of MPI.
obtained by blood flow measurements the beat-to-beat variability was reduced by averaging 4-5 cardiac cycles\textsuperscript{243}.

Another interesting finding is that both manually and automatically evaluated Am and Sm velocities in the LV were lower than their septal counterparts in our material. This was slightly unexpected as it has not been demonstrated by other studies. This could be due to technical issues, such as the slight shadowing of the LV wall by the spine in late pregnancy with the position of the baby mostly being with the spine up. However, this is to our knowledge the largest study describing fetal cardiac function with TDI at this gestational age and it is possible that this could also be an accurate description of the situation in late pregnancy.

There were also differences between groups with adverse and normal perinatal outcomes with regards to RV post-ejection time, RV cMPI and septal pre-ejection time when using automated analysis. The meaning of these differences is unclear and cannot be interpreted using this small group of patients but needs to be investigated in larger populations. It is, however, interesting that, hypothetically, changes in certain cardiac variables in late pregnancy could constitute risk factors for adverse outcomes during labor. Alsolai et al. recently demonstrated that term fetuses that develop intrapartum compromise, defined as undergoing emergency operative delivery, showed signs of lower LV and higher RV cardiac outputs compared to fetuses that did not need emergency delivery. If this is somehow linked to lower myocardial velocities in the LV wall is unknown. Associations between cTDI variables and perinatal outcomes are not confirmatory at this stage and can only serve for the generation of hypotheses for future studies.

14.2.3 Study III – reference ranges in normal pregnancy

This study demonstrated an increase in myocardial velocities with advancing gestational age as previously demonstrated with both cTDI and sTDI\textsuperscript{131, 142, 143, 149}. The increase in myocardial velocities were more prominent during ventricular ejection and early diastole, but to a lesser degree during atrial contraction. This illustrates fetal cardiac maturation throughout pregnancy, where the relative contribution of the atrial contraction gets less prominent as relaxation and, thus, rapid/early ventricular filling gradually increases. Cardiac cycle time intervals generally remained more stable throughout gestation. It is logical to think that these would vary less with gestational age as they are not equally influenced by the size of the heart as are the velocities\textsuperscript{143, 244}.

Interestingly, there was a difference between female and male fetuses in myocardial velocities during ventricular ejection and in the RV and septal wall during atrial contraction. Male fetuses demonstrated higher peak myocardial velocities. There were also differences in septal and RV atrial contraction time, LV pre-ejection and septal ventricular ejection time. Most of these time intervals were ones that demonstrated changes with gestational age. These gender differences observed in variables describing late diastolic and systolic fetal cardiac
function might be due to differences in cardiac size, but other mechanisms influencing myocardial function and maturation cannot be ruled out.

Possible differences due to cardiac size might be adjusted if measurements were normalized by fetal size instead of gestational age. Comas et al. demonstrated a progressive increase of myocardial velocities with increasing fetal weight and presented weight adjusted reference ranges for sTDI, which would be important when evaluating fetuses with IUGR. Another, maybe more tempting alternative, would be to adjust to cardiac size in analogy to what we did in Study I by adjusting ROI size to septal length. The longitudinal diameter of the heart has also been used to compare measurements between fetuses with IUGR and normal controls.

It is also worth noting that there were differences between the velocities obtained in Study II in week 41+ and the calculated reference ranges for week 42+ in Study III. The myocardial velocities in the LV were higher in the reference material than in Study II. There might be several reasons for this. In Study II, medians are displayed, whereas the reference ranges are shown as fitted mean obtained from the regression equation. The number of patients is also considerably different between the two studies and, furthermore, cTDI data were obtained using two different ultrasound machines.

The evaluation of coefficients of variation (CVs) in Study III, included the entire procedure from echocardiography to automated analysis and was substantial (8.3-43.1%), as expected. When interpreting this variation, it is important to consider biological variation, varying fetal state as well as the subjectivity of ROI placement. However, there was no difference between the inter- and intra-variability assessments, suggesting that the variation was not primarily operator dependent. As the ROI placement was performed by one operator and the automated analysis does not show any variability, a large part of variation might be due to biological variation. In our opinion, this substantial variation proves the importance of automated analysis that will minimize variation. An important aspect to determine is whether this variation will influence the possibility to detect differences between normal and pathological conditions. However, we speculate that this might not be the case as the beat-to-beat variability could be less in pathological conditions, in analogy to the silent CTG patterns present in situations with suspected asphyxia.

