### Abstract:
3-D/4-D ultrasound techniques in antenatal screening are commonplace, but not routinely used for perinatal post-mortem ultrasound. In this technical innovation, we performed both 2-D and 3-D post-mortem ultrasound on 11 foetuses (mean gestation: 23 weeks; range: 15 – 32 weeks) to determine if there was any benefit in 3-D methods over conventional 2-D ultrasound. In 1 case of osteogenesis imperfecta, both 2-D and 3-D ultrasound were non-diagnostic due to small foetal size. Of the remaining 10 foetuses, 7 were normal at imaging and autopsy; and 3 had abnormalities detected on both 2-D and 3-D ultrasound. There were no false positive diagnoses by 2-D or 3-D ultrasound. Whilst 3-D post-mortem ultrasound was a feasible technique, it did not provide additional information over 2-D ultrasound. Routine 3-D post-mortem ultrasound cannot therefore be routinely recommended based on our findings.

### Author Comments:
Dear Dr Oystein Olsen and members of the Editorial Board,

Thank you for inviting us to resubmit our attached manuscript entitled “3D versus 2D post-mortem ultrasound: Feasibility and additional value in perinatal death investigation” for publication as a technical innovation article in Pediatric Radiology.

We had previously submitted this as an original article on 27 January 2020, however given our small cohort and heterogenous population it was advised that we reproduce our article for the technical innovation. The previously suggested changes by the authors, including removal of abbreviations and acronyms as well as addition of keywords within the text and reformating of reference list has now been implemented.
This work was supported by the Guy Sebag research grant, awarded by the ESPR in 2017. The funds from the grant supported the use of a 3D ultrasound machine which we used to acquire the images and results presented in this paper. A preliminary version of this work was presented as an oral presentation at the ESPR annual conference in Helsinki, June 2019. The manuscript has not been published, and is not being considered for publication elsewhere. The authors have no conflicts of interest to disclose.

Yours sincerely,

Dr. Susan Shelmerdine
Paediatric Radiology Research Fellow
On behalf of all authors
Title:
3-dimensional (3-D) versus 2-dimensional (2-D) post-mortem ultrasound: Feasibility and additional value in perinatal death investigation

Short Title:
3-dimensional versus 2-dimensional perinatal post-mortem ultrasound

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Keywords:
Ultrasound, autopsy, perinatal, post-mortem, pathology
Declarations:

Funding information:
This work was supported by the European Society of Paediatric Radiology (ESPR) 2017 Guy Sebag Research Grant. SCS is supported by a RCUK/UKRI Innovation Fellowship and Medical Research Council (MRC) Clinical Research Training Fellowship (Grant Ref: MR/R002118/1). This award is jointly funded by the Royal College of Radiologists (RCR). OJA is funded by a National Institute for Health Research (NIHR) Career Development Fellowship (NIHR-CDF-2017-10-037).

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Conflicts of interest:
None of the authors have conflicts to declare

Ethics approval:
Ethical approval was granted for this single centre, prospective cohort study (IRAS ID:13195; REC reference: 13/LO/1494). All parents signed written consent allowing for post-mortem imaging to be conducted as part of the autopsy.

Consent to participate and publication:
All patients included in this article have a signed parental consent form for use of imaging for research/ audit/ educational purposes.

Availability of data and material:
All relevant clinical information is already provided within the manuscript. Additional information is available from the corresponding author upon reasonable request.

Code availability:
Not applicable

Authors’ contributions:
SCS performed the data collection, analysis and primary write up of the manuscript. NJS and OJA conceived the idea of the research project. All authors have had an input in reviewing and editing the final draft of this manuscript.
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Code availability:
Not applicable.

Authors’ contributions:
SCS performed the data collection, analysis and primary write up of the manuscript. NJS and OJA conceived the idea of the research project. All authors have had an input in reviewing and editing the final draft of this manuscript.
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In this report we aimed to test the feasibility of performing perinatal 3-dimensional (3-D) post-mortem ultrasound at a range of different gestations, and assess whether this technique can provide additional information over our conventional 2-dimensional (2-D) PMUS imaging.

