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BULLETED STATEMENTS

What's already known about this topic?

- Less invasive alternatives for a fetal autopsy are gaining popularity given the low parental consent and parental acceptability rates for invasive methods.
- Different types of post-mortem radiological imaging techniques are available, however each have advantages and disadvantages and the choice and availability of these in different centres is variable.

What does this study add?

- We clarify the commonly used medical terms for describing different forms of non-invasive and less invasive autopsy methods.
- We summarise the latest research in the different types of post-mortem imaging techniques and provide suitable clinical usages for each.

ABSTRACT

A sustained decline in parental consent rates for perinatal autopsies has driven the development of less-invasive methods for death investigation. A wide variety of imaging modalities have been developed for this purpose and include post-mortem whole body MRI, ultrasound, CT and micro-focus CT techniques. These are also vital for 'minimally invasive' methods which include potential for tissue sampling, such as image guidance for targeted biopsies and laparoscopic assisted techniques.

In this article we address the range of imaging techniques currently in clinical practice, and those under development. Significant advances in high field MRI and micro-focus CT imaging show particular promise for smaller and earlier gestation fetuses. We also review how MRI biomarkers such as diffusion weighted imaging and organ volumetric analysis may aid diagnosis and image interpretation in the absence of autopsy data. 3D printing and augmented reality may help make imaging findings more accessible to parents, colleagues and trainees.

INTRODUCTION

Conventional autopsy techniques have shaped our understanding of human anatomy, development, pathology and thus, much of what we currently understand about modern medicine. Traditionally considered the 'gold standard' in diagnostic approach for investigating perinatal deaths[1], it has suffered a marked decline in acceptance rates amongst parents, stemming from a variety of personal, social and religious reasons[2]. This has led to an increase in demand for non-invasive imaging alternatives[3, 4].

The main objectives of post-mortem imaging are much the same as those of the 'internal examination' aspect of the conventional autopsy – to define and characterize structural fetal anomalies, determine their significance, and thus enable a more comprehensive approach to parental counseling – which in fetal autopsy - particularly focuses on determining the recurrence risk for future pregnancies[5]. Detailed visualization of anatomical structures is vital, and the different advantages of imaging techniques allow a more focused approach[6]. By understanding the clinical indication, knowledge of 'expected' post-mortem changes and potential pathology, appropriate imaging pathways can be proposed.

In this article we outline the clinical utility of current imaging modalities, their diagnostic accuracy and merits for their use in the perinatal post-mortem setting. Advanced imaging techniques using high-field MRI, micro-focus CT (micro-CT) imaging and the use of radiological biomarkers (such as organ volumes and diffusion coefficients) are further explored, including how imaging can assist in tissue sampling. Finally we describe some challenges and methods in communicating imaging results to non-radiological colleagues and parents.

The Less Invasive Autopsy (LIA)

With new techniques, comes new terminology. The literature is rife with terms relating to 'imaging autopsy', and unfortunately these are not routinely standardized or consistently used and could mean slightly different things with respect to the degree of 'invasiveness'. For ease of reference and understanding, we have collated the examples of commonly used terms with their explanations in **Table 1**.

What determines the level of invasiveness?

Although a non-invasive autopsy (NIA) is the preferred situation for most parents, the level of 'invasiveness' of an autopsy may not be entirely parental choice dependent. This primarily depends on the circumstances of the death (e.g. 'natural' versus suspicious deaths / those having a medico-legal component) as well as the availability of imaging techniques and likely contribution of tissue sampling investigation according to clinical presentation.

In forensic work, fetal post-mortem CT (PMCT) imaging and skeletal radiographs have been widely used in stillbirth[7] and in identifying underlying lethal skeletal dysplasia[8] (e.g. osteogenesis imperfecta[9]). Where imaging effectively provides the diagnosis, the need for invasive autopsy can be avoided in many cases, however other situations may not be so easily defined. At present, the Royal College of Pathologists (RCPath) support the usage of PMCT for coroner's inquests and in the investigations for stillbirths and sudden unexplained deaths in infants[10, 11], mostly for fracture detection, although further tissue sampling and invasive testing may be refined based on the imaging findings. In the Netherlands, for older children, PMCT is also recommended for unnatural deaths[12].