14.2.4 Study IV – cardiac function before and after IUT

This study demonstrated that cTDI with automated analysis can be used to evaluate fetal cardiac function and adaptation to changing loading conditions. It showed that, in addition to hemodynamic changes, the alteration in fetal cardiac function related to the hyperdynamic state caused by fetal anemia and its cessation following IUT is also reflected in myocardial wall motion.

The increase in both systolic and diastolic myocardial velocities before IUT are in accordance with the hyperdynamic circulation associated with anemia, as has been described by other studies investigating blood flow velocities. Interestingly, myocardial velocity during
rapid ventricular filling (Em) seemed to be increasingly enhanced compared to myocardial velocity during atrial contraction (Am) with increasing degree of anemia. This resulted in a significant negative correlation (rho = -0.82) between hemoglobin and the LV Em/Am ratio before first IUTs. This could be interpreted as a sign of improved myocardial relaxation but might also be secondary to better emptying of the ventricle during systole because of a decrease in afterload or an effect of increased filling, possibly due to adaptation and/or remodeling. In analogy with this finding, Rizzo et al. have shown a trend towards increased blood flow E/A ratios before transfusion in previously transfused fetuses\(^1\). In our material when evaluating all IUTs, the LV Em/Am ratio before IUT showed a z-score of 0.20 (95% CI of -0.05-0.44). Considering the heterogeneity of the group, this might also be considered a trend even though not statistically significant. Further studies are needed to establish if the LV Em/Am ratio could be used to refine the assessment of fetal cardiac function in anemic fetuses undergoing transfusion.

The decrease in several of the peak myocardial velocities after IUT is most likely caused by the cessation of the hyperkinetic circulation as hemoglobin level and, consequently, oxygen carrying capacity increases. However, the reduction of myocardial velocities after transfusion was greater than expected. One possible explanation to this exaggerated decrease in cardiac performance could be the fast volume expansion with packed red blood cells leading to an immediate increase in afterload\(^2\), which is known to be poorly tolerated by the fetal heart. A decrease in cardiac output immediately after IUT has previously been described in other studies\(^3,4\). Interestingly, there was also a significant increase in LV Em/Am ratio after IUT, in accordance with findings reported using blood flow variables\(^2\). If the decrease in systolic velocities observed after IUT represents increase in afterload, with a negative effect on myocardial velocity during ventricular ejection, this might secondarily affect the filling during diastole. A ventricle that is less empty and did not manage to eject the same amount of blood would probably not experience problems during the rapid ventricular filling (Em) but rather during the last part of ventricular filling during the atrial contraction (Am). Hypothetically, this could explain the increase in Em/Am ratio seen shortly after IUT.

Less is known about cardiac cycle time intervals in this situation. Before IUT, there was a significant shortening of the LV post-ejection time, supporting the idea of enhanced myocardial performance in anemia\(^5,6\). IUT is expected to affect preload, afterload, heart rate and, in severely ill fetuses, possibly also contractility. Consequently, it is not surprising that the changes in cardiac time intervals were more diverse and difficult to interpret. Hypothetically, the prolongation of pre- and post-ejection time and cMPI in the RV wall after transfusion might indicate a dysfunction immediately following the IUT. Although this explanation is speculative and needs to be further investigated, it is known from experimental data, that the right ventricle is less able to respond to an increase in preload and more sensitive to an increase in afterload\(^7\).

When interpreting results from this study, it is also important to bear in mind that the assessment of cardiac function was performed close in time to the IUT and what we detected
was likely to be acute/immediate changes that might be transient and gradually disappear. Several investigators did not demonstrate changes in cardiovascular variables when more time had elapsed since the transfusion\textsuperscript{87, 177, 207}.

