ULTRASOUND in Obstetrics & Gynecology isuog

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

Journal:	*Ultrasound in Obstetrics and Gynecology*
Manuscript ID	Draft
Wiley - Manuscript type:	Original Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Herling, Lotta; Karolinska Institutet, Department of Clinical Science, Intervention and Technology - CLINTEC Johnson, Jonas; Center for Fetal Medicine, Department of Obstetrics and Gynecology, Karolinska University Hospital; School of Technology and Health, KTH Royal Institute of Technology, Department of Medical Engineering Ferm-Widlund, Kjerstin; Center for Fetal Medicine, Department of Obstetrics and Gynecology, Karolinska University Hospital Bergholm, Fredrik; School of Technology and Health, KTH Royal Institute of Technology, Department of Medical Engineering Elmstedt, Nina; School of Technology and Health, KTH Royal Institute of Technology, Department of Medical Engineering Lindgren, Peter; Center for Fetal Medicine, Department of Obstetrics and Gynecology, Karolinska University Hospital Sonesson, Sven-Erik; Pediatric Cardiology Unit, Department of Women's and Children's Health, Karolinska University Hospital Acharya, Ganesh; Karolinska Institutet, Department of Clinical Science, Intervention and Technology - CLINTEC; Karolinska University Hospital, Center for Fetal Medicine, Department of Obstetrics and Gynecology; UiT-The Arctic University of Norway, Women´s Health and Perinatology Research Group, Department of Clinical Medicine Westgren, Magnus; Karolinska Institutet, Department of Clinical Science, Intervention and Technology - CLINTEC; Center for Fetal Medicine, Department of Obstetrics and Gynecology, Karolinska University Hospital
Manuscript Categories:	Obstetrics
Keywords:	Fetal cardiac function, Tissue Doppler imaging, TDI, Automated analysis, Fetal echocardiography, Reference ranges, Fetal gender

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

Herling L[1,2], Johnson J[2,3], Ferm-Widlund K[2], Bergholm F[3], Elmstedt N[3], Lindgren P[2], Sonesson S-E[4], Acharya G[1,2,5], Westgren M[1,2]

[1] Department of Clinical Science, Intervention and Technology - CLINTEC, Karolinska Institute, Stockholm, Sweden

[2] Center for Fetal Medicine, Department of Obstetrics and Gynecology, Karolinska University Hospital, Stockholm, Sweden

[3] Department of Medical Engineering, School of Technology and Health, KTH Royal Institute of Technology, Stockholm, Sweden

[4] Pediatric Cardiology Unit, Department of Women's and Children's Health, Karolinska University Hospital, Stockholm, Sweden

[5] Women´s Health and Perinatology Research Group, Department of Clinical Medicine, UiT-The Arctic University of Norway, Tromsø, Norway

Email addresses:

LH: lotta.herling@sll.se

Center for Fetal Medicine, Department of Obstetrics and Gynecology, Karolinska University Hospital, Stockholm, Sweden. Telephone +46 703996968.

JJ: jjohn@kth.se

KFW: kjerstin.ferm-widlund@sll.se

FB: fredrik.bergholm@kth.se

NE: ninae@kth.se

PL: peter.lindgren@sll.se

SES: sven-erik.sonesson@ki.se

GA: ganesh.acharya@ki.se

MW: magnus.westgren@ki.se

Key words (5-8 words):

Fetal cardiac function, tissue Doppler imaging, automated analysis, fetal echocardiography, reference ranges, fetal gender

INTRODUCTION

Fetal echocardiography is established as a method of choice to detect structural heart anomalies *in utero*. It could also be used to assess fetal cardiac function and, consequently, improve our understanding of fetal cardiovascular physiology, cardiac maturation throughout gestation and fetal adaptation to various insults, for instance hypoxia. However, there is a need to develop simple, reliable methods to noninvasively evaluate fetal cardiac function both in research and in clinical practice.

Tissue Doppler imaging (TDI) is an echocardiographic technique that measures myocardial velocities providing a quantitative assessment of myocardial motion[1]. In adults, TDI is used as an early marker of cardiac dysfunction, and to predict mortality and morbidity associated with cardiovascular disease[2]. In fetuses, TDI has been attempted in order to assess fetal cardiac function both in normal pregnancies[3-6] and in pregnancies with complications such as intra-uterine growth restriction[7-9], maternal diabetes[10, 11] and intra-amniotic infection[12].

To improve the understanding of maturational changes in the fetal myocardium and to adequately evaluate normal and pathological function throughout pregnancy, reference ranges adjusted to gestational age are needed. There are two main forms of TDI, i.e. spectral TDI (sTDI) and color TDI (cTDI). There are several publications describing myocardial velocities throughout pregnancy using sTDI[3, 4, 6, 13] but the available information on cTDI is less. Increasing myocardial velocities with advancing gestational age have been described using cTDI and there have been attempts to describe cardiac cycle time intervals[5, 14, 15]. However, the off-line analysis of myocardial velocity traces generated using cTDI cine-loops is cumbersome and time-consuming, and there is a need to improve and simplify the analysis. Our group has previously demonstrated the feasibility of an automated algorithm for the analysis of cTDI myocardial velocity traces[16] and used this to evaluate fetal cardiac function in postdate pregnancies[17]. In the present study, we hypothesized that automated analysis could be used throughout the second half of pregnancy to measure parameters describing fetal cardiac function.

The aim of this study was to evaluate maturational changes in fetal cardiac function and construct gestational age specific reference intervals in the second half of pregnancy, using a newly developed automated method to analyze cTDI recordings.

METHODS

This prospective, cross-sectional study was conducted between September 2009 and August 2011 at the Center for Fetal Medicine, Karolinska University Hospital, Sweden. A part of this material was previously used to construct reference ranges using a manual method of assessment[5]. Women with uncomplicated, singleton pregnancies were included between 18 and 42 weeks of gestation. Exclusion criteria were maternal complications such as pre-eclampsia, chronic hypertension or diabetes at inclusion or fetal chromosomal or major structural abnormalities discovered during pregnancy or at a postnatal examination. Pregnancies were dated by measuring biparietal diameter in the second trimester according to local guidelines[18, 19]. Each fetus was included only once.

cTDI recordings were performed by an experienced ultrasonographer (KFW) by using a GE Vivid-i ultrasound imaging system with a 3S-RS (1.9 - 3.8 MHz) phased array transducer (GE Vingmed, Horten, Norway). An apical or basal four-chamber view of the fetal heart was acquired and cine-loops of consecutive cardiac cycles were recorded using cTDI. The insonation angle was kept as close to the long-axis of the heart as possible (always <30^{o}) and the image adjusted to obtain as high frame rate as possible. Later off-line analysis was performed in EchoPAC version 201 (GE Vingmed Ultrasound AS, Horten, Norway). The region of interest (ROI) was adjusted in height and width to gestational age according to previous research[16] and placed at the atrioventricular plane (AV-plane) in the left ventricular (LV), right ventricular (RV) and septal walls of the fetal heart (Figure 1). All ROIs were placed by one operator (LH). Usually 10-11 (range 3-25) cardiac cycles, depending on the heart rate and the length of the cine-loops, were analyzed using a three-sample moving average filter, to construct myocardial velocity traces.