Description of Innovation

Ethical approval was granted for this single centre, prospective cohort study (IRAS ID:13195; REC reference: 13/LO/1494). Eleven consecutive, unselected fetuses were prospectively included over a 6-month study period (1 July 2018 – 1 December 2018). No inclusion or exclusion criteria were applied. Of these 6/11 (54.5%) were male. The mean gestation age was 23 weeks (range 15-32 weeks) and mean body weight was 469g (47-1375g). Six fetuses (54.5%) were stillborn, 5/11 (45.4%) were terminations of pregnancy (1 for foetal hydrops, 2 for oligohydramnios, 1 for suspected absent corpus callosum, 1 for bladder outlet obstruction). There was an average time between fetal delivery and ultrasound examination of 5 days (range 1 – 18 days), and average time between ultrasound examination and autopsy or post-mortem MRI was 13 days (1 – 36 days).
The 2-D ultrasound examinations were first performed, followed by 3-D ultrasound imaging by the same operator - a paediatric radiology research fellow (4 years post-mortem imaging; 6 years paediatric radiology experience), according to published guidelines [1, 5]. All ultrasound examinations were performed on machines used only for post-mortem imaging, based in the hospital mortuary. The imaging was performed with the fetus submerged in a cold water bath (approximately 5°C) to ensure maximal sonographic wave transmission from probe to body surface [1].

The 2-D imaging was performed on a UGEO HM70A (Samsung Medison, Gangwon, Republic of Korea) scanner, equipped with a high frequency linear transducer, model L7-16 (bandwidth, 7-16MHz). The 3-D PMUS imaging was performed on a Voluson E8 (GE Medical Systems, Zipf, Austria) scanner, equipped with a 3D/4D wide band convex volume transducer, model RAB4-8-D (bandwidth, 2-8 MHz; field of view 70°: V, 70° × 85°; Wide). 3-D volume acquisitions were taken of the head, thorax and abdomen for all fetuses. The total scanning and image interpretation time per fetus was approximately 20 minutes for 2-D ultrasound, and with an additional 30 minutes for 3-D ultrasound. Whilst acquisition of images for 3-D ultrasound were more rapid (taking only approximately 5 minutes), additional time was required to review the different imaging planes and to create diagnostic post-processed images for PACS storage. The findings from the 2-D and 3-D examinations were inserted into a predefined report template, in Microsoft Excel (Microsoft, Seattle, USA) for five anatomical regions: brain, spine, cardiac, thorax and abdomen, as outlined previously [1,6]. For each field, the radiologist allocated either a ‘normal’ or ‘abnormal’ label, with further description of the abnormality and organ involved within the body area if abnormal [1,6].

Where parents provided consent for autopsy, these findings were used as the reference standard against the ultrasound findings. All invasive (‘full’) autopsies were performed according to Royal College of Pathologists autopsy guidelines [6, 7], minimally invasive autopsy methods were performed using laparoscopic guided internal examination techniques [8], and all non-invasive autopsies received a whole body post-mortem MRI examination co-reported by two paediatric radiologists (with 10 and 4 years of paediatric post-mortem imaging experience each). The MRI techniques have also been previously published [9].

Post-mortem Ultrasound Findings
In one foetus, a 15 week gestation foetus with osteogenesis imperfecta (OI), weighing only 47g, both the 2-D and 3-D ultrasound were non-diagnostic due to small foetal size.

For the remaining 10 foetuses, 7 had 11 cases (63.6%) there were no abnormalities identified at autopsy or at 2-D or 3-D ultrasound is shown in Figure 1. In 3/11 (27.3%) cases, abnormalities were identified at autopsy including foetal hydrops, bladder outlet obstruction and partial agenesis of the corpus callosum and osteogenesis imperfecta (OI).

Both 2-D and 3-D ultrasound imaging were able to identify all three of these diagnoses. The 3-D technique did not demonstrate additional findings that were not seen at 2-D ultrasound for any cases (Figures 2–4). In one case of a 15 week gestation fetus with OI, osteogenesis, weighing only 47g, both 2-D and 3-D ultrasound were non-diagnostic due to small patient size.

Discussion

3-D post-mortem ultrasound imaging is a feasible technique but did not provide additional information over the conventional 2-D imaging in this small cohort. Based on these preliminary findings, there is insufficient evidence to recommend routine usage of 3-D ultrasound volume acquisitions in addition to 2-D ultrasound imaging, particularly given the additional scanning time, cost of equipment and software.