For stillbirths in which there are no medico-legal or suspicious circumstances, the causes of death often remain elusive, even when an autopsy has been performed. One study found that approximately half of all autopsies for stillbirth concluded in an 'unexplainable cause of death'[13]. Interestingly a large population study conducted in the United States[14] found that the yield in explainable diagnoses for stillbirths was similar for cases that had undergone an invasive autopsy (56%) versus no autopsy (58%); and that for most families – given the low chance of an explainable cause - a less invasive method of investigation such as performing imaging only in conjunction with placental examination, would have been preferable.

Given this sentiment, many centres performing post-mortem imaging will now use it as a method of triaging cases into two groups – those that could benefit from further invasive examination, and those that do not. This is of particular importance when considering that the perinatal autopsy examination comprises of many parts (e.g. placental examination, clinical history, microbiology testing, etc) – each

of which could provide a plausible explanation for cause of death before the body is even examined[15]. In at least two large recent cohort studies, the most useful component of the perinatal autopsy (with respect to providing overall cause of death or main diagnosis) was placental pathology (32%[16] – 65%[17]). The internal examination (i.e. invasive component of the autopsy) only provided the diagnosis for an additional 1% of cases when other investigations were non-contributory[13, 18]. Therefore, one pragmatic approach to avoid invasive testing would be to only perform these for cases where imaging demonstrates a complex abnormality requiring further confirmation (such as complex congenital cardiac anomalies). Invasive examination is unlikely to yield additional information where the imaging is normal, and where the placental examination already presents an explanation for cause of death.

For cases in which tissue samples from internal organs are required for histopathological, molecular, metabolic or genetic testing but a less invasive approach is preferred (for example a 'minimally invasive autopsy' (MIA), using a smaller incision than a fully invasive examination), several methods may be feasible. One approach involves extraction and macroscopic examination of the organs via a keyhole surgical approach (also coined 'MinImAL' – Minimally Invasive Autopsies with Laparoscopically assisted tissue sampling). In this scenario, a single small incision is made to the anterior abdominal wall (usually left subchondral region) to allow entry for a laparoscope and forceps, and through which tissue can be extracted[19]. A recent study revealed that this technique was feasible in 97.8% (91/93) of the cases recruited, with an organ sampling success rate of >80% for the majority of organs, and took a similar amount of time as a standard autopsy[20].

An alternative approach is to conduct image-guided tissue biopsies. A recently pioneered method for avoiding any incisions is the INcision-less Targeted Core Tissue (INTACT) biopsy method[21]. Here, the biopsy needle is inserted into the abdominal cavity via the umbilical cord, where the needle can be then directed to various organs under the direction of ultrasound imaging, negating the need for a skin incision. In a small sample of thirty cases, the biopsy sampling success rate for all organs was 76.1%, although this may have been lowered by using macerated fetuses.

Although both these methods of MIA are more appealing than a fully invasive autopsy, one challenging area is the assessment of the brain and spinal cord. Post-mortem MRI (PMMR) is the

preferred choice for fetal intracranial abnormalities[22], although any uncertainties at imaging will likely require whole brain extraction and fixation, rather than brain biopsies. This is particularly in the perinatal cohort when assessing developmental structural anomalies, as complex intracranial abnormalities identified on antenatal ultrasound can be missed on PMMR[23].

What imaging techniques are available?