The myocardial velocity traces generated were transferred to MATLAB (R2015a, MathWorks, MA, USA) where they were analyzed by an automated algorithm developed by FB and JJ[20-23]. Once the velocity traces were transferred this was a fully automated procedure that did not require any manual marking of the traces. The algorithm was constructed as a rule-based system that involves pattern recognition and several adaptive processes. The beginning and the end of the cardiac cycle is estimated by the algorithm and it identifies cardiac time intervals based on shifts in mechanical work according to the *Dynamic Adaptive Piston Pump (DAPP)* technology that describes the heart as a mechanical pump controlled by its inflow[24, 25]. The cardiac phases are called mechanical cardiac time intervals referring to the mechanical definition of the delineating time points/events. The mechanical cardiac time intervals are identified without the need for a concurrent ECG signal. According to *DAPP* technology the movement of the AV-piston (AV-plane) initiates the mechanical functioning of the heart and, therefore, the atrial contraction is considered as the starting point of the cardiac cycle in analogy to the ECG starting with the p-wave. The cardiac cycle was divided into six mechanical cardiac time intervals defined as atrial contraction, pre-ejection, ventricular ejection, post-ejection, rapid filling/early diastole and slow filling/diastasis (Figure 2). The terms pre-and post-ejection are used instead of isovolumic contraction and relaxation as the definition of the time intervals is based on shifts in myocardial mechanical work rather than blood flow events. The atrial contraction, pre-ejection, ventricular ejection and post-ejection periods were measured. The myocardial performance index derived from cTDI (cMPI) was calculated as (pre-ejection + post-ejection)/ventricular ejection time. The peak myocardial velocities during atrial contraction (Am), ventricular ejection/systole (Sm) and rapid filling/early diastole (Em) were automatically derived by the software and the ratio of Em/Am was calculated. All measurements were performed for the LV, RV and septal walls separately.

All cardiac cycles available were evaluated and an average of all recorded cycles was used for analysis. The algorithm was run repeatedly to verify that the exactly same results were obtained each time.

The inter- and intra-observer variability of the ultrasound examination was assessed using a Vivid S6 ultrasound imaging system with a M4S-RS (1.9 - 4.1 MHz) phased array transducer (GE CV Ultrasound, Haifa, Israel). The inter-observer variability was evaluated by two ultrasonographers (KFW and LH) examining 25 patients from December 2016 to February 2017. The intra-observer variability was evaluated by comparing two separate recordings made by the same operator (KFW) approximately 5-10 minutes apart in 22 patients. Patients were examined between 19 and 41 weeks of gestation. All ROIs were placed by one operator and all generated myocardial velocity traces were analyzed by the automated algorithm.

Statistical analysis

Data analysis was performed using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, N.Y., USA) and MATLAB (R2015a, MathWorks, MA, USA). Continuous variables were presented as mean (± SD) or median (interquartile range) as appropriate. Categorical variables were presented as absolute values and percentage (n (%)). We used the statistical analysis described by Royston and Wright to create reference intervals[26]. Box-Cox power transformation was used to decide adequate type of transformation. The chosen transformations were none, decadic logarithm and square root transformation. Linear polynomial regression was used to estimate the relationship between the studied variables and gestational age (GA), expressed in exact weeks (decimal days). Normal distribution of absolute standardized residuals was checked with Shapiro-Wilk's test. Mean and SD curves as a function of GA were calculated and plotted. The equations of the polynomial regression were used to calculate the fitted mean, 5th and 95th centiles for the corresponding GA as fitted mean ± 1.645 SD. Confidence intervals were calculated for the fitted mean.

Spearman's rho was used to assess correlation between cardiac parameters and GA. A sub-analysis using Mann-Whitney *U*-test was performed comparing cardiac parameters between female and male fetuses. Coefficients of variation (CV) within subjects were calculated[27].

Ethical approval of the study was obtained from the Stockholm Regional Ethics Committee (DNr 2009/1617-31/2 and 2017/539-32). Informed oral as well as written consents were obtained from all participants.

RESULTS

Maternal baseline characteristics and pregnancy outcome are presented in Table 1. A total of 202 women were included. In three women outcome data were not available because of delivery in other hospitals. One baby had muscular ventricular septal defects and a bicuspid aortic valve detected postnatally and was excluded from analysis, resulting in a final study population of 201 women.

The four-chamber view was possible to obtain in all pregnancies examined. The fetal heart was apical in 107 (53.2%) and basal in 94 (46.8%) of the analyzed cine-loop recordings. The mean frame rate was 208 (±6.8) frames/sec with 7 (3.5%) <200 frames/sec and the minimum 171 frames/sec. The mean heart rate was 139 (±11.0) beats/min. A total of 603 myocardial velocity traces (201 each from LV, RV and septal walls) were available for analysis. After visual inspection one myocardial velocity trace was omitted completely 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 the RV Em in eleven cases due to fusion of the Em and Am waves. The final number of observations per parameter are presented in Tables 2 and 3.

At 18 weeks of gestation the peak myocardial velocities (cm/s) presented as mean with 95% confidence intervals were: LV Am 3.39 (3.09-3.70), LV Sm 1.62 (1.46-1.79), LV Em 1.95 (1.75-2.15), septal Am 3.07 (2.80-3.36), septal Sm 1.93(1.81-2.06), septal Em 2.57 (2.32-2.84), RV Am 4.89 (4.59-5.20), RV Sm 2.31 (2.16-2.46) and RV Em 2.94 (2.69-3.21). At 42 weeks of gestation the peak myocardial velocities (cm/s) had increased to: LV Am 4.25 (3.87-4.65), LV Sm 3.53 (3.19-3.89), LV Em 4.55 (4.18-4.94), septal Am 4.49 (4.17-4.82), septal Sm 3.36 (3.17-3.55), septal Em 3.76 (3.51-4.03), RV Am 6.52 (6.09-6.96), RV Sm 4.95 (4.59-5.32) and RV Em 5.42 (4.99-5.88).

The best model for all parameters was a first-degree linear polynomial after normalization. All myocardial velocities showed an increase with GA (Figure 3). The LV and RV Em/Am ratio also increased with GA. The mechanical cardiac time intervals generally remained more stable throughout pregnancy. There was an increased duration with GA in septal and RV atrial contraction, LV pre-ejection and septal and RV ventricular ejection time. There was a decrease in duration in septal post-ejection (Figure 4). Septal cMPI decreased with gestation whereas LV and RV walls remained stable (Figure 5). Details and regression equations are demonstrated in Tables 2 and 3. Scatterplots of peak myocardial velocities, Em/Am ratios, mechanical cardiac time intervals and cMPI, plotted against GA with mean, 5^{th}, 95^{th} centiles are displayed in Figures 3, 4 and 5. Z-scores and Q-Q plots are provided in the supporting information.

All peak myocardial velocities showed positive correlations with GA. The weakest correlation was seen in LV Am (rho=0.172; $p<0.05$). The remainder of myocardial velocities showed positive correlations with rho=0.303-0.598; $p<0.001$. The LV and RV Em/Am ratios showed positive correlations with GA (rho=0.444 and 0.294; $p<0.001$). Positive correlations with GA were also seen among a few cardiac time intervals, i.e. LV pre-ejection (rho=0.209; $p<0.05$), septal atrial contraction (rho=0.248; $p<0.001$), septal ventricular ejection (rho=0.278; $p<0.001$), RV atrial contraction (rho=0.168; $p<0.05$) and RV post-ejection (rho=0.193; $p<0.05$). Only the septal post-ejection time (rho=-0.164; $p<0.05$) and the septal cMPI (rho=-0.255; $p<0.001$) were negatively correlated with GA.

There was a difference between female and male fetuses in septal and RV Am, Sm at all locations, septal and RV atrial contraction time, LV pre-ejection and septal ventricular ejection time. Details are presented in supporting information (Table S1).

When the algorithm was run repeatedly exactly the same results were obtained.

The inter- and intra-observer variability of ultrasound examination showed the lowest CVs for both inter- and intra-variability for ventricular ejection time (8.3-13.7%). The pre-ejection period was the time interval with the highest CV (28.0-35.2%). The lowest CVs for peak myocardial velocities were

found in intra-variability in the septal Sm (11.8%) followed by inter-variability in RV Am (13.0%). CVs for peak myocardial velocities were highest in the intra-variability LV Am and LV Sm (43.1 and 34.5%). Details about inter- and intra-variability are supplied in the supporting information (Table S2).

DISCUSSION

This study shows that it is possible to use an automated algorithm to facilitate the analysis of myocardial velocity traces obtained by cTDI to evaluate fetal cardiac function throughout the second half of pregnancy as it was previously shown for term fetuses[17]. We provide reference ranges adjusted to gestational age for peak myocardial velocities, Em/Am ratios, mechanical cardiac time intervals and cMPI, that were calculated using the automated method. All myocardial velocities showed an increase with gestation.

