<https://helda.helsinki.fi>

Effect of hypoxemia on fetal ventricular deformation in a chronically instrumented sheep model

Bhide, Amarnath

2017-05

Bhide , A , Räsänen , J , Huhta , H , Junno , J , Erkinaro , T , Ohtonen , P , Haapsamo , M & Acharya , G 2017 , ' Effect of hypoxemia on fetal ventricular deformation in a chronically instrumented sheep model ' , *Ultrasound in Medicine & Biology* , vol. 43 , no. 5 , pp. 967-973 . <https://doi.org/10.1016/j.ultrasmedbio.2017.01.010>

<http://hdl.handle.net/10138/313130>

<https://doi.org/10.1016/j.ultrasmedbio.2017.01.010>

cc_by_nc_nd

acceptedVersion

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.

The effect of hypoxemia ~~and metabolic acidemia~~ on fetal ventricular deformation in a chronically instrumented sheep model

Amarnath Bhide¹, Juha Rasanen^{2, 3, 4}, Heikki Huhta⁴, Juulia Junno⁴, Tiina Erkinaro⁵,
Pasi Ohtonen⁶, Mervi Haapsamo⁷, Ganesh Acharya^{1,8}

Women's Health & Perinatal Research Group, Department of Clinical Medicine, UiT-
The Arctic University of Norway, Tromsø, Norway (1),

Department of Obstetrics and Gynecology, Kuopio University Hospital and
University of Eastern Finland, Kuopio, Finland (2),

Department of Obstetrics and Gynecology, Helsinki University Hospital, Helsinki,
Finland (3),

Department of Obstetrics and Gynecology (4), Anaesthesiology (5) and Statistics (6),
Oulu University Hospital, Oulu, Finland.

Department of Obstetrics and Gynecology, Lapland Central Hospital, Finland (7).

Department of Clinical Science, Intervention and Technology (CLINTEC),
Karolinska Institutet, Stockholm, Sweden (8)

Address for correspondence:

Dr. Amar Bhide, MD, FRCOG
Fetal Medicine Unit
4th Floor, Lanesborough Wing
St. George's Hospital, Blackshaw Road
SW17 0QT
United Kingdom
Tel: +44 20 87250080
Fax: +44 20 87250079
e-mail: abhide@sgul.ac.uk

Running title: Hypoxemia and ~~myocardial strain~~ventricular deformation
in fetal sheep model

Abstract

We hypothesized that in ~~a~~-near-term sheep ~~pregnancy-fetuses~~ hypoxaemia changes myocardial function that ~~is detectable by~~areis reflected in altered ventricular deformation on speckle-tracking echocardiography. Fetuses were instrumented in 21 pregnant sheep. After 4-day recovery, fetal cardiac function was assessed by ~~speckle-tracking~~ echocardiography at baseline, after 30 and 120 minutes of induced fetal hypoxemia and following its reversal. Left (LV) and right ventricular (RV) cardiac outputs, myocardial strains were measured. Baseline mean (SD) LV and RV global longitudinal strains were -18.7% (3.8) and -14.3% (5.3). Baseline RV global longitudinal and circumferential ~~strains-deformations~~ were ~~lower-lesser~~ ~~(p = 0.016 and p < 0.005)~~ compared to LV (p=0.016 and p<0.005). LV, but not RV global longitudinal strain reduced (p=0.003) compared to baseline with hypoxemia. Circumferential and radial strains did not show significant changes. In near-term sheep fetus, LV global longitudinal and circumferential strains are more negative compared to RV. Acute hypoxemia leads to LV rather than RV dysfunction as demonstrated by decreased deformation.

Word count: ~~149~~148

Keywords - Speckle tracking echocardiography; hypoxemia; sheep model

1 Introduction –

2 Fetal cardiovascular protective response to moderate isocapnic
3 ~~hypoxia~~hypoxaemia involves chemoreflex, endocrine and local components. Acute
4 fetal hypoxemia is associated with increased blood flow to the myocardium, brain and
5 adrenal glands, but the fetal autonomic response to hypoxemia varies by gestational
6 age (Iwamoto, et al. 1989). At 0.6-0.7 gestation in fetal sheep (87-102 days), the
7 arterial blood pressure (BP) does not change significantly and fetal heart rate may
8 remain unchanged or increase slightly (Iwamoto, et al. 1989), whereas at 0.9 gestation
9 vagally mediated bradycardia and a rise of BP is observed (Giussani, et al. 1993).
10 Hemodynamic changes related to acute and chronic hypoxemia in sheep fetuses have
11 been well described (Carter 2015, Jensen, et al. 1999, Rudolph 1985). Chronic
12 hypoxemia without acidemia does not affect the fetal cardiac output (Block, et al.
13 1990). ~~Band~~ blood flow to the placenta, carcass, kidney, and spleen are maintained
14 remain unchanged with chronic hypoxaemia until fetal acidosis develops ~~-(without~~
15 ~~acidaemia), but it decreases with fetal acidemia~~acidemia (Jensen, et al. 1999). We
16 have ~~previously~~ reported that acute hypoxemia in near-term fetal sheep leads to an
17 increase in the pulmonary arterial vascular impedance, as well as an increase in the
18 right ventricular cardiac output (Mäkitallio, et al. 2006). Peak myocardial velocities
19 measured by tissue Doppler technique appear to decrease during hypoxemia/acidemia
20 especially during the isovolumic periods of cardiac cycle when tissue Doppler
21 technique is used (Acharya, et al. 2008). However, changes in myocardial strain that
22 may occur during fetal hypoxemia have not been elucidated. Two-dimensional (2-D)
23 strain imaging uses standard B-mode images and speckle-tracking for the evaluation
24 of myocardial deformation. Lagrangian strain is defined as the instantaneous
25 lengthening or shortening compared to the initial muscle fiber length, which, in the
26 myocardium, is commonly determined by the end of diastole (D'Hooge, et al. 2000).
27 The relative independence of the angle of ultrasound beam and direction of movement
28 makes this technique less prone to errors. Speckle tracking echocardiography is still a
29 research tool, and its place value in clinical practice is being debated ~~at~~
30 ~~present~~ (Huang and Orde 2013, Orde, et al. 2016, Tanaka and Hirata 2016). This
31 technique has been used to evaluate cardiac function in normal fetuses (Kapusta, et al.
32 2012), those of diabetic mothers (Kulkarni, et al. 2016), fetuses with twin to twin
33 transfusion syndrome and those with congenital diaphragmatic hernia (Van Mieghem,
34 et al. 2011). To our knowledge, response of fetal cardiac function to

~~hypoxaemia has not been evaluated before using speckle-tracking echocardiography~~
~~has not been reported before.~~

The present experiment on near-term fetal sheep was designed to test the hypothesis that a decrease in fetal oxygenation leads to compensatory changes in the myocardial function that can be detected by 2-D speckle-tracking echocardiography. Specifically, ~~w~~We aimed to ~~The~~evaluateasked two questions in this study: 1) are ~~whether potential uses of~~ left and right ventricular deformations similar in normoxemic conditions and 2) are their responses to prolonged hypoxemia different? ~~this technique has a potential is to develop be used as a new and more sensitive tests to detect myocardial dysfunction caused by fetal hypoxaemia, so that surveillance of compromised fetuses and appropriate interventions are possiblecould be improved.~~

Material and methods

All experiments were performed in accordance with the 'European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes' (Council of Europe 1986) and the European Union Directive ETS 123 (1997). The Animal Care and Use Committee of the University of Oulu approved the study protocol.

Animal preparation

Data from 21 chronically instrumented pregnant sheep were used for this report. Mean (SD) weight of the ewes and the fetuses was 53.9 (SD=8.1) kg and 2401 (256) g, respectively.

Surgery was performed at 115–129 gestational days (term 145 days). Ewes were fasted overnight and pre-medicated with intramuscular ketamine (2 mg/kg) and midazolam (0.2 mg/kg). The maternal external jugular vein was cannulated and Ringer's lactate solution was infused at a rate of 200 ml/h. General anesthesia was induced with intravenous Propofol (4–7 mg/kg) and maintained with Isoflurane (1.5–2.5%) in an oxygen–air mixture delivered via an endotracheal tube. Intravenous boluses of fentanyl (0.05–0.15 mg) were administered as required.

A laparotomy was performed under general anesthesia and endotracheal intubation. The fetal head and neck were delivered through a small uterine incision. Catheters were introduced in the internal jugular vein and the carotid artery to allow access to arterial and venous circulations, and to collect blood samples.