Given the variety of modalities described, the best imaging modality may be based on clinical indication, but be restricted by availability. A European survey exploring the status of paediatric (including older children as well as fetuses) post-mortem imaging in 2013[24] found that of the 47 centres providing this service, most used plain radiography (38/47, 81%) or computed tomography (CT; 24/47, 51%), with only a few centres offering MRI (18/47, 38%) and hardly any performing ultrasound (4/47, 8.5%). Information on such services in North America and Asia are not available, and presumed to be lower given lack of published scientific material from centres in those regions. This demonstrates that many real and assumed barriers still exist for wider adoption, including lack of resources, time, interpretive expertise and the opinion amongst healthcare professionals and parents that the imaging could potentially 'miss' clinically important information[2, 25] despite widely published work to the contrary. As such, much work has focused on determining the clinical relevance and accuracy for these techniques, summarized here.

Post-mortem Skeletal Surveys, PMSS

For many years, the most prevalent form of perinatal post-mortem imaging was a whole body radiograph of the fetus (commonly referred to as a skeletal survey). This is intended to provide the pathologist with a general overview of fetal skeletal maturity, estimation of gestational age and appreciation for any potential genetic bone disorders[26]. Nevertheless, routine radiographic acquisition has been shown to be very of low diagnostic value (<5% yield[26, 27]), given the increased specificity in prenatal ultrasonography in ruling out underlying skeletal dysplasias[28]. Skeletal radiography should be targeted towards those with antenatally diagnosed skeletal abnormalities, to maximise efficiency and diagnostic yield.

Post-mortem Magnetic Resonance Imaging, PMMR

The first large diagnostic accuracy study using PMMR (MAgnetic Resonance Imaging in Autopsy Study; MARIAS, reported in 2013), showed that it could provide excellent image resolution and a high diagnostic accuracy across a range of fetal miscarriages, terminations of pregnancy and stillbirths[29] (**Figure 1**). Since this time, several advances have been made focusing on developing PMMR as a clinical tool, with published recommendations on suitable PMMR sequences[30] and normal PMMR appearances[31]. We outline a suggested full and abbreviated imaging protocol in **Table 2**. The time taken for a full protocol is in the region of 60-90 minutes, whereas those of a shortened protocol can be completed in less than 20 minutes. The decision regarding which protocol to follow will largely depend on scanner time availability, the size of the fetus (fetuses <500g are less likely to yield diagnostic quality imaging and therefore it would be futile to waste excess scanning time for a poor result) and indication for the study (e.g. a fetus terminated for a suspected cardiac anomaly would warrant more detailed high resolution imaging through the heart, included in the full protocol). Whilst fetal immersion in a gadolinium based solution (to provide an increased signal to noise ratio through contrast enhancement) has been trialed, this has so far not demonstrated any added benefit over non-contrast PMMR[32].

In terms of generalizability of technique and reporting, it has been shown that given appropriate experience and training, a single radiologist can learn PMMR with similar accuracy to radiologists with body-part specific experience[33]. Reasonable levels of diagnostic accuracy from smaller cohorts performed in other European[34, 35] and Indian centres[36] are also being published. Whilst sensitivity rates for whole body diagnoses have been reported as high as 89%[33], a recently published systematic review incorporating several international studies has estimated the pooled sensitivity for paediatric PMMR at 0.73 (95% CI 0.56 – 0.84)[37].

When counseling parents with regards to 'usefulness of an invasive autopsy', recent work has shown that when PMMR results were concordant with the prenatal ultrasound results, an invasive autopsy was only of added clinical benefit in 4.5% cases[38], suggesting little benefit and the potential for avoiding this procedure altogether. Nevertheless, where the findings are discordant or only partially concordant further discussion is warranted and the benefits are less clear cut. Furthermore, where an

antenatally detected complex intracranial brain pathology is identified, intrauterine MRI (iuMR) has been found to be more accurate in characterizing all underlying phenotypical features rather than PMMR[23, 39], possibly due to the resultant hypoxic oedematous changes that mask subtle findings in the post-mortem period.