Larger cohort studies from antenatal (i.e. intra-uterine) fetal imaging have reported some benefits of 3-D ultrasound imaging over 2-D techniques, although the size of improvement is variable. Merz et al [10] reviewed over 3000 second and third trimester pregnancies and found that 3-D ultrasound identified 42/1012 (4.2%) congenital malformations which were not seen on 2-D ultrasound. Xu et al [11] corroborate these findings, and found that 3-D ultrasound identified 9/61 (14.8%) additional diagnoses not seen on 2-D imaging alone. Possible reasons why 3-D ultrasound may have offered a benefit for obstetric over post-mortem fetal imaging may be because the additional information seen in-utero is already possible by external examination and plain radiography at perinatal autopsy, without the need for ultrasound. Pathologies such as skeletal dysplasias, cleft palates, neural tube defects, dysmorphic features (e.g. unusual facial appearances, isolated limb anomalies,
polydactyly) can all be assessed by inspecting the fetus after delivery, compared to during pregnancy when imaging with 3-D surface rendering and multiplanar reconstructions are more helpful.

Despite this, larger studies covering a greater variety of fetuses with congenital anomalies could identify whether there are specific clinical situations where 3-D ultrasound could be helpful. These may include cases where the fetal head has been distorted by extraction. In such cases, post-mortem intracranial imaging is typically difficult due to sutural laxity causing overlapping skull bones, deformed cranial features and a reduced ability to obtain a satisfactory sonographic window via the fontanelles [1]. A 3-D volume, possibly using a micro-convex volume transducer, could help provide multiplanar views of the brain. This has been shown to be useful in utero, particularly for midline structures such as the corpus callosum [12] which can sometimes be difficult to identify at 2-D ultrasound. It may also prove beneficial in the identification and characterisation of complex congenital cardiac diseases, a particularly challenging diagnosis for post-mortem ultrasound. Previous studies assessing the benefit of 3-D versus 2-D fetal echocardiography have found that the addition of 3-D volumes was helpful in assessing the anatomical relationship of the main outflow tracts and their diameters [13], as well as informing the cardiac anatomy for parental counselling in fetuses with double outlet right ventricle [14]. Unfortunately it was not possible to assess this benefit in our cohort, given the lack of any cases with cardiac anomalies.

As with all studies, ours had several limitations - the main one relates to our small sample size, with only a few fetuses with structural abnormalities. A larger sample size covering a greater variety of different congenital pathologies would have allowed us to better assess whether certain clinical scenarios or anomalies are better suited for additional 3-D ultrasound review. Secondly, we acknowledge the highly operator dependent nature of sonography. Our ultrasound examinations were performed by an experienced paediatric radiologist at a tertiary centre, who already regularly performs 2-D ultrasound in a variety of live and post-mortem paediatric settings. It may be that this experience with 2-D imaging reduced the potential of finding any benefit for 3-D methods which may not be the case for other less experienced operators.

Finally our 2-D and 3-D imaging were performed on different ultrasound machines with transducers of differing bandwidths. This could have conferred an advantage for the 2-D ultrasound technique given the higher frequency of the linear probe which is better suited to smaller patient size. This was determined by the
availability of ultrasound equipment and transducers at the time of the study. Future work using a single ultrasound scanner with both 2-D and 3-D capabilities would be better suited in accurately assessing the true added value/benefit of the 3-D method.

In conclusion, this study has found that 3-D ultrasound imaging did not provide useful additional information over performing 2-D ultrasound alone. Future studies covering a wider variety of perinatal pathologies could help us better understand whether there may be a role for 3-D post-mortem ultrasound for specific abnormalities or clinical scenarios.
Figure Captions

**Fig 1** Normal abdominal appearances in a 24 week gestational aged female foetus. (a) 2-D PMUS imaging of the abdomen, at the level of the adrenal glands in transverse view and (b) sagittal 2-D panoramic imaging of the thorax and abdomen. (c) 3-D PMUS of the abdomen was acquired in the sagittal view (top left corner) with axial (top right), coronal reconstructions (bottom left) and volume rendered image of the anterior abdominal wall (bottom right). With a 3-D volumetric acquisition of the abdomen, it is possible to reconstruct axial views through the adrenal glands (white arrows) and also identify the thoracolumbar vertebral appearances (dashed arrows).