1 A small left lateral thoracotomy was performed at the level of 3rd intercostal
2 space and the ~~fetal~~ ductus arteriosus was identified and dissected. A 4 mm ultrasonic
3 transit-time flow probe was secured around the vessel and tunneled outside for the
4 measurement of ductus arteriosus blood flow. Thoracotomy was closed.
5 Electrocardiogram (ECG) leads were placed subcutaneously to obtain fetal ECG. A
6 catheter was anchored to the fetal skin to measure intra amniotic pressure. After the
7 replacement of amniotic fluid by 0.9% warm saline and closure of the surgical
8 wounds, all catheters and probes were tunneled subcutaneously and exteriorized
9 through a small skin incision in the ewe's flank. Postoperative analgesia was provided
10 with a fentanyl patch (50 mcg/h) attached to the ewe's tail, with additional
11 intramuscular injections of fentanyl 1.5 to 2 mg/kg twice daily.

12 After a 4-day recovery, general anesthesia was induced again as described
13 above. A 16-gauge polyurethane catheter was inserted into the maternal descending
14 aorta through a femoral artery. The ewe was placed supine with a right lateral tilt and
15 allowed to stabilize for 30 min before the baseline measurements. Thereafter,
16 maternal and fetal hypoxemia was induced by replacing inhaled oxygen with medical
17 air in the re-breathing circuit to reach maternal oxyhemoglobin saturation level of 75–
18 80%. Hypoxemia phase data were obtained at 30 minutes and 120 minutes after
19 reaching desired maternal saturation level. After the data collection for the last
20 hypoxemia phase was completed, oxygen was substituted, and re-breathing
21 discontinued to allow recovery from hypoxemia, and recovery phase data was
22 obtained 15 minutes after maternal normoxemia was established. Fetal heart rate was
23 monitored with a cardiometer triggered from the arterial pressure waves. Fetal
24 arterial blood pressure was referenced to amniotic fluid pressure. Fetal blood samples
25 were obtained to measure the acid-base status, and blood gases at the end of each
26 phase. At the end of the experiment, the animals were euthanized with an intravenous
27 overdose (1.0 mg/kg) of pentobarbital sodium. Fetal weight was recorded.

28
29 ~~Two-dimensional speckle tracking echocardiography~~ Fetal
30 echocardiography was performed using Vivid 7 ultrasound system (GE medical
31 systems, Horten, Norway) with a ~~10-MHz phased-array transducer 7S-phased array~~
32 ~~probe~~ to obtain 2-D gray-scale images with a high frame-rate. Cine-loop images of
33 four-chamber view and short-axis view at the level of papillary muscles were
34 obtained by a single investigator (JR). The imaging depth was 5 -10 cm and the

1 mechanical index (MI) and thermal index (TI) were kept below 1.5 and 1.0,
2 respectively. Two stable well-defined consecutive cardiac cycles from each view were
3 used for speckle tracking analysis. The scan-line digital data for these images were
4 processed using acoustic-tracking software (EchoPAC, GE medical systems, Horten,
5 Norway), allowing off-line semi-automated analysis of speckle-based strain.

6 The off-line analysis was performed by a single operator (AB) who was
7 blinded to the fetal arterial blood gas values and hemodynamic data. Endocardial
8 surface was manually traced at the end-systolic frame, and an automated tracking
9 algorithm outlined the myocardium in successive frames throughout the cardiac cycle
10 (Figure 1). Tracking quality was verified automatically for each segment. Manual
11 adjustment of the region of interest was performed if necessary. Myocardial global
12 longitudinal strain was measured for left and the right ventricles on a four-chamber
13 view. These measurements were repeated in another cardiac cycle from the second
14 stored cine-loop of the same fetus. Mean of these two measurements were used for
15 data analysis. Interventricular septum was not excluded while measurement of right
16 ventricular the RV global longitudinal strain was performed. Global circumferential
17 and radial strains for the left and the right ventricle (Hayabuchi, et al. 2015) were
18 calculated from the short-axis view at the level of the papillary muscles (Figure 2). An
19 average of segmental values was calculated to obtain global values.

20
21 ~~Measurement of fetal cardiac output~~—Blood flow velocity waveforms were
22 obtained at the level of aortic and pulmonary valves ~~from the left and the right~~
23 ~~ventricular outflow tracts~~ keeping the angle of insonation close to zero degrees
24 (always <15 degrees), and the mean velocities were determined (mean velocity =
25 time-velocity integral x heart rate). Inner diameters of the aortic and pulmonary
26 valves were measured using a B-mode cine loop images keeping the insonation angle
27 perpendicular to the vessels as far as possible (Kiserud, et al. 2006). An average of
28 three measurements was used for calculating cardiac outputs. The left (LVCO) and
29 the right ventricular cardiac outputs (RVCO) were computed separately as: cardiac
30 output (CO) = mean velocity x $3.14 \text{ (diameter of the outflow tract/2)}^2$. The combined
31 cardiac output (CCO) was calculated as the sum of LVCO and RVCO.

~~Measurement of pulmonary blood flow and impedance~~ The pulmonary volume blood flow was calculated as the difference between the RVCO and the ductus arteriosus volume blood flow. Doppler blood flow velocity waveforms were obtained from the right pulmonary artery (RPA) as described previously (Rasanen, et al. 1996). Pulsatility index (PI) was calculated as: (peak systolic velocity – end-diastolic velocity)/time-averaged maximum velocity (Deane 2000) as a surrogate for pulmonary arterial vascular impedance.

~~Statistical analysis~~

Data were expressed as mean and standard deviation (SD) unless stated otherwise. Linear Mixed Model (LMM) was used for repeatedly measured data. If LMM showed a significant difference between measurement points ($P_{\text{time}} < 0.05$), then a pairwise comparison between relevant points was performed. Paired samples t-test was used to compare LV and RV global longitudinal, circumferential and radial strains. Mean differences with 95% confidence intervals (95% CI) are presented for paired samples t-test. Statistical analyses were performed using SPSS (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.) and SAS (version 9.3. SAS Institute Inc., Cary, NC, USA). Two-tailed p value < 0.05 was considered statistically significant.

Results

~~Mean weight of the ewes and the fetuses was 53.9 (SD: 8.1) kg and 2401 (256) g respectively.~~

~~Blood gas values and hemodynamic data~~

~~As expected, m~~Maternal hypoxemia led to a significant reduction in fetal pO_2 , and during recovery phase it returned back to baseline level (Table 1). Fetal pH decreased significantly at 120-minute hypoxemia phase ~~significantly~~ and remained significantly lower during recovery phase than at baseline. In addition, fetal lactate level was significantly higher during hypoxemia and recovery phases than at baseline. Fetal pCO_2 did not change during the experiment. Hypoxemia decreased fetal arterial blood pressures significantly (Table 1). Fetal heart rate was significantly lower at the recovery phase as compared to baseline and hypoxemia phases. During hypoxemia,

Formatted: Font: Not Italic

Formatted: Font: Not Italic

1 there was a significant increase in fetal RPA PI values and a decrease in LVCO.
2 Pulmonary blood flow decreased with hypoxemia compared to the baseline, but the
3 reduction did not reach statistical significance. Fetal RVCO and CCO remained stable
4 during the entire experiment (Table 1).

6 *2-D strain data*

7 Ultrasound recordings were obtained at a mean frame rate of 167 (Range: 126-
8 235) frames/sec. At baseline, there was significantly more deformation in the LV
9 global longitudinal ($p = 0.016$) and circumferential ($p < 0.005$) strains than in the
10 corresponding parameters of the RV (Table 2). Hypoxemia at 30 and 120 minutes led
11 to a significant reduction (less deformation) in the global longitudinal strain of the
12 LV, while the global circumferential and radial strains were not affected by fetal
13 hypoxemia (Table 2, Figure 32). During the recovery period, LV global longitudinal
14 strain returned back to baseline level. Right ventricular global longitudinal,
15 circumferential and radial strains did not change statistically significantly. Baseline
16 left ventricular strain rate was significantly higher than the right ($p = 0.003$). -during
17 the experiment There were no significant changes in left or right ventricular global
18 longitudinal strain rates during the experiment. (Table 2).

