1.1. Intrauterine Programming of Adult Disease

1.1.1. What is Intrauterine Programming? What are Its Mechanisms?

Fetal programming or intrauterine programming is the phenomenon describing deviations from normal developmental patterns. These deviations can increase risks for diseases later in life and are an example of phenotypic plasticity seen throughout nature (1). For instance, infants born with low birth weight, as a marker of an unfavorable intrauterine environment, are programmed differently and may have an increased risk for multiple diseases in adulthood. These risks include coronary heart disease, increased insulin resistance, hypertension, and imbalances in the immune system. The ‘‘Barker Hypothesis’’ demonstrated the correlation between low birth weight (LBW) and death at adult age from ischemic heart disease (2). Birth weight (BW) was inversely related to adult hypertension (3). Other studies have reproduced similar effects.

Normally, the fetus experiences critical periods of vulnerability in-utero. Any suboptimal condition during the perinatal period can result in permanent effects that can continue over generations. There is increasing evidence in the literature supporting this hypothesis and suggesting a correlation between LBW and pathologies that occur later in life. To state a few examples; LBW has strong associations with mortality from diabetes in adult females (3). There is a consistent relationship between mortality from cardiovascular events and BW. Individuals whose BW was less than 2.25 kg experienced twice the mortality compared with individuals born with normal BW. BW >4.5 kg was also associated with higher mortality rates (2-4). Correlations also exist between glucose intolerance, high blood pressure, and BW. Small for gestational-age (SGA) children born prematurely carried increased risks for glucose intolerance and hypertension compared with those born prematurely at appropriate weight for gestational age, as well as those born at term (5-7).

There are many proposed mechanisms for programming. “*Deficient cell proliferation secondary to deficient nutrients*” is particularly important in the obstetrical setting. The placenta provides all blood and nutrients supplied during fetal life. Uteroplacental insufficiency is a major cause of growth restriction where the fetus is trying to

compensate for insufficient nutrition. This may lead to programming of organs to different responses during the vulnerable periods of fetal life. Each organ or system has a peak of development during fetal life with increased cell proliferation and increased nutrient demand. If even a transient decrease of blood flow from placenta occurs during this ‘‘peak’’ period, the corresponding organ could be programmed on a different pathway, maybe via a different gene expression or indirectly due to a lack of cell proliferation (7). Mechanisms other than deficient cell proliferation involve in fetal programming are among others “*altered gene expression*” and “*apoptotic cell death caused by oxidative stress*”. Each mechanism involved in programming interacts with one another, one being a trigger for a whole cascade of other mechanisms. Complex interactions make it difficult to depict the whole picture of programming. Exploration of these mechanisms and their influences on in-utero life opens a wide scope in research. Knowing whether this cascade is triggered and when its effects take place may be instrumental in deciding which fetus needs to be delivered prematurely, thus decreasing morbidity in neonatal period, later childhood or adulthood. The standard practice is to allow a fetus with intrauterine growth restriction to grow inside the mother’s womb, unless the obstetrician has a distinct indication to deliver the infant prematurely. The presence of reverse blood flow in the umbilical artery or the development of severe maternal complications could be valid reasons for premature delivery. Although postponing prematurity is definitely a reasonable goal, allowing further delay in the growth of the fetus may program different responses with an increased risk for several diseases. Therefore, fetal programming may be considered more frequent in the future when calculating risks and benefits associated with delaying delivery of a fetus with low estimated weight as our knowledge expands and data accumulate in this area.

1.1.2. Fetal Programming and Its Potential Impact on Fetal Cardiac State

The concept “programming” involves structural changes in important organs; altered cell number, impaired function of the organ, imbalance in distribution of different cell types within the organ, and altered blood supply or receptor numbers (8). Environmental factors like abnormal chemicals such as alcohol or nicotine, which predominantly may lead to fetal hypoxia, abnormal physical forces such as high blood pressure or low-protein intake of mother cause undernutrition and stress of the fetus and interact with genes inducing the above mentioned alterations. In the unfavorable environment, the

developing baby makes attempts to compensate for deficiencies. Following compensation, birth weight may be normal or only slightly decreased. This conception can be advocated since it has been shown that factors which might induce fetal programming may not necessarily be related to birth weight (9, 10). This suggests that suboptimal maternal conditions may specifically impair organ development without affecting fetal growth (11). This makes the assessment of fetal heart function in the presence of various maternal preexisting conditions particularly interesting and sophisticated. Although there are many studies in the literature, especially animal studies, which deal with certain variations at molecular (alterations in cardiac gene expression, amount of ligands and receptors) and cellular (loss of myocytes, myocyte hypertrophy, estimation of tension in isolated papillary muscle, recapillarization of vessels) level, it is sparsely investigated whether the overall contractile function of myocardium changes in a disadvantageous setting or if there is a compensatory effort at the organ level (12). Most of the human studies conducted are based on fetal hemodynamics and can be considered as a reflection of myocardial state only. It certainly is a point of major interest to investigate the true contractile capacity of fetal heart in favorable and unfavorable circumstances and to look for the very first signs of adverse cardiac function in adult (Figure 1).

