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Fetal speckle-tracking echocardiography: a comparison between two-dimensional and electronic spatio-temporal image correlation (e-STIC) technique

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1 ABSTRACT

2 Objectives

3 Speckle tracking technology has been applied to assess ventricular deformation
4 throughout the cardiac cycle. Reproducibility is still a matter of concern because this
5 technique mostly depends on the quality of acquisition. An electronic 4D probe that
6 allows rapid acquisition of electronic spatio-temporal image correlation volumes (eSTIC)
7 has been recently introduced. The aim of our study was to investigate whether e-STIC
8 acquisition improves deformation analyses reproducibility.

9 Methods

10 We recruited fetuses between 20 and 40 weeks of gestation. We obtained a 2D video clip
11 and an e-STIC volume of a four-chamber view. An expert operator did the 2D and 4D
12 measurements twice for each fetus. Other two operators, with a specific training on the
13 software, made the 2D and 4D measurements once respectively. We focused on left
14 ventricular global strain (LV-GS) and left ventricular ejection fraction (LV-FE).
15 Intraobserver, interobserver and intermethod agreement were assessed by means of
16 intraclass correlation coefficient (ICC) and illustrated by Bland-Altman plots. Systematic
17 differences between measurements were assessed using a paired t-test.

18 Results

19 The mean difference between LV-GS values obtained with e-STIC and 2D analysis was
20 -0.10 (95% CI $-2.28, 2.08$). No systematic differences were found between the two
21 techniques for LV-GS values (p -value = 0.927), the two methods agreed equally through
22 the range of measurements. The mean difference between LV-FE values obtained with e-
23 STIC and 2D analysis was 7.55 (95% CI $4.16, 10.95$). This difference was statistically

24 significant (p-value < 0.001), indicating the presence of fixed bias between the two
25 techniques. The inter-rater reliability of LV-GS was moderate-to-substantial for both e-
26 STIC and 2D. On the contrary, the inter-rater reliability of LV-FE obtained via e-STIC
27 was superior to that obtained via 2D analysis. The intra-rater reliability of LV-GS
28 obtained with e-STIC was superior to that obtained with 2D analysis (ICC 0.857; 95% IC
29 0.761-0.917). Similarly, the intra-rater reliability of LV-FE obtained via e-STIC was
30 superior to that obtained via 2D analysis (ICC 0.647; IC 0.51-0.783).

31 **Conclusion**

32 e-STIC has been proved to be a better technique than 2D analysis for intra-rater reliability
33 of LV-GS. 2D-STE and e-STIC are not interchangeable when applied to measure LV-FE,
34 given the presence of intermethod fixed bias. 4D acquisition might improve intrinsic
35 limitations of STE.

36 **Introduction**

37 Echocardiography has emerged as the mainstay of fetal cardiovascular assessment ¹⁻³.
38 Primarily focused on identifying congenital heart defects, echocardiography has recently
39 renewed by the increasing interest on fetal myocardial function ⁴. However, objective
40 methods to assess the presence and the degree of fetal cardiac dysfunction have not been
41 thoroughly validated. Recently, 2-dimensional speckle tracking echocardiography, (STE)
42 a technology based upon the principle of deformation (strain and strain rate) has been
43 developed in order to obtain information regarding segmental and global cardiac function
44 ⁵. This approach is currently used in adults and children by cardiologists ^{6, 7} and it has
45 recently been applied to the fetus ⁸⁻¹⁰, providing a non-invasive measure and
46 representation of myocardial contractility in several pregnancy-related complications ¹¹⁻
47 ¹⁵. Deformation imaging directly measure the lengthening and shortening of the
48 myocardium throughout the cardiac cycle ^{5, 16 17, 18}. Several issues limit its routinely use
49 in fetal echocardiography. Spatial resolution, angle independence and the frame rate are
50 questioned, and reproducibility is still a matter of concern because the technique mostly
51 depends on the quality of acquisition ^{19, 20}. Moreover, several commercially available
52 speckle-tracking software applications can reduce the comparing of analysis. Recently,
53 three-dimensional (3D) STE has been introduced to adult and pediatric practice to
54 overcome B-mode imaging limitations and there are only limited reports of its use in fetal
55 cardiology. Previous studies have demonstrated that four-dimensional (4D) ultrasound
56 technologies, such as spatiotemporal image correlation (STIC) ²¹, facilitate both
57 examination and documentation of sonographic datasets ²². However, acquisition of
58 diagnostic volumes can be limited by fetal movements. With the standard mechanical
59 probes available thus far, acquisition of a e-STIC volume of good quality requires 7.5-15