20 **Conclusion**

21 ~~We have shown that the fetal heart function and its response to hypoxemia can~~
22 ~~be evaluated using speckle tracking echocardiography. At a gestational age equivalent~~
23 ~~to 0.8 term, the global longitudinal and circumferential strains are less negative in the~~
24 ~~RV as compared to the LV. Fetus responds to acute hypoxemia by reduced~~
25 ~~deformation of the LV, but not the RV, and this response is reversed by reversal of~~
26 ~~hypoxemia. This recovery of fetal cardiac function is seen even in the face of~~
27 ~~worsening metabolic acidemia.~~

29 **Discussion**

30 The present study was designed to investigate the effects of fetal hypoxemia
31 ~~and metabolic acidemia~~ on LV and RV strain formation. The experiments were done
32 in fetal sheep at 0.8 gestation that corresponds to approximately 30-32 weeks
33 gestation of human pregnancy. Blood gas values demonstrated that after 30 minutes
34 of hypo-oxygenation the fetuses were hypoxemic without any significant metabolic

Formatted: None, Indent: First
line: 1.27 cm

1 component, but prolonged hypoxemia (120 minutes) led to significant fetal metabolic
2 acidemia. In the recovery phase, fetal oxygenation returned back to baseline level, but
3 there was still significant metabolic acidemia. We found that at baseline there was
4 more global longitudinal and circumferential deformation (strain) in the LV than in
5 the RV. Fetal hypoxemia was associated with reduced LV global longitudinal strain
6 that returned back to baseline level when fetal normoxemia was restored, even though
7 the fetus still had a significant metabolic acidemia. Fetal hypoxemia, even with and/or
8 metabolic ~~acidemia~~acidemia, had no effect on RV strains.

9
10 A reduction in the LV global longitudinal deformation with hypoxemia may
11 be a result of reduced LV preload, direct LV myocardial dysfunction or a combination
12 of the two. It has been shown that pulmonary circulation is sensitive to fetal
13 oxygenation during the last trimester of pregnancy (Rasanen, et al. 1996), and that
14 hypoxemia leads to pulmonary vasoconstriction (Arraut, et al. 2013). Indeed, in the
15 current experiment RPA PI values increased significantly with hypoxemia suggesting
16 that impedance to pulmonary blood flow increased during hypoxemia, although the
17 reduction in the estimated pulmonary volume blood flow was not statistically
18 significant. It has been shown in a near-term sheep model of acute ductus arteriosus
19 occlusion that the fetus is unable to increase volume blood flow across the foramen
20 ovale (Tulzer, et al. 1991), thus the reduction in the LVCO is most likely~~must be~~ a
21 consequence of a drop in pulmonary volume blood flow leading to a decrease in LV
22 preload. It has been shown in human fetuses that global longitudinal strain is similar
23 in both ventricles at 20-24 weeks of gestation (Kapusta, et al. 2012). Later in
24 pregnancy LV global longitudinal strain does not change, however, corresponding RV
25 strain or deformation decreases. This is explained by an increase in the RV preload. If
26 the change in preload would be the major factor in the strain formation, we should
27 have observed an opposite finding in the LV global longitudinal strain. Therefore, our
28 results ~~suggest that the decrease in the LV global longitudinal strain during~~
29 ~~hypoxemia was caused by LV myocardial dysfunction.~~indicate that the direct effect of
30 hypoxemia on ~~inherent~~intrinsic contractile properties of the LV is -likely responsible
31 for decreased global longitudinal strain.~~play a role in addition to the effect of preload~~
32 and afterload.

Fetal global LV longitudinal deformation already decreased at 30-minute hypoxemia phase, when significant metabolic acidemia had not yet developed. At 120-minute hypoxemia phase fetal pO₂ was comparable to 30-minute hypoxemia phase, however, a significant metabolic acidemia had developed between these two time points. Interestingly, we found no further deterioration in any of the strain parameters. We have previously reported that fetal LV is more susceptible to hypoxemia as compared to fetal RV. However, in that study, fetal cardiac function and hemodynamics were assessed by tissue Doppler and pulsed Doppler imaging, rather than speckle tracking. The findings of this study corroborate our previous observations. We have also found that the fetal LV myocardial performance index (MPI) significantly worsens with hypoxemia (Bhide, et al. 2016), which also supports the findings of this study. At recovery phase, when fetal oxygenation was returned back to baseline conditions, the LV global longitudinal strain was similar to baseline values, even though fetuses were still suffering from significant metabolic acidemia. Furthermore, LVCO, RVCO or CCO did not show significant changes with progressive metabolic acidemia. This shows that the fetal myocardial function was not further deteriorated despite worsening metabolic acidemia. Our results suggest that LV function appears to be more sensitive to oxygenation than metabolic acidosis. Experimental intravenous infusion of lactic acid to sheep fetuses led to reduction in fetal pH, increase in the heart rate but no change in blood pressure (Hohimer and Bissonnette 1991). This also suggests that lactic acid does not influence cardiac function.

Di Salvo et al (Di Salvo, et al. 2005) were the first to describe fetal LV and RV function using ultrasound based strain and strain rate imaging. They used myocardial color Doppler imaging for quantification of strain. Doppler technology is known to be angle dependent, which speckle tracking can eliminate (Biswas, et al. 2013). There are several software programs to assess myocardial deformation using speckle tracking. However, they are not necessarily comparable. The velocity vector imaging mainly tracks myocardial motion in a narrow area just underneath the endocardium. In contrast, the EchoPAC automated functional imaging software tracks myocardial deformation in a relatively wider area, specified by the operator (Biswas, et al. 2013).

Strengths and weaknesses –

At least 60–110 frames per second are recommended when imaging the adult heart (Mondillo, et al. 2011), and even higher rates are desirable to provide enough frames per heart beat in the fetus (Matsui, et al. 2011). We studied the fetus at a mean frame rate of 167 Hz, and this is likely to have led to reliable results. The surgical procedures may constitute a significant stress on the sheep fetuses, and it may be argued that the conditions are quite different from human fetuses exposed to hypoxemia. Normal arterial blood gas values at the baseline stage suggest conditions close to physiologic circulatory state (Acharya, et al. 2004). The study was carried out in a narrow gestational age window of 115-129 days, and may limit the validity and significance outside this time period. Extrapolation of these results to human pregnancy should be done cautiously. The sheep model has been extensively used for research in fetal hemodynamics, and the myocardial strain curves are similar in normal ovine and human pregnancy. We did not monitor cerebral blood flow during the experiment and it could be argued that the reduction in LV global longitudinal strain could just reflect a drop in cerebral vascular resistance during hypoxemia. Indeed, carotid artery blood pressure decreased during hypoxemia. Furthermore, it has been shown that carotid artery volume blood flow increases from about 85 ml/min (normoxemia) to about 95-100 ml/min during isocapnic hypoxemia (Bennet, et al. 1998)(ref). We can estimate that there was about 24 % reduction in the carotid artery vascular resistance during hypoxemia. However, based on these estimations, over 80% of LVCO bypasses brain circulation during prolonged hypoxemia and is affected by lower body vascular resistance. Thus, we believe that reduced carotid artery vascular resistance is not the main determinant of reduced LV global longitudinal strain during hypoxemia. effects shown could have just as much of as much an effect on the preload as on the afterload of the LV, and cerebrovascular resistance should have been studied. The blood pressure was obtained by a catheter placed in the carotid artery of the fetus, which . Therefore, the it is a directly reflection of the cerebrovascular resistance. Both systolic and diastolic blood pressures showed a reduction with the onset of hypoxaemia, reflecting the cerebral vasodilatation as a fetal response to hypoxaemia. If hypoxemia affected only the cerebrovascular resistance and not the left ventricularLV contractility, the left ventricularLV output should not have changed. THowever, he LV output showed a reductiondecreased

1 ~~with hypoxaemia, anwhichd is highly suggestive of a reduction in LV contractility.~~
2 ~~The green symbol obtained for the automatic evaluation of myocardial speckle~~
3 ~~tracking strain solution indicates the highest level of minimization of the sum of the~~
4 ~~absolute difference by the algorithm, and indicates the highest level of confidence in~~
5 ~~the speckle tracking computations and in the strain solutions.~~

6 Conclusion

7 We have shown that hypoxemia leads to changes in fetal cardiac function that
8 is reflected in altered ventricular deformation on the fetal heart function and its
9 response to hypoxemia can be evaluated using speckle-tracking echocardiography. At
10 a gestational age equivalent to 0.8 term, the global longitudinal and circumferential
11 strains are less negative in the RV as compared to the LV. Fetus responds to acute
12 hypoxemia by reduced deformation of the LV, but not the RV, and this response is
13 reversed by reversal of hypoxemia. This recovery of LV fetal cardiac function is seen
14 even in the face of worsening presence of metabolic acidemia.

15
16 Acknowledgement: This study was funded by the Regional Health Authority of
17 Northern Norway.