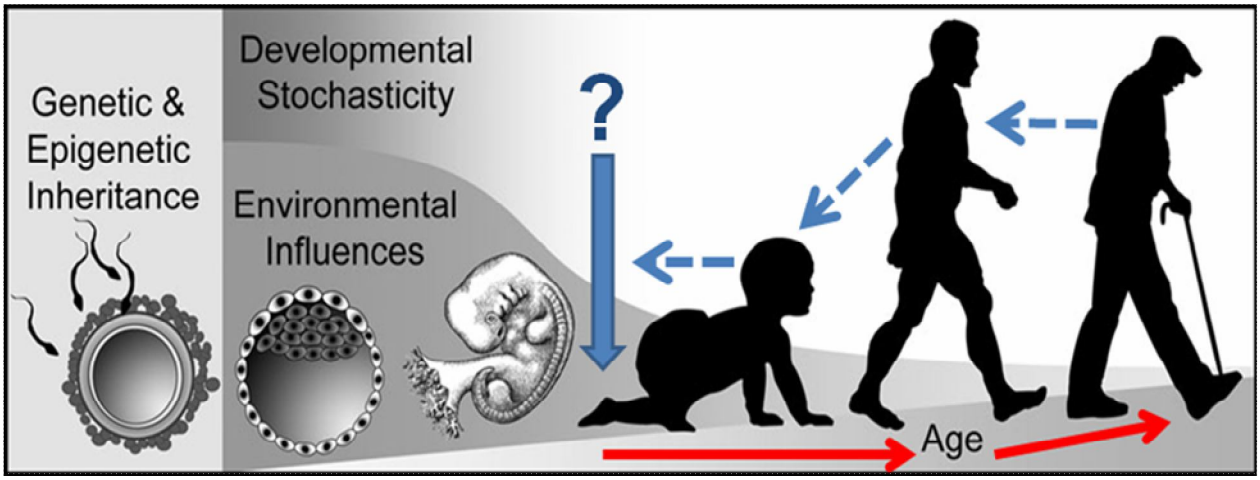

Fig.1. Epigenetic gene regulation and intrauterine milieu play the major role in programming projecting pathologic health conditions in postnatal life. The programming of physiological systems occurs at the gene, cell, tissue, organ and system levels and causes permanent structural and functional changes, which can lead to overt disease, particularly with increasing age (13). Are pathologic states determinable prenatally or is there any potential to determine some deviations from the norm at the level of myocardial function? Is it possible to define norm values for intrauterine cardiac function? What is the contractile status of the fetal heart? Can we find traces of the „future“ „now“? Figure adapted from (14).

1.1.3. Myocardial Function of Fetuses in Disadvantageous Intrauterine Milieu and The Need for Establishing Standards for Future Studies

The fetal heart is fully developed and functioning by 55 days post-fertilisation and subsequent development is achieved by cell division, maturation of the cardiomyocyte, development of the conduction system, formation of gap junctions and calcium handling to enable optimal myocyte function and appropriate ventriculo-vascular coupling (15). The maturation of baroreceptor function and growth of conduit arteries and peripheral vascular beds and their regulation are determined during intrauterine life and in the early postnatal months. These processes can all be deregulated by fetal responses to an adverse environment including reduction in the provision of essential nutrients during critical periods of development, particularly in the last trimester of pregnancy. In an adverse state like this there is an increase in the proportion of cardiomyocytes with two nuclei that are incapable of further division (16) and thus it has been suggested that the total number of cardiomyocytes will be reduced for life in individuals suffered from certain factors which can lead to growth restriction. In addition to the fewer number of cardiomyocytes in adult life it is postulated that there is an in-utero implication of chronic hypoxemia characterized by reduction of long axis myocardial function that may be an early indicator of “in-utero” heart failure (15).

Virtually any maternal condition can cause a low estimated fetal weight via many pathways. Smoking, anemia, undernutrition, chronic systemic diseases, alcohol and infections can lead to impaired fetal growth and may trigger the mechanisms discussed earlier. Our main focus of interest will be to determine global longitudinal myocardial function with a novel approach “Feature Tracking”, which is validated in adult echocardiography (17, 18) and found its place in fetal echocardiography recently (19, 20), in an unselected cohort of fetuses. To ascertain possible variations between fetuses who follow a standart growth curve and fetuses compromised due to several conditions in future studies there are a) thorough investigation of the utilized methodology and its pitfalls and b) evaluation of non-diseased fetal hearts mandatory. In order to establish a reliable first step towards future research our work will function as a study which tries to depict factors possibly influencing global heart function and shed light on “normal” fetal cardiac state.

In the second half of the introduction we present the course fetal cardiac function assessment ran over the past decades and give a brief summary about steps taken towards more sophisticated evaluation methods. We will finish this chapter by outlining our study aims and proceed to the section “Methods and Study Population” where we explain the tissue tracking algorithm we used and describe our study collective in detail.

1.2. Current State of Ultrasonographic Evaluation of the Fetal Heart Function

1.2.1. Advances in Cardiac Function Assessment – Early Stages

M-Mode

The evaluation of fetal heart functional parameters has been a challenge for fetal echocardiographers as the journey of cardiac health assessment in-utero begun in the early 1970s when the first fetal M-Mode study was conducted (21). M-mode ultrasound was used to illustrate fetal arrhythmias and to measure ventricular chamber size, wall thickness, atrioventricular (AV) valve size and the dimensions of out-flow tracts (Figure 2).