60 s. Recently, General Electric and Philips Ultrasound introduced an electronic 4D probe
61 that allows acquisition of electronic STIC (e-STIC) volumes in a much shorter time. Our
62 group has already proved that e-STIC volumes of good quality could be obtained in more
63 than 90% of cases within the time frame of a standard examination of fetal anatomy ²³.
64 The aim of our study was to investigate whether e-STIC acquisition improves
65 deformation analyses reproducibility.

66

67 **Materials and methods**

68 This observational cohort study included fetuses with accurate first and/or second-
69 trimester dating ultrasound (US) scans, examined between 20- and 40-weeks' gestation,
70 once during the study period. Fetuses were not at risk for congenital heart abnormalities
71 or other fetal anomalies and the mothers did not have clinical conditions (diabetes
72 mellitus, chronic hypertension, and preeclampsia).

73 *Image acquisition and analysis*

74 All measurements and acquisitions were performed by an expert examiner (G.P.). Two-
75 dimensional images of the 4-chamber view were obtained with either an RM6C or EM6C
76 transducer from a Voluson E10 US system (GE Healthcare, Milwaukee, WI).

77 The examiner identified the 4-chamber view within the chest, filling most of the
78 ultrasound screen, with the apex perpendicular or tangential to the ultrasound beam.

79 Images were optimized to enhance the borders between the blood pool and endocardium
80 ²⁴. Three-second cine clips of the 4-chamber view were stored as Digital Imaging and
81 Communications in Medicine files and exported to an offline cloud database. The Digital

82 Imaging and Communications in Medicine image frame rate was equivalent to the frame
83 rate acquisition at the time of the examination.

84 Once a cine clip of the 2D image was saved, the examiner immediately activated the
85 eSTIC sweep to acquire the volume.
86 E-STIC volumes were obtained using an electronic 4D probe, EM6C, using the option
87 maximal quality, as we previously reported ²³. On the multiplanar display the examiner
88 could manipulate the images to align the 4-chamber view in the optimal position and to
89 mirror the 2D image previously acquired. The examiner compared each paired of datasets
90 (2D image and eSTIC volume) before analysis to determine if the speckle tracking is
91 altered by the type of image acquisition.

92 Once the 2D images and 4D e-STIC volumes of the 4-chamber view were obtained and
93 stored in the Digital Imaging and Communications in Medicine format, they were
94 examined using fetalHQ software (GE Healthcare; Zipf, Austria) using criteria that have
95 been previously described ²⁴. Briefly, the endocardial border for each ventricle was traced
96 from the base of the lateral wall to the apex and from the apex to the base of the septal
97 wall at end diastole and end systole. After the tracing, automated analysis detected the
98 endocardial borders during diastole and systole. An M-Mode derived from the 2-
99 dimensional image of the four-chamber view was used to identify a single cardiac cycle
100 (end-diastole, end-systole, end-diastole) used for speckle tracking analysis ²⁴. Following
101 selection of one cardiac cycle, the automated software was activated to detect the
102 endocardial border for each ventricle at end-systole and end-diastole. Adjustments were
103 made to the end-systolic and end-diastolic contours, as needed, before the final analysis.
104 Using the equation of Hadlock et al ^{25, 26}, the estimated fetal weight (computing the

105 measurements of the biparietal diameter, head circumference, abdominal circumference,
106 and femur length) was expressed using z-score²⁷.