Formatted: Not Highlight

References

- Acharya G, Erkinaro T, Makikallio K, Lappalainen T, Rasanen J. Relationships among Doppler-derived umbilical artery absolute velocities, cardiac function, and placental volume blood flow and resistance in fetal sheep. *American journal of physiology. Heart and circulatory physiology* 2004; 286:H1266-72.
- Acharya G, Rasanen J, Makikallio K, Erkinaro T, Kavasmaa T, Haapsamo M, Mertens L, Huhta JC. Metabolic acidosis decreases fetal myocardial isovolumic velocities in a chronic sheep model of increased placental vascular resistance. *American journal of physiology. Heart and circulatory physiology* 2008; 294:H498-504.
- Arraut AM, Frias AE, Hobbs TR, McEvoy C, Spindel ER, Rasanen J. Fetal pulmonary arterial vascular impedance reflects changes in fetal oxygenation at near-term gestation in a nonhuman primate model. *Reprod Sci* 2013; 20:33-8.
- Bennet L, Peebles DM, Edwards AD, Rios A, Hanson MA. The cerebral hemodynamic response to asphyxia and hypoxia in the near-term fetal sheep as measured by near infrared spectroscopy. *Pediatric research* 1998; 44:951-7.
- Bhide A, Vuolteenaho O, Haapsamo M, Erkinaro T, Rasanen J, Acharya G. Effect of Hypoxemia with or without Increased Placental Vascular Resistance on Fetal Left and Right Ventricular Myocardial Performance Index in Chronically Instrumented Sheep. *Ultrasound in medicine & biology* 2016; 42:2589-98.
- Biswas M, Sudhakar S, Nanda NC, Buckberg G, Pradhan M, Roomi AU, Gorissen W, Houle H. Two- and three-dimensional speckle tracking echocardiography: clinical applications and future directions. *Echocardiography* 2013; 30:88-105.
- Block BS, Schlafer DH, Wentworth RA, Kreitzer LA, Nathanielsz PW. Intrauterine asphyxia and the breakdown of physiologic circulatory compensation in fetal sheep. *American journal of obstetrics and gynecology* 1990; 162:1325-31.
- Carter AM. Placental Gas Exchange and the Oxygen Supply to the Fetus. *Compr Physiol* 2015; 5:1381-403.
- D'Hooge J, Heimdal A, Jamal F, Kukulski T, Bijnens B, Rademakers F, Hatle L, Suetens P, Sutherland GR. Regional strain and strain rate measurements by cardiac ultrasound: principles, implementation and limitations. *European journal of echocardiography : the journal of the Working Group on Echocardiography of the European Society of Cardiology* 2000; 1:154-70.

- 1 Deane C. Doppler Ultrasound: principles and practice, In: Nicolaides K, Rizzo G, Hecher
2 K., ed. *Placental and fetal Doppler*. London: The Parthenon Publishing
3 Group, 2000. 22.
- 4 Di Salvo G, Russo MG, Paladini D, Pacileo G, Felicetti M, Ricci C, Cardaropoli D,
5 Palma M, Caso P, Calabro R. Quantification of regional left and right
6 ventricular longitudinal function in 75 normal fetuses using ultrasound-based
7 strain rate and strain imaging. *Ultrasound in medicine & biology* 2005;
8 31:1159-62.
- 9 Giussani DA, Spencer JA, Moore PJ, Bennet L, Hanson MA. Afferent and efferent
10 components of the cardiovascular reflex responses to acute hypoxia in term
11 fetal sheep. *The Journal of physiology* 1993; 461:431-49.
- 12 Hayabuchi Y, Sakata M, Kagami S. Right ventricular myocardial deformation
13 patterns in children with congenital heart disease associated with right
14 ventricular pressure overload. *Eur Heart J Cardiovasc Imaging* 2015; 16:890-
15 9.
- 16 Hohimer AR, Bissonnette JM. Vascular lactic acid infusions do not alter the incidence
17 of fetal breathing movements or their inhibition by acute hypoxemia. *Pediatric*
18 *research* 1991; 29:483-6.
- 19 Huang SJ, Orde S. From speckle tracking echocardiography to torsion: research tool
20 today, clinical practice tomorrow. *Curr Opin Crit Care* 2013; 19:250-7.
- 21 Iwamoto HS, Kaufman T, Keil LC, Rudolph AM. Responses to acute hypoxemia in
22 fetal sheep at 0.6-0.7 gestation. *The American journal of physiology* 1989;
23 256:H613-20.
- 24 Jensen A, Garnier Y, Berger R. Dynamics of fetal circulatory responses to hypoxia
25 and asphyxia. *European journal of obstetrics, gynecology, and reproductive*
26 *biology* 1999; 84:155-72.
- 27 Kapusta L, Mainzer G, Weiner Z, Deutsch L, Khoury A, Haddad S, Lorber A. Second
28 trimester ultrasound: reference values for two-dimensional speckle tracking-
29 derived longitudinal strain, strain rate and time to peak deformation of the
30 fetal heart. *Journal of the American Society of Echocardiography : official*
31 *publication of the American Society of Echocardiography* 2012; 25:1333-41.
- 32 Kiserud T, Ebbing C, Kessler J, Rasmussen S. Fetal cardiac output, distribution to the
33 placenta and impact of placental compromise. *Ultrasound in obstetrics &*

gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2006; 28:126-36.

Kulkarni A, Li L, Craft M, Nanda M, Lorenzo JM, Danford D, Kuttly S. Fetal Myocardial Deformation in Maternal Diabetes Mellitus and Obesity. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2016.

Mäkikallio K, Erkinaro T, Niemi N, Kavasmaa T, Acharya G, Pääkilä M, Räsänen J. Fetal oxygenation and Doppler ultrasonography of cardiovascular hemodynamics in a chronic near-term sheep model. American journal of obstetrics and gynecology 2006; 194:542-50.

Matsui H, Germanakis I, Kulinskaya E, Gardiner HM. Temporal and spatial performance of vector velocity imaging in the human fetal heart. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2011; 37:150-7.

Mondillo S, Galderisi M, Mele D, Cameli M, Lomoriello VS, Zacà V, Ballo P, D'Andrea A, Muraru D, Losi M, Agricola E, D'Errico A, Buralli S, Sciomer S, Nistri S, Badano L, Echocardiography Study Group Of The Italian Society Of Cardiology (Rome Ia). Speckle-tracking echocardiography: a new technique for assessing myocardial function. Journal of ultrasound in medicine : official journal of the American Institute of Ultrasound in Medicine 2011; 30:71-83.

Orde S, Huang SJ, McLean AS. Speckle tracking echocardiography in the critically ill: enticing research with minimal clinical practicality or the answer to non-invasive cardiac assessment? Anaesth Intensive Care 2016; 44:542-51.

Rasanen J, Wood DC, Weiner S, Ludomirski A, Huhta JC. Role of the pulmonary circulation in the distribution of human fetal cardiac output during the second half of pregnancy. Circulation 1996; 94:1068-73.

Rudolph AM. Distribution and regulation of blood flow in the fetal and neonatal lamb. Circ Res 1985; 57:811-21.

Tanaka H, Hirata K. Is speckle tracking actually helpful for cardiac resynchronization therapy? J Echocardiogr 2016; 14:53-60.

Tulzer G, Gudmundsson S, Rotondo KM, Wood DC, Yoon GY, Huhta JC. Acute fetal ductal occlusion in lambs. American journal of obstetrics and gynecology 1991; 165:775-8.

1 Van Mieghem T, Deprest J, Verhaeghe J. Fetal and maternal hemodynamics in
2 pregnancy: new insights in the cardiovascular adaptation to uncomplicated
3 pregnancy, twin-to-twin transfusion syndrome and congenital diaphragmatic
4 hernia. Facts Views Vis Obgyn 2011; 3:205-13.
5
6

Legend to figures

Figure 1. Automated tracking algorithm outlining the myocardium and calculation of global strain and strain rate using Echopac software.

Figure 2. Automated tracking algorithm outlining the myocardium and calculation of right ventricular global circumferential strain using Echopac software.

Figure ~~32~~. Global left (solid lines) and right ventricular (dotted line) longitudinal strain with hypoxemia and recovery

The effect of hypoxemia on fetal ventricular deformation in a chronically instrumented sheep model

Amarnath Bhide¹, Juha Rasanen^{2, 3, 4}, Heikki Huhta⁴, Juulia Junno⁴, Tiina Erkinaro⁵, Pasi Ohtonen⁶, Mervi Haapsamo⁷, Ganesh Acharya^{1,8}

Women's Health & Perinatal Research Group, Department of Clinical Medicine, UiT- The Arctic University of Norway, Tromsø, Norway (1),

Department of Obstetrics and Gynecology, Kuopio University Hospital and University of Eastern Finland, Kuopio, Finland (2),

Department of Obstetrics and Gynecology, Helsinki University Hospital, Helsinki, Finland (3),

Department of Obstetrics and Gynecology (4), Anaesthesiology (5) and Statistics (6), Oulu University Hospital, Oulu, Finland.