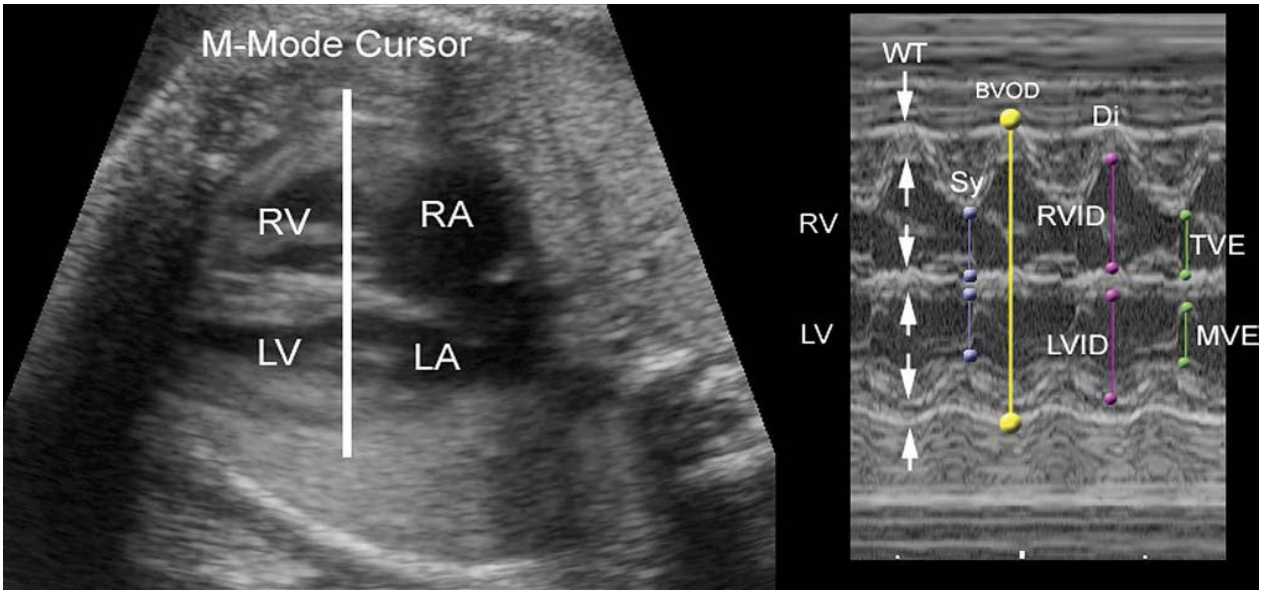

Fig.2. Recording of the M-mode from the four-chamber view. Measurements are made from the M-mode tracing at end-diastole (Di), which is identified as the point when the tricuspid and mitral valves close. End-systole (Sy) is defined as the maximal inward movement of the ventricular walls. Measurement of the maximal opening of the tricuspid valve (TVE) and mitral valve (MVE) occurs at the end of the rapid filling phase. BVOD, biventricular outer dimension; RVID, right ventricular inner dimension; LVID, left ventricular inner dimension; WT, wall thickness; RV, right ventricle; LV, left ventricle; RA, right atrium; and LA, left atrium. Figure and annotation from DeVore (22).

Measurements of cardiac dimensions were used to derive “fractional shortening” [(end-diastolic dimension - end-systolic dimension) / (end-diastolic dimension)] as an index of ventricular contractility, too. (23-25)

Cross-sectional Echocardiography

As the frequency of the transducers increased and the ability to record and evaluate B-mode images with the cine loop function evolved, accurate measurements utilizing B-mode real-time ultrasound became possible. (Figure 3) (26-29)

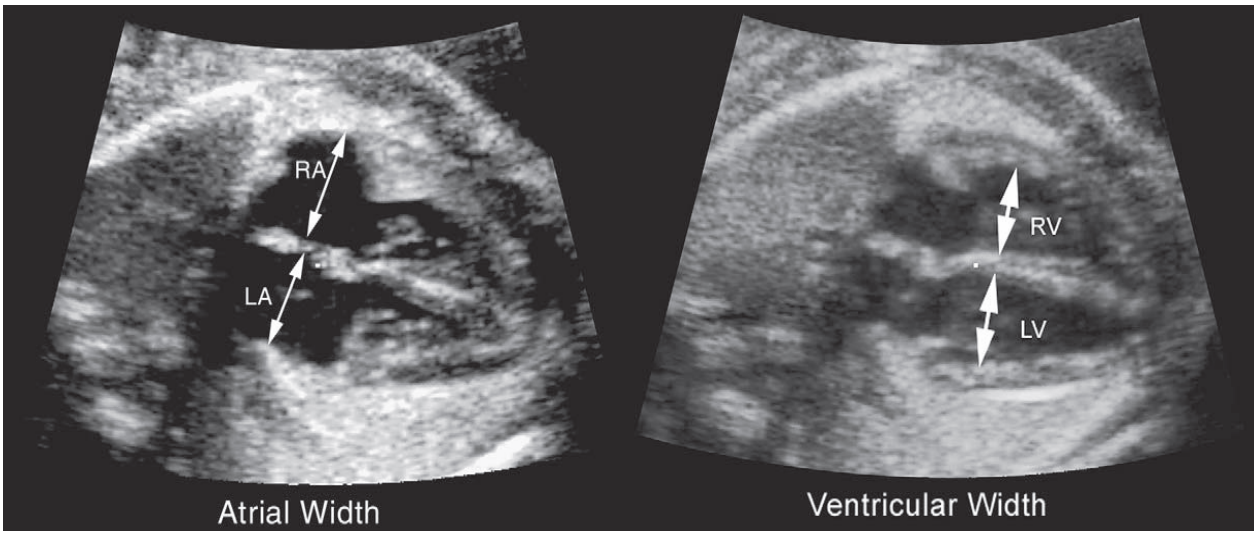

Fig.3. Measurements of the right atrial (RA), left atrial (LA), right ventricular (RV) and left ventricular (LV) chamber width. Atrial measurements are obtained at end-ventricular systole. Ventricular measurements are obtained at end-ventricular diastole Figure from Shapiro et al. (28).

1.2.2. Intracardiac Blod Flow Estimation with Pulsed Doppler as an Indicator of Diastolic Cardiac Function and Outflow Tract Doppler Examination as a Measure of Systolic Ventricular Function

The establishment of new transducer technologies and ability to receive Doppler signals from moving red blood cells resulted in great interest in flow velocity studies from early 1990s on. These studies were reflecting the myocardial properties indirectly in determining waveforms of the characteristic biphasic Doppler signal and reporting the progress in velocities over gestation (30, 31) (Figure 4). The well known E/A ratio was established in this era and determined as the relative rate of E component of the wave (rapid filling phase of diastole) to the A component (result of atrial contraction). Studies also have shown changes in the E/A ratio as a result of intra-uterine growth retardation, fetal inflammatory response syndrome, maternal diabetes and cardiac malformations (32-35).