107 The expert operator (G.P., operator 1) did the 2D and 4D measurements twice for each
108 fetus. Other two operators (M.G.D. and E.M., operator 2 and 3), with a less expertise but
109 with a specific training on the software made the 2D and 4D measurements once
110 respectively.

111 Once the analysis was complete, the raw data were exported to a CSV file. This file was
112 imported into an Excel® spreadsheet (Microsoft Corp., Redmond, WA, USA). In this
113 study, we focused on left ventricular global strain (LV-GS) and left ventricular ejection
114 fraction (LV-FE).

115

116 **Ethics**

117 The study protocol was approved by the local Ethics Committee of Sant' Orsola-Malpighi
118 Hospital and a consent form signed at recruitment was obtained from each eligible patient
119 (575/2018/Oss/AOUBo). The study protocol conforms to the ethical guidelines of the
120 "World Medical Association (WMA) Declaration of Helsinki-Ethical Principles for
121 Medical Research Involving Human Subjects" adopted by the 18th WMA General
122 Assembly, Helsinki, Finland, June 1964 and amended by the 59th WMA General
123 Assembly, Seoul, South Korea, October 2008

124

125 **Statistical analyses**

126 Continuous variables were summarized as mean \pm standard deviation; discrete and
127 categorical variables were summarized as frequencies and percentages. Agreement
128 between 2D and 4D measurements made by Operator 1 was assessed using a paired 2-

129 sample t-test, and illustrated using the Bland-Altman plot. Inter-rater reliability of
130 measurements made by the 3 operators was assessed with intra-class correlation
131 coefficient (ICC) estimates and 95% confidence intervals (CIs) based on a single-rating,
132 absolute-agreement, 2-way random-effects model ²⁸. Intra-rater reliability of
133 measurements made by Operator 1 was assessed with ICC estimates and 95% CIs based
134 on a single-rating, absolute-agreement, two-way mixed-effects model. As a rule of thumb,
135 values between 0.01 and 0.20 indicate “slight” agreement, values between 0.21 and 0.40
136 indicate “fair” agreement, values between 0.41 and 0.60 indicate “moderate” agreement,
137 values between 0.61 and 0.80 indicate “substantial” agreement, and values between 0.81
138 and 1.00 indicate “almost perfect” agreement ²⁸.

139 All data were analyzed using the Stata 15 software (StataCorp. 2017. Stata Statistical
140 Software: Release 15. College Station, TX: StataCorp LP).

141

142 **Results**

143 The study sample included 49 patients recruited between October 2018 and February
144 2019. Mean gestational age (GA) was 30±5 weeks (range: 19 to 36), mean z-score of
145 estimated fetal weight (EFW) was 0.17±1.34, and mean z-score of abdominal
146 circumferences (CA) was 0.45±1.16. One patient exhibited increased umbilical artery
147 pulsatility index.

148 *Comparison between e-STIC and 2D*

149 The mean LV-GS values obtained with e-STIC and 2D analysis were -22.50±7.14 and -
150 22.40±8.04, respectively. The mean difference between LV-GS values obtained with e-
151 STIC and 2D analysis was -0.10 (95% CI -2.28, 2.08). Based on a paired 2-sample t-test,

152 there were no systematic differences between the two techniques ($t = -0.09$, p -value =
153 0.927). As shown in Figure 1, there was no evidence of proportional bias, i.e., the two
154 methods agreed equally through the range of measurements. The 95% limits of agreement
155 illustrated in Figure 1, which are defined as the mean difference ± 1.96 times the standard
156 deviation of the differences and indicate how far apart measurements are likely to be for
157 most individuals, were -14.98 (95% CI $-18.71, -11.25$) and 14.78 (95% CI $11.05, 18.51$).