Department of Obstetrics and Gynecology, Lapland Central Hospital, Finland (7).

Department of Clinical Science, Intervention and Technology (CLINTEC), Karolinska Institutet, Stockholm, Sweden (8)

Address for correspondence:

Dr. Amar Bhide, MD, FRCOG

Fetal Medicine Unit

4th Floor, Lanesborough Wing

St. George's Hospital, Blackshaw Road

SW17 0QT

United Kingdom

Tel: +44 20 87250080

Fax: +44 20 87250079

e-mail: abhide@sgul.ac.uk

Running title: Hypoxemia and ventricular deformation in fetal sheep

1 **Abstract**

2 We hypothesized that in near-term sheep fetuses hypoxemia changes
3 myocardial function that is reflected in altered ventricular deformation on speckle-
4 tracking echocardiography. Fetuses were instrumented in 21 pregnant sheep. After 4-
5 day recovery, fetal cardiac function was assessed by echocardiography at baseline,
6 after 30 and 120 minutes of induced fetal hypoxemia and following its reversal. Left
7 (LV) and right ventricular (RV) cardiac outputs, myocardial strains were measured.
8 Baseline mean (SD) LV and RV global longitudinal strains were -18.7% (3.8) and -
9 14.3% (5.3). Baseline RV global longitudinal and circumferential deformations were
10 less compared to LV ($p=0.016$ and $p<0.005$). LV, but not RV global longitudinal
11 strain reduced ($p=0.003$) compared to baseline with hypoxemia. Circumferential and
12 radial strains did not show significant changes. In near-term sheep fetus, LV global
13 longitudinal and circumferential strains are more negative compared to RV. Acute
14 hypoxemia leads to LV rather than RV dysfunction as demonstrated by decreased
15 deformation.

16
17 **Word count: 148**

18
19 **Keywords** - Speckle tracking echocardiography; hypoxemia; sheep model
20
21

1 **Introduction –**

2 Fetal cardiovascular protective response to moderate isocapnic hypoxemia
3 involves chemoreflex, endocrine and local components. Acute fetal hypoxemia is
4 associated with increased blood flow to the myocardium, brain and adrenal glands,
5 but the fetal autonomic response to hypoxemia varies by gestational age (Iwamoto, et
6 al. 1989). At 0.6-0.7 gestation in fetal sheep (87-102 days), the arterial blood pressure
7 (BP) does not change significantly and fetal heart rate may remain unchanged or
8 increase slightly (Iwamoto, et al. 1989), whereas at 0.9 gestation vagally mediated
9 bradycardia and a rise of BP is observed (Giussani, et al. 1993). Hemodynamic
10 changes related to acute and chronic hypoxemia in sheep fetuses have been well
11 described (Carter 2015, Jensen, et al. 1999, Rudolph 1985). Chronic hypoxemia
12 without acidemia does not affect the fetal cardiac output (Block, et al. 1990). Blood
13 flow to the placenta, carcass, kidney, and spleen are maintained with chronic
14 hypoxemia until fetal acidosis develops (Jensen, et al. 1999). We have reported that
15 acute hypoxemia in near-term fetal sheep leads to an increase in the pulmonary
16 arterial vascular impedance, as well as an increase in the right ventricular cardiac
17 output (Mäkikallio, et al. 2006). Peak myocardial velocities measured by tissue
18 Doppler technique appear to decrease during hypoxemia/acidemia especially during
19 the isovolumic periods of cardiac cycle (Acharya, et al. 2008). However, changes in
20 myocardial strain that may occur during fetal hypoxemia have not been elucidated.
21 Two-dimensional (2-D) strain imaging uses standard B-mode images and speckle-
22 tracking for the evaluation of myocardial deformation. Lagrangian strain is defined as
23 the instantaneous lengthening or shortening compared to the initial muscle fiber
24 length, which, in the myocardium, is commonly determined by the end of diastole
25 (D'Hooge, et al. 2000). The relative independence of the angle of ultrasound beam
26 and direction of movement makes this technique less prone to errors. Speckle tracking
27 echocardiography is still a research tool, and its value in clinical practice is being
28 debated (Huang and Orde 2013, Orde, et al. 2016, Tanaka and Hirata 2016). This
29 technique has been used to evaluate cardiac function in normal fetuses (Kapusta, et al.
30 2012), those of diabetic mothers (Kulkarni, et al. 2016), fetuses with twin to twin
31 transfusion syndrome and those with congenital diaphragmatic hernia (Van Mieghem,
32 et al. 2011). To our knowledge, response of fetal myocardium to hypoxemia has not
33 been evaluated using speckle-tracking echocardiography.

1 The present experiment on near-term fetal sheep was designed to test the
2 hypothesis that a decrease in fetal oxygenation leads to compensatory changes in the
3 myocardial function that can be detected by 2-D speckle-tracking echocardiography.
4 Specifically, we asked two questions in this study: 1) are left and right ventricular
5 deformations similar in normoxemic conditions and 2) are their responses to
6 prolonged hypoxemia different?

8 **Material and methods**

9 All experiments were performed in accordance with the ‘European
10 Convention for the Protection of Vertebrate Animals used for Experimental and Other
11 Scientific Purposes’ (Council of Europe 1986) and the European Union Directive ETS
12 123 (1997). The Animal Care and Use Committee of the University of Oulu approved
13 the study protocol.

14 Data from 21 chronically instrumented pregnant sheep were used for this
15 report. Mean (SD) weight of the ewes and the fetuses was 53.9 (8.1) kg and 2401
16 (256) g, respectively.

17 Surgery was performed at 115–129 gestational days (term 145 days). Ewes
18 were fasted overnight and pre-medicated with intramuscular ketamine (2 mg/kg) and
19 midazolam (0.2 mg/kg). The maternal external jugular vein was cannulated and
20 Ringer’s lactate solution was infused at a rate of 200 ml/h. General anesthesia was
21 induced with intravenous Propofol (4–7 mg/kg) and maintained with Isoflurane (1.5–
22 2.5%) in an oxygen–air mixture delivered via an endotracheal tube. Intravenous
23 boluses of fentanyl (0.05–0.15 mg) were administered as required.

24 A laparotomy was performed under general anesthesia and endotracheal
25 intubation. The fetal head and neck were delivered through a small uterine incision.
26 Catheters were introduced in the internal jugular vein and the carotid artery to allow
27 access to arterial and venous circulations, and to collect blood samples.

28 A small left lateral thoracotomy was performed at the level of 3rd intercostal
29 space and the ductus arteriosus was identified and dissected. A 4 mm ultrasonic
30 transit-time flow probe was secured around the vessel and tunneled outside for the
31 measurement of ductus arteriosus blood flow. Thoracotomy was closed.
32 Electrocardiogram (ECG) leads were placed subcutaneously to obtain fetal ECG. A
33 catheter was anchored to the fetal skin to measure intra amniotic pressure. After the
34 replacement of amniotic fluid by 0.9% warm saline and closure of the surgical

wounds, all catheters and probes were tunneled subcutaneously and exteriorized through a small skin incision in the ewe's flank. Postoperative analgesia was provided with a fentanyl patch (50 mcg/h) attached to the ewe's tail, with additional intramuscular injections of fentanyl 1.5 to 2 mg/kg twice daily.

After a 4-day recovery, general anesthesia was induced again as described above. A 16-gauge polyurethane catheter was inserted into the maternal descending aorta through a femoral artery. The ewe was placed supine with a right lateral tilt and allowed to stabilize for 30 min before the baseline measurements. Thereafter, maternal and fetal hypoxemia was induced by replacing inhaled oxygen with medical air in the re-breathing circuit to reach maternal oxyhemoglobin saturation level of 75–80%. Hypoxemia phase data were obtained at 30 minutes and 120 minutes after reaching desired maternal saturation level. After the data collection for the last hypoxemia phase was completed, oxygen was substituted, and re-breathing discontinued to allow recovery from hypoxemia, and recovery phase data was obtained 15 minutes after maternal normoxemia was established. Fetal heart rate was monitored with a cardiometer triggered from the arterial pressure waves. Fetal arterial blood pressure was referenced to amniotic fluid pressure. Fetal blood samples were obtained to measure the acid-base status, and blood gases at the end of each phase. At the end of the experiment, the animals were euthanized with an intravenous overdose (1.0 mg/kg) of pentobarbital sodium. Fetal weight was recorded.