**Fig 2** Fetal hydrops in a 22 week gestational aged female foetus. (a, b) 2-D PMUS imaging of the thorax and abdomen in transverse views show bilateral large pleural effusions and ascites. (c) 3-D PMUS of the thorax and abdomen, acquired in transverse views (top left corner) with sagittal (top right), coronal (bottom left) and volume rendered reconstructions (bottom right) demonstrate the same abnormalities.

**Fig 3** Bladder outlet obstruction secondary to scaphoid megalourethra in a 20 week gestational aged male foetus. (a) 2-D PMUS imaging of the upper abdomen, taken with foetus in a water bath, demonstrates a large left suprarenal septated cyst (asterisk), while (b) transverse and (c) sagittal views of the pelvis demonstrate a distended urinary bladder (white arrow), and dilated penile urethra (dashed arrow). (d) The 3-D PMUS of the abdomen, acquired in sagittal view (top left corner) with axial (top right), coronal (bottom left) and volume rendered (bottom right) views show the same key findings.

**Fig 4** Partial agenesis of the corpus callosum in a 20 week gestational aged male foetus. (a) 2-D PMUS imaging of the brain in coronal, (b) trans-temporal and (c) sagittal views shows some callosal tissue anteriorly (white arrow), although it was difficult to visualise posteriorly. (d) 3-D PMUS imaging acquired in the coronal and sagittal planes with resultant multiplanar reconstructions showed similar appearances as the 2-D PMUS.
References


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help us better understand whether there may be a role for 3-D post-mortem ultrasound for specific abnormalities.
Figure Captions

**Fig 1** Normal abdominal appearances in a 24 week gestational aged female foetus. (a) 2-D PMUS imaging of the abdomen, at the level of the adrenal glands in transverse view and (b) sagittal 2-D panoramic imaging of the thorax and abdomen. (c) 3-D PMUS of the abdomen was acquired in the sagittal view (top left corner) with axial (top right), coronal reconstructions (bottom left) and volume rendered image of the anterior abdominal wall (bottom right). With a 3-D volumetric acquisition of the abdomen, it is possible to reconstruct axial views through the adrenal glands (white arrows) and also identify the thoracolumbar vertebral appearances (dashed arrows).

**Fig 2** Foetal hydrops in a 22 week gestational aged female foetus. (a, b) 2-D PMUS imaging of the thorax and (c) abdomen in transverse views show bilateral large pleural effusions and ascites. (d) 3-D PMUS of the thorax and (e) abdomen, acquired in transverse views (top left corner) with sagittal (top right), coronal (bottom left) and volume rendered reconstructions (bottom right) demonstrate the same abnormalities.

**Fig 3** Bladder outlet obstruction secondary to scaphoid megalourethra in a 20 week gestational aged male foetus. (a) 2-D PMUS imaging of the upper abdomen, taken with foetus in a water bath, demonstrates a large left suprarenal septated cyst (asterisk), while (b) transverse and (c) sagittal views of the pelvis demonstrate a distended urinary bladder (white arrow), and dilated penile urethra (dashed arrow). (d) The 3-D PMUS of the abdomen, acquired in sagittal view (top left corner) with axial (top right), coronal (bottom left) and volume rendered (bottom right) views show the same key findings.

**Fig 4** Partial agenesis of the corpus callosum in a 20 week gestational aged male foetus. (a) 2-D PMUS imaging of the brain in coronal, (b) trans-temporal and (c) sagittal views shows some callosal tissue anteriorly (white arrow), although it was difficult to visualise posteriorly. (d) 3-D PMUS imaging acquired in the coronal and (e) sagittal planes with resultant multiplanar reconstructions showed similar appearances as the 2-D PMUS.
References


Table 1:
Demographic and imaging findings for all cases included in this study. M = male, F = female, IUD = intrauterine death, TOP = termination of pregnancy, MIA = minimally invasive autopsy (i.e. laparoscopic assisted internal autopsy examination)

