# PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

## ARTICLE DETAILS

| | |
|---|---|
| **TITLE (PROVISIONAL)** | Feasibility of 2-D ultrasound shear wave elastography of fetal lungs in case of threatened preterm labor: a study protocol |
| **AUTHORS** | MOTTET, Nicolas; Aubry, Sébastien; Vidal, Chrystelle; Boiteux, Guillaume; Metz, Jean-Patrick; Riethmuller, Didier; Pazart, Lionel; Ramanah, Rajeev |

## VERSION 1 - REVIEW

| | |
|---|---|
| **REVIEWER** | Edgar Hernandez Andrade<br>Wayne State University<br>Detroit USA |
| **REVIEW RETURNED** | 02-Apr-2017 |

| | |
|---|---|
| **GENERAL COMMENTS** | Comments on the manuscript: Feasibility and diagnostic value of shear wave elastography of fetal lungs in case of threatened preterm labor: a protocol study<br>The authors aim to evaluate the feasibility and reproducibility of shear wave elatography (SWE) in the evaluation of fetal lung maturity.<br>The authors propose a study of two groups of fetuses; 1) those at risk of preterm delivery, and 2) those from uncomplicated pregnancies, both groups at 24 to 34 weeks of gestation. As a secondary outcome the authors also aim to evaluate changes in lung elasticity using SWE after steroid administration. They propose to evaluate 10 fetuses per gestational week, five complicated and five controls (10 per week, 100 in total: 50 complicated and 50 controls)<br>In the complicated group the authors will apply SWE in the fetal lungs (both) after the first administration of steroids (day 0), within 24 hours after the second administration of steroids (day 2), and 5 to 7 days after the first administration (day 5-7). In control fetuses the authors will perform only two SWE lung evaluations with an interval of 2 days.<br>The authors will evaluate the elastic Young modulus (units: kilopascals) in three regions of each fetal lung and in three regions of the fetal liver. They will use a standard region of interest (ROI) of 5 mm for each SWE estimation and will register the distance between the lung area and the US probe<br>For reproducibility analysis a second operator will obtain SWE readings in 30 fetuses<br>Clinical and respiratory data will be collected at delivery, and a |

| | |
|---|---|
| | follow-up clinical evaluation will be done at three months after birth<br>Comments<br>The main comment of this protocol is about safety and approval from the ethics committee. Shear wave elastography has not been approved for fetal evaluation, is still not clear the biological impact that the acoustic radiation force impulse can produce when it is directly aimed to fetal tissues. This is an important aspect as the Journals might request the informed consent and authorization of the participants.<br>Study design<br>According to the title, the authors aim to evaluate “Feasibility and diagnostic value” of SWE and lung maturity. How they will do it? Are they going to compare SWE kilopascals with lung maturity tests evaluated in amniotic fluid obtained by amniocentesis? Are they going to correlate SWE with the perinatal outcome? But, many of these fetuses will not have a preterm delivery, or at least they will not be delivered immediately after SWE; how they will control the time-interval between SWE measurements and the delivery? How they will correlate SEW measurements with respiratory complications? Are they going to include patients with rupture of membranes? Are they going to adjust for infection or reduced amniotic fluid?<br>Comparison with controls: The first SWE in complicated cases will be done after the first shot of steroids, this cannot be compared with uncomplicated pregnancies; a basal SWE before steroids administration should be performed. Differences between controls and complicated cases per week might not be easy to estimate, as only five fetuses per group will be compared. The variation among measurements within each group might preclude an accurate analysis of the differences. The reduced number of cases per week might not allow estimating clear differences among the different gestational weeks.<br>Confounding Factors, The authors should describe confounding factors that can affect their results such as anterior placentas, reduced amniotic fluid, fetal position, maternal body mass index.<br>How controls will be selected? they cases and controls should be matched by maternal BMI, gestational age, maternal age.<br>How he authors will control bias? SWE measurements will be done by operators knowing that the patient received steroids before.<br>What is the information that this study will provide? How the SWE evaluation of the fetal lung can be used in the clinical management of these fetuses?<br>Minor comments: please define “major pregnant women” page 10 line 20<br>Discussion: Page 14 lines 6-7 “SWE can be considered as a new noninvasive interesting tool for evaluating FLM based on evaluation of mechanical properties of lung during pregnancy”, this has not been proved, the authors cannot use this statement<br>A clear objective is needed; the authors mentioned: (page 14 lines 20-21) “The main objective is to evaluate feasibility and |

