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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 <sup>1-3</sup>.  
38 Primarily focused on identifying congenital heart defects, echocardiography has recently  
39 renewed by the increasing interest on fetal myocardial function <sup>4</sup>. 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 <sup>5</sup>. This approach is currently used in adults and children by cardiologists <sup>6, 7</sup> and it has  
45 recently been applied to the fetus <sup>8-10</sup>, providing a non-invasive measure and  
46 representation of myocardial contractility in several pregnancy-related complications <sup>11-</sup>  
47 <sup>15</sup>. Deformation imaging directly measure the lengthening and shortening of the  
48 myocardium throughout the cardiac cycle <sup>5, 16 17, 18</sup>. 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 <sup>19, 20</sup>. 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) <sup>21</sup>, facilitate both  
57 examination and documentation of sonographic datasets <sup>22</sup>. 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 <sup>23</sup>.  
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 <sup>24</sup>. 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 <sup>23</sup>. 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 <sup>24</sup>. 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 <sup>24</sup>. 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 <sup>25, 26</sup>, the estimated fetal weight (computing the

105 measurements of the biparietal diameter, head circumference, abdominal circumference,  
106 and femur length) was expressed using z-score<sup>27</sup>.

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 <sup>28</sup>. 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 <sup>28</sup>.

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  $\pm 1.96$  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  $60.65 \pm 10.34$  and  
159  $53.10 \pm 8.41$ , 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 model<sup>28</sup>. 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 model<sup>28</sup>. 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 interactions<sup>29</sup>. 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 strain<sup>30</sup>.

191 Since first measures of myocardial strain in the healthy fetus were reported, many  
192 concerns remains about the reliability of these measurements<sup>20</sup>. Some studies have  
193 proved that calculation of strain parameters from bidimensional fetal images have good  
194 inter and intra-observer reproducibility<sup>4, 31</sup>, 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<sup>5, 19</sup>, but overall the agreement of 2D-  
197 STE appears to be good, and equal or superior to Doppler techniques<sup>4, 19</sup>.

198 However, 2D imaging has several limitations<sup>20</sup>: 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 interest<sup>32</sup>. 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<sup>33, 34</sup>. 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 outlined<sup>21-23</sup>. 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<sup>22, 23</sup>. 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 <sup>22,24</sup>. 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 <sup>20, 29, 35</sup>.  
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 gestation <sup>19</sup> and SGA or IUGR fetuses could show altered myocardial  
232 deformation values <sup>11, 15</sup>. 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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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.

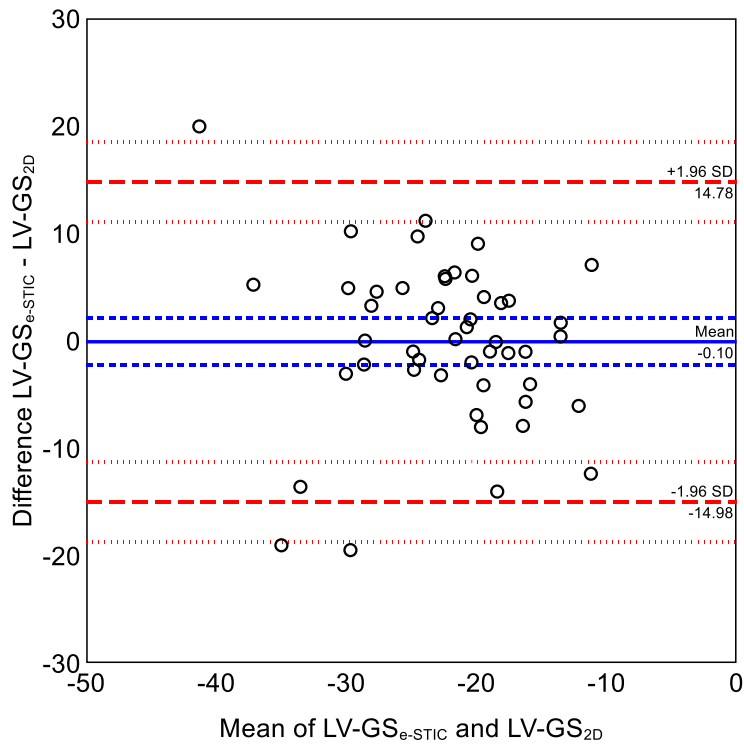
364 **Table 2** Intra-rater reliability of e-STIC acquisition and 2D analysis for heart evaluation  
 365 (LV-GS and LV-FE)

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.

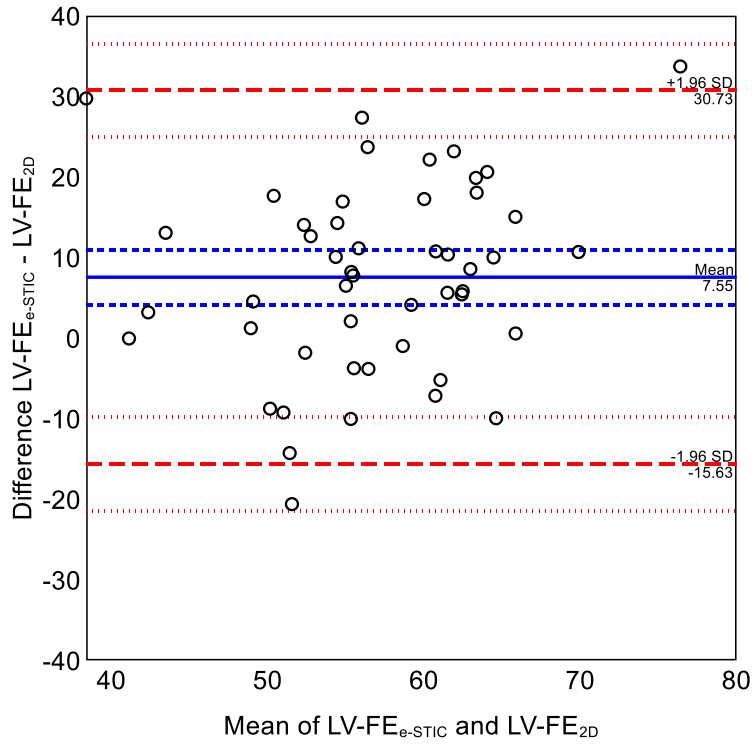
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368 **Figure 1** Bland-Altman plot of left ventricular global strain (LV-GS) measured with e-  
369 STIC acquisition versus 2D analysis. Short-dashed lines indicate the 95% confidence  
370 interval for the mean difference. Dotted lines indicate the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