<table>
<thead>
<tr>
<th>Foetus</th>
<th>Gestation (weeks)</th>
<th>Gender</th>
<th>Mode of Demise</th>
<th>Antenatal history</th>
<th>2-D Ultrasound</th>
<th>3-D Ultrasound</th>
<th>Autopsy Type</th>
<th>Autopsy Results (foetal findings)</th>
<th>Autopsy Results (placental findings)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>M</td>
<td>IUD</td>
<td>Dichorionic, diamniontic twin pregnancy.</td>
<td>Unremarkable</td>
<td>Unremarkable</td>
<td>MRI / External examination</td>
<td>No major congenital malformations</td>
<td>Acute funisitis and chorioamnionitis</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>F</td>
<td>IUD</td>
<td>Dichorionic, diamniontic twin pregnancy.</td>
<td>Unremarkable</td>
<td>Unremarkable</td>
<td>MRI / External examination</td>
<td>No major congenital malformations</td>
<td>Acute funisitis and chorioamnionitis</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>M</td>
<td>TOP</td>
<td>Oligohydramnios (secondary to spontaneous rupture of membranes)</td>
<td>Unremarkable</td>
<td>Unremarkable</td>
<td>MIA</td>
<td>No major congenital malformations</td>
<td>Normal</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>F</td>
<td>TOP</td>
<td>Oligohydramnios (low grade vaginal bleeding throughout pregnancy)</td>
<td>Unremarkable</td>
<td>Unremarkable</td>
<td>MRI / External examination</td>
<td>No major congenital malformations</td>
<td>Mild chorioamnionitis with large placental haematoma</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>M</td>
<td>TOP</td>
<td>Prenatal ultrasound at 20 weeks showed total absence of corpus callosum, small interhemispheric cysts.</td>
<td>Partial absence of the corpus callosum</td>
<td>As per 2-D Ultrasound findings</td>
<td>MIA + Micro- CT imaging</td>
<td>Partial absence of the corpus callosum with small interhemispheric cyst</td>
<td>Normal</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>M</td>
<td>TOP</td>
<td>Prenatal ultrasound at 18 weeks showed oligohydramnios, talipes, thickened nuchal fold. Bladder was enlarged and there was a large ventricular septal defect.</td>
<td>Hypoplastic left ventricle, large right atrium. Septal defects difficult to visualise. Left suprarenal cyst. Distended urethra and bladder; bilateral dilated kidneys.</td>
<td>As per 2-D Ultrasound findings</td>
<td>Full</td>
<td>Megalourethra with obstructive uropathy. Microscopic renal cystic dysplasia. Left adrenal gland is necrotic. Associated talipes and positional hand deformities on external assessment.</td>
<td>Normal placenta, single umbilical artery.</td>
</tr>
<tr>
<td>#</td>
<td>Age</td>
<td>Gender</td>
<td>IUD Type</td>
<td>Clinical Findings</td>
<td>Ultrasound Findings</td>
<td>Outcome</td>
<td>Diagnoses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----</td>
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<td>---------------------</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>F</td>
<td>IUD</td>
<td>Thickened soft tissue folds along left side of neck.</td>
<td>Hydropic foetus (large pleural effusions, pericardial effusion, ascites, hypoplastic lungs)</td>
<td>Full</td>
<td>Hydroptic foetus, hypoplastic lungs (secondary to hydrops), normal heart. Cause for hydrops not determined. Stromal villous haemorrhage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>32</td>
<td>M</td>
<td>IUD</td>
<td>Unremarkable pregnancy. IUD confirmed on prenatal ultrasound, labour induced at 32 weeks gestation.</td>
<td>Unremarkable</td>
<td>Full</td>
<td>No major congenital malformations Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>22</td>
<td>M</td>
<td>IUD</td>
<td>Recurrent maternal urinary tract infections during pregnancy. Spontaneous labour with rupture of membranes. Foetus delivered without heartbeat.</td>
<td>Unremarkable</td>
<td>Full</td>
<td>No major congenital malformations Acute subchorionitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>25</td>
<td>F</td>
<td>IUD</td>
<td>Unremarkable pregnancy.</td>
<td>Unremarkable</td>
<td>Full</td>
<td>No major congenital malformations Normal</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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**Conflict of Interest Form**
COI form 3D PMUS paper.pdf
27th August 2020

Editor, Pediatric Radiology

Dear Dr Oystein Olsen,

Re: Submission ID: PRAD-D-20-00199

Thank you for considering our manuscript entitled “3-dimensional (3-D) versus 2-dimensional (2-D) post-mortem ultrasound: Feasibility in perinatal death investigation” for publication in Pediatric Radiology as a Technical Innovation paper.

We are grateful for your thorough reading of our paper and have addressed the reviewer and editorial comments, with our responses (in bold text) below and made amendments to our article as suggested.