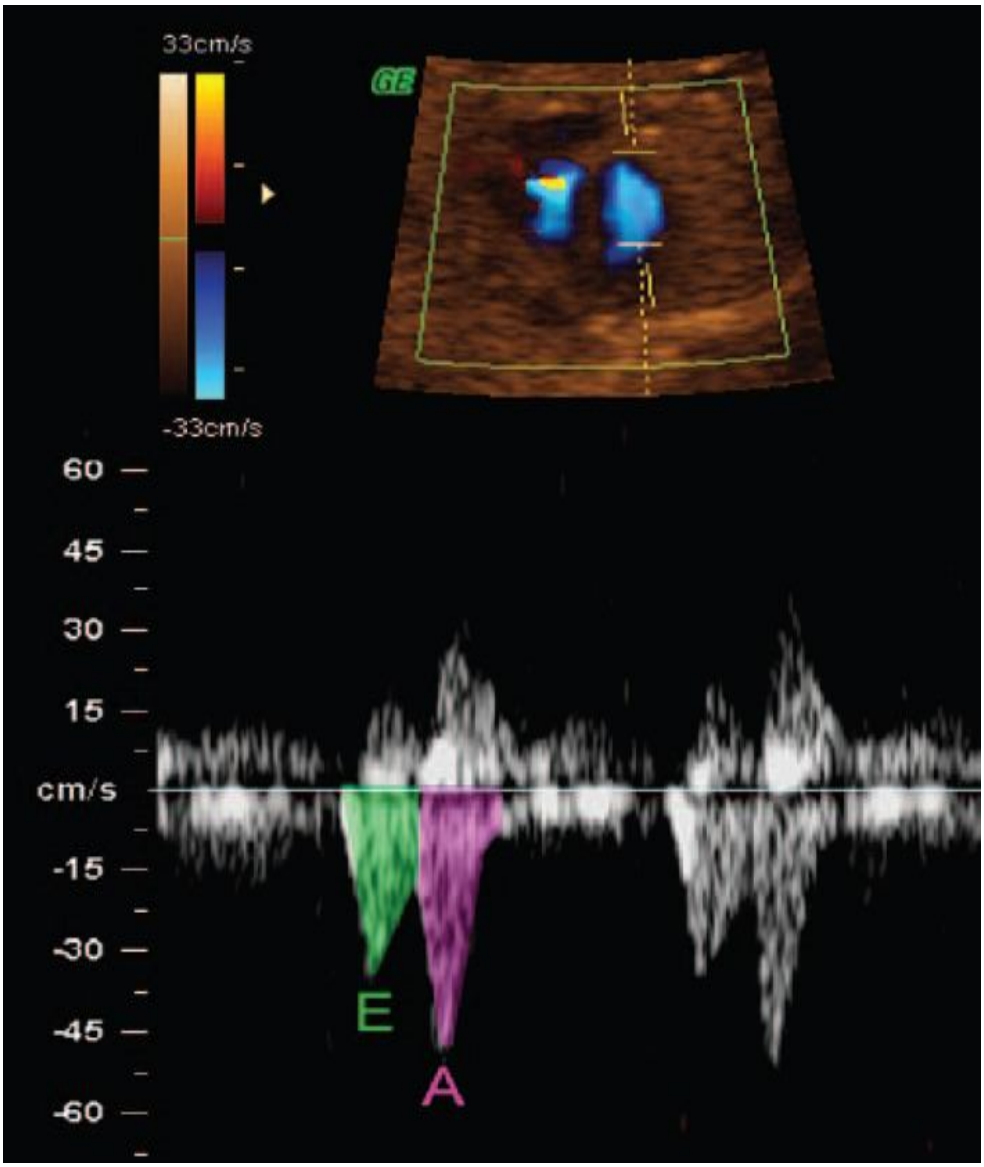

Fig.4. The sample volume is placed on to left ventricle. The spine is at 1 o`clock, the blue color of the Doppler signal indicates flow moving away from the transducer. E and A waves shown schematically. Figure from (22).

Parallel to AV valve Doppler recordings peak velocities for aorta and main pulmonary artery have been collected and it has been shown that maximum values increase as a function of gestational age. Time to peak velocity (TPV) (also known as acceleration time), which can be measured from the beginning of the waveform to the point of peak velocity, was increased in the aorta over the course of pregnancy, whereas TPV of pulmonary artery was shortened, attributed to the increase of resistance in the pulmonary trunk (35) (Figure 5). Investigators also calculated the cardiac output from the aorta and main pulmonary artery and found that it was increased during gestation (36, 37).