14.3 METHODOLOGICAL CONSIDERATIONS AND LIMITATIONS

14.3.1 Study I – feasibility of automated analysis

This was a study where women were randomly chosen from a larger study with a longitudinal design. When evaluating the effect of different ROI sizes, the longitudinal design might be considered an advantage as it allows for serial evaluation of the same fetus as the heart grows and matures. This could possibly limit confounding by other maternal-fetal factors. A limitation, however, is the small number of participants (n = 5) included in the evaluation of the effect of ROI sizes at different gestational ages. However, the number of myocardial velocity traces available for assessment of the feasibility of the automated algorithm (n = 261) was judged adequate for initial evaluation of this method.

Furthermore, the placement of ROIs and the evaluation of acceleration traces were both subjective. Although we aimed to minimize variation by only one operator positioning the ROIs and attempting to classify acceleration traces according to definitions, there is always a risk of misclassification bias. However, the aim was primarily to get an idea about whether it was at all possible to use the automated algorithm and if it were worthwhile pursuing the development to facilitate algorithm function by adjusting ROI sizes. It was judged that increasing ROI sizes made algorithm function more stable as the ROIs would average a larger area of the myocardium and, as such, not be too sensitive to disturbances. The drawback, however, being the risk that it might be more difficult to assess differences between normal and abnormal cardiac function. Considering all other technical aspects and difficulties when using cTDI in the fetus, such as high heart rate, fetal movement, depth in the abdomen, etc., we judged that at this stage the priority should be to develop and optimize the algorithm to facilitate its use.

14.3.2 Study II – automated analysis at ≥ 41 weeks

This was a cross-sectional study where women were prospectively included during week 41+. All included participants were dated based on ultrasound in the first or second trimester, which makes the gestational age quite reliable. This gestational week was chosen as it is traditionally considered as high-risk, advanced gestational age may make scanning the fetal heart difficult and because it was easy to recruit participants as our policy is to perform a routine ultrasound examination during that gestational week. The sample size (n = 107) was judged to be adequate for assessing the feasibility of the algorithm and comparing manual and automated methods, which were the main aims. However, for assessing association with adverse outcomes, the sample size is too small as adverse outcomes, such as peripartum
death, fortunately are uncommon events in our setting. The definition of adverse outcome is, therefore, wide and results regarding perinatal outcomes should be interpreted with caution.

Moreover, one general issue concerning the analysis is how to correctly and adequately display and interpret cardiac cycle time intervals. One option could have been to display them as percentage of the total cardiac cycle length or to correct them with the heart rate. However, this is not as simple as it seems as different time intervals would change differently with a change of the cycle length, such as in brady- or tachycardia. Therefore, we decided to present absolute values in Studies II-IV.

14.3.3 Study III – reference ranges in normal pregnancy

This was a prospective cross-sectional study aiming to construct reference ranges for the second half of pregnancy. Limitations of this study are the relatively low number of observations per gestational week included for constructing the reference ranges and the uneven distribution of participants throughout gestation. However, this is to our knowledge the largest study describing fetal cardiac function using cTDI\textsuperscript{131, 149, 151}.

Concerning the internal validity, there is of course a risk of selection bias even though care was taken to obtain a random sample representing the general pregnant population in our setting. All examinations were performed by one single experienced operator, eliminating inter-observer variability and only one ultrasound machine was used, which could be expected to increase internal validity, but might reduce the generalizability of results. Different results can be obtained using machines from different manufacturers\textsuperscript{245}, but to our knowledge differences between machines from the same manufacturer have not been evaluated in fetuses.

Further aspects that could influence results relate to the handling of outliers and the risk of information bias. When extreme outliers were found, the individual myocardial velocity traces were inspected and in cases where these were not correctly analyzed by the algorithm the values were omitted. It was considered appropriate to exclude these cases as the aim was to establish normal reference values.

14.3.4 Study IV – cardiac function before and after IUT

In this study, women referred to the fetal medicine clinic due to suspected fetal anemia were included. This cohort was collected during a longer period and women were not consecutively recruited. Several factors complicate the interpretation of results. The study group was heterogeneous with varied etiologies of the fetal anemia and different degrees of anemia (in some cases no anemia) at the time of fetal blood sampling and IUT. Consequently, cardiac adaptive mechanisms and reaction to the IUT might have differed between fetuses, and this could have influenced measurements. However, when all measurements were included the myocardial velocities were consistently higher than the reference values before blood sampling and IUT. A similar consistent decrease in velocities was also observed following IUT. Furthermore, as multiple measurements were performed in the same fetus
when evaluating all IUTs, we evaluated first IUTs separately even though the numbers were few.