| | |
|---|---|
| | reproducibility of SWE in human fetal lung according to a strict protocol of measures between 24 and 34 WG", and then they mentioned (page 16 lines 11-12): "To sum up, we hope that the results of this study will contribute to clarify applicability of SWE as a new means to evaluate fetal lung maturity". The authors should adjust the protocol to answer a specific research question, as the design of the study is described, there is no certainty that the association between SWE and lung maturation, or differences in SWE between normal and abnormal fetuses will be reliably obtained |

| | |
|---|---|
| **REVIEWER** | Shaun Kunisaki<br>University of Michigan, C.S. Mott Children's Hospital, Ann Arbor, Michigan, USA |
| **REVIEW RETURNED** | 01-May-2017 |

| | |
|---|---|
| **GENERAL COMMENTS** | This is a pilot study describing a very interesting concept paper on non-invasive measurement of fetal lung maturity using shear wave elastography. The rationale and protocol are well written and quite detailed. Unfortunately, there is very little data or even preliminary results to report- only values measured at two different time points. Therefore, I think the manuscript is to preliminary to be published as is. I look forward to future work by this group. |

## VERSION 1 – AUTHOR RESPONSE

Comments
The main comment of this protocol is about safety and approval from the ethics committee. Shear wave elastography has not been approved for fetal evaluation, is still not clear the biological impact that the acoustic radiation force impulse can produce when it is directly aimed to fetal tissues. This is an important aspect as the Journals might request the informed consent and authorization of the participants.

**We agree with this remark. There is to date no publication regarding its use on human fetuses. The Food and Drug Administration (FDA) has so far, not yet approved use of SWE for obstetric clinical applications because of the paucity of data in the literature. However, setting parameters respect mechanical and thermal indexes for soft-tissues and bone, which are necessary to allow an obstetric examination defined by the FDA.**

**Moreover, interesting and reassuring results are available concerning use of Shear Wave Elastography (SWE) on developing organs, especially on premature infants and animals models. Principals organs explored are the liver, brain and lungs. In this study, fetuses will be included from 24 to 34 WG, over the period of organogenesis. In the patient information sheet, it is specified that previous studies explored liver or brain of premature infants with SWE. Alison et al. studied liver stiffness in preterm neonates with intra uterine growth restriction (IUGR) and gestational age at birth between 26-31 WG [31]. Franchi-Abella et al evaluate feasibility and diagnostic accuracy of SWE for the assessment of liver stiffness in 96 patients**

**and 7 were preterm newborns between 30-35 WG [42]. Both studies did not report adverse outcomes and were approved by institutional review board. Moreover, consent form mentions conclusions of an experimental study of the biological effects associated with SWE in brains of neonatal mice. Results indicated that using SWE doe not cause detectable histologic changes. Potential effects on intracellular signaling pathways were detected when the SWE scanning lasted more than 30 min [25].**

**Thus, patients will be informed that our study is focused on fetuses with a gestational age similar to preterm newborn included in the above-mentioned studies. Moreover, exploration with SWE will concern only lung and liver with duration of less than 10 min.**

Study design.

According to the title, the authors aim to evaluate "Feasibility and diagnostic value" of SWE and lung maturity. How they will do it? Are they going to compare SWE kilopascals with lung maturity tests evaluated in amniotic fluid obtained by amniocentesis?