Off-line analysis of PMMR also allows for some functions of the autopsy to be performed from imaging findings. For example, organ segmentation and volume analysis can be extracted from imaging data, and have a high correlation with autopsy organ weights, including abdominal organs and brain[40-42]. As organ ratios between brain and liver may indicate severity of fetal growth restriction, imaging surrogates may be equally useful where parents decline invasive organ assessment[43]. More advanced MRI techniques such as diffusion weighted imaging (DWI) may give additional information regarding time or mode of death[44].

Post-mortem Computed Tomography, PMCT

Relatively few post-mortem CT (PMCT) studies have been performed for fetuses, although one study comparing PMCT and PMMR in 53 fetuses, found that PMCT had a lower overall concordance rate with autopsy than PMMR (for fetuses <24 weeks gestation concordance with autopsy was 38.1% [20.8 – 59.1% CI] for PMCT versus 51.6% [34.8 – 68.0% CI] for PMMR)[8]. This is likely due to a lack of fetal body fat to generate differences between organ imaging contrast, as well as the densely consolidated unaerated lung appearances.

Techniques such as ventilated PMCT[45] and contrast enhanced PMCT[46] can provide added value, although this is more labour intensive and would require additional training and resources to be built into routine clinical practice. The largest cohort study to determine the diagnostic accuracy of PMCT in older children[47] showed that it was most accurate for fracture detection and intracranial pathologies, and therefore should remain a useful tool in older children and adults particularly in medicolegal proceedings[47].

Post-mortem Ultrasound, PMUS

Although not currently routinely performed in many centres, PMUS has been shown to be a feasible technique for post-mortem imaging[48, 49] with good concordance rates compared to autopsy of up to 81.4% [73.2% - 89.6% CI][50-53] (**Figure 2**). The techniques described in the literature commonly utilise high frequency linear probes for the majority of body parts (e.g. frequencies of 6-18MHz or 6-12MHz for GE Voluson E8 model[50]; 7-16MHz for Samsung HM70A model[50]; 10-15MHz for Toshiba Aplio 500 model[52]). Lower frequency curvilinear and micro-convex probes have also been reported also for larger subjects and cranial imaging (e.g. 5-8MHz micro-convex for Philips iU22 model[53]; 5-9MHz for GE Voluson E8 model[50,51]; 2.5 – 8MHz GE Logiq E9 model[50]; 7-10MHz for Toshiba Aplio 500 model[52]).

New data has also shown that where images are of diagnostic quality, the ability to diagnose congenital anomalies is comparable to 3T PMMR[54] (PMUS 81.8 – 96.5% vs PMMR 81.6 – 99.1%). However, there was a greater likelihood of non-diagnostic PMUS studies compared to PMMR. This is particularly notable for brain and cardiac imaging where PMUS was non-diagnostic in a third of cases compared to PMMR which was only non-diagnostic in less than 5% ($p<0.001$)[54]. The reasons for this may be partly due to overlapping cranial sutures from maceration or extraction methods, precluding a clear sonographic window, and the difficulty in delineating cardiac anomalies generated by the lack of Doppler flow in the post-mortem population. In cases where feticide has occurred, there may be both intracardiac gas and pneumothoraces[55], which could further obscure the transmission of sonographic waves. Nevertheless, when diagnostic, it could provide an alternative method for imaging where PMMR is not available or access is limited, and as above mentioned, it could aid in organ visualisation for targeted organ biopsies[21].

Challenges in Imaging Small Fetuses

Smaller fetal cases are more challenging to image, particularly at 1.5T PMMR where the post-mortem weight is less than 500g[56]. Higher field strength MRI scanners can help the situation. When imaging the same cohort of fetuses at 3T PMMR compared to 1.5T PMMR, imaging was less likely to be non-diagnostic and better for fetuses <24 weeks gestation[57]. Specialist research centres have taken this further, trialing fetal post-mortem imaging with higher magnetic field strengths of 7T[58] and 9.4T[59].

These studies showed improved detailed anatomy, but the diagnostic improvement was unknown, and widespread adoption and availability of such scanners are limited.