Fetal echocardiography was performed using Vivid 7 ultrasound system (GE medical systems, Horten, Norway) with a 10-MHz phased-array transducer to obtain 2-D gray-scale images with a high frame-rate. Cine-loop images of four-chamber view and short-axis view at the level of papillary muscles were obtained by a single investigator (JR). The imaging depth was 5–10 cm and the mechanical index (MI) and thermal index (TI) were kept below 1.5 and 1.0, respectively. Two stable well-defined consecutive cardiac cycles from each view were used for speckle tracking analysis. The scan-line digital data for these images were processed using acoustic-tracking software (EchoPAC, GE medical systems, Horten, Norway), allowing off-line semi-automated analysis of speckle-based strain.

The off-line analysis was performed by a single operator (AB) who was blinded to the fetal arterial blood gas values and hemodynamic data. Endocardial surface was manually traced at the end-systolic frame, and an automated tracking

1 algorithm outlined the myocardium in successive frames throughout the cardiac cycle
2 (Figure 1). Tracking quality was verified automatically for each segment. Manual
3 adjustment of the region of interest was performed if necessary. Myocardial global
4 longitudinal strain was measured for left and the right ventricles on a four-chamber
5 view. These measurements were repeated in another cardiac cycle from the second
6 stored cine-loop of the same fetus. Mean of these two measurements were used for
7 data analysis. Interventricular septum was not excluded while measurement of the RV
8 global longitudinal strain was performed. Global circumferential and radial strains for
9 the left and the right ventricle (Hayabuchi, et al. 2015) were calculated from the short-
10 axis view at the level of the papillary muscles (Figure 2). An average of segmental
11 values was calculated to obtain global values.

12
13 Blood flow velocity waveforms were obtained at the level of aortic and
14 pulmonary valves keeping the angle of insonation close to zero degrees (always <15
15 degrees), and the mean velocities were determined (mean velocity = time-velocity
16 integral x heart rate). Inner diameters of the aortic and pulmonary valves were
17 measured using a B-mode cine loop images keeping the insonation angle
18 perpendicular to the vessels as far as possible (Kiserud, et al. 2006). An average of
19 three measurements was used for calculating cardiac outputs. The left (LVCO) and
20 the right ventricular cardiac outputs (RVCO) were computed separately as: cardiac
21 output (CO) = mean velocity x $3.14 (\text{diameter of the outflow tract}/2)^2$. The combined
22 cardiac output (CCO) was calculated as the sum of LVCO and RVCO.

23
24 The pulmonary volume blood flow was calculated as the difference between
25 the RVCO and the ductus arteriosus volume blood flow. Doppler blood flow velocity
26 waveforms were obtained from the right pulmonary artery (RPA) as described
27 previously (Rasanen, et al. 1996). Pulsatility index (PI) was calculated as: (peak
28 systolic velocity – end-diastolic velocity)/time-averaged maximum velocity(Deane
29 2000) as a surrogate for pulmonary arterial vascular impedance.

30
31 Data were expressed as mean and standard deviation (SD) unless stated
32 otherwise. Linear Mixed Model (LMM) was used for repeatedly measured data. If
33 LMM showed a significant difference between measurement points ($P_{\text{time}} < 0.05$), then
34 a pairwise comparison between relevant points was performed. Paired samples t-test

was used to compare LV and RV global longitudinal, circumferential and radial strains. Mean differences with 95% confidence intervals (95% CI) are presented for paired samples t-test. Statistical analyses were performed using SPSS (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.) and SAS (version 9.3. SAS Institute Inc., Cary, NC, USA). Two-tailed p value < 0.05 was considered statistically significant.

Results

Maternal hypoxemia led to a significant reduction in fetal pO₂, and during recovery phase it returned back to baseline level (Table 1). Fetal pH decreased significantly at 120-minute hypoxemia phase and remained significantly lower during recovery phase than at baseline. In addition, fetal lactate level was significantly higher during hypoxemia and recovery phases than at baseline. Fetal pCO₂ did not change during the experiment. Hypoxemia decreased fetal arterial blood pressures significantly (Table 1). Fetal heart rate was significantly lower at the recovery phase as compared to baseline and hypoxemia phases. During hypoxemia, there was a significant increase in fetal RPA PI values and a decrease in LVCO. Pulmonary blood flow decreased with hypoxemia compared to the baseline, but the reduction did not reach statistical significance. Fetal RVCO and CCO remained stable during the entire experiment (Table 1).

2-D strain data

Ultrasound recordings were obtained at a mean frame rate of 167 (Range: 126-235) frames/sec. At baseline, there was significantly more deformation in the LV global longitudinal (p = 0.016) and circumferential (p < 0.005) strains than in the corresponding parameters of the RV (Table 2). Hypoxemia at 30 and 120 minutes led to a significant reduction (less deformation) in the global longitudinal strain of the LV, while the global circumferential and radial strains were not affected by fetal hypoxemia (Table 2, Figure 3). During the recovery period, LV global longitudinal strain returned back to baseline level. Right ventricular global longitudinal, circumferential and radial strains did not change statistically significantly. Baseline left ventricular strain rate was significantly higher than the right (p = 0.003). There were no significant changes in left or right ventricular global longitudinal strain rates during the experiment (Table 2).

1 **Discussion**

2 The present study was designed to investigate the effect of fetal hypoxemia on
3 LV and RV strain formation. The experiments were done in fetal sheep at 0.8
4 gestation that corresponds to approximately 30-32 weeks gestation of human
5 pregnancy. Blood gas values demonstrated that after 30 minutes of hypo-oxygenation
6 the fetuses were hypoxemic without any significant metabolic component, but
7 prolonged hypoxemia (120 minutes) led to significant fetal metabolic acidemia. In the
8 recovery phase, fetal oxygenation returned back to baseline level, but there was still
9 significant metabolic acidemia. We found that at baseline there was more global
10 longitudinal and circumferential deformation (strain) in the LV than in the RV. Fetal
11 hypoxemia was associated with reduced LV global longitudinal strain that returned
12 back to baseline level when fetal normoxemia was restored, even though the fetus still
13 had a significant metabolic acidemia. Fetal hypoxemia, even with metabolic
14 acidemia, had no effect on RV strains.

15
16 A reduction in the LV global longitudinal deformation with hypoxemia may
17 be a result of reduced LV preload, direct LV myocardial dysfunction or a combination
18 of the two. It has been shown that pulmonary circulation is sensitive to fetal
19 oxygenation during the last trimester of pregnancy (Rasanen, et al. 1996), and that
20 hypoxemia leads to pulmonary vasoconstriction (Arraut, et al. 2013). Indeed, in the
21 current experiment RPA PI values increased significantly with hypoxemia suggesting
22 that impedance to pulmonary blood flow increased during hypoxemia, although the
23 reduction in the estimated pulmonary volume blood flow was not statistically
24 significant. It has been shown in a near-term sheep model of acute ductus arteriosus
25 occlusion that the fetus is unable to increase volume blood flow across the foramen
26 ovale (Tulzer, et al. 1991), thus the reduction in the LVCO is most likely a
27 consequence of a drop in pulmonary volume blood flow leading to a decrease in LV
28 preload. It has been shown in human fetuses that global longitudinal strain is similar
29 in both ventricles at 20-24 weeks of gestation (Kapusta, et al. 2012). Later in
30 pregnancy LV global longitudinal strain does not change, however, corresponding RV
31 strain or deformation decreases. This is explained by an increase in the RV preload. If
32 the change in preload would be the major factor in the strain formation, we should
33 have observed an opposite finding in the LV global longitudinal strain. Therefore, our

1 results indicate that the direct effect of hypoxemia on intrinsic contractile properties
2 of the LV is likely responsible for decreased global longitudinal strain.

3
4 Fetal global LV longitudinal deformation already decreased at 30-minute
5 hypoxemia phase, when significant metabolic acidemia had not yet developed. At
6 120-minute hypoxemia phase fetal pO_2 was comparable to 30-minute hypoxemia
7 phase, however, a significant metabolic acidemia had developed between these two
8 time points. Interestingly, we found no further deterioration in any of the strain
9 parameters. We have previously reported that fetal LV is more susceptible to
10 hypoxemia as compared to fetal RV. However, in that study, fetal cardiac function
11 and hemodynamics were assessed by tissue Doppler and pulsed Doppler imaging,
12 rather than speckle tracking. The findings of this study corroborate our previous
13 observations. We have also found that the fetal LV myocardial performance index
14 significantly worsens with hypoxemia (Bhide, et al. 2016), which also supports the
15 findings of this study. At recovery phase, when fetal oxygenation was returned back
16 to baseline conditions, the LV global longitudinal strain was similar to baseline
17 values, even though fetuses were still suffering from significant metabolic acidemia.
18 Furthermore, LVCO, RVCO or CCO did not show significant changes with
19 progressive metabolic acidemia. This shows that the fetal myocardial function was
20 not further deteriorated despite worsening metabolic acidemia. Our results suggest
21 that LV function appears to be more sensitive to oxygenation than metabolic acidosis.
22 Experimental intravenous infusion of lactic acid to sheep fetuses led to reduction in
23 fetal pH, increase in the heart rate but no change in blood pressure (Hohimer and
24 Bissonnette 1991). This also suggests that lactic acid does not influence cardiac
25 function.