The increasing myocardial velocities especially during ventricular ejection and during early diastole but to a lesser degree during atrial contraction confirm previous findings using TDI[3-5, 13, 14]. This illustrates fetal cardiac maturation throughout pregnancy where the relative contribution of the atrial contraction/kick gets less prominent as the relaxation and, thus, early ventricular filling gradually increases. In contrast to our study, a previous report has suggested a non-linear maturation[13] of some fetal cardiac function parameters. These differences in results could be explained by the different TDI techniques used, the population studied and the choice of regression equations.

The mechanical cardiac time intervals did not change throughout pregnancy in the same way as the myocardial velocities. Less is known about the fetal cardiac cycle time intervals, but 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[28]. Previous studies exploring cardiac time intervals using TDI have also used other definitions than the ones we used with the automated algorithm, which at least partly explains the longer pre- and post-ejection periods in the present study[5, 15].

There was a difference between female and male fetuses in peak myocardial velocities during ventricular ejection and in the RV and septal wall during atrial contraction/kick where male fetuses demonstrated higher peak myocardial velocities. Differences were also seen in septal and RV atrial contraction time, LV pre-ejection and septal ventricular ejection time. The majority of these time intervals were the same that demonstrated changes with GA. These gender differences observed in 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.

In this study, we have constructed gestational age specific, cross-sectional reference ranges using an automated analysis of myocardial velocity traces in normal pregnancies. The major advantage is that the exactly same results are obtained when the algorithm is run repeatedly. Another advantage of using automated analysis is that it diminishes the subjectivity in the delineation of cardiac time intervals which is often a problem with TDI as well as with MPI derived from investigation of blood flow[29]. Furthermore, when using the algorithm considerably less time is required for the analysis (from minutes to milliseconds) which would facilitate the use of cTDI in larger groups of patients.

The CV for the whole procedure from the echocardiography to the automated analysis was substantial (8.3-43.1%) as expected. When interpreting this variation, it is important to take into consideration the biological variation, varying fetal state as well as the subjectivity of ROI placement. This variation proves the importance of automated analysis that will minimize variation in the assessment of the myocardial velocity trace. It also emphasizes the need for further development of automation procedure, such as automated ROI placement. Further refinements of the algorithm, and the possibility of on-line use in the ultrasound machine could improve results and reduce the variability in the assessment of variables describing fetal cardiac function. As the automated method of analysis progressively gets more stable the aim is to assess if cTDI with automated analysis can be used to discriminate between normal and pathological conditions.

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 the gestation. However, this is to our knowledge the largest study describing cardiac function using cTDI[5, 14, 15].

CONCLUSION

This study shows that it is possible to use an automated algorithm for the analysis of myocardial velocity traces throughout gestation and that it can facilitate the construction of reference ranges describing cardiac function and its evolution during pregnancy. However, further development and refinements are needed to evaluate if this method could be used to differentiate between normal and pathological fetal condition/cardiac function.

ACKNOWLEDGMENTS

This study was supported by grants provided by the Swedish Heart-Lung Foundation and Stockholm County Council (ALF project). The *DAPP* technology was invented by the recently deceased Stig Lundbäck.

CONFLICT OF INTEREST

JJ is a member of the Board in Gripping Heart AB.

REFERENCES

1. Comas M, Crispi F. Assessment of fetal cardiac function using tissue Doppler techniques. *Fetal Diagn Ther*. 2012;**32**(1-2):30-8.
2. Boyd AC, Schiller NB, Thomas L. Principles of transthoracic echocardiographic evaluation. *Nat Rev Cardiol*. 2015;**12**(7):426-40.
3. Comas M, Crispi F, Gomez O, Puerto B, Figueras F, Gratacos E. Gestational age- and estimated fetal weight-adjusted reference ranges for myocardial tissue Doppler indices at 24-41 weeks' gestation. *Ultrasound Obstet Gynecol*. 2011;**37**(1):57-64.
4. Chan LY, Fok WY, Wong JT, Yu CM, Leung TN, Lau TK. Reference charts of gestation-specific tissue Doppler imaging indices of systolic and diastolic functions in the normal fetal heart. *Am Heart J*. 2005;**150**(4):750-5.

5. Elmstedt NN, Johnson JJ, Lind BB, Ferm-Widlund KK, Herling LL, Westgren MM, Brodin LA. Reference values for fetal tissue velocity imaging and a new approach to evaluate fetal myocardial function. *Cardiovasc Ultrasound*. 2013;**11**(1):29.
6. Harada K, Tsuda A, Orino T, Tanaka T, Takada G. Tissue Doppler imaging in the normal fetus. *Int J Cardiol*. 1999;**71**(3):227-34.
7. Larsen LU, Petersen OB, Sloth E, Uldbjerg N. Color Doppler myocardial imaging demonstrates reduced diastolic tissue velocity in growth retarded fetuses with flow redistribution. *Eur J Obstet Gynecol Reprod Biol*. 2011;**155**(2):140-5.
8. Larsen LU, Sloth E, Petersen OB, Pedersen TF, Sorensen K, Uldbjerg N. Systolic myocardial velocity alterations in the growth-restricted fetus with cerebroplacental redistribution. *Ultrasound Obstet Gynecol*. 2009;**34**(1):62-7.
9. Comas M, Crispi F, Cruz-Martinez R, Martinez JM, Figueras F, Gratacos E. Usefulness of myocardial tissue Doppler vs conventional echocardiography in the evaluation of cardiac dysfunction in early-onset intrauterine growth restriction. *Am J Obstet Gynecol*. 2010;**203**(1):45 e1-7.
10. Balli S, Pac FA, Ece I, Oflaz MB, Kibar AE, Kandemir O. Assessment of cardiac functions in fetuses of gestational diabetic mothers. *Pediatr Cardiol*. 2014;**35**(1):30-7.
11. Hatem MA, Zielinsky P, Hatem DM, Nicoloso LH, Manica JL, Piccoli AL, Zanettini J, Oliveira V, Scarpa F, Petracco R. Assessment of diastolic ventricular function in fetuses of diabetic mothers using tissue Doppler. *Cardiol Young*. 2008;**18**(3):297-302.
12. Di Naro E, Cromi A, Ghezzi F, Giocolano A, Caringella A, Loverro G. Myocardial dysfunction in fetuses exposed to intraamniotic infection: new insights from tissue Doppler and strain imaging. *Am J Obstet Gynecol*. 2010;**203**(5):459.e1-7.
13. Gardiner HM, Pasquini L, Wolfenden J, Barlow A, Li W, Kulinskaya E, Henein M. Myocardial tissue Doppler and long axis function in the fetal heart. *Int J Cardiol*. 2006;**113**(1):39-47.
14. Nii M, Roman KS, Kingdom J, Redington AN, Jaeggi ET. Assessment of the evolution of normal fetal diastolic function during mid and late gestation by spectral Doppler tissue echocardiography. *J Am Soc Echocardiogr*. 2006;**19**(12):1431-7.
15. Willruth A, Steinhard J, Enzensberger C, Axt-Fliedner R, Gembruch U, Doelle A, Gorissen W, Fimmers R, Bahlmann F. Fetal colour tissue Doppler imaging (cTDI): biventricular reference ranges for the time segments of the cardiac cycle in second and third trimesters of gestation. *Arch Gynecol Obstet*. 2016.
16. Herling L, Johnson J, Ferm-Widlund K, Lindgren P, Acharya G, Westgren M. Automated analysis of color tissue Doppler velocity recordings of the fetal myocardium using a new algorithm. *Cardiovasc Ultrasound*. 2015;**13**:39.
17. Herling L, Johnson J, Ferm-Widlund K, Bergholm F, Lindgren P, Sonesson SE, Acharya G, Westgren M. Automated analysis of fetal cardiac function using color tissue Doppler imaging. *Ultrasound Obstet Gynecol*. 2017.
18. Saltvedt S, Almstrom H, Kublickas M, Reilly M, Valentin L, Grunewald C. Ultrasound dating at 12-14 or 15-20 weeks of gestation? A prospective cross-validation of established dating formulae in a population of in-vitro fertilized pregnancies randomized to early or late dating scan. *Ultrasound Obstet Gynecol*. 2004;**24**(1):42-50.
19. Selbing A, Kjessler B. Conceptual dating by ultrasonic measurement of the fetal biparietal diameter in early pregnancy. *Acta Obstet Gynecol Scand*. 1985;**64**(7):593-7.
20. Lundback S, Johnson J, Bergholm F. A cardiac state system. US patent WO 2016022052 A1. 2016.
21. Lundback S, Johnson J. State machine user and validation interface system. US patent 8,943,429. 2008.
22. Bergholm F. En algoritm för att finna maxima och minima i "periodisk" kolvliknande rörelse. ISBN: 978-91-7729-245-6. Stockholm, Sweden: KTH Technology and Health; 2016.
23. Bergholm F. Kommentarer kring hastighetssignaler från AV-planets rörelse som är uppmätta i foster. ISBN: 978-91-7729-246-3. Stockholm, Sweden: KTH Technology and Health; 2016.