### 14.3.5 Limitations related to application of methodology

#### Limitations of using cTDI in the fetus

When using cTDI, general technical aspects need to be considered. One of these is the angle-dependency, which means that the assessment of velocities is very sensitive to the angle of insonation. With an increased angle of insonation there is a loss of velocity information. Important to consider is also that the IVS, LV and RV walls do not move in identical directions, likely due to differences in myocardial fiber orientation. Consequently, when assessing the movement of the IVS, it is likely that a lateral view of the heart would give additional, and maybe better, information on movement as septal motion is not necessarily longitudinal.

Other limitations in fetuses relate to fetal and maternal movements, small size of the fetal heart, high fetal heart rate and scanning depth, which could be further complicated in cases of maternal obesity. An increased scanning depth will decrease lateral resolution as the ultrasound beams diverge at increasing depths. Small fetal heart size will further decrease resolution and decrease the amount of detailed information obtained from the ROI. The high fetal heart rate also makes it important to obtain high frame rates, especially when analyzing time intervals with short duration, such as the pre- and post-ejection phases.

The manual placement of ROIs influences the measurements. Care was taken in all studies to minimize subjectivity by only one operator placing the ROIs. The aim was to place the ROI with the AV-plane reaching approximately one third into the ROI at end-systole (then in the rest of the cycle, the ROI would cover the area in the myocardium just below the AV-plane). However, a certain degree of subjectivity was unavoidable.

Furthermore, it is important to bear in mind that, as with many techniques in fetuses, this method has not yet been experimentally validated.

#### Variability associated with using two different ultrasound systems

cTDI data were obtained using different ultrasound machines. Using different machines could be considered a limitation as they might give different results\(^{245}\). However, we have used machines from the same manufacturer, which would probably diminish that risk. Nevertheless, this corresponds to the clinical situation where machines and software within the machines are changed regularly.
The automated algorithm and aspects on automation

The general issue of automation also needs to be considered and discussed. It is important to understand that this is an automated procedure once the velocity traces have been transferred to MATLAB. Considering the whole procedure from echocardiography to analysis of images, it is obviously a semi-automated procedure as the ultrasound imaging to acquire cTDI velocity recordings and ROI placement are not automated. There are further issues relating to algorithm function and discussion of details, which are out of the scope of this thesis, but a few aspects are discussed below.

In this thesis the analysis of time intervals has been restricted to four out of six available intervals. There are two main reasons for doing so: a) these are likely to be the most important cardiac cycle time intervals as they involve the transitional phases (pre- and post-ejection) and b) the function of the algorithm is not yet stable enough for evaluating the rapid and slow ventricular filling phases.

The definition of the cardiac time intervals is also different compared to other studies, which used crossing of the zero line for definition. In fetuses the zero line is not very stable as there is often substantial drift, which will influence the position of the velocity trace relative to the zero line. Furthermore, the definition of time intervals with the automated algorithm is based on several aspects where the acceleration traces and the shift in cardiac work load are important. Consequently, time intervals are slightly different resulting in, for instance, longer post-ejection phases.
15 CONCLUSIONS

The heart is central in fetal adaptation to various pregnancy complications, such as intrauterine growth restriction, anemia and hypoxemia/acidemia. There is a lack of reliable and robust methods to clinically assess fetal well-being, as well as fetal cardiac function, dysfunction and adaptation to different pregnancy complications. cTDI has the potential to assess fetal cardiac function and add information about the function of the myocardium itself. However, the technique has limitations and the image analysis is cumbersome and time-consuming.