Reviewer #1:

1. In the discussion, please explain why it takes 30 minutes for 3D ultrasound vs 20 minutes for 2D ultrasound. Is this amount of time all acquisition time (which I would have assumed it would be faster for 3D with single acquisitions per body station)? Does the 30 vs 20 min time difference include post-acquisition processing time?

The additional time for the 3-D ultrasound is due to the lengthy image post-processing time. Whilst it only takes a matter of 5 minutes to acquire a volume of each foetal body region (i.e. head, thorax, abdomen), the reconstruction and creation of meaningful and interpretable images for recording in our PACS system and for interpretation requires additional time. This is in contrast to the 2-D ultrasound acquisition where the operator is acquiring and interpreting the imaging as they perform the study. We have now explained this more fully in our methodological description.

2. I suggest including a table of each subject with gestational age, gender, pre-autopsy presumed cause of death (and how it was made, e.g. ultrasound, x-ray, etc), 2D postmortem ultrasound findings, 3D postmortem ultrasound findings, and autopsy findings.

We have now included a single table in this revised submission detailing the 11 foetuses in this study.
3. In the body of the results, I would make it explicit that the 1 case that did not detect an 
abnormality (i.e. the 25% “false negative”) was the OI case but it was due to patient being too 
small. In all patients where you were able to do an ultrasound, it sounds like you were able to 
distinguish normal from abnormal. It was only in the case that you couldn't scan that 
ultrasound was not helpful.

This is correct. We have amended this in the results section to reiterate that the one 
case we could not diagnose was due to non-diagnostic imaging, and small foetal size 
and amended our abstract to fit this rewording.

4. I suggest abandoning percentages with such small sample size. Just list it out: 10 patients 
scanned, 7 normal, 3 abnormal, concordant autopsy. 1 patient not scanned due to OI.

We have now removed all percentages from our results in the main manuscript and 
listed the abnormalities out of 10 (not 11 cases).

5. For readers like me that are not familiar with the topic, I suggest including another table listing 
common diagnoses that you think post-mortem ultrasound should be able to detect. Clearly 
abnormalities that result in fluid accumulation are accessible. This table should be based on 
existing literature, the results of your study, and your expert opinion.

Thank you for this suggestion. We have published a very comprehensive review of our 
post-mortem ultrasound technique, template reporting (including specific anatomy and 
areas for review) and potential abnormalities for perinatal losses in a separate pictorial 
review/guidance document within the journal ‘Insights into Imaging’ as a two part, 
open access series.

We have now included this as a reference in our manuscript to guide readers who are 
interested in learning more about postmortem ultrasound imaging. As this is only a 
short technical innovation article, it may be quite lengthy to incorporate all possible 
details. If, however, the editor feels it would be helpful to reproduce these tables, we 
can seek the necessary permissions from the original journal for reproduction.

6. In the figures, you included the 3 abnormal cases. That's great. I suggest also including 1 
normal case with annotations showing the level of detail that one can achieve with postnatal 
ultrasound.
We have now included an additional image of a normal foetus in Figure 1 to demonstrate the normal appearances seen at ultrasound. The previous figures have been renumbered accordingly.

7. Does your report template only include 5 fields, brain, spine, cardiac, thorax, abdomen? Or does it include subcategories (e.g. liver, bladder, etc.)? If subcategories are included, I suggest describing your template in detail in a table or appendix, so that readers have a sense of what your survey covers. Perhaps this information could be tied into my suggestion #5, helping the readers understand what diagnoses you are looking for in each anatomic region.

Thank you for this suggestion. Please see our response above to point 5. Our template is for just the 5 body regions, however if there is any abnormality within any of these areas, we would expand to include positive and negative findings per body organ within these regions.

Reviewer #2:

8. Thank you for this article. I applaud the authors for reporting a "negative" observation. Certainly the use of 3D ultrasound is not new in pediatrics and fetal imaging. In the last 10-15 years it has become apparent that 3D provides very few benefits with regard to accuracy and sensitivity. It like most advanced visualization techniques is helpful in presenting anatomy to non-imagers who might better understand the findings if presented in 3D or multiple planes. If a benefit has been shown it would be in complex disease that may be difficult to understand with a single plane, such as congenital heart disease. This being said and certainly agreed upon by the authors, the report lacks sufficient specificity and power to provide scientific guidance. Given the sample of 11 and the variety of pathology presented it is hard to justify the conclusion that 3D is either beneficial or not. I agree with the authors that the technique is feasible. If the report were to focus on a specific pathology it may be more valuable.