24. Lundback S. Cardiac pumping and function of the ventricular septum. *Acta Physiol Scand Suppl*. 1986;**550**:1-101.
25. Carlsson M, Ugander M, Mosen H, Buhre T, Arheden H. Atrioventricular plane displacement is the major contributor to left ventricular pumping in healthy adults, athletes, and patients with dilated cardiomyopathy. *Am J Physiol Heart Circ Physiol*. 2007;**292**(3):H1452-9.
26. Royston P, Wright EM. How to construct 'normal ranges' for fetal variables. *Ultrasound Obstet Gynecol*. 1998;**11**(1):30-8.
27. Hyslop NP, White WH. Estimating precision using duplicate measurements. *J Air Waste Manag Assoc*. 2009;**59**(9):1032-9.
28. Batterham A, Shave R, Oxborough D, Whyte G, George K. Longitudinal plane colour tissue-Doppler myocardial velocities and their association with left ventricular length, volume, and mass in humans. *Eur J Echocardiogr*. 2008;**9**(4):542-6.
29. Maheshwari P, Henry A, Welsh AW. The Fetal Modified Myocardial Performance Index: Is Automation the Future? *Biomed Res Int*. 2015;**2015**:215910.

Table 1 Maternal characteristics and pregnancy outcome (n=201)

Parameter	
Maternal data	
Maternal age (years)	30.1 ± 5.2
Maternal BMI (kg/m^2)	23.7 ± 3.9
Nulliparous	81 (40.3)
Smoker	12 (6.0)
Pregnancy outcomes	
GA at delivery (weeks)	40.0 ± 1.4
Mode of delivery:	
Normal vaginal delivery	147 (74.2)
Vacuum extraction	22 (11.1)
Cesarean section	29 (14.6)
Birth weight (g)	3576 ± 501
Female babies	96 (48.5)
Cord arterial pH (n=138)	7.25 ± 0.09
Cord venous pH (n=49)	7.32 ± 0.07
5-min Apgar score <7	0 (0.0)
Pre-eclampsia	4 (2.0)
Pre-term delivery <37 weeks	4 (2.0)

Data are given as mean ± SD or *n* (%). BMI, body mass index; GA, gestational age.

Table 2 Regression equations for peak myocardial velocities obtained by color tissue Doppler imaging and automatically analyzed

Parameter	*n*	*Fitted equation*	*a*	*b*	*SE-b*	*p-b*	R^2*adj.*
Mean of peak myocardial velocities (cm/s)							
Am							
LV	193	a + b GA	1.675	0.009	0.003	0.005	0.036
Sept	201	a + b GA	1.478	0.015	0.003	<0.001	0.121
RV	187	a + b GA	1.955	0.014	0.003	<0.001	0.119
Sm							
LV	193	a + b GA	0.817	0.025	0.003	<0.001	0.285
Sept	201	a + b GA	1.059	0.018	0.002	<0.001	0.357
RV	199	a + b GA	0.991	0.029	0.002	<0.001	0.431
Em							
LV	193	a + b GA	0.840	0.031	0.003	<0.001	0.365
Sept	201	a + b GA	1.352	0.014	0.003	<0.001	0.116
RV	187	a + b GA	1.255	0.026	0.003	<0.001	0.264
SD of peak myocardial velocities (cm/s)							
Am							
LV	193	a + b GA	0.172	0.005	0.002	0.032	
Sept	201	a + b GA	0.253	0.001	0.002	0.724	
RV	187	a + b GA	0.109	0.006	0.002	0.007	
Sm							
LV	193	a + b GA	0.005	0.010	0.002	<0.001	
Sept	201	a + b GA	0.100	0.002	0.001	0.109	
RV	199	a + b GA	-0.054	0.010	0.001	<0.001	
Em							
LV	193	a + b GA	0.081	0.007	0.002	0.002	
Sept	201	a + b GA	0.335	-0.002	0.002	0.280	
RV	187	a + b GA	0.153	0.005	0.002	0.055	
Mean of Em/Am ratio							
LV	193	a + b GA	-0.413	0.011	0.002	<0.001	0.166
Sept	201	a + b GA	-0.081	0.000	0.002	0.939	-0.005
RV	187	a + b GA	-0.314	0.005	0.002	<0.001	0.060
SD of Em/Am							
LV	193	a + b GA	0.206	-0.001	0.001	0.291	
Sept	201	a + b GA	0.224	-0.002	0.001	0.081	
RV	187	a + b GA	0.118	0.001	0.001	0.552	

n = number of observations; LV, left ventricular wall; Sept, septal wall; RV, right ventricular wall; Am, peak myocardial velocity during atrial contraction; Sm, peak myocardial velocity during ventricular ejection (systole); Em, peak myocardial velocity during early diastole; GA, gestational age; SE, standard error; SD, standard deviation; p, p-value; R2adj., adjusted R-squared.
Peak myocardial velocities were square root transformed and Em/Am log transformed (base 10).

Table 3 Regression equations for mechanical cardiac time intervals and myocardial performance index obtained by color tissue Doppler imaging and automatically analyzed

Parameter	*n*	*Fitted equation*	*a*	*b*	*SE-b*	*p-b*	*R^2adj.*
Mean of mechanical cardiac time intervals (ms)							
Atrial contraction							
LV	191	a + b GA	60.452	-0.057	0.133	0.668	-0.004
Sept	201	a + b GA	48.159	0.788	0.220	<0.001	0.056
RV	198	a + b GA	60.348	0.287	0.108	0.008	0.030
Pre-ejection							
LV	192	a + b GA	40.112	0.418	0.151	0.006	0.034
Sept	201	a + b GA	42.407	-0.238	0.153	0.121	0.007
RV	198	a + b GA	45.262	0.203	0.169	0.231	0.002
Ventricular ejection							
LV	191	a + b GA	157.672	-0.256	0.171	0.136	0.007
Sept	195	a + b GA	132.539	0.819	0.198	<0.001	0.077
RV	195	a + b GA	147.209	0.471	0.159	0.004	0.038
Post-ejection							
LV	191	a + b GA	85.050	-0.281	0.191	0.144	0.006
Sept	195	a + b GA	101.032	-0.568	0.253	0.026	0.020
RV	187	a + b GA	65.826	0.174	0.130	0.183	0.004
SD of of mechanical cardiac time intervals (ms)							
Atrial contraction							
LV	191	a + b GA	11.049	0.067	0.081	0.508	
Sept	201	a + b GA	23.194	-0.022	0.129	0.894	
RV	198	a + b GA	8.076	0.106	0.060	0.161	
Pre-ejection							
LV	192	a + b GA	23.576	-0.286	0.095	0.017	
Sept	201	a + b GA	8.407	0.252	0.088	0.024	
RV	198	a + b GA	4.104	0.443	0.105	0.001	
Ventricular ejection							
LV	191	a + b GA	12.107	0.154	0.107	0.251	
Sept	195	a + b GA	17.292	0.090	0.119	0.547	
RV	195	a + b GA	-6.845	0.824	0.092	<0.001	
Post-ejection							
LV	191	a + b GA	23.231	-0.186	0.129	0.251	
Sept	195	a + b GA	33.366	-0.273	0.156	0.162	
RV	187	a + b GA	5.764	0.210	0.085	0.049	
Mean of myocardial performance index (cMPI)							
LV	189	a + b GA	-0.098	0.001	0.001	0.285	0.001
Sept	196	a + b GA	0.076	-0.006	0.002	<0.001	0.067
RV	187	a + b GA	-0.115	0.000	0.001	0.901	-0.005
SD of myocardial performance index (cMPI)							
LV	189	a + b GA	0.166	-0.001	0.001	0.154	
Sept	196	a + b GA	0.139	0.000	0.001	0.689	
RV	187	a + b GA	-0.005	0.004	0.001	<0.001	