The work presented in this thesis shows the potential of an automated algorithm to facilitate the analysis of fetal myocardial velocity traces obtained by cTDI. The feasibility of using an automated algorithm in fetal examinations was demonstrated. Moreover, the adjustment of ROI sizes according to gestational age was proposed to improve the function of the automated algorithm. Feasibility of using the automated analysis in advanced gestations close to labor was also demonstrated. Comparison of the automated and manual methods of analysis sometimes demonstrated substantial bias and poor precision for some variables measured. However, the advantage of the automated method of analysis is that it can average a large number of cardiac cycles, and that it gives the exact same results on repeated analysis, thus, minimizing variability. Gestational age specific reference ranges were constructed for the second half of normal pregnancy, which is a pre-requisite to discriminate normal from abnormal cardiac function. Finally, cTDI with the automated analysis was tested in fetuses before and after IUT where cardiac dysfunction and adaptation is anticipated. The effect of the hyperdynamic circulation and its reversal following transfusion on the fetal myocardium could be detected using this technique. Taken together, these studies support the idea that cTDI can be used to evaluate cardiac function in different pregnancy complications and automated analysis may facilitate its application.

To conclude, cTDI with the automated analysis shows promise as a feasible method to study fetal cardiac function as well as to detect cardiac dysfunction and adaptation to pathological situations. The use of automated analysis could also facilitate the application of cTDI in research and clinical practice.
16 FUTURE PERSPECTIVES, CLINICAL AND SCIENTIFIC IMPLICATIONS

Automated analysis of myocardial velocity traces gives the ability to evaluate the use of cTDI in a larger population of fetuses with different kinds of pregnancy complications as it has the potential to simplify its use. However, the process still involves several steps, with transfer of data between different software/systems and manual ROI placement. The optimal situation would be to have software with the algorithm incorporated in the ultrasound machine to use it on-line during the examination. This would greatly improve the quality of traces/data as the examiner could directly evaluate whether the algorithm performs accurately, and in cases of disturbance or suboptimal functioning the traces could be cropped, or a new trace acquired. This would then be analogous to the assessment of velocities and pulsatility index in the umbilical artery, middle cerebral artery or ductus venosus, where sometimes many recordings need to be performed before an optimal waveform is obtained that can be easily and automatically measured. Furthermore, automated ROI placement would be preferable. Work towards achieving these two goals are on-going.

When evaluating the method in the future, care needs to be taken when deciding what parameters should be evaluated. Hypothetically, other variables describing acceleration, time from acceleration to peak velocity or aspects describing variability might be equally important. Importantly, the algorithm facilitates simpler use of such additional information.

In order to assess its value in clinical practice the method needs to be validated. The work to achieve this is currently being performed in animal experiments. Prospective studies of adequate sample size in high- and low-risk pregnancies will be required to assess the value of cTDI in the assessment of fetal well-being and in predicting perinatal and long-term outcomes.
Hjärtat är centralt i fostrets anpassning till olika komplikationer under graviditeten, som exempelvis hypoxi (låg syrehalt) och anemi (lågt blodvärde). Det är också möjligt att tidiga och diskreta symtom vid komplikationer först skulle kunna visa sig som en ändrad hjärtfunktion hos fostret. Vi har idag begränsade möjligheter att bedöma och övervaka fostrets hjärtfunktion och allmänna mående i livmodern. Color tissue Doppler imaging (cTDI) är en ultraljudsteknik som kan mäta hjärtväggens (myokardiets) rörelse och ge information om förflytning och hastigheter i väggen. cTDI har provats på foster men har varit svår att använda, delvis för att analysen av de hastighetskurv ur som erhålls är tidskrävande och omständlig. Huvudmålet med den här avhandlingen har varit att värdera en algoritm för automatisk analys av hastighetsdata, framtagen vid Kungliga Tekniska Högskolan (KTH) i Stockholm, med målsättningen att kunna förenkla och förkorta tiden för analysprocessen och därmed underlätta användningen av cTDI under graviditet.

I alla fyra studierna som ingår i den här avhandlingen har fostrets hjärta bedömts i en fyrkammarbild med samtidig användning av cTDI, där cine-loopar med efterföljande hjärtslag har registrerats, sparats och sedan överförts till separata system för analys. Mätområden (region of interest, ROI) har placerats i nivå med det atroventrikulära planet i hjärtat, dvs i hjärtväggen strax under klaffarna, som öppnar sig in mot hjärtats kammare. Dessa ROI:er har placerats i vänster och höger kammarvägg, liksom i septum som skiljer kamrarna åt. Från dessa registreringar har sedan erhållits hastighetskurvor, som efter vidare analys (manuell eller automatisk) gett information om hastigheter, duration av olika hjärtecykelintervall samt förflytning.