158 The mean LV-FE values obtained with e-STIC and 2D analysis were 60.65 ± 10.34 and
159 53.10 ± 8.41 , respectively. The mean difference between LV-FE values obtained with e-
160 STIC and 2D analysis was 7.55 (95% CI $4.16, 10.95$). Based on a paired 2-sample t -test,
161 this difference was significantly different from zero ($t = 4.47$, p -value < 0.001), indicating
162 the presence of fixed bias between the two techniques (the e-STIC tends to give higher
163 values). As shown in Figure 2, there was no evidence of proportional bias. The 95% limits
164 of agreement illustrated in Figure 2 were -15.63 (95% CI $-21.43, -9.83$) and 30.73 (95%
165 CI $24.93, 36.53$).

166 *Inter-rater reliability*

167 Intra-class correlation coefficient (ICC) estimates and their 95% confidence intervals
168 were calculated based on a single-rating ($k = 3$), absolute-agreement, two-way random-
169 effects model²⁸. Results are shown in Table 1. The inter-rater reliability of LV-GS was
170 moderate-to-substantial for both e-STIC and 2D. On the contrary, the inter-rater
171 reliability of LV-FE obtained via e-STIC was superior to that obtained via 2D analysis
172 (moderate-to-substantial versus slight-to-fair).

173 *Intra-rater reliability*

174 ICC estimates and their 95% confidence intervals were calculated based on a single-rating
175 ($k = 2$), absolute-agreement, two-way mixed-effects model²⁸. Results are shown in Table
176 2. The intra-rater reliability of LV-GS obtained via e-STIC was superior to that obtained
177 via 2D analysis (substantial-to-almost perfect versus fair-to-moderate). Similarly, the
178 intra-rater reliability of LV-FE obtained via e-STIC was superior to that obtained via 2D
179 analysis (moderate-to-substantial versus slight-to-fair).

180

181 **Discussion**

182 We report the first observational study on 2D-STE and e-STIC for the evaluation of LV-
183 GS and LV-FE parameters. Although these techniques are feasible, their reproducibility
184 is limited.

185 STE is a semi-automated process, performed offline on previously acquired two-
186 dimensional images, using small stable myocardial footprints, or speckles, generated by
187 ultrasound-myocardial tissue interactions²⁹. These bright myocardial areas can be tracked
188 frame-by-frame using specific image-processing algorithm to measure strain and strain
189 rate. The post processing software can automatically divide the myocardium into equal
190 segments, giving also a quantification of regional strain³⁰.

191 Since first measures of myocardial strain in the healthy fetus were reported, many
192 concerns remains about the reliability of these measurements²⁰. Some studies have
193 proved that calculation of strain parameters from bidimensional fetal images have good
194 inter and intra-observer reproducibility^{4, 31}, partially as a consequence of the semi-
195 automated nature of the technique. Some others have reported a lower interobserver

196 variability at 24 weeks gestation compared to 20^{5, 19}, but overall the agreement of 2D-
197 STE appears to be good, and equal or superior to Doppler techniques^{4, 19}.

198 However, 2D imaging has several limitations²⁰: fetal position can vary the orientation of
199 four chamber view to the transducer; magnification and the foreshortening risk of the
200 acquired images can affect the analysis. Moreover, myocardial function may be altered
201 beat by beat during maternal breathing or fetal movements. The whole heart moves
202 through the 2D plane of interest³². Therefore, 3D acquisitions have the potential to
203 overcome the limitation of plane-dependency of 2D imaging. This has only recently
204 experimented and there are limited reports of its use in fetal cardiology^{33, 34}. Aiming to
205 lower the times of acquisition, as fetal movements are a major limiting factor in these
206 methods, we decided to apply e-STIC technique. The advantages of 4D over traditional
207 2-dimensional sonography of the fetal heart have been previously outlined²¹⁻²³. In our
208 study e-STIC has been proved to be a better technique than 2D analysis for intra-rater
209 reliability of LV-GS. Similarly, the intra-rater reliability of LV-FE obtained via e-STIC
210 was superior to that obtained via 2D analysis. Agreement between observers showed
211 moderate-to-substantial for both e-STIC and 2D measurements of LV-GS. On the
212 contrary, the inter-rater agreement of LV-FE obtained via e-STIC was superior to that
213 obtained via 2D analysis.