Thank you for these comments. We agree with the reviewer's advice. In our conclusion we have now reworded our suggestion for future larger studies, to include a recommendation for studies covering a wider range of positive pathologies to understand what specific conditions the 3-D technique may be helpful. It is true that for some congenital cardiac malformations, a 3-D volume may be helpful to reconstruct different planes (as is the case in intrauterine/prenatal imaging). In post-mortem fetal cardiac ultrasound we suffer from poor diagnostic 2-D accuracy results given the lack
of circulation to aid diagnosis and collapse of the cardiac chambers with presence of thrombus. It would be interesting to know whether 3-D imaging may be helpful in these circumstances. This issue has been added to our discussion.

**Editor:**

9. Title cannot have acronyms (2-D, 3-D; also please note the hyphens)

   This has been amended in the title. The hyphenation has been amended in the abstract and throughout the document.

10. Title page: fix the phone number

   This has been amended

11. I would have removed all mention of 'additional value' from title and objective description, because how can such a broad term be defined based on n=11?

   The term 'added value' has been removed from the title and abstract and manuscript.

12. In my opinion the study has not been convincingly justified. To me there is not obvious benefit of 3-D US, which is why we never use it clinically although we could easily do so. Therefore, rather than stating the obvious theoretical benefits, which pathological entities in particular would you say might be more precisely detected and described by 3-D US? Could you add this to Introduction?

   Thank you for the suggestion. We believe there may be some benefit in complex congenital cardiac or neurological diseases and have now added this to our introduction to build a more convincing case.

13. How cold is a 'cold water bath'?

   We have added this detail to the description (approximately 5 degrees Celsius)

14. We don’t need to know which spreadsheet what used, that is too much detail

   This detail has been removed.
15. But we would like to know how the scans were done

A full description of the image acquisition parameters and methodology are provided under subheading ‘description of innovation’, third paragraph. We explain the 2-D and 3-D scanners used, bandwidth of the transducers and the body parts we acquired for the acquisition.

16. Essentially, you have only looked at n=4 since 7/11 were entirely normal anatomically. Why have you not added any description of normal anatomy at 2-D v 3-D? Wouldn't that be of some interest? E.g. luminal volumes, femoral lengths?

As per suggestion of reviewer 1, we have now included an additional figure (Figure 1) of normal anatomy seen at 2-D ultrasound and 3-D ultrasound.

In post-mortem imaging (regardless of CT/MRI or ultrasound) we are mainly looking to confirm antenatally detected anomalies or congenital anomalies (and associated pathologies) that may have been missed. We do not routinely measure femoral lengths (already done on skeletal radiographs prior to cross-sectional imaging), nor any luminal volumes, organ volumes etc. unless these are obviously abnormal or cause for concern has been raised. Therefore our main priority is in identification of major structural anomalies, so if none is apparent then we do not have anything further to comment upon and do not record this level of detail.

17. Are dead foetuses ‘patients’?

We have removed the term patients/cases in our manuscript and only use the term foetuses for consistency.

18. Recommending larger studies seems completely futile to me. What is the point if there isn’t even some theoretical possibility of any diagnostic benefit? If you think there are possible advantages, then what are those, and why?

We have removed suggestion of conducting further larger studies, given that 3-D imaging did not identify anything that was missed by 2-D. In keeping with reviewer 2 we suggest studies focussing on a wider range of pathologies, where the 3-D imaging may be helpful for reconstructing different imaging planes for better visualisation of complex anatomy.
19. Legend fig 3: male or female?
   This is a male foetus, we have added this detail.

20. Figure should as a general rule not be composite. I would accept the MPR/VR grids, but I think 1a, 2a and 3a should be split.

   We have now split figures for 1a, 2a and 3a into separate files and provide the new files with this submission. The figure legends have also been amended accordingly to the new numbering and split images.

We hope that you will now find our revised work in a more publishable state.

Thank you once again for your time and help.

Yours sincerely,

Dr. Susan Shelmerdine
Paediatric Radiology Research Fellow

On behalf of all authors