I Studie III konstruerades referensvärden för variabler erhållna med cTDI och analyserade med den automatiska analysmetoden under graviditets andra hälft. Den slutgiltiga studiegruppen inkluderade 201 kvinnor från graviditetsvecka 18 till och med vecka 42. Studien visade på ökande hastigheter i hjärtväggen med ökande graviditetslängd medan durationen av hjärtykelintervallen var mer stabila över tiden.

I Studie IV undersöktes 32 foster som genomgick 70 intrauterina blodtransfusioner. Av dessa hade 27 foster, som tillsammans genomgick 63 transfusioner, anemi till följd av alloimmunisering, vilket är ett tillstånd där mamman har bildat antikroppar mot fostrets röda blodkroppar som förstörs, varpå fostret utvecklar anemi. I hela studiegruppen konstaterades, före blodtransfusionen, att samtliga myokardhastigheter var ökade i vänster kammarvägg och septum, medan det i högerväggen bara sågs ökad hastighet under hjärtats utpumpningsfas i jämförelse med normalmaterialet. Efter blodtransfusionen konstaterades sedan en minskning av samma hastigheter. Vid analys av de tillfällen då fostret erhöll sin första blodtransfusion noterades en signifikant negativ korrelation mellan hemoglobinvärdet och den vänstersidiga hastigheten under den tidiga delen av hjärtats fyllnad, liksom av kvoten mellan hastigheterna i tid och sen fyllnadsfas.

Den här avhandlingen visar att det är möjligt att använda denna algoritm för att automatiskt kunna analysera hastighetskurvor erhållna från hjärtnuskulväggen hos foster för att bedöma fostrets hjärtafunktion under graviditets andra hälft.

Utifrån resultaten från Studie I har vi föreslagit att mätområden (ROI) ska ökas i storlek under graviditeten trots att förlust av hastighetsinformation uppstår, då detta förefaller förbättra tydligheten i accelerationskurvorna som delvis ligger till grund för den automatiska analysen.

Det har dessutom visats att man kan använda den automatiska analysen av hastighetskurvor i sen graviditet ≥ 41 veckor och då i nära anslutning till förlossningen. Jämförelsen mellan den manuella och automatiska analysmetoden uppvisade ibland större skillnader. Ingen av metoderna är dock att betrakta som standardmetod, varför den automatiska analysmetoden, som ger upprepat identiskt resultat vid analys av hastighetskurvor och som snabbt kan bedöma ett flertal hjärtycklar, borde prioriteras då den potentiellt skulle kunna underlättas användning av cTDI inom forskning och klinisk verksamhet.

Referensvärden anpassade till graviditetslängden för variabler erhållna med cTDI har också skapats, vilket är en förutsättning för att diskriminera mellan normal och avvikande hjärtafunktion hos fostret.

Slutligen utvärderades den automatiska analysmetoden av hastighetskurvor hos foster som genomgår intrauterina blodtransfusioner p.g.a. misstänkt anemi, vilket visade på en möjlig användning i klinisk verksamhet. Studien visade på ökade hastigheter i myokardiet innan blodtransfusion, vilka minskade signifikant efter blodtransfusion jämfört med normalvärden. Detta stämmer väl överens med de fynd man sett hos foster vid undersökning av
blodflödeshastigheter och illusterar den hyperdynamiska situation som uppstår i samband med anemi hos fostret.

Sammanfattningsvis uppvisar den automatiska analysen av hastighetskurvor erhållna med cTDI hos foster en potential att kunna bedöma och utvärdera fostrets hjärtfunktion samt att kunna detektera avvikelser och anpassning till avvikande tillstånd. Användningen av denna skulle även potentiellt kunna förenkla och effektivisera användningen av cTDI inom forskning och klinisk verksamhet.
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19 REFERENCES


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211. Socialstyrelsen. Statistik om graviditeter, förlossningar och nyfödda barn 2016. 208