214 According to these results, our study proved that 2D-STE and e-STIC cannot be
215 interchangeably used, especially when used to measure LV-FE. Technical factors may
216 account for this poor correlation. Cardiac volumes of optimal diagnostic quality are easily
217 obtained with e-STIC because this approach is faster than 2D analysis^{22, 23}. E-STIC
218 acquisition stitches together sub-volumes and this results in a higher resolution real time
219 image. Moreover, the multiplanar display allow the operator to improve the acquisition,

220 manipulating it in A, B and C planes and observing corresponding changes in the
221 perpendicular images before exporting the volume for initiating the strain software
222 analysis^{22,24}. This advantage has already been outlined for STE compared to alternative
223 methods of assessing fetal cardiac function, even if some studies still debate on it^{20, 29, 35}.
224 The multiplanar approach give a less angle dependent acquisition than conventional 2D
225 method, allowing more flexibility.

226 The main strength of our study is that, for the first time, the performance of an electronic
227 4D probe was compared with 2-dimensional acquisition method for the myocardial
228 deformation analyses. We do acknowledge some limitations. First, the small number of
229 our cohort study composed by fetuses between second and third trimester of gestation.
230 Second, we included fetuses of different estimated weight. Strain measurements vary
231 through gestation¹⁹ and SGA or IUGR fetuses could show altered myocardial
232 deformation values^{11, 15}. These factors could have been affected our results.

233 To conclude in our experience, speckle tracking made on 3D-STE volume showed a better
234 reliability and this characteristic is crucial for a diagnostic tool. The main advantage of e-
235 STIC analysis is the faster acquisition and the ability to modify the volumes, according
236 to the desired plans of investigation, obtaining good analysis even in fetuses with non-
237 optimal positions. Moreover, we observed that with the STIC technique the borders
238 between the cavities of cardiac chambers and the endocardium are enhanced and this is
239 fundamental for strain evaluation. One limitation for the wide spreading of this method
240 is that e-STIC probe is quite expensive, but in a referral center with this tool available we
241 recommend it to improve the reproducibility of the evaluations.

242 We believe that 4D acquisition might improve intrinsic limitations of STE. E-STIC
243 acquisition need to be standardized and longitudinally experimented on a larger cohort of
244 normal fetuses on both ventricles. Further studies are necessary also through gestational
245 ages, relating to estimated fetal weight.

246 Supplementary material S1 contains video abstract illustrating fetal speckle-tracking
247 technique and the main results of our study.

248

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359

360

361 **Table 1** Inter-rater reliability of e-STIC acquisition and 2D analysis for heart evaluation
 362 (LV-GS and LV-FE).

Heart evaluation	Technique	ICC*	95% confidence interval of ICC	
			Lower boundary	Upper boundary
LV-GS	e-STIC	0.562	0.403	0.704
	2D	0.581	0.424	0.718
LV-FE	e-STIC	0.544	0.382	0.689
	2D	0.183	0.029	0.362

363 *ICC, intra-class correlation coefficient.