26
27 Di Salvo et al (Di Salvo, et al. 2005) were the first to describe fetal LV and
28 RV function using ultrasound based strain and strain rate imaging. They used
29 myocardial color Doppler imaging for quantification of strain. Doppler technology is
30 known to be angle dependent, which speckle tracking can eliminate (Biswas, et al.
31 2013). There are several software programs to assess myocardial deformation using
32 speckle tracking. However, they are not necessarily comparable. The velocity vector
33 imaging mainly tracks myocardial motion in a narrow area just underneath the
34 endocardium. In contrast, the EchoPAC automated functional imaging software tracks

1 myocardial deformation in a relatively wider area, specified by the operator (Biswas,
2 et al. 2013).

3 4 Strengths and weaknesses

5 At least 60–110 frames per second are recommended when imaging the adult
6 heart (Mondillo, et al. 2011), and even higher rates are desirable to provide enough
7 frames per heart beat in the fetus (Matsui, et al. 2011). We studied the fetus at a mean
8 frame rate of 167 Hz, and this is likely to have led to reliable results. The surgical
9 procedures may constitute a significant stress on the sheep fetuses, and it may be
10 argued that the conditions are quite different from human fetuses exposed to
11 hypoxemia. Normal arterial blood gas values at the baseline stage suggest conditions
12 close to physiologic circulatory state (Acharya, et al. 2004). The study was carried out
13 in a narrow gestational age window of 115-129 days, and may limit the validity and
14 significance outside this time period. Extrapolation of these results to human
15 pregnancy should be done cautiously. The sheep model has been extensively used for
16 research in fetal hemodynamics, and the myocardial strain curves are similar in
17 normal ovine and human pregnancy. We did not monitor cerebral blood flow during
18 the experiment and it could be argued that the reduction in LV global longitudinal
19 strain could just reflect a drop in cerebral vascular resistance during hypoxemia.
20 Indeed, carotid artery blood pressure decreased during hypoxemia. Furthermore, it has
21 been shown that carotid artery volume blood flow increases from about 85 ml/min
22 (normoxemia) to about 95-100 ml/min during isocapnic hypoxemia (Bennet, et al.
23 1998). We can estimate that there was about 24 % reduction in the carotid artery
24 vascular resistance during hypoxemia. However, based on these estimations, over
25 80% of LVCO bypasses brain circulation during prolonged hypoxemia and is affected
26 by lower body vascular resistance. Thus, we believe that reduced carotid artery
27 vascular resistance is not the main determinant of reduced LV global longitudinal
28 strain during hypoxemia.

29 **Conclusion**

30 We have shown that hypoxemia leads to changes in fetal cardiac function that
31 is reflected in altered ventricular deformation on speckle-tracking echocardiography.
32 At a gestational age equivalent to 0.8 term, the global longitudinal and circumferential
33 strains are less negative in the RV as compared to the LV. Fetus responds to acute
34 hypoxemia by reduced deformation of the LV, but not the RV, and this response is

1 reversed by reversal of hypoxemia. This recovery of LV function is seen even in the
2 presence of metabolic acidemia.

3

4 Acknowledgement: This study was funded by the Regional Health Authority of
5 Northern Norway.

6

7

References

- Acharya G, Erkinaro T, Makikallio K, Lappalainen T, Rasanen J. Relationships among Doppler-derived umbilical artery absolute velocities, cardiac function, and placental volume blood flow and resistance in fetal sheep. *American journal of physiology. Heart and circulatory physiology* 2004; 286:H1266-72.
- Acharya G, Rasanen J, Makikallio K, Erkinaro T, Kavasmaa T, Haapsamo M, Mertens L, Huhta JC. Metabolic acidosis decreases fetal myocardial isovolumic velocities in a chronic sheep model of increased placental vascular resistance. *American journal of physiology. Heart and circulatory physiology* 2008; 294:H498-504.
- Arraut AM, Frias AE, Hobbs TR, McEvoy C, Spindel ER, Rasanen J. Fetal pulmonary arterial vascular impedance reflects changes in fetal oxygenation at near-term gestation in a nonhuman primate model. *Reprod Sci* 2013; 20:33-8.
- Bennet L, Peebles DM, Edwards AD, Rios A, Hanson MA. The cerebral hemodynamic response to asphyxia and hypoxia in the near-term fetal sheep as measured by near infrared spectroscopy. *Pediatric research* 1998; 44:951-7.
- Bhide A, Vuolteenaho O, Haapsamo M, Erkinaro T, Rasanen J, Acharya G. Effect of Hypoxemia with or without Increased Placental Vascular Resistance on Fetal Left and Right Ventricular Myocardial Performance Index in Chronically Instrumented Sheep. *Ultrasound in medicine & biology* 2016; 42:2589-98.
- Biswas M, Sudhakar S, Nanda NC, Buckberg G, Pradhan M, Roomi AU, Gorissen W, Houle H. Two- and three-dimensional speckle tracking echocardiography: clinical applications and future directions. *Echocardiography* 2013; 30:88-105.
- Block BS, Schlafer DH, Wentworth RA, Kreitzer LA, Nathanielsz PW. Intrauterine asphyxia and the breakdown of physiologic circulatory compensation in fetal sheep. *American journal of obstetrics and gynecology* 1990; 162:1325-31.
- Carter AM. Placental Gas Exchange and the Oxygen Supply to the Fetus. *Compr Physiol* 2015; 5:1381-403.
- D'Hooge J, Heimdal A, Jamal F, Kukulski T, Bijnens B, Rademakers F, Hatle L, Suetens P, Sutherland GR. Regional strain and strain rate measurements by cardiac ultrasound: principles, implementation and limitations. *European journal of echocardiography : the journal of the Working Group on Echocardiography of the European Society of Cardiology* 2000; 1:154-70.

1 Deane C. Doppler Ultrasound: principles and practice, In: Nicolaides K, Rizzo G, Hecher
2 K., ed. *Placental and fetal Doppler*. London: The Parthenon Publishing
3 Group, 2000. 22.

4 Di Salvo G, Russo MG, Paladini D, Pacileo G, Felicetti M, Ricci C, Cardaropoli D,
5 Palma M, Caso P, Calabro R. Quantification of regional left and right
6 ventricular longitudinal function in 75 normal fetuses using ultrasound-based
7 strain rate and strain imaging. *Ultrasound in medicine & biology* 2005;
8 31:1159-62.

9 Giussani DA, Spencer JA, Moore PJ, Bennet L, Hanson MA. Afferent and efferent
10 components of the cardiovascular reflex responses to acute hypoxia in term
11 fetal sheep. *The Journal of physiology* 1993; 461:431-49.

12 Hayabuchi Y, Sakata M, Kagami S. Right ventricular myocardial deformation
13 patterns in children with congenital heart disease associated with right
14 ventricular pressure overload. *Eur Heart J Cardiovasc Imaging* 2015; 16:890-
15 9.

16 Hohimer AR, Bissonnette JM. Vascular lactic acid infusions do not alter the incidence
17 of fetal breathing movements or their inhibition by acute hypoxemia. *Pediatric*
18 *research* 1991; 29:483-6.

19 Huang SJ, Orde S. From speckle tracking echocardiography to torsion: research tool
20 today, clinical practice tomorrow. *Curr Opin Crit Care* 2013; 19:250-7.

21 Iwamoto HS, Kaufman T, Keil LC, Rudolph AM. Responses to acute hypoxemia in
22 fetal sheep at 0.6-0.7 gestation. *The American journal of physiology* 1989;
23 256:H613-20.

24 Jensen A, Garnier Y, Berger R. Dynamics of fetal circulatory responses to hypoxia
25 and asphyxia. *European journal of obstetrics, gynecology, and reproductive*
26 *biology* 1999; 84:155-72.