n = number of observations; LV, left ventricular wall; Sept, septal wall; RV, right ventricular wall; GA, gestational age; SE, standard error; SD, standard deviation; p, p-value; R^2adj., adjusted R-squared. Myocardial performance index was log transformed (base 10).

Table S1 Comparison between female (n=96) and male fetuses (n=102) using Mann-Whitney *U*-test

Parameter	*Males*	*Females*	*p-value*	*Difference between gender*
Peak myocardial velocities (cm/s)				
Am				
LV	4.005	3.455	0.055	No
Sept	4.084	3.513	0.020	Yes
RV	5.912	5.318	0.001	Yes
Sm				
LV	2.669	2.305	0.027	Yes
Sept	2.688	2.404	0.008	Yes
RV	3.718	3.229	0.017	Yes
Em				
LV	3.453	2.747	0.148	No
Sept	3.143	3.007	0.250	No
RV	4.292	3.875	0.084	No
Em/Am ratio				
LV	0.834	0.860	0.684	No
Sept	0.779	0.851	0.127	No
RV	0.693	0.736	0.353	No
Mechanical cardiac time intervals (ms)				
Atrial contraction				
LV	57.050	57.127	0.918	No
Sept	76.547	69.389	0.027	Yes
RV	72.200	66.329	0.008	Yes
Pre-ejection				
LV	55.859	50.027	0.044	Yes
Sept	35.422	38.540	0.290	No
RV	53.860	51.380	0.179	No
Ventricular ejection				
LV	146.85	153.134	0.132	No
Sept	162.400	153.014	0.040	Yes
RV	157.319	159.112	0.270	No
Post-ejection				
LV	73.036	71.960	0.842	No
Sept	81.760	78.852	0.665	No
RV	71.875	70.669	0.646	No
Myocardial performance index (cMPI)				
LV	0.923	0.843	0.259	No
Sept	0.778	0.785	0.282	No
RV	0.780	0.773	0.388	No

Data are given as median. LV, left ventricular wall; Sept, septal wall; RV, right ventricular wall; Am, peak myocardial velocity during atrial contraction; Sm, peak myocardial velocity during ventricular ejection (systole); Em, peak myocardial velocity during early diastole.

Table S2 Inter- and intra-variability expressed as coefficients of variation (CV)

	LV		*Sept*		*RV*	
Parameter	*Inter CV (%)*	*Intra CV (%)*	*Inter CV (%)*	*Intra CV (%)*	*Inter CV (%)*	*Intra CV (%)*
Am	33.4	43.1	20.4	21.1	13.0	23.4
Sm	32.1	34.5	13.8	11.8	18.6	21.8
Em	25.3	26.1	14.1	14.5	18.1	29.3
Atrial contraction	15.9	21.3	17.5	23.4	14.1	15.5
Pre-ejection	31.0	35.1	28.0	30.3	34.3	35.2
Ventricular ejection	12.5	13.7	10.0	8.3	9.2	10.2
Post-ejection	19.9	18.4	25.7	23.9	15.8	16.8
cMPI	27.8	30.5	24.9	26.7	22.6	29.3

LV, left ventricular wall; Sept, septal wall; RV, right ventricular wall; Am, peak myocardial velocity during atrial contraction; Sm, peak myocardial velocity during ventricular ejection (systole); Em, peak myocardial velocity during early diastole; cMPI, myocardial performance index obtained by color tissue Doppler imaging.

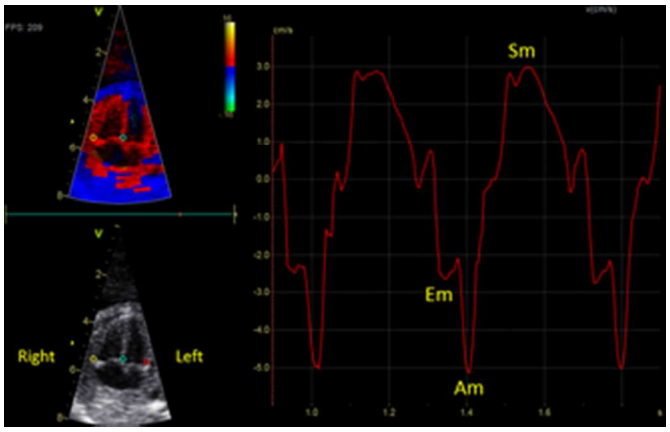


Figure 1 Positioning of regions of interest (ROIs) to obtain myocardial velocity traces in a fetus at 22 weeks of gestation. The left panel indicates the position of ROIs in the four-chamber view of the fetal heart. The right panel shows the myocardial velocity trace derived from the left ventricular wall. Sm, peak myocardial velocity during ventricular ejection (systole); Em, peak myocardial velocity during early diastole; Am, peak myocardial velocity during atrial contraction.

27x17mm (300 x 300 DPI)

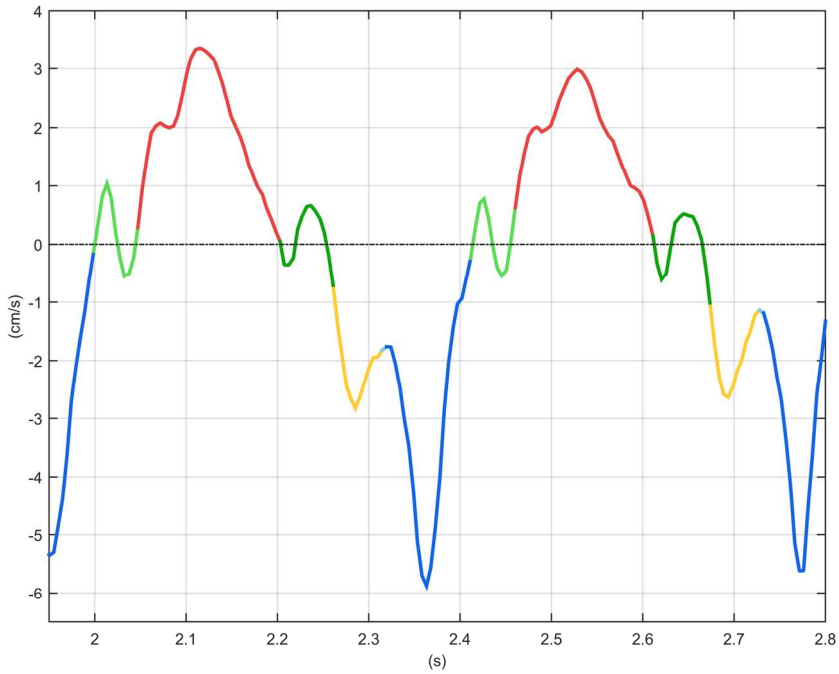


Figure 2 Automated analysis of a myocardial velocity trace from the right ventricular wall obtained with color tissue Doppler imaging (cTDI) demonstrating phases of the cardiac cycle in a fetus at 24 weeks of gestation. The colors indicate the phases defined by the automated algorithm. Dark blue, atrial contraction; light green, pre-ejection; red, ventricular ejection; dark green, post-ejection; yellow, rapid filling; light blue, slow filling.