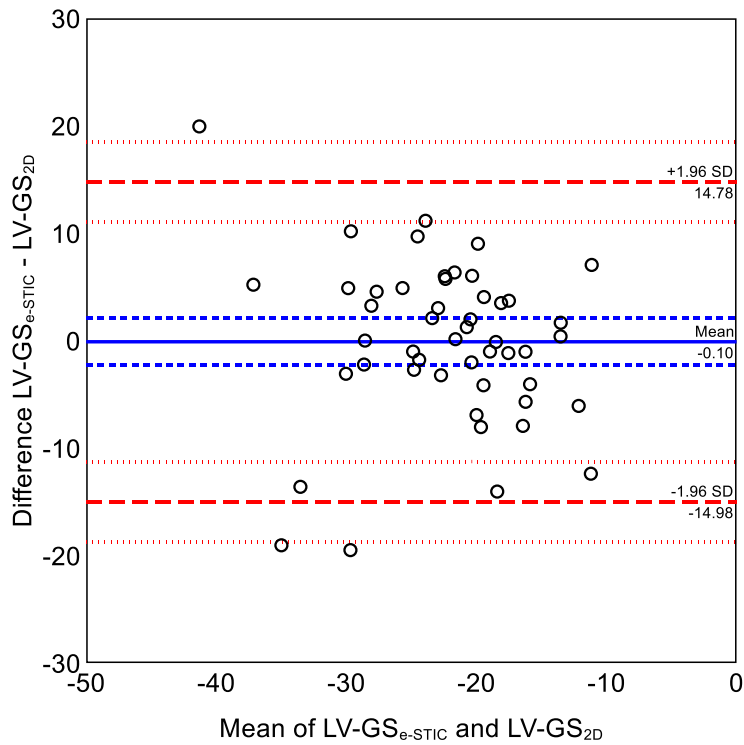
364 **Table 2** Intra-rater reliability of e-STIC acquisition and 2D analysis for heart evaluation
 365 (LV-GS and LV-FE)

Heart evaluation	Technique	ICC*	95% confidence interval of ICC	
			Lower boundary	Upper boundary
LV-GS	e-STIC	0.857	0.761	0.917
	2D	0.507	0.268	0.688
LV-FE	e-STIC	0.647	0.451	0.783
	2D	0.114	0.000	0.359

366 *ICC, intra-class correlation coefficient.

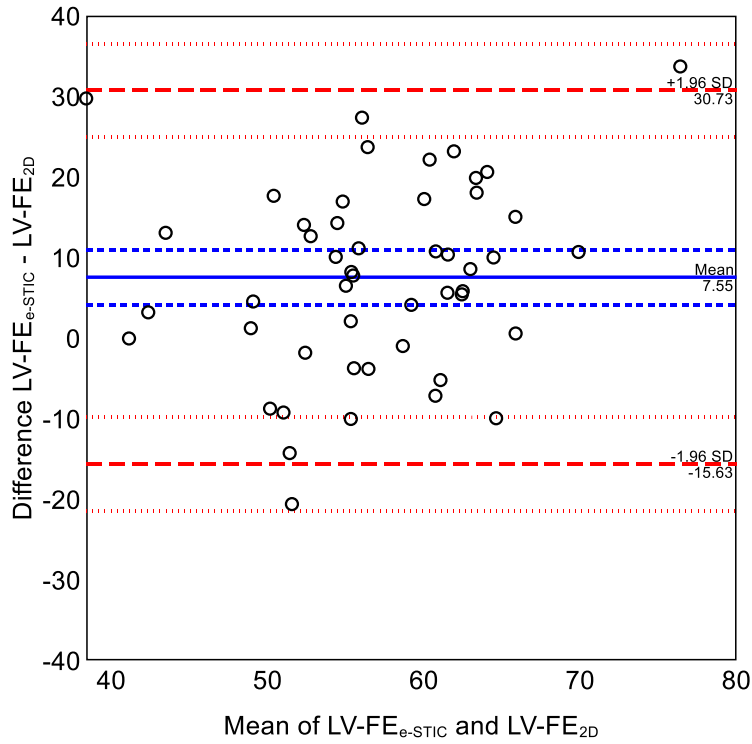
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368 **Figure 1** Bland-Altman plot of left ventricular global strain (LV-GS) measured with e-
369 STIC acquisition versus 2D analysis. Short-dashed lines indicate the 95% confidence
370 interval for the mean difference. Dotted lines indicate the 95% confidence intervals of the
371 limits of agreement.



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381 **Figure 2** Bland-Altman plot of LV-FE (%) measured with e-STIC acquisition versus 2D
382 analysis. Short-dashed lines indicate the 95% confidence interval for the mean difference.
383 Dotted lines indicate the 95% confidence intervals of the limits of agreement.



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386 **Supplementary material S1** Video abstract illustrating fetal speckle-tracking technique
387 and the main results of our study.