A different type of imaging modality using CT technology, termed 'micro-focus computed tomography' (i.e. micro-CT) could provide a solution. It has long been used for small animal research trials and only recently specially adapted for post-mortem fetal work by addition of an iodine based contrast medium (**Figure 3**). The time taken for fetal preparation by immersion in the iodine contrast can vary with the size of the fetus and concentration of the iodine solution, ranging between 3-10 days in our local experience. Whilst it does mean an additional process and a slightly longer delay to the imaging time, it has allowed for the internal structures of small fetuses to be visualised at high resolution. One study has reported this technique to have a sensitivity of 93.8% and specificity of 100%[60], in a cohort of 20 fetuses below 22 weeks gestation. It has even been possible to image the internal organs of small embryos as early as 7 weeks gestational age[61] at a similar resolution to light microscopy (**Figure 4**). In certain cases it may alter parental counseling and change the suspected antenatal diagnosis; such as in Bardet Biedl syndrome[62]. In that case report, multiple renal cysts and a complex congenital cardiac malformation were subsequently discovered for a fetus that was originally suspected of a ciliopathy, given the antenatally detected polydactyly[62]. Nevertheless, it is important to remember that the immersion of the fetus in the iodine contrast can cause brown discolouration of the skin, and although techniques to reverse the staining are possible (e.g. with further immersion in a sodium thiosulphate solution for an additional 1-2 days[60]), this reversal may not be complete. In our practice we therefore always ask parents to additionally consent to this possibility, prior to proceeding with the study.

Whilst not necessarily preventing an invasive autopsy per se, the analysis of fetal pathological specimens can also be performed using micro-CT, enabling pathologists to understand the organ they intend to dissect before causing any destruction to the tissue. This has been demonstrated for ex vivo organ in fetuses including the brain[63], heart[64, 65] and kidneys[66]. This can be useful for identification of lesions not readily seen at microscopy or potentially destroyed during dissection, such as rhabdomyomas in a case of tuberous sclerosis[67].

Post-mortem Interval

It remains unclear whether current post-mortem imaging techniques can reliably provide data on post-mortem interval (PMI) i.e. retrospectively estimating the time that has passed since death based on imaging. This would be most useful in forensic cases, or intrapartum deaths, although fetal cases are further complicated by the additional effect of maceration on tissue decomposition and autolysis[68].

Current literature on this topic has suggested that fluid shifts within the body may be surrogate markers of PMI, but imaging these has been met with limited accuracy. For example, fluid shifts in the chest mean that volume of pleural effusion[69] and the amount of restricted diffusion in different organs[70, 71] could be informative, but as yet none of these have proven to be robust markers to support routine measurement. Whilst the total volume of gas within the body alters with PMI and can be detected with PMCT, this work has so far focused on animal[72] and adult studies with variable results[73, 74].

Parental Attitudes

This article began by outlining the lack of acceptance by parents for traditional autopsy techniques and therefore it is of upmost importance that we also understand their attitudes towards imaging to understand whether this is a viable alternative from a 'patient's' perspective, and also of the associated healthcare professionals. Whilst many parents prefer non-invasive to conventional autopsy, a minimally invasive autopsy (utilizing either needle or laparoscopic guided biopsies) was interestingly viewed in a similar manner to non-invasive[75]. In addition, Muslims and Jews who have a strict religious criteria regarding burial proceedings also find the non-invasive work acceptable[75, 76]. One major barrier to uptake, particularly amongst healthcare professionals consenting parents[25], includes the fear of 'missing something'[2, 25] thus highlighting the importance of appropriate evidence based counselling and need for further development of accurate techniques, and the availability of these.

Future Work and Directions

Much groundwork has been laid in the development of perinatal post-mortem imaging techniques, however there is still a lot of work and progress to be made. We now understand the benefits and drawbacks of many imaging modalities, however robust protocols and imaging pathways as well as

evidence based referral guidelines are lacking. The ESPR[6] and SPR[77] have established post-mortem taskforces which are aiming to tackles these issues.