152x114mm (300 x 300 DPI)

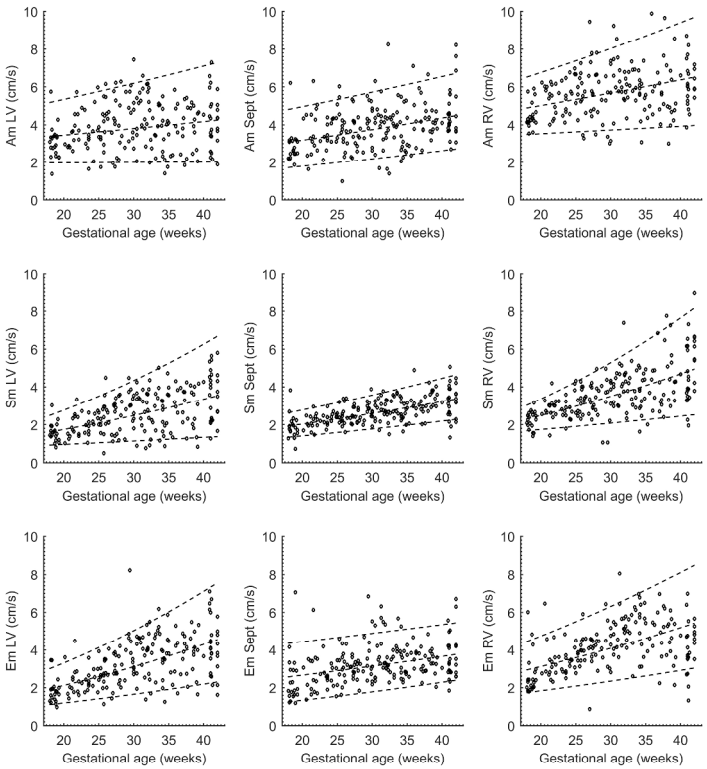


Figure 3 Scatterplots of the left (LV), septal (Sept) and right (RV) peak myocardial velocities obtained by color tissue Doppler imaging and plotted against gestational age. The dotted lines indicate the estimated 5th, 50th and 95th centiles. Am, peak myocardial velocity during atrial contraction; Sm, peak myocardial velocity during ventricular ejection (systole); Em, peak myocardial velocity during early diastole.

297x420mm (300 x 300 DPI)

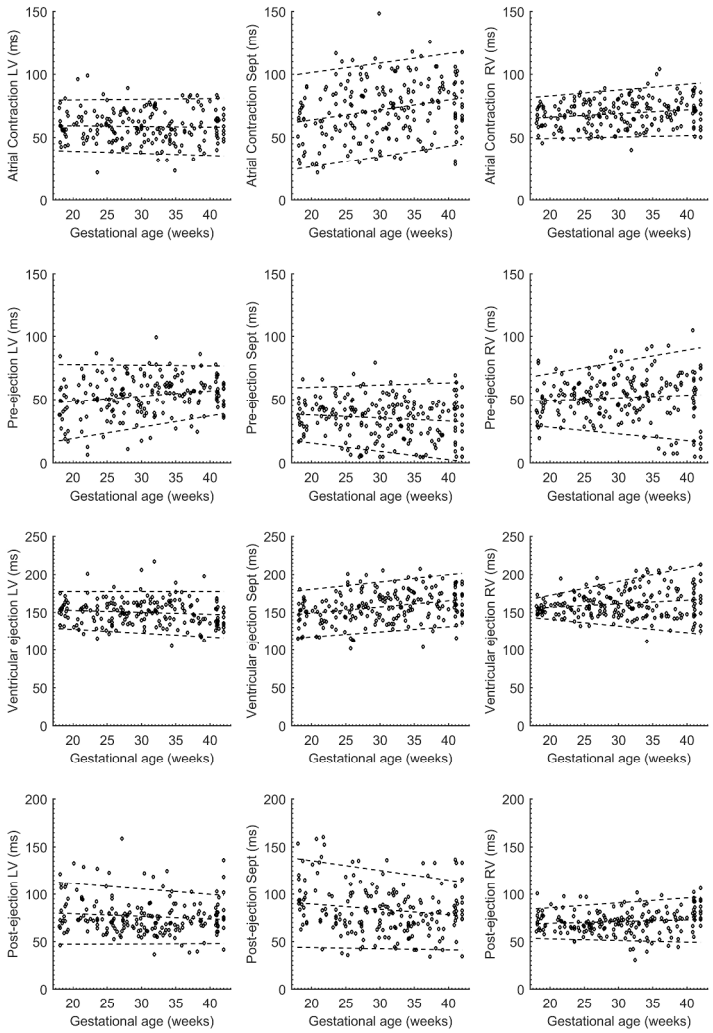


Figure 4 Scatterplots of the left (LV), septal (Sept) and right (RV) mechanical cardiac time intervals obtained by color tissue Doppler imaging and plotted against gestational age. The dotted lines indicate the estimated 5th, 50th and 95th centiles.

297x420mm (300 x 300 DPI)

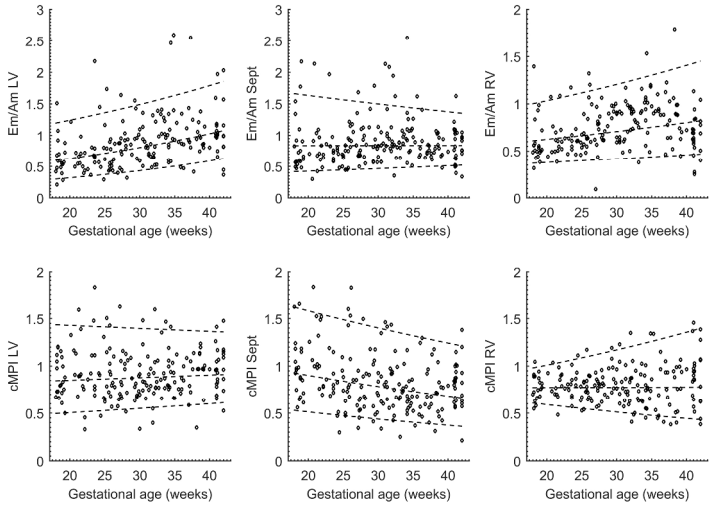
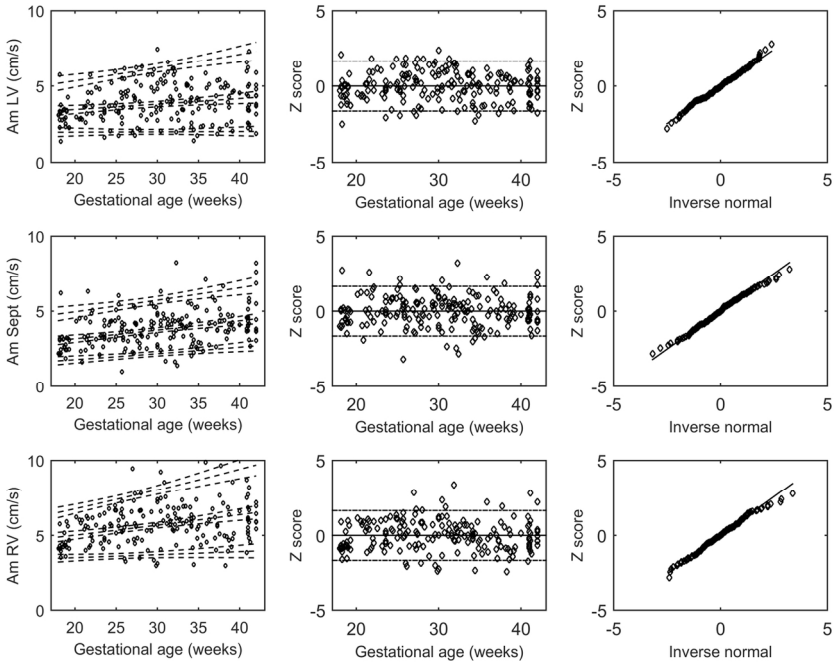
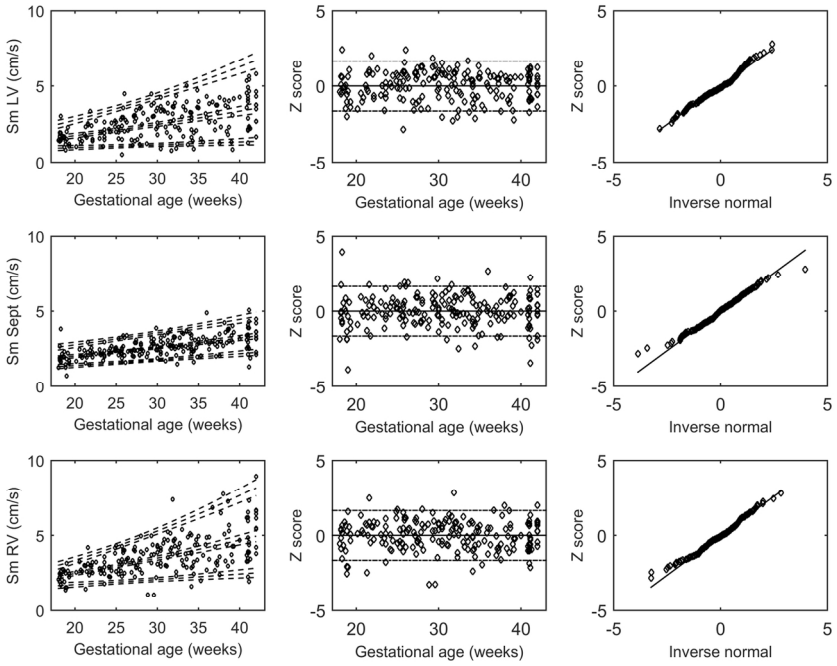