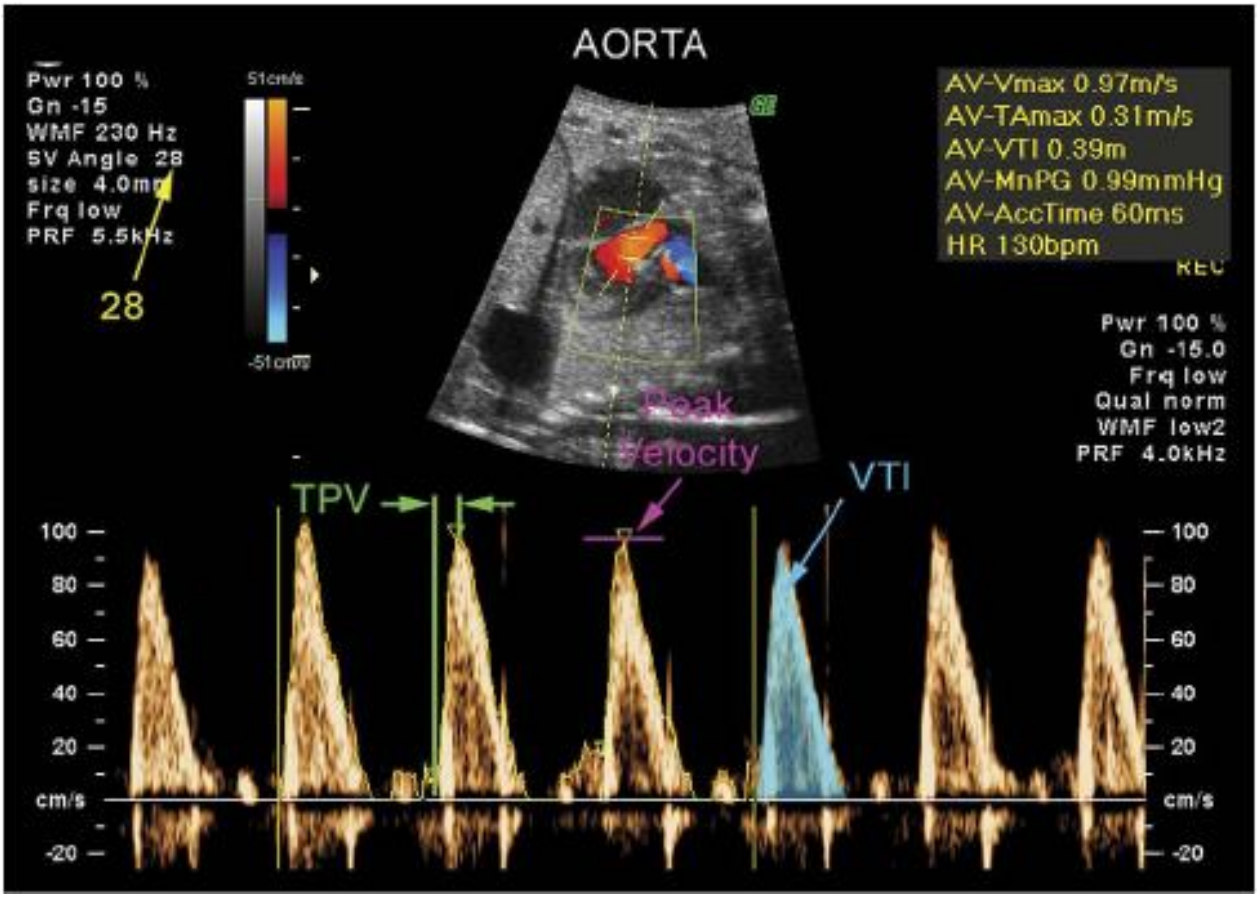

Fig.5. Representative figure for pulsed Doppler recording. Blood flow across the ascended aorta is being sampled. TPV; time to peak velocity or acceleration time and VTI, velocity time integral (a component of cardiac output). Notice the angle correction for aligning the blood flow to the Doppler beam (yellow arrow). Figure from (22).

The cardiac performance in growth restriction and fetal anemia constituted the curiosity of researchers once again, and it has been demonstrated that there is a decrease in cardiac output in fetuses with IUGR and prolonged hypoxia (38, 39).

1.2.3. Estimation of Myocardial Performance Index (MPI) as a Derivative of Doppler Studies

MPI is a echocardiographic measure of global (systolic and diastolic) ventricular performance and is independent of ventricular geometry and can be applied to both left and right ventricular function. It is calculated as follows; (ICT + IRT)/ET and is schematized on Figure 6.