**Our first version was not clear on objectives and we clarify aims of this study in this revised version. The main objective of this pilot study is to evaluate feasibility of SWE in human fetal lung between 24 and 34 WG and to modelize fetal lung-to-liver elastography ratio (LLE ratio) from the most reproducible regions of both target organs, between 24 and 34 WG. Secondary objective is to assess //( UDWLR¶V YDULDWLRQV EHWZHHQ fetal lungs with different supposed biomechanical properties: normal lung and lung surfactant-enriched after a corticosteroids course indicated for a threatened preterm labor. In this pilot study, corticosteroids generate an effect model interesting for assessment of LLE ratio variation. We take advantage of the action of corticosteroids to explore another medium with different supposed biomechanical properties. Indeed, corticosteroids accelerated development of type 2 pneumocytes leading to surfactant production. Similarly, there is a significant increase in saturated phosphatidylcholine content in fetal lung, modifying propagation of shear waves potentially.**

**Thus we shall not attempt to define reference value of fetal lung elasticity during pregnancy or to correlate SWE value with respiratory function at birth. This pilot study will focus on technical performances before considering clinical applications.**

Comparison with controls: The first SWE in complicated cases will be done after the first shot of steroids, this cannot be compared with uncomplicated pregnancies; a basal SWE before steroids administration should be performed.

**This is a mistake in our formulation. In the literature, an artificial delay of 24h seems to be necessary to produce surfactant in pneumocytes type 2, given the placental transfer and time to induce cellular mechanism of transcription. Thus clinical benfits are expected 24h after the first**

| | |
|---|---|
| | Although the methodology is well planned<br>1. Proximal and distal lungs are not clearly defined<br><br>2. Because of the difference in depth, there is a difference between the proximal and distal lung tissues ? How were averaged (separate or combined)<br><br>3. Location of the figures in the article should be checked again.<br><br>4. Intra and inter-observer variability should be reported statistically in terms of the reliability of the protocol.<br><br>5. It is understandable how the lung maturation is used without evaluating the results. It should only be rewritten according to the summary.<br><br>6. This is a protocol study and I am not sure that the title matches the content of the study. It is thought that results can be retrieved and used with evaluating lung maturity when the title is read. However, this was not evaluated in the article.<br><br>7. I think it would be more appropriate to write an evaluation with the results as a research paper |

## VERSION 2 – AUTHOR RESPONSE

**Reviewer: 1**
Reviewer Name: Elisenda Bonet-Carne, PhD - Research Associate
Institution and Country: UCL - Microstructure Imaging Group, Centre for Medical Image Computing (CMIC)
Gower Street, London, UK; UCL Metabolism & Experi Therapeutics, Centre for Medical Imaging (CMI), London, UK
Competing Interests: None declared

In this work, the authors propose a trial to test the feasibility and applicability of Shear Wave Elastography in human fetal lungs. In different moments they measure that they do not attempt to define reference values of elasticity or to correlate with function at birth. I think that results from this study will be of interest to the community, making this work of interest for publication.

I have some comments for the authors:

,Q WKH DEVWUDFW WKH DLP VKRXOG model a fetal lung-to-liver EH PRUH FOHDU DQG PRYH ³D
HODVWRJUDSK\ UDWLR´ WR D VHFRQGDU\ REMHFWLYH 6HFRQG SDUDJ
the methods, should be moved in the introduction or removed. In methods the clinical data to test invasiveness of SWE and the intra-inter experiments should be mentioned.

Response: Changes are made in the abstract.

2. In one figure you show kpa changes from one lung to the other (proximal to distal) where there is not that much difference in terms of depth, in the discussion section you say that the small differences
LQ GHSWK VKRXOGQ¶W QRW DIIHFW H[SHFW ORZ YDULDWLRQV EHWZ
explain this changes? How will you try to address this differences? How adding liver could help as

liver KPa would also change depending on the fetal position. Is this going to be a problem when there is no proximal or distal lung (i.e.spine located in the distal position for example).