Figure 5 Scatterplots of the left (LV), septal (Sept) and right (RV) Em/Am ratios and myocardial performance index (cMPI) obtained by color tissue Doppler imaging and plotted against gestational age. The dotted lines indicate the estimated 5th, 50th and 95th centiles.

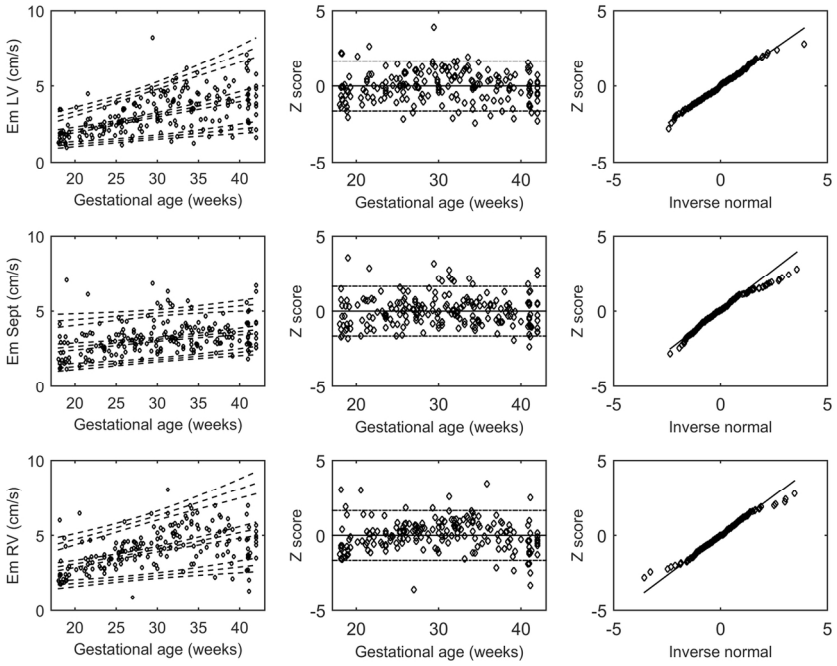
297x420mm (300 x 300 DPI)



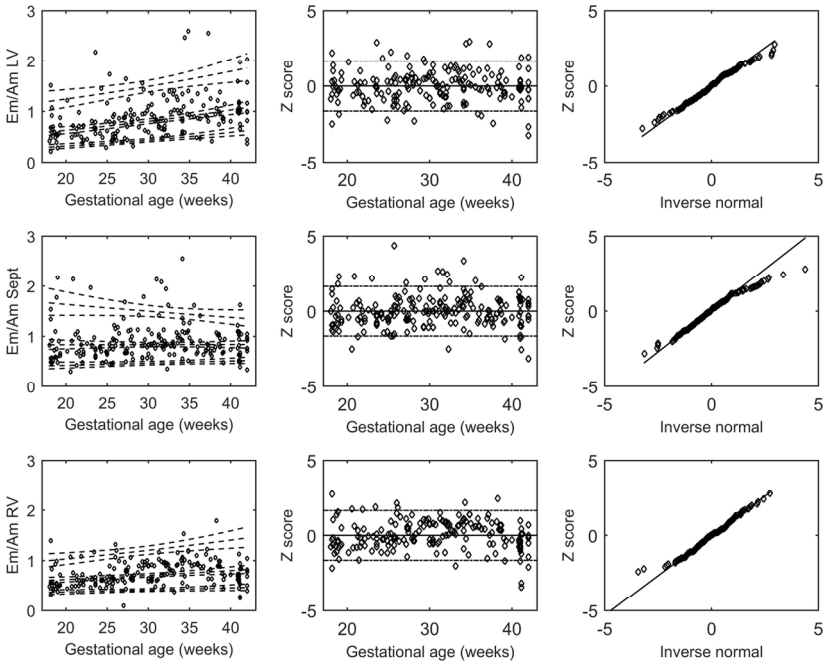
152x114mm (300 x 300 DPI)



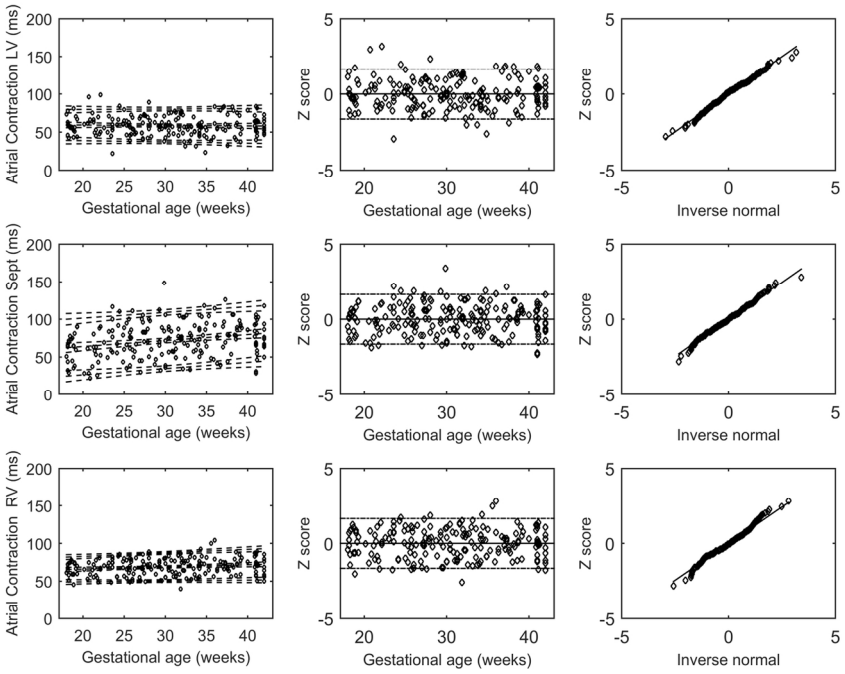
152x114mm (300 x 300 DPI)