Training future radiologists in this field will be required if it is to increase expertise and care. Establishing normative values at post-mortem imaging at various gestations, and also creating an embryological library using high resolution techniques for training, as has been done using digital imaging techniques[78] and MRI[79]. In addition, the use of advanced computer visual tools such as augmented reality using HoloLens[80] and 3D printing methods[81] (**Figure 5**) could enable interactive learning of post-mortem imaging datasets and trainee engagement, as well as parental understanding of their child's congenital abnormalities. Similar work to explain congenital cardiac anomalies to patients and families has been well-received[82].

Finally, on a societal level to allow for equal access to healthcare, particularly in a government funded system, this work will need to undergo a full economic healthcare cost analysis. This may not be as simple as comparing the cost of an invasive autopsy versus costs of imaging, since many parents who would decline an invasive autopsy would consent to an imaging-based autopsy. The long term psychological distress faced by parents and the impact this has on the economy (between those that felt they had to make an 'all or nothing' choice with regards to invasive autopsy versus those who were offered an imaging based option) is difficult to quantify but clearly presents another interesting avenue for future research.

In conclusion, post-mortem imaging in the perinatal setting offers high diagnostic quality to parents who decline conventional autopsy. It adds value to antenatal findings, and image-guided autopsy may become the standard in the future. Further economic evaluation is required, but with a well-informed, evidence-based approach, using the right imaging for the right clinical indication will generate the highest yield for parents and clinicians alike.

REFERENCES

1. Ernst LM. A pathologists perspective on the perinatal autopsy. Semin Perinatol. 2015;39(1):55-63.

2. Lewis C, Hill M, Arthurs OJ et al. Factors affecting uptake of postmortem examination in the prenatal, perinatal and paediatric setting. BJOG. 2018;125(2):172-81.

3. Kang X, Cos T, Guizani M et al. Parental acceptance of minimally invasive fetal and neonatal autopsy compared with conventional autopsy. Prenat Diagn. 2014;34(11):1106-10.

4. Ben-Sasi K, Chitty LS, Franck LS et al. Acceptability of a minimally invasive perinatal/paediatric autopsy: healthcare professionals' views and implications for practice. Prenat Diagn. 2013;33(4):307-12.

5. McPherson E, Nestoridi E, Heinke D et al. Alternatives to Autopsy for Fetal and Early Neonatal (Perinatal) Deaths: Insights from the Wisconsin Stillbirth Service Program. Birth Defects Res. 2017;109(18):1430-41.

6. Arthurs OJ, van Rijn RR, Whitby EH et al. ESPR postmortem imaging task force: where we begin. Pediatr Radiol. 2016;46(9):1363-9.

7. Chen Y. State of the art in post-mortem forensic imaging in China. Forensic Sci Res. 2017;2(2):75-84.

8. Arthurs OJ, Guy A, Thayyil S et al. Comparison of diagnostic performance for perinatal and paediatric post-mortem imaging: CT versus MRI. Eur Radiol. 2016;26(7):2327-36.

9. Zou DH, Shao Y, Zhang JH et al. Determination of a Newborn with Lethal Type II Osteogenesis Imperfecta and Other Anomalies Using Autopsy and Postmortem MSCT--A Case Report. Fa Yi Xue Za Zhi. 2016;32(1):69-73.

10. Royal College of Pathologists (RCPath) Guidelines for the Investigation of Sudden Unexplained Death in Infancy 2016 https://www.rcpath.org/discover-pathology/news/new-guidelines-for-the-investigation-of-sudden-unexpected-death-in-infancy-launched.html. Accessed 15 July 2019.