Response: We agree with this remark. In this figure, there is a change for elasticity from one lung to the other. Aim of this figure is to illustrate possibility to obtain an homogeneous quantitative elastogram in both proximal and distal lungs. Elasticity value cannot be evaluated on a single measure. According to studies concerning use of 2D SWE on non-human primate fetuses, 3 valid measurements are performed on lungs/liver and the mean value of these measurements is used to evaluate elasticity. To avoid confusion, we decide to remove elasticity values in this figure.
Concerning impact of depth, we expected low variation between proximal and distal lung because lungs may be considered as soft tissues. In previous biomechanical studies, the effect of target depth on shear wave velocity has been reported in elasticity phantoms. These studies show that shear-wave velocity is gradually under estimated with increasing depth. Nevertheless, the effect is greatest in hard targets, whereas little effect of depth is seen in soft targets. This phenomenon might be explained by a damping of the acoustic push pulse that generates the shear waves by both increased attenuation and increased stiffness of the target.
In this study, exploration of the liver is important to ensure that SWE is able to detect variations in elasticity between lung and liver. Moreover, if depth influences elasticity value, it would be interesting to see if this variation is more important for the lung or the liver.
Concerning fetal position, if the spine is located is a distal position, operator can move the fetus in order to lateralize the back and obtain a proximal and a distal lung. Thus, fetal lungs will be voluntarily approached laterally to systematically obtain proximal and distal lungs regarding the distance to the probe.

3. For the control group you say that you will have 24-25 and 30-34 samples. There is a gap from 25 to 30 weeks. How are you going to fill this samples?

Response: In this study, control group can be recruited during ultrasounds OR prenatal visits. To fill the sample between 25 to 30 WG, patients will be informed during routine prenatal visits and a SWE exam will be offered if they agree to participate

Comment: Why depth is fixed? Fetuses will grow from week 24 to week 34, should depth not be fixed to some lung landmark?

Response: The depth for SWE exam is not fixed. Depth of 6 cm concerns harmonic imaging parameter for the 2D-Mode used in obstetrics ultrasounds.

4. In methods you say that the ROIs are done after seeing the waves, would not be better the other way around to avoid bias?

Response: In methods, we precise that elasticity will be sampled in a ROI of 5 mm. Operators will adjust the position of the ROI after obtaining an homogeneous elastogram. According to our main objective, feasibility will be evaluated by assessing the number of exams performed and the number of exams with interpretable results. Thus we need to obtain an homogeneous elastogram before doing ROI.

5. When is the second observer going to do the ROIs? Online? Just after the first one? Or are you storing the US and he will do it off-line. Should be explained.

Response: The second observer will perform measurements just after the first one and will be blinded to the feasibility and results obtained by the first one.

This remark is added in the manuscript

6. In technical validation you say that reduced amniotic fluid, fetal position and maternal body mass index will be evaluated. You should say all the variables and data that will be stored as it is not clear. Maybe adding a table would help.

Response: We add a new table
All the variables and data that are stored and summarized in table 1

Comment: Finally, why are you testing until 34WG instead of 37 or 39? It would be great if it is possible to expand it (at least until 36WG).

Response: Actually, corticosteroids are recommended for threatened preterm labor between 24 and 34 WG, and the study design is a prospective case-control study. As explained in the discussion, we shall not attempt to define reference value of fetal lung elasticity during pregnancy or to correlate SWE value with respiratory function at birth (Nevertheless, we think that new results from this study will be of interest to the community). The main objective is to evaluate feasibility and reproducibility of SWE in human fetal lung.
According to the result of this study, a prospective study until 41 WG will be considered.
Due to the budget allocated to the study, we have to limit inclusion between 24-34 WG. Each case that comes back for an SWE exam performed outside current medical visit will receive financial compensation for travel expenses.

And some minor notes:

1. Reference 13,14 do not show comparison with real risk of neonatal respiratory morbidity, add a newer reference for that.

Response: These new references are added

13. Bonet-Carne E, Palacio M, Cobi T et al. Quantitative ultrasound texture analysis of fetal lungs to predict neonatal respiratory morbidity. Ultrasound Obstet Gynecol 2015;45(4):427-33.
14. Palacio M, Bonet-Carne E, Cobo T et al. Prediction of neonatal respiratory morbidity by quantitative ultrasound lung texture analysis: a multicenter study. Am J Obstet Gynecol. 2017; Epub ahead of print.