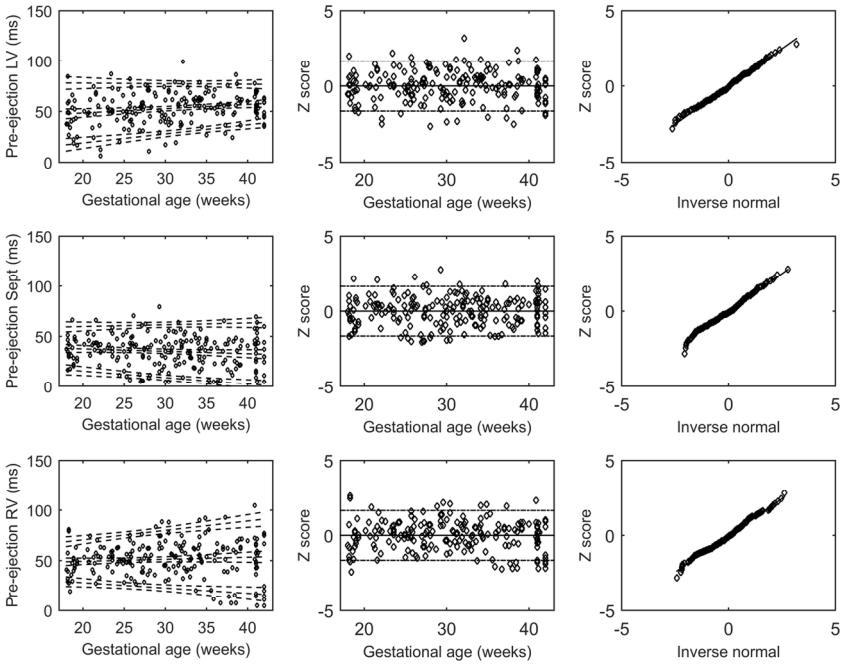
152x114mm (300 x 300 DPI)



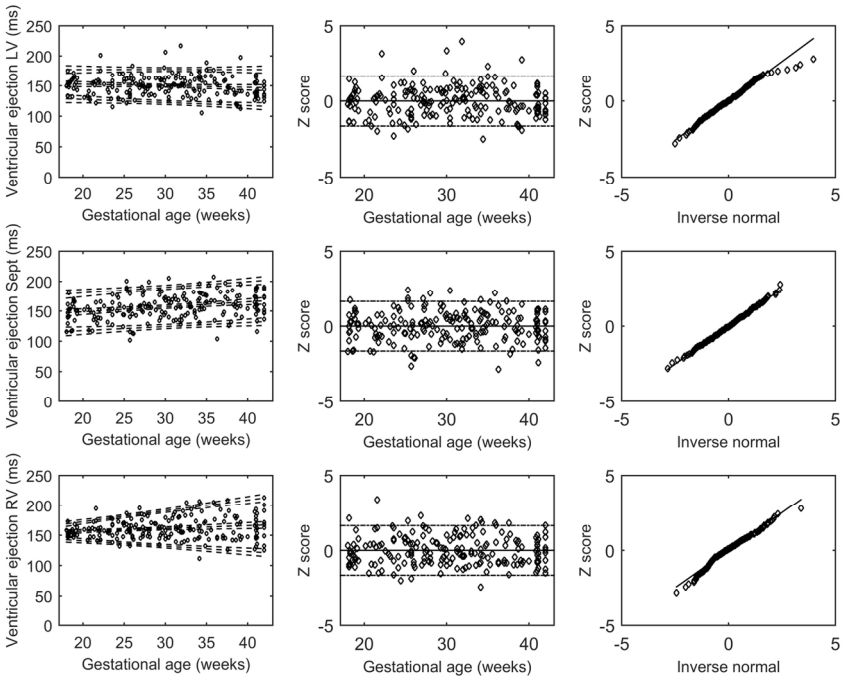
152x114mm (300 x 300 DPI)



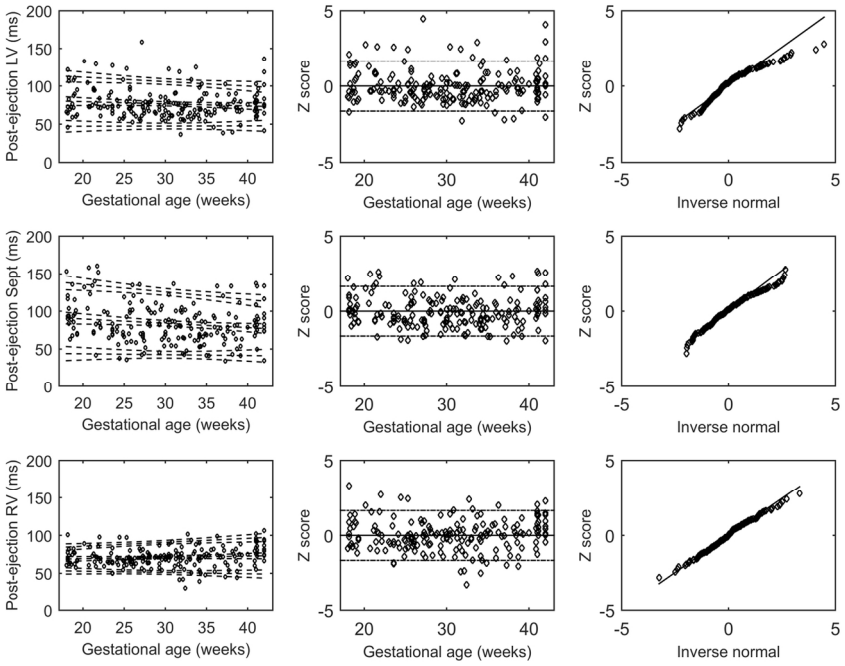
152x114mm (300 x 300 DPI)



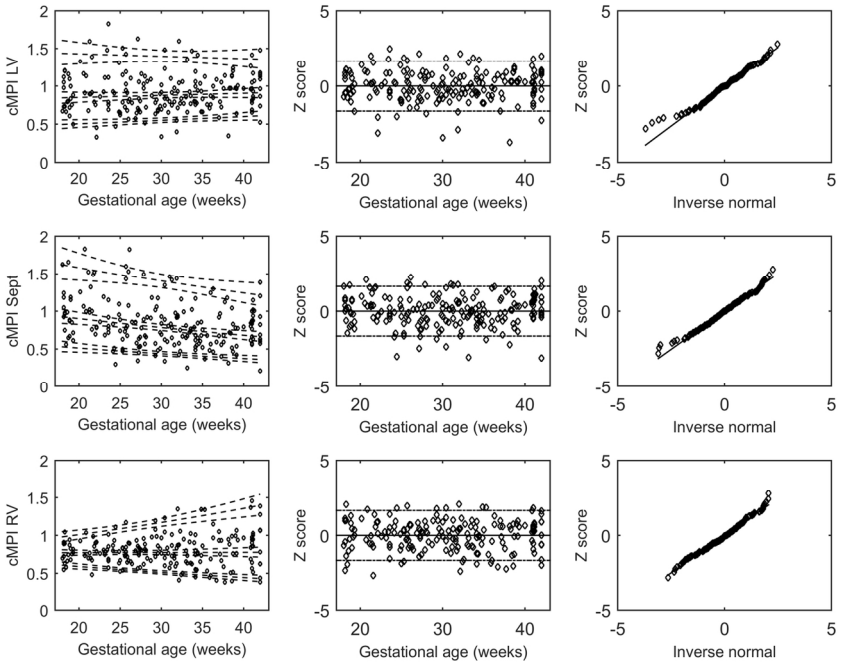
152x114mm (300 x 300 DPI)



152x114mm (300 x 300 DPI)



152x114mm (300 x 300 DPI)



152x114mm (300 x 300 DPI)