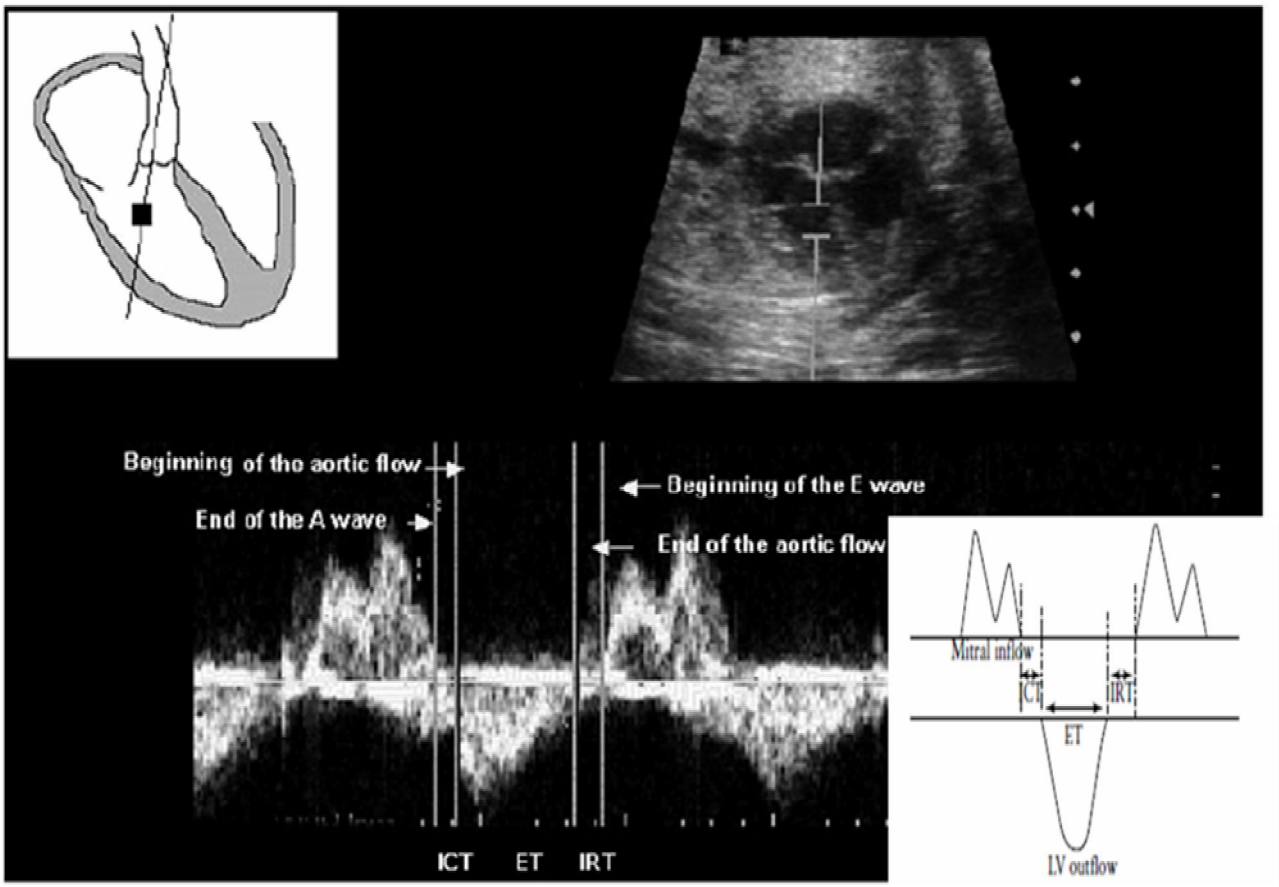

Fig.6. Doppler recording and schema of the myocardial performance index described by Friedman et al. (40). The sample volume is located in the left ventricle below the mitral valve. References for the time-period estimations are as follows: isovolumetric contraction time (ICT) from the end of the E/A waveform to the beginning of the aortic flow (AF), ejection time (ET) from the beginning to the end of the AF, and isovolumetric relaxation time (IRT), from the end of the AF to the beginning of the E/A waveform. The E/A waveform is always displayed as positive flow. Figure adapted from (40) and (41).

From the introduction of MPI by Tei et al in the mid 1990 on (42) there has been a continuously increasing number of fetal MPI studies with different study collectives (43-47). As a reproducible and relatively easily obtainable measure MPI is still popular in the arena of perinatal research. (48, 49).

1.2.4. Newer Imaging Modalities – 3D Fetal Echocardiography, Tissue Doppler Imaging, Doppler-Independent Tissue Tracking Modalities (Speckle Tracking and Feature Tracking)

3D Echocardiograhy for Fetal Ventricular Volume Estimation

While Doppler flow nomograms have been establish in 2D fetal echocardiography, many adult echocardiographic measures such as stroke volume, ejection fraction and cardiac output are based on end-systolic and end-diastolic ventricular volumes and are not validated in prenatal sonography (20, 50). Current applications for fetal ventricular volumetry include real-time volumetric data acquisition using matrix-array transducer technology, motion artifact elimination using Spatio-Temporal Image Correlation (STIC) and various display options which evaluated in normal and disordered hearts in great details in many studies. (50-56). Figure 7 illustrates an example to calculate ventricular volumes in fetal heart.