11. The Lancet. Progress on investigating stillbirths. Lancet 2019;393(10179):1386.

12. Sonnemans LJP, Vester MEM, Kolsteren EEM et al. Dutch guideline for clinical foetal-neonatal and paediatric post-mortem radiology, including a review of literature. Eur J Pediatr. 2018;177(6):791-803.

13. Man J, Hutchinson JC, Heazell AE et al. Stillbirth and intrauterine fetal death: factors affecting determination of cause of death at autopsy. Ultrasound Obstet Gynecol. 2016;48(5):566-73.

14. McPherson E, Nestoridi E, Heinke D et al. Alternatives to Autopsy for Fetal and Early Neonatal (Perinatal) Deaths: Insights from the Wisconsin Stillbirth Service Program. Birth defects research. 2017;109(18):1430-41.

15. Nijkamp JW, Sebire NJ, Bouman K et al. Perinatal death investigations: What is current practice? Semin Fetal Neonatal Med. 2017;22(3):167-75.

16. Man J, Hutchinson JC, Heazell AE et al. Stillbirth and intrauterine fetal death: role of routine histopathological placental findings to determine cause of death. Ultrasound Obstet Gynecol. 2016;48(5):579-84.

17. Page JM, Christiansen-Lindquist L, Thorsten V et al. Diagnostic Tests for Evaluation of Stillbirth: Results From the Stillbirth Collaborative Research Network. Obstet Gynecol. 2017;129(4):699-706.

18. Man J, Hutchinson JC, Ashworth M et al. Stillbirth and intrauterine fetal death: role of routine histological organ sampling to determine cause of death. Ultrasound Obstet Gynecol. 2016;48(5):596-601.

19. Sebire NJ, Weber MA, Thayyil S et al. Minimally invasive perinatal autopsies using magnetic resonance imaging and endoscopic postmortem examination ("keyhole autopsy"): feasibility and initial experience. J Matern Fetal Neonatal Med. 2012;25(5):513-8.

20. Hutchinson C, Shelmerdine SC, Lewis C et al. Feasibility and experience of the MinImAL procedure: Minimally Invasive perinatal and paediatric Autopsies with Laparoscopically assisted tissue sampling. Ultrasound Obstet Gynecol. 2019 Jan 8. Doi: 10.1002/uog.20211 [Epub ahead of print].

21. Shelmerdine SC, Hutchinson JC, Ward L et al. INTACT (INcision-less TArgeted Core Tissue) biopsy procedure for perinatal autopsy: initial feasibility. Ultrasound Obstet Gynecol. 2019 Jul 4. Doi:10.1002/uog.20387

22. Shelmerdine SC, Hutchinson JC, Al-Sarraj S et al. British Neuropathological Society and International Society of Forensic Radiology and Imaging expert consensus statement for post mortem neurological imaging. Neuropathol Appl Neurobiol. 2018;44(7):663-72.

23. Goergen SK, Alibrahim E, Govender N et al. Diagnostic assessment of foetal brain malformations with intra-uterine MRI versus perinatal post-mortem MRI. Neuroradiology. 2019 Aug; 61(8):921-934

24. Arthurs OJ, van Rijn RR, Sebire NJ. Current status of paediatric post-mortem imaging: an ESPR questionnaire-based survey. Pediatr Radiol. 2014;44(3):244-51.

25. Lewis C, Hill M, Arthurs OJ et al. Health professionals' and coroners' views on less invasive perinatal and paediatric autopsy: a qualitative study. Arch Dis Child. 2018;103(6):572-8.

26. Olsen EO, Espeland A, Maartmann-Moe H et al. Diagnostic value of radiography in cases of perinatal death: a population based study. Arch Dis Child Fetal Neonatal Ed. 2003;88(6):F521-4.

27. Arthurs OJ, Calder AD, Kiho L et al. Routine perinatal and paediatric post-mortem radiography: detection rates and implications for practice. Pediatr Radiol. 2014;44(3):252-7.