27 Kapusta L, Mainzer G, Weiner Z, Deutsch L, Khoury A, Haddad S, Lorber A. Second
28 trimester ultrasound: reference values for two-dimensional speckle tracking-
29 derived longitudinal strain, strain rate and time to peak deformation of the
30 fetal heart. *Journal of the American Society of Echocardiography : official*
31 *publication of the American Society of Echocardiography* 2012; 25:1333-41.

32 Kiserud T, Ebbing C, Kessler J, Rasmussen S. Fetal cardiac output, distribution to the
33 placenta and impact of placental compromise. *Ultrasound in obstetrics &*

1 gynecology : the official journal of the International Society of Ultrasound in
2 Obstetrics and Gynecology 2006; 28:126-36.

3 Kulkarni A, Li L, Craft M, Nanda M, Lorenzo JM, Danford D, Kutty S. Fetal
4 Myocardial Deformation in Maternal Diabetes Mellitus and Obesity.
5 Ultrasound in obstetrics & gynecology : the official journal of the
6 International Society of Ultrasound in Obstetrics and Gynecology 2016.

7 Mäkikallio K, Erkinaro T, Niemi N, Kavasmaa T, Acharya G, Pääkilä M, Räsänen J.
8 Fetal oxygenation and Doppler ultrasonography of cardiovascular
9 hemodynamics in a chronic near-term sheep model. American journal of
10 obstetrics and gynecology 2006; 194:542-50.

11 Matsui H, Germanakis I, Kulinskaya E, Gardiner HM. Temporal and spatial
12 performance of vector velocity imaging in the human fetal heart. Ultrasound
13 in obstetrics & gynecology : the official journal of the International Society of
14 Ultrasound in Obstetrics and Gynecology 2011; 37:150-7.

15 Mondillo S, Galderisi M, Mele D, Cameli M, Lomoriello VS, Zacà V, Ballo P,
16 D'Andrea A, Muraru D, Losi M, Agricola E, D'Errico A, Buralli S, Sciomer S,
17 Nistri S, Badano L, Echocardiography Study Group Of The Italian Society Of
18 Cardiology (Rome Ia. Speckle-tracking echocardiography: a new technique
19 for assessing myocardial function. Journal of ultrasound in medicine : official
20 journal of the American Institute of Ultrasound in Medicine 2011; 30:71-83.

21 Orde S, Huang SJ, McLean AS. Speckle tracking echocardiography in the critically
22 ill: enticing research with minimal clinical practicality or the answer to non-
23 invasive cardiac assessment? Anaesth Intensive Care 2016; 44:542-51.

24 Rasanen J, Wood DC, Weiner S, Ludomirski A, Huhta JC. Role of the pulmonary
25 circulation in the distribution of human fetal cardiac output during the second
26 half of pregnancy. Circulation 1996; 94:1068-73.

27 Rudolph AM. Distribution and regulation of blood flow in the fetal and neonatal
28 lamb. Circ Res 1985; 57:811-21.

29 Tanaka H, Hirata K. Is speckle tracking actually helpful for cardiac resynchronization
30 therapy? J Echocardiogr 2016; 14:53-60.

31 Tulzer G, Gudmundsson S, Rotondo KM, Wood DC, Yoon GY, Huhta JC. Acute
32 fetal ductal occlusion in lambs. American journal of obstetrics and gynecology
33 1991; 165:775-8.

1 Van Mieghem T, Deprest J, Verhaeghe J. Fetal and maternal hemodynamics in
2 pregnancy: new insights in the cardiovascular adaptation to uncomplicated
3 pregnancy, twin-to-twin transfusion syndrome and congenital diaphragmatic
4 hernia. Facts Views Vis Obgyn 2011; 3:205-13.
5
6

1 **Legend to figures**

2 Figure 1. Automated tracking algorithm outlining the myocardium and calculation of
3 global strain and strain rate using Echopac software.

4

5 Figure 2. Automated tracking algorithm outlining the myocardium and calculation of
6 right ventricular global circumferential strain using Echopac software.

7

8 Figure 3. Global left (solid lines) and right ventricular (dotted line) longitudinal strain
9 with hypoxemia and recovery

Table 1. Fetal parameters during the experiment

Parameter	Baseline	Hypoxemia (30 min)	Hypoxemia (120 min)	Recovery	P _{time}
Fetal heart rate (BPM)	169 (38)	166 (26)	170 (24)	136 (16)	0.004
Fetal SBP (mm Hg)	60 (10.3)	52 (8.5)	54 (7.4)	56 (8.7)	0.0008
Fetal DBP (mm Hg)	40 (7.5)	35 (8.8)	36 (7.3)	37 (8.4)	0.034
Fetal MAP (mm Hg)	49 (10.7)	43 (11.2)	47 (10.9)	48 (10.3)	0.050
Fetal pO ₂ (kPa)	2.79 (0.32)	1.59 (0.40)	1.49 (0.16)	2.80 (0.41)	<0.0001
Fetal pCO ₂ (kPa)	6.51 (1.06)	6.68 (0.48)	7.05 (0.99)	6.81 (0.48)	0.56
Fetal pH	7.32 (0.05)	7.30 (0.04)	7.14 (0.12)	7.17 (0.06)	<0.0001
Fetal BE (mM/ml)	-1.39 (2.35)	-2.05 (2.92)	-10.70 (6.07)	-9.63 (3.25)	<0.0001
Fetal lactate (mM/ml)	2.42 (1.63)	4.10 (1.84)	10.17 (3.94)	9.91 (4.20)	<0.0001
Left ventricular output (LVCO) <u>ml/min</u>	644 (198)	501 (122)	545 (123)	548 (157)	0.010
Right ventricular output (RVCO) <u>ml/min</u>	644 (159)	638 (229)	651 (179)	619 (147)	0.60
Combined cardiac output (CCO) <u>ml/min</u>	1288 (294)	1125 (246)	1184 (240)	1158 (229)	0.19
Right pulmonary artery PI	12.54 (11.75)	66.97 (26.88)	57.08 (23.94)	69.42 (56.93)	0.006**
Pulmonary flow (ml/min)	467 (177)	311 (127)	303 (204)	299 (179)	0.089

BPM = Beats per minute, MAP = Mean arterial pressure SBP = Systolic blood pressure, DBP = Diastolic blood pressure, BE = Base excess, PI = pulsatility Index

FHR significantly different with recovery as compared to all other phases.

Significant difference between baseline compared to 30 min hypoxemia as well as baseline compared to 120 min hypoxemia for SBP and DBP.

Significant difference between baseline compared to 30 and 120 min hypoxemia for pO₂.

Significant difference between baseline and all the other three phases for pH, LVCO, BE, lactate.

**RPA PI was log transformed. Significant difference between baseline compared to 30, 120 min hypoxemia and recovery for Right pulmonary artery PI.

Table 2

Table 2. 2-D strain data using speckle tracking echocardiography

Parameter	Baseline	Hypoxemia (30 min)	Hypoxemia (120 min)	Recovery	P _{time}
LV global longitudinal strain (%)	-18.7 (3.8)	-13.9 (3.3)	-15.4 (4.3)	-17.8 (2.9)	0.005*
LV global longitudinal strain rate (1/s)	-2.50 (0.55)	-2.58 (0.64)	-2.67 (0.86)	-2.70 (0.79)	0.91
LV circumferential strain (%)	-21.0 (5.9)	-18.5 (6.7)	-19.3 (6.6)	-19.3 (5.7)	0.69
LV Radial strain (%)	17.1 (7.0)	12.8 (6.3)	14.5 (5.4)	13.7 (4.7)	0.31
RV Global longitudinal strain (%)	-14.3 (5.4)	-14.8 (2.7)	-14.7 (3.9)	-15.3 (2.2)	>0.9
RV global longitudinal strain rate (1/s)	-2.00 (0.48)	-2.27 (0.50)	-2.11 (0.86)	-2.07 (0.37)	0.57
RV Circumferential strain (%)	-11.1 (5.3)	-13.6 (5.6)	-11.7 (6.3)	-11.7 (4.6)	0.20
RV Radial strain (%)	14.3 (4.8)	10.8 (2.0)	10.3 (5.1)	14.1 (4.8)	0.15

* p = 0.0011 between baseline and 30 minute hypoxemia, p = 0.026 between baseline and 120 min hypoxemia and p = 0.013 between 30 min hypoxemia and recovery.

p = 0.016 between baseline left and right ventricular baseline global longitudinal strain.

p < 0.005 between baseline left and right ventricular baseline global circumferential strain, p = 0.003 between baseline left and right ventricular global longitudinal strain rate

Figure 1
[Click here to download high resolution image](#)

Figure 2
[Click here to download high resolution image](#)

Figure 3
[Click here to download high resolution image](#)