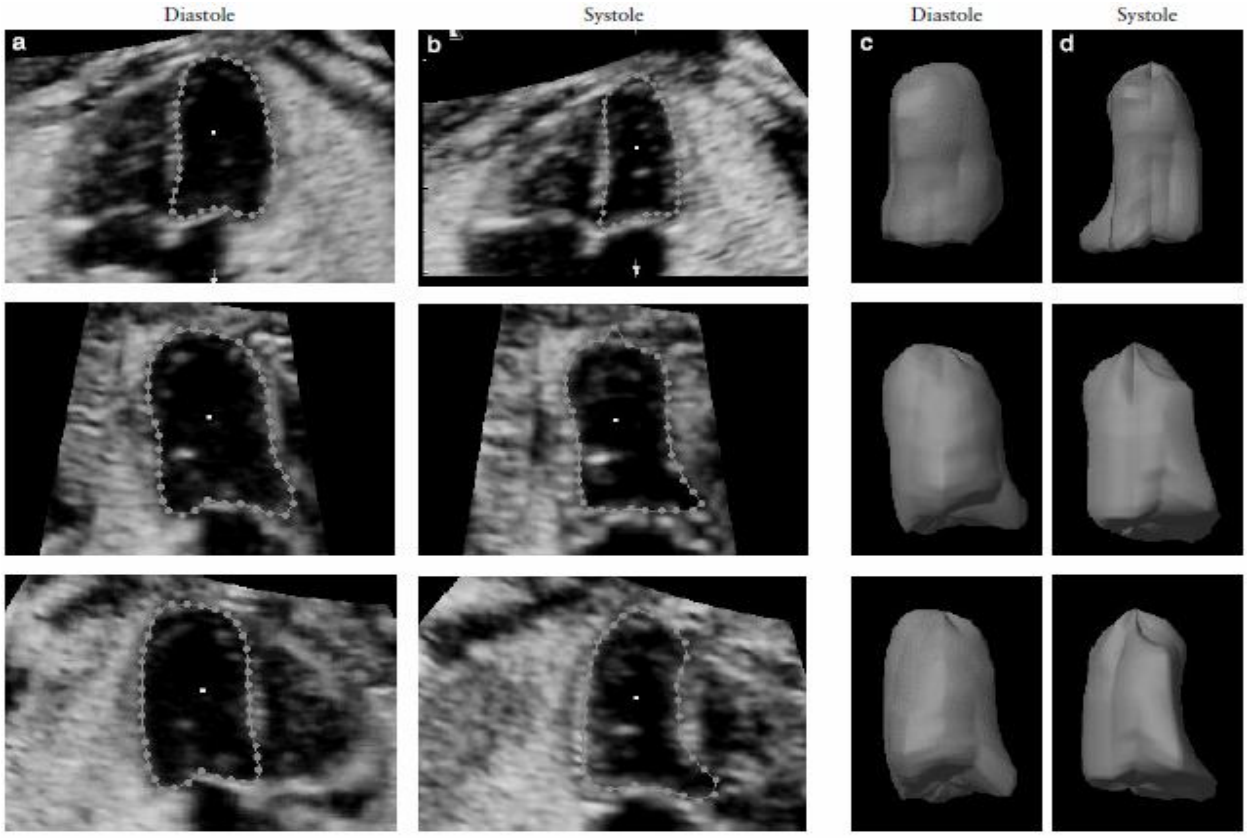

Fig.7. Three-dimensional ultrasound images of three different sections of the left ventricle in diastole (a) and systole (b) and the cardiac volumes derived from them using VOCAL (Virtual Organ Computer-aided AnaLysis) (c and d, respectively). Figure from Molina et al. (54).

Tissue Doppler Echocardiography (TDE)

This modality is used to derive cardiac indices for myocardial motion and contractility like velocity, strain and strain rate and expanded the ability to evaluate the function of the fetal heart. In the basic obstetrical setting, Doppler devices are designed to detect Doppler shifts from blood flow which is rapid in speed (around 20–200 cm/s) and weak in amplitude. Doppler shifts from slower-moving and stronger-echoing parts, such as the cardiac walls (with speed < 20 cm/s), are filtered to avoid interference. On the contrary, Doppler tissue imaging is designed to detect the Doppler shifts of relatively slow moving parts while filtering high-speed flow signals (57) (Figure 8).

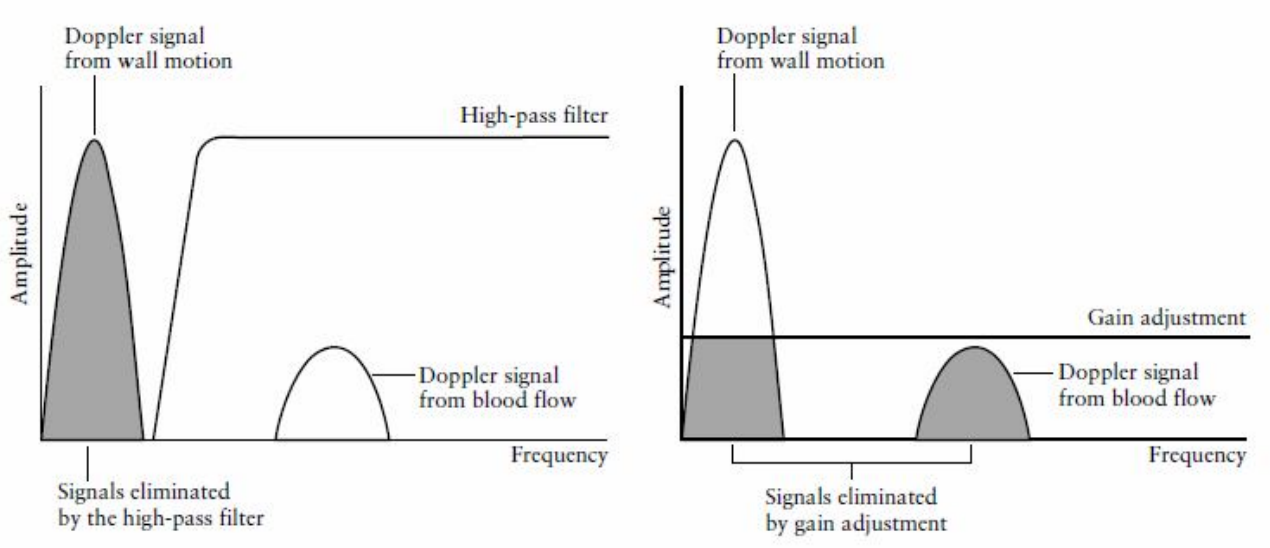

Fig.8. Doppler signals can be analyzed for frequency shifts, indicating the velocity and direction of motion (e.g. in blood flow Doppler velocimetry) or for the reflected signal amplitude (in power Doppler mode). The peak velocities of blood flow in the fetus vary, but are usually above 10 cm/s. As in B-mode, in color Doppler fluids reflect little energy. To display fetal blood flow Doppler signals, high receive gain settings and so-called 'wall' or 'high-pass' filters must be applied, the latter being required to eliminate high-intensity signals received from cardiac structures. In order to obtain TDE signals, the high-pass filter has to be reduced or switched off and only high amplitude signals (i.e. those requiring a low gain setting) should be displayed. Annotation and Figure from Tutschek et al. (57).

There are many studies in the literature dealing with the TDI in the fetal heart (57-66). First studies focused on demonstrating fetal heart movement and structure as a sequence of color changes [Color Doppler Myocardial Imaging (CDMI)] in the myocardium and derivation of velocities (Pulsed Wave Doppler) from basal and mid-portion free walls of left ventricle (LV) and right ventricle (RV) and basal and mid-part of

interventricular septum (57-60). After the initial publications, the first studies with great case numbers (62, 65) and a paper came on ventricular function in Twin to Twin Transfusion Syndrome and cardiomyopathy (61). In 2005 a new era has opened in prenatal research. Concepts such as Strain and Strain Rate Imaging by means of TDE were introduced into fetal echocardiography (63, 64). With this novel approach it was possible to quantify myocardial “deformation” properties since studies on velocity estimation were indicating the motion fetal heart showed but not reflecting the true contractility of the myocardium (65). Although these indices provide information on intrinsic myocardial properties, their application on fetal heart muscle was regional due to inherent dependency of the Doppler method on insonation angle and limitations in computational steps. These facts find their mirroring in publications in different sampling sizes and regions of interest utilized, which make the comparison of the data difficult (Figure 9).