2. In the introduction E should be defined. It is defined first time in prenatal variables.

Response: E for Elasticity is defined in the background

3. WG is not defined in the main text before using it.

Response: WG is defined in the background

4. Add a scale in the colorers of the figures.

Response: A scale is added for each figure

5. Figure caption should be better H [ S O D L Q H G  Z L W K  V X E I L J X U H  O D E H O V  D V  D  E « D
understand each sub image without the text.

Response: More explanations are gave in figure captions

6. In the formula, EPF is not defined. All the acronyms should be checked.

Response: Estimation of fetal weight (EFW) is described in methods

**Reviewer: 2**
Reviewer Name: Figen Bakirtas Palabiyik
Institution and Country: Health Science University. Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Department of Radiology, Istanbul, Turkey
Competing Interests: None declared

This article type is protocol study
The english of article is clear and understanding
The ethic committee approval is available.

General comments
When we look at the literature, we see that the studies related to the evaluation of the fetus with SWE are related to the evaluation of premature infants at postnatal period and the experimental level. Especially studies concerning the evaluation of the placenta is common.
SWE is a method of measuring the elasticity of structures. I think that the SWE used in the study can be a suitable method for the functional evaluation of lung maturation. SWE is a personally independent method that operator this to measure numerically. It may be thought that the elasticity of the fetus may change theoretically as the surfactant becomes adequate in the lung development process.

I think that there may be some problems with the use of SWE when assessing the fetus.

Comment and Responses:
1. Technique problems: How does the depth of the fetus and the amniotic fluid in between affect the reliability of the measurement? In addition, the fetus is an organ in motion in amniotic fluid. How much does this affect SWE measurements?

Response: As explained to the first reviewer concerning impact of depth, we expected low variation between proximal and distal lung because lungs may be considered as soft tissues. In previous biomechanical studies, the effect of target depth on shear wave velocity has been reported in elasticity phantoms. These studies show that shear-wave velocity is gradually under estimated with increasing depth. Nevertheless, the effect is greatest in hard targets, whereas little effect of depth is seen in soft targets. This phenomenon might be explained by a damping of the acoustic push pulse that generates the shear waves by both increased attenuation and increased stiffness of the target. According to our experience of obstetrics ultrasounds, fetal movements are important especially during the second trimester. During the third trimester, mobility of fetuses is more limited and they can be maintained between the probe and the other hand of the operator to facilitate exploration of the fetal thorax.

2. Are there any negative effects that SWE may have on the developing fetus? (In the study it has been informed by literature number 25-27)

Response: There is actually debate concerning safety of SWE is fetal medicine. In the section ethical consideration, we cite studies that report reassuring results concerning use of Shear Wave Elastography (SWE) on developing organs, especially on premature infants and animals models. If we considered biomechanical studies, there is a risk of tissue heating as soon as the thermal index

(TI) is greater than 0.7. The duration of the examination must therefore be reduced. Herman and Harris studied the models and regulatory considerations for transient temperature rise during diagnostic ultrasound pulses and found that any transient increase in temperature caused by pulse bursts might still be within the safe limits determined by the Food and Drug Administration (FDA). Some authors stated that the generation of micron-scale displacements using acoustic radiation force in tissue requires the use of high-intensity acoustic beams, must be evaluated. They showed that transducer heating was below 1°C for the current clinical applications. According to experimental studies that simulate the heating of soft tissue during acoustic radiation force examination (ARFI), they demonstrate that ARFI imaging of soft tissue is safe, provided that thermal index must be monitored.

In our study, we decide to respect thresholds for Biophysical safety indices according to the British PHGLFDO XOWUDVRXQGV VRFLHW\ DQG )'$ . 7, ” DQG 0, :H

Indeed BMUS recommended TI < 0,7 only during the embryonic period.

These remarks are added in the background section.

3. The fetus is a constantly growing and developing organism and can change the SWE values of the structures measured over days or weeks (Which works studied lung tissue over are particularly 24-34 weeks in the period of very rapid growth and maturation)

In this study, authors tried to standardize the SWE values of fetal lung tissue and ratio fetal liver and fetal lung in 24-34 weeks fetuses and to establish a protocol for SWE measurements before and after corticosteroid treatment in maturation incomplete fetuses.

This is a protocol study and I am not sure that the title matches the content of the study. It is thought that results can be retrieved and used with evaluating lung maturity when the title is read. However, this was not evaluated in the article.

Since there is no previous study and methodology in the related literature, could it be more accurate to write a separate article on the evaluation and results of pre- and post-treatment lung maturation and normal fetal pulmonary tissue by SWE?

Response: In this paper, we want to describe our reasoning and methodology to assess feasibility of SWE on fetal lung. We also think it would more accurate to write two papers at the end of this study: The first one to describe feasibility of the technique with the modelization of LLE ratio and a second one concerning results of pre and post treatement for lung maturation.

In the title, we precise that our objective is to assess feasibility of SWE on fetal lung during threatened preterm labor. Why do we choose a limited period between 24 to 34 WG? We want to take advantage of the action of corticosteroids to explore another medium with different supposed biomechanical properties. In this study, corticosteroids generate an effect model interesting for assessment of LLE ratio variation. Indeed, corticosteroids accelerated development of type 2 pneumocytes leading to surfactant production. Similarly, there is a significant increase in saturated phosphatidylcholine content in fetal lung, modifying propagation of shear waves potentially.

Although the methodology is well planned

1. Proximal and distal lungs are not clearly defined

Response: In our protocol, fetal lungs will be voluntarily approached laterally to systematically obtain proximal and distal lungs regarding the distance to the probe .

We add this remark in methods

2. Because of the difference in depth, there is a difference between the proximal and distal lung tissues ? How were averaged (separate or combined)

Response: $FWXDOO\ ZH GRQ¶W NQRZ LI WKHUH LV D GLIIHUHQFH between the proximal and distal lung.
According to Quarello et al on non-human primate fetuses, Depth seems to play a role as demonstrated by the different ICC in fetal lungs, proximally or distally located in relation with the ultrasound probe.
Differences will be average separately : means for the proximal lung versus means for the distal lung.

3. Location of the figures in the article should be checked again.

Response: We check location of the figures

4. Intra and inter-observer variability should be reported statistically in terms of the reliability of the protocol.

Response: All the variables and data that will be stored for each SWE exam are summarized in table 1. Intra and inter-observer reproducibility will be evaluated by the intra-class correlation coefficient (ICC) with 95% IC. Intra-observer reproducibility will be calculated using the two cycles of measure, inter observer reproducibility between the operators will be calculated for means of the two cycles of measures if intra-observer is high. Only repeatable and reproducible values of each ROI will be considered to modelize LLE ratio.

5. It is understandable how the lung maturation is used without evaluating the results. It should only be rewritten according to the summary.

Response: We rewrite according the summary

6. This is a protocol study and I am not sure that the title matches the content of the study. It is thought that results can be retrieved and used with evaluating lung maturity when the title is read.

Response: However, this was not evaluated in the article.

7. I think it would be more appropriate to write an evaluation with the results as a research paper.

Response: SWE is a promising tool and journals increasingly accept articles reporting use of SWE in pregnant women. It important to explain how ethic committee approval has been obtained to encourage others teams to work on this topic with respect of ethical rules. Moreover, in order to reach more comprehensive and explanatory data regarding the use of SWE in fetal medicine, we need to standardize the method before publishing results in a research paper.

## VERSION 3 – REVIEW

| | |
|---|---|
| **REVIEWER** | Elisenda Bonet-Carne, PhD<br>UCL - Microstructure Imaging Group, Centre for Medical Image Computing (CMIC),<br>London, United Kingdom<br><br>UCL Metabolism & Experi Therapeutics, Centre for Medical Imaging (CMI),<br>London, United Kingdom |
| **REVIEW RETURNED** | 23-Aug-2017 |

| | |
|---|---|
| **GENERAL COMMENTS** | In this work, the authors propose a trial to test the feasibility and applicability of Shear Wave Elastography in human fetal lungs. In different moments they measure that they do not attempt to define reference values of elasticity or to correlate with function at birth. I think that results from this study will be of interest to the community, making this work of interest for publication.<br><br>The authors have addressed the main previous concerns and there is just one minor comment regarding the STROBE checklist (item #5). Authors should include the relevant dates, including periods of recruitment.<br><br>Some additional typos/comments:<br>- "Clinical trial.gov" should be "clinicaltrials.gov"<br>- In table1 where it says fetal weight it should say estimated fetal weight<br>- Figures 1 and 3 would benefit from adding different image subindices (i.e. a), b),...) |

| | |
|---|---|
| **REVIEWER** | Figen Bakirtas Palabiyik<br>Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Department of Pediatric Radiology, Istanbul, Turkey<br>Competing: Pediatric Radiology |
| **REVIEW RETURNED** | 26-Aug-2017 |

| | |
|---|---|
| **GENERAL COMMENTS** | 1. The aim of the study was to report variations between normal lung and lung surfactant enriched after corticosteroids therapy but how can we understand this before results are reported in the study?<br>2. Key words should be changed as stifness-elasticty, prenatal-fetal and "ultrasound" shoul be removed. Because shear wave elastography is used instead shear wave ultrasound.<br>3. LLE ratio is used to increase the reliability of the study and to standardize the study should be added. |

## VERSION 3 – AUTHOR RESPONSE

**Reviewer: 1**
Reviewer Name: Elisenda Bonet-Carne, PhD
Institution and Country: UCL - Microstructure Imaging Group, Centre for Medical Image Computing (CMIC),
London, United Kingdom; UCL Metabolism & Experi Therapeutics, Centre for Medical Imaging (CMI), London, United Kingdom
Competing Interests: None declared

Comment: In this work, the authors propose a trial to test the feasibility and applicability of Shear Wave Elastography in human fetal lungs. In different moments they measure that they do not attempt to define reference values of elasticity or to correlate with function at birth. I think that results from this study will be of interest to the community, making this work of interest for publication.

The authors have addressed the main previous concerns and there is just one minor comment regarding the STROBE checklist (item #5). Authors should include the relevant dates, including periods of recruitment.

Response: The first eligible patient was enrolled in May 2016 and we plan to enroll for 18 months. End of follow up of children will be completed approximately six months after the last birth.

Some additional typos/comments:
Comment: "Clinical trial.gov" should be "clinicaltrials.gov"

Response: We add this correction

Comment: In table1 where it says fetal weight it should say estimated fetal weight

Response: :H FKDQJH ³IHWDO ZHLJKW´ IRU ³HVWLPDWHG IHWDO ZHLJKW´ L

Comment: Figures 1 and 3 would benefit from adding different image subindices (i.e. a), b),...)

Response: We add different image subindices

**Reviewer: 2**
Reviewer Name: Figen Bakirtas Palabiyik
Institution and Country: Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Department of Pediatric Radiology, Istanbul, Turkey
Competing Interests: Pediatric Radiology

Comment: 1. The aim of the study was to report variations between normal lung and lung surfactant enriched after corticosteroids therapy but how can we understand this before results are reported in the study?

Response: In this study, secondary objective is to modelize fetal lung-to-liver elastography ratio (LLE ratio) and to assess variations between normal lung and lung surfactant-enriched after a corticosteroids course.

We suppose that increase of surfactant production in the lung could modify propagation of shear waves. Indeed, administration of corticosteroids to pregnant women results in a significant increases of phosphatidylcholine content in fetal lungs and could increase viscosity, leading to shear wave attenuation.

Comment 2. Key words should be changed as stifness-elasticty, prenatal-fetal and "ultrasound" shoul be removed. Because shear wave elastography is used instead shear wave ultrasound.

Response: We change key words

Comment 3. LLE ratio is used to increase the reliability of the study and to standardize the study should be added.

Response: We add this sentence in the discussion