28. Rossi AC, Prefumo F. Correlation between fetal autopsy and prenatal diagnosis by ultrasound: A systematic review. Eur J Obstet Gynecol Reprod Biol. 2017;210:201-6.

29. Thayyil S, Sebire NJ, Chitty LS et al. Post-mortem MRI versus conventional autopsy in fetuses and children: a prospective validation study. Lancet. 2013;382(9888):223-33.

30. Norman W, Jawad N, Jones R et al. Perinatal and paediatric post-mortem magnetic resonance imaging (PMMR): sequences and technique. Br J Radiol. 2016;89(1062):20151028.

31. Arthurs OJ, Barber JL, Taylor AM, Sebire NJ. Normal perinatal and paediatric postmortem magnetic resonance imaging appearances. Pediatr Radiol. 2015;45(4):527-35.

32. Kang X, Cannie M, Jani JC. Effect of staining using gadolinium and formaldehyde on fetal whole-body postmortem 3-T magnetic resonance imaging. Ultrasound Obstet Gynecol. 2019 May 27. Doi:10.1002/uog.20356 [Epub ahead of print]

33. Ashwin C, Hutchinson JC, Kang X et al. Learning effect on perinatal post-mortem magnetic resonance imaging reporting: single reporter diagnostic accuracy of 200 cases. Prenat Diagn. 2017;37(6):566-74.

34. Vullo A, Panebianco V, Cannavale G et al. Post-mortem magnetic resonance foetal imaging: a study of morphological correlation with conventional autopsy and histopathological findings. Radiol Med. 2016;121(11):847-56.

35. Hellkvist A, Wikstrom J, Mulic-Lutvica A et al. Postmortem magnetic resonance imaging vs autopsy of second trimester fetuses terminated due to anomalies. Acta Obstet Gynecol Scand. 2019 Ju; 98(7):865-876

36. Shruthi M, Gupta N, Jana M et al. Conventional vs virtual autopsy with postmortem MRI in phenotypic characterization of stillbirths and fetal malformations. Ultrasound Obstet Gynecol. 2018;51(2):236-45.

37. Ahmad MU, Sharif KA, Qayyum H et al. Assessing the use of magnetic resonance imaging virtopsy as an alternative to autopsy: a systematic review and meta-analysis. Postgrad Med J. 2017;93(1105):671-8.

38. Shelmerdine SC, Arthurs OJ, Gilpin I et al. Is traditional perinatal autopsy needed after detailed fetal ultrasound and post-mortem MRI? Prenat Diagn. 2019 Mar 20. Doi:10.1002/pd.5448. [Epub ahead of print]

39. Izzo G, Talenti G, Falanga G et al. Intrauterine fetal MR versus postmortem MR imaging after therapeutic termination of pregnancy: evaluation of the concordance in the detection of brain abnormalities at early gestational stage. Eur Radiol. 2019 Jun; 29(6):2740-2750

40. Orasanu E, Melbourne A, Cardoso MJ et al. Brain volume estimation from post-mortem newborn and fetal MRI. NeuroImage Clinical. 2014;6:438-44.

41. Thayyil S, Schievano S, Robertson NJ et al. A semi-automated method for non-invasive internal organ weight estimation by post-mortem magnetic resonance imaging in fetuses, newborns and children. Eur J Radiol. 2009;72(2):321-6.

42. Breeze AC, Gallagher FA, Lomas DJ et al. Postmortem fetal organ volumetry using magnetic resonance imaging and comparison to organ weights at conventional autopsy. Ultrasound Obstet Gynecol. 2008;31(2):187-93.

43. Shelmerdine SC, Chung KL, Hutchinson JC et al. Feasibility of Postmortem Imaging Assessment of Brain: Liver Volume Ratios with Pathological Validation. Fetal Diagn Ther. 2019:1-8.

44. McDowell AR, Shelmerdine SC, Carmichael DW, Arthurs OJ. High resolution isotropic diffusion imaging in post-mortem neonates: a feasibility study. Br J Radiol. 2018;