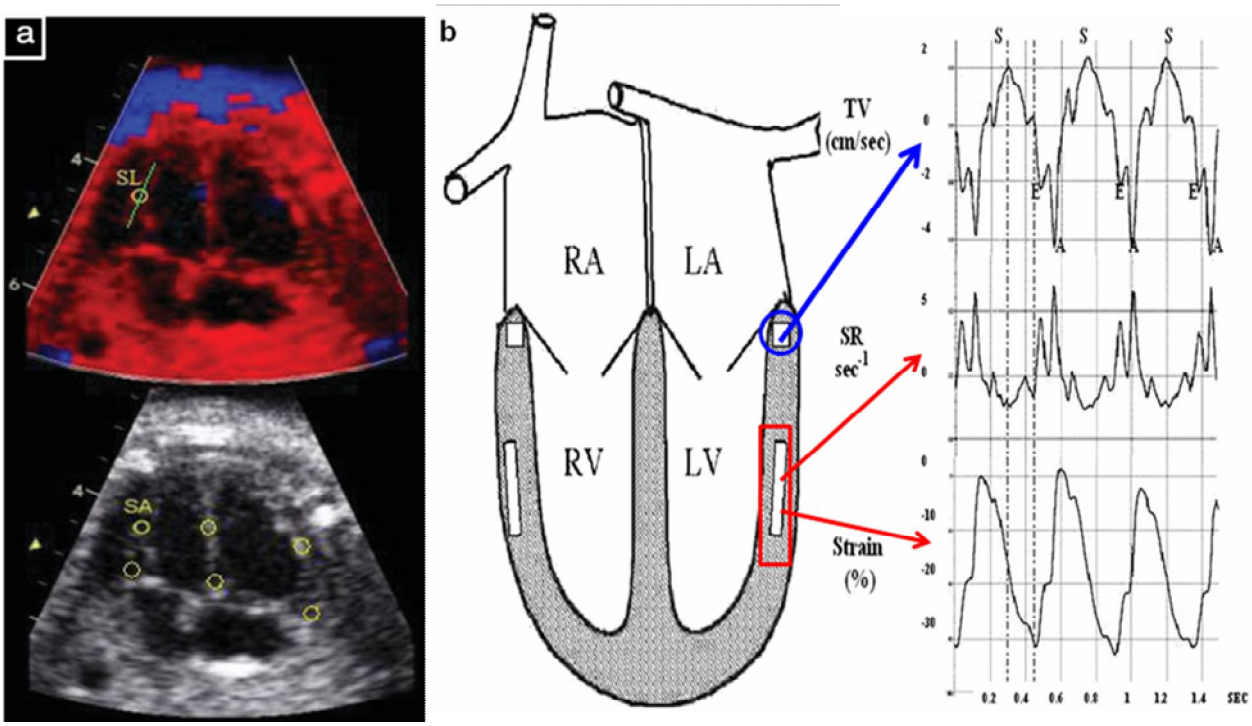

Fig 9. (a) 4Ch-view in B-mode with CDMI superimposed. Red indicates movement toward the transducer and blue away from the transducer. SA, sample area; SL, strain length. Yellow circles indicate several Regions of Interest (ROI). Although it is possible to deduce Velocity (TV) and Displacement from basal and mid-portion of ventricular walls, it is not feasible to calculate Strain and Strain Rate (SR) in the basal segments of the ventricle. Figure from (64). (b) Same limitation seen in this draft. Squares, the TV-ROI. Rectangles, S and SR. Despite superimposed curves note that they represent different ROIs. Figure from (66). Notice the different lengths of SA for computation.

2D Grey-scale Doppler-Independent Tissue Tracking Algorithms

To overcome the limitations Doppler-dependent studies exhibited, both in adult and in prenatal life, newer approaches are implemented into fetal echocardiography. (19, 20, 67 and 68) These innovative methods are “Speckle Tracking” and “Feature Tracking” algorithms. They both are independent of insonation angle, and with the help of them it is possible to obtain information in any chosen segment even in apical regions of the heart as shown in a pilot study from Barker et al. (20) In the light of the fact being able to gain information from every region, one may hypothesize that this holistic approach can provide more accurate information of the whole heart and give one a better insight into overall cardiac status, thus intrauterine well-being of the fetus.

1.3. Purposes of the Study

Our aims were:

1. To evaluate the feasibility and reproducibility of feature tracking algorithm in the assessment of global LV and RV function in a large cohort of hemodynamically stable fetuses.
2. To derive reference values for global peak longitudinal systolic and diastolic velocities, global peak longitudinal systolic strain, global peak longitudinal systolic and diastolic strain rate and global maximal longitudinal displacement of LV and RV in normal fetuses using tissue tracking echocardiography (TTE).
3. To assess the changes in parameters of motion and deformation during gestation.
4. To investigate the effect heart rate has on motion and deformation parameters.
5. To determine the relationship between ejection fraction and Strain as a measure of contractility hence as an indicator of pump function.
6. To evaluate the possibility whether TTE can be implemented to a routine fetal echocardiographic examination.

In the following section we will go into the details of our work by describing the properties of the study collective, analyzing the Feature Tracking Algorithm and highlighting the theoretical background and parameters investigated in this study.