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Left ventricular function evaluation of the fetal heart: reference intervals and inter-observer variability of 2D speckle-tracking echocardiography measurements

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Abstract

Aims: Measurement of myocardial strain using 2D speckle-tracking echocardiography can successfully quantify ventricular function, being considered superior to conventional echocardiography. This study aimed to establish reference intervals, interobserver agreements and reliability of two fetal echocardiographic parameters which reflect left ventricular myocardial function, left ventricular apical 4 chamber endo peak strain (AP4pLS) and ejection fraction (EF). **Material and methods:** We conducted a prospective study on 103 healthy fetuses. In each case, cardiac ultrasound images obtained were stored and afterwards were subject to offline 2D speckle-tracking echocardiographic analyses. In 15 randomly chosen subjects a second examiner also carried out an offline analysis of the 4-chamber view and the archived images, in order to assess inter-observer reproducibility and agreement level. Our study group was sub-divided into four different gestational age groups. **Results:** Reference ranges were established for the two measured parameters, AP4pLS and EF, which did not differ significantly between four different gestational age groups (p=0.98 and p=0.64) and neither correlated with gestational age progression (p=0.37 and p=0.08). An excellent level of agreement between the two examiners was found for the echocardiographic measurements, expressed through an intra-class correlation coefficient (ICC) value of 0.85 (0.62-0.94 for 95%CI) for AP4pLS and 0.78 (0.47-0.92 for 95% CI) for EF. **Conclusions:** Speckle tracking AP4pLS and EF parameters are useful for assessment of ventricular myocardial function in healthy fetuses and can be reliably reproduced by two different skilled examiners. Further studies conducted on larger populations are required to standardize reference values of fetal speckle-tracking measurements.

Keywords: fetal echocardiography; speckle-tracking; myocardial strain; reference values; inter-observer variability

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Introduction

Fetal echocardiography was, until recently, used exclusively for the identification of cardiac malformations. In recent years, however, special attention has been paid to the evaluation of the cardiac ventricular function in the fetus. The accurate assessment of fetal cardiac function is still challenging, given the small size and motion of the fetal heart, the moving fetus and the negative influence of high maternal body mass index [1].

The cardiopulmonary transition from fetal to neonatal circulation represents the most important adaptative process during life. This process is responsible for dramatic changes in the left ventricular (LV) loading conditions, generating progressive modification of the LV geometry and function.

Myocardial strain measured using 2D speckle-tracking echocardiography represents an innovative parameter for the quantification of ventricular function. In infants it has demonstrated a specificity and sensitivity which is superior to conventional echocardiographic parameters [2].

Ventricular global longitudinal strain with the advantage of being relatively angle-independent, speckle-tracking echocardiography is easily applicable in the moving fetus, making it a promising and reproducible tool for the assessment of cardiac function [1,3–5].

The disadvantage of speckle-tracking is that it is highly dependent upon temporal resolution. During a cardiac cycle, the ultrasound signal pattern is tracked frame-by-frame. In children the recommended frame rate which is considered adequate to evaluate myocardial deformation is approximately 80 frames per second (fps) [6]. In the small-sized fetal heart, the optimal frame rate is not well defined. Due to an increased fetal heart rate, the European Association of Cardiovascular Imaging (EACVI) and the American Society of Echocardiography (ASE) suggest using the highest frame rate possible, preferably above 90 fps. [7–9].

The present study aims to establish reference intervals of the fetal echocardiographic parameters which reflect left ventricular myocardial function, namely left ventricular apical 4 chamber endo peak strain (AP4pLS) and ejection fraction (EF), to establish the interobserver agreement and reliability of these two left ventricular function parameters and to comparatively evaluate the variation of the parameters within fetal life in relation to maternal gestational age.

Material and methods

Study population

We conducted a unicentric prospective study over a period of 27 months (May 2020-September 2022), time during which 103 fetuses were enrolled and underwent an echocardiographic evaluation, performed in the Pediatric Cardiology Clinic from the Emergency Institute of Cardiovascular Disease and Heart Transplantation from Targu Mures, Romania by a pediatric cardiologist with experience in the field of fetal cardiology. The mothers of the subjects enrolled in the study had at least a fetal echocardiography performed during their pregnancy, as recommended by the national obstetric guidelines and a fetal biometry evaluation was performed in each of them 2-7 days prior to the fetal examination.

Inclusion criteria consisted of fetuses with normal weight for their gestational age, belonging to singleton, physiological, periodically monitored pregnancies. Fetuses with a diagnosis of cardiovascular disorder, congenital cardiac or extracardiac malformation, with intrauterine growth restriction, as well as those belonging to mothers with twin pregnancies, diagnosed with diabetes, obesity, arterial hypertension, arrythmias or conduction disorders were excluded from the study. Moreover, those subjects in whom the echocardiographic images obtained were of poor quality, or in whom the apex down image acquisition did not allow an appropriate analysis of the 4-chamber view were also omitted from the study.

Echocardiography examinations and ultrasound image analysis

All echocardiographic recordings were made using an Epiq ultrasound machine, in accordance with the recommendations provided by the ASE [7-9]. An optimization of the image was carried out in each subject and the depth and the sector width were reduced in order to obtain the high frame rates. The minimal accepted frame rate was 80 Hz and for each fetus a minimal number of four 2D images of several cardiac cycles from 4-chamber view of each ventricle. The images were stored in raw Digital Imaging and Communication in Medicine (DICOM) format and provided for offline 2D speckle-tracking echocardiographic analyses, using a Philips Qlab13 software, according to a previously established protocol. The cardiac cycle was validated from the operator. In the absence of an ECG, the cardiac cycle was determined from the mechanical movement of the mitral valve followed in time. End-diastole was defined by the complete closure of the atrioventricular valve. The time cursor was placed just before the mitral valve closure and again just after a consequent closure.

A correct marking of the cardiac cycle was supposedly able to accurately determine the automatically calculated heart rate, with a value very close to the one assessed through Doppler examination. The marking of three endocardium points (base of the septum, lateral wall of the mitral valvular annulus and apical) was followed by the automatic virtual reconstruction of the left-ventricular endocardial margin. Afterwards, the software was able to automatically calculate end-diastolic volume, end-systolic left ventricular volume, EF and AP4pLS, as represented in figure 1.

The ultrasound examinations were carried out by a single, experienced pediatric cardiologist in most cases. In 15 randomly chosen subjects a second examiner also

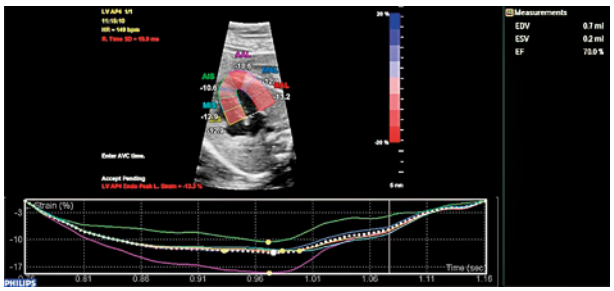


Fig 1. Left ventricle speckle tracking analysis (denoted by left ventricular AP4pLV - peak longitudinal strain of the left ventricle from apical 4 chamber view) and ejection fraction (EF) calculated using left ventricular volumes

carried out the offline analysis of the 4-chamber view and archived images, following the same protocol. The involvement of a second examiner ensured the possibility of inter-observer variability and agreement analysis for a part of the study population.

The entire research protocol was conducted in accordance with the principles stated in the declaration of Helsinki. A signed, informed consent detailing the methodology of the study was provided for each pregnant woman enrolled in the study. The study was approved by the Local Ethical Committee (1276/25.02.2021).

Statistical analysis

Descriptive statistics were represented as arithmetic mean, median and standard deviation (SD). Departure from normal distribution of median value was represented through the interquartile range (IQR), corresponding to the 25th percentile and 75th percentile, respectively. Assessment of normal/abnormal distribution of the numeric data involved application of the Kolmogorov-Smirnov test.

Linear relationship between EF/ AP4pLS and gestational age was tested through Pearson's or Spearman's correlation coefficients, depending on the Gaussian/non-Gaussian distribution of the variables. In case of the 15 subjects who were examined by two pediatric cardiologists, a mean comparison of the studied echocardiographic parameters was also conducted to assess differences in the obtained measurements. This comparison implied the use of paired t test. Furthermore, mean comparison was carried out to investigate differences in AP4pLS and EF values between four different gestational age groups (22-27 gestational weeks, 28-31 gestational weeks, 32-35 gestational weeks and 36-40 gestational weeks), by applying the one-way ANOVA test.

Inter-examiner reproducibility of AP4pLS and EF was analyzed with the help of the intra-class correlation coefficient (ICC) and its subsequent 95% confidence interval (CI), derived from the two-way random effects model applied for single-rater agreement. Reliability of ICC was judged in accordance with a previously proposed classification, in which a value<0.40 is regarded as poor agreement, a value ranging between 0.40 and 0.59 represents a fair agreement, one between 0.60 and 0.74 is suggestive of good reliability and a ICC of 0.75-1.00 is judged as excellent agreement [10,11]. Global agreement between the two examiners was evaluated through the help of the Bland-Altman method, which delivered plots with visual representation of the differences between the measurements obtained and their corresponding 95% upper limit and lower limit (UL and LL) of agreement. The same method was used for quantification of systematic bias (systematic error of measurement). Passing-Bablock regression line was afterwards used to assess proportional bias of echocardiographic measurements and its intercept and slope parameters were represented in table format, together with their 95% CIs. The presence of the value 0 among the CI of the intercept was suggestive of a lack of significant constant difference between the measurements obtained by the two examiners, whereas the inclusion of the value 1 among the CI of the slope was indicative of a lack of proportional difference between the two measurements [12].

For the estimation of the reference interval (RI), ROUT test was initially used to identify outliers. RI was then represented before and after outlier removal. The representation of RI for the echocardiographic measurements was separately provided for the aforementioned four gestational age groups, by applying the parametric and robust method to variables complying to a Gaussian distribution and the non-parametric method for those with abnormal/non-Gaussian distribution. For the parametric method, UL and LL of RI were calculated as mean+1.96xSD and mean–1.96xSD [13]. In case of the robust method, recommended for small sample sizes, a Box and Cox transformation of the data was initially carried out [14,15]. The non-parametric method defined the 2.5th and 97.5th percentiles as the LL and UL of the RI. Independently of the applied method, 90% CIs of the LL and UL were calculated in each case, which involved bootstrapping in case of the robust and non-parametric method, with 1000 samples [16].

The entire statistical analysis was carried out using GraphPad Prism version 9.0.2, MedCalc trial version and Excel (Microsoft Office Professional Plus 2016). All results obtained were considered significant at a p value threshold of 0.05 (95% CI).

Results

There were 103 pregnant women (mean age 28.98±0.37 years) enrolled in the study, with gestational

ages ranging between 22 and 40 weeks (mean gestational age of 29.17±4.55 weeks) which were separately assessed on four different gestational age intervals as follows: 22-27 weeks, 28-31 weeks, 32-35 weeks and 36-40 weeks The descriptive statistics and distribution of the echocardiographic measurements has been depicted in table I. A lack of normal distribution of the data was identified only in the case of AP4pLS, for the 22-27 weeks range, after applying the Kolmogorov-Smirnov test ($p<0.01$). Comparative assessment of mean values of the two measurements between the gestational age groups revealed no significant differences, neither for AP4pLS ($p=0.98$), nor for EF ($p=0.64$). Furthermore, a linear relationship was tested to address a possible correlation between AP-4pLS or EF and gestational age, with the non-parametric Spearman test yielding insignificant results ($r=0.08$, $p=0.37$ and $r=-0.17$, $p=0.08$, respectively).

Based on the distribution of the fetal echocardiography measurements, we aimed to identify RIs for the four gestational age groups, after applying the non-parametric method or both parametric and robust method, as seen in table II. The RIs were provided both before and after outlier removal, if those were found among the studied data. Broader RIs can be seen in most cases after applying the robust method, as opposed to the conventional parametric method.

For those 15 fetuses who underwent ultrasound examinations carried out by two different observers, inter-

Table I. Descriptive statistics and distribution of fetal echocardiographic measurements, divided based on gestational age intervals.

Gestational age	Measurement	Mean (± SD)	Range (Min, Max)	Median (IQR)	Distribution
22-27 weeks	AP4pLS	-15.59 (±4.84)	-36.00 to -10.60	-13.90 (-18.50, -12.40)	Non-Gaussian
	EF (%)	58.61 (±9.88)	32.50 to 80.30	59.70 (53.10, 66.70)	Gaussian
28-31 weeks	AP4pLS	-14.53 (±2.39)	-20.10 to -10.90	-14.50 (-15.80, -12.50)	Gaussian
	EF (%)	56.33 (±8.98)	37.80 to 75.40	58.80 (49.10, 62.75)	Gaussian
32-35 weeks	AP4pLS	-15.26 (±4.00)	-23.20 to -9.70	-13.60 (-18.65, -12.13)	Gaussian
	EF (%)	56.99 (±8.74)	41.30 to74.20	57.10 (50.10, 64.63)	Gaussian
36-40 weeks	AP4pLS	-14.80 (±3.80)	-20.40 to -10.30	-14.50 (-18.85, -11.20)	Gaussian
	EF (%)	54.70 (±11.35)	34.90 to 74.10	58.50 (44.95, 61.10)	Gaussian

AP4pLS - left ventricular apical 4 chamber endo peak strain; EF - ejection fraction; IQR - interquartile range; SD - standard deviation.

Table II. Reference intervals of fetal echocardiographic measurements, divided based on gestational age intervals.

Gestational age (n)	Measurement	RI type	95% RI after Outlier removal			95% RI without Outlier removal		
			RI (LL-UL)	90% CI for LL	90% CI for UL	RI (LL-UL)	90% CI for LL	90% CI for UL
22-27 weeks (n=39)	AP4pLS	Non-parametric	-26.40 to -11.20	-26.40 to -20.70	-11.20 to -10.60	-36.00 to -11.20	-36.00 to -26.40	-11.20 to -10.60
	EF (%)	Parametric	NA	NA	NA	39.22 to 77.98	34.67 to 43.77	73.43 to 82.53
		Robust	NA	NA	NA	38.76 to 79.57	33.94 to 44.17	74.71 to 83.82
28-31 weeks (n=33)	AP4pLS	Parametric	NA	NA	NA	-19.22 to -9.83	-20.42 to -18.02	-11.03 to -8.63
		Robust	NA	NA	NA	-19.45 to -9.54	-20.53 to -17.94	-10.65 to -8.24
	EF (%)	Parametric	NA	NA	NA	38.72 to 73.93	34.22 to 43.22	69.43 to 78.43
		Robust	NA	NA	NA	37.33 to 75.97	31.27 to 42.79	71.63 to 79.64
32-25 weeks (n=18)	AP4pLS	Parametric	NA	NA	NA	-23.09 to -7.41	-25.83 to -20.35	-10.15 to -4.67
		Robust	NA	NA	NA	-23.02 to -4.17	-26.77 to -19.53	-8.41 to -2.31
	EF (%)	Parametric	NA	NA	NA	39.86 to 74.12	33.88 to 45.84	68.14 to 80.10
		Robust	NA	NA	NA	38.00 to 76.12	32.56 to 44.90	70.34 to 81.54
36-40 weeks (n=13)	AP4pLS	Parametric	NA	NA	NA	-22.25 to -7.34	-25.34 to -19.16	-10.43 to -4.25
		Robust	NA	NA	NA	-23.25 to -7.34	-26.67 to -19.33	-8.78 to -1.86
	EF (%)	Parametric	NA	NA	NA	32.45 to 76.94	23.24 to 41.66	67.73 to 86.15
		Robust	NA	NA	NA	27.73 to 82.26	19.61 to 41.05	71.88 to 88.76

AP4pLS - left ventricular apical 4 chamber endo peak strain; CI - confidence interval; EF - ejection fraction; n - number of fetuses; NA - not applicable; RI - reference interval; UL - upper limit

Table III. Interobserver agreement and reproducibility analysis

Echo-cardiographic measurements	Examiner 1 (mean ± SD)	Examiner 2 (mean ± SD)	ICC (95% CI)	Interobserver reproducibility		
				Bland-Altman bias (95% CI)	Passing-Bablock intercept (95% CI)	Passing-Bablock slope (95% CI)
AP4pLS	-16.02 ± 4.48	-16.63 ± 3.51	0.85 (0.62-0.94)	0.60 (-0.61,1.82)	5.42 (-1.31,20.58)	1.30 (0.87,2.20)
EF (%)	59.67 ± 8.75	60.04 ± 10.32	0.78 (0.47-0.92)	0.37 (-3.91,13.17)	7.66 (-21.12,23.09)	0.84 (0.57,1.27)

AP4pLS - left ventricular apical 4 chamber endo peak strain; CI - confidence interval; EF - ejection fraction; ICC - intra-class correlation coefficient; SD - standard deviation

observer agreement and reproducibility were analyzed (table III). Mean measurements obtained by the two examiners did not differ significantly, as suggested by the comparison conducted with the help of the paired t test (AP4pLS: p=0.30; EF: p=0.82). An excellent level of agreement between the two examiners was found for the echocardiographic measurements: ICC value of 0.85 (0.62-0.94 for 95%CI) for AP4pLS and 0.78 (0.47-0.92 for 95%CI) for EF (table III). Passing-Bablock regression revealed no significant systematic and proportional difference between the measurements performed by the two examiners.

The Bland-Altman analysis of AP4pLS measurements revealed a systematic mean bias of 0.6 (-0.61 to 1.82 for 95% CI), represented by the green dotted line in figure 2. 95% LL and UL of agreement were -3.70 (-5.82 to -1.57 for 95%CI) and 4.91 (2.78 to 7.04 for 95%CI), as represented by the red dotted lines in the same figure 2.

In the case of EF, Bland-Altman analysis revealed a systematic mean bias of 0.37 (-3.91 to 13.17 for 95%CI), visually represented in figure 3 (green dot line). A 95% LL of agreement of -12.17 (-5.97 to -18.36 for 95%CI) was obtained and a 95% UL of agreement of 12.91 (6.71 to 19.11 for 95%CI) was found. The LL and UL were represented by the red dotted lines in figure 3.

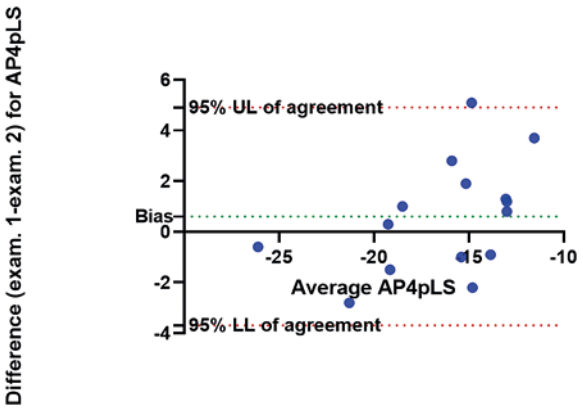


Fig 2. Bland-Altman plot comparing the AP4pLS measurements obtained by the two examiners. AP4pLS - left ventricular apical 4 chamber endo peak strain; exam. - examiner; LL - lower limit; UL - upper limit

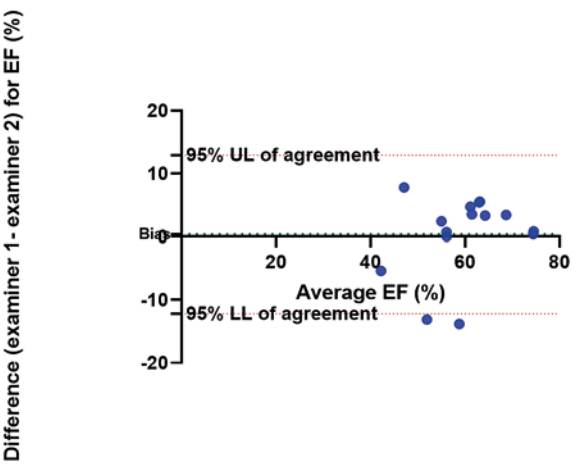


Fig 3. Bland-Altman plot comparing the EF measurements obtained by the two examiners. EF - ejection fraction; exam. - examiner; LL - lower limit; UL - upper limit

Discussion

The establishment of normal strain values in a healthy fetal cohort is a mandatory prerequisite for their use in evaluating pathologic changes in the function of both ventricles and for a better understanding of cardiac structural diseases during fetal period. The current study's main strong point is the identification of gestational-age dependent reference ranges of two parameters which are representative of left ventricular myocardial function, volumetrically measured EF and AP4pLS, reference ranges which might guide future research on the matter and might aid daily practice for pediatric cardiologists due to their facile reproducibility. Global longitudinal myocardial deformation of the left ventricle seems to be indicative of possible onset of fetal cardiac disfunction and declines gradually in the second and third trimester of the pregnancy, according to Lee-Tannock et al [17]. Schubert et al described stable values of global longitudinal strain until around the 38th week of pregnancy, with their significant decrease following afterwards [6]. On the other hand, one retrospective study which included fetuses with gestational ages ranging between 22 and 35 weeks revealed a significant increase in left ventricular global longitudinal strain with the progression of gesta-

tion [18]. However, the two parameters significantly decrease during the last trimester and the transitional period due to the marked hemodynamic changes [19]. After birth, biventricular pre- and afterload increase, and as a result a decrease in biventricular longitudinal strain takes place [5,20]. The global longitudinal strain is defined as a change in the length of a myocardial segment, in comparison to its initial length. According to a theory in which longitudinal strain has a value independent of gestational age, as cardiac growth occurs through increase in myocyte volume, and myocyte number remains unchanged, it is possible that the global longitudinal strain value is stable from the moment when the maximum number of myocytes has been reached [21–23]. Similarly, our study did not identify any association between AP4pLS and gestational age.

In our study, the inter-examiner reproducibility of AP4pLS and EF revealed an excellent level of agreement between the two examiners with no significant difference between the measurements performed by the two examiners. In particular, the level of agreement for AP4pLS was slightly higher than the one found in one of our previous studies, in which interobserver agreement analysis was also performed to evaluate reproducibility of longitudinal and regional strains measurements performed on newborn hearts [24]. Moreover, Patey et al also demonstrated good to excellent interobserver repeatability correlation of all fetal speckle-tracking measurements, with good agreement irrespective of the ultrasound platform used [5]. In their study, Huntley et al. described only good interobserver correlation and agreement for LV and RV global strain fetal measurements [25]. Several parameters can influence measurement reproducibility, with Lorch et al attributing an important inter-observer variability in healthy pediatric populations to the "learning curve" related to the technique and software used [26].

However, establishment of reference intervals requires a significantly larger cohort than the one enrolled in our study, as previously highlighted in systematic reviews conducted on the subject [20] and a longitudinal prospective study can give the benefit of possible correlations between left ventricle parameters, gestational age and neonatal outcome. Therefore, the main limitation of our study remains the limited number of cases and the even smaller study samples of subgroups obtained after gestational age-dependent division.

The utility of left ventricle function evaluation through longitudinal strain assessment has been proven by a series of studies. During intrauterine life, the right ventricular function prevails, which leads to a difficulty in left ventricular dysfunction recognition. After birth, a shift in dominant ventricle function occurs, and even the lightest dysfunction can become obvious. It is known though that strain values vary depending on the analysis software [9,20]. Moreover, the miscellaneous results obtained by published studies so far in the literature might have been influenced by the different ultrasound machines used, the suboptimal, small scale studies conducted and the varying study algorithms applied, as suggested by a systematic review published by van Oostrum et al [20]. Furthermore, frame rate and insonation angle greatly impact AP4pLS measurement, representing a potential source of bias [27]. Therefore, each research conducted so far that evaluated fetal AP4pLS, needs to be interpretated in light of its acquisition protocol and future prospective studies are warranted to yield the identification of reference ranges.

Conclusions

Speckle tracking AP4pLS and EF parameters are useful in the evaluation of ventricular myocardial function in healthy fetuses. Through this study we identified reference ranges for AP4pLS (-16.02 ± 4.48 SD) and EF (59.67 ± 8.75 SD) within a fetal cohort. None of these left ventricular parameters varied with gestational age progression. Moreover, an excellent inter-observer agreement was identified for both AP4pLS and EF.

Conflict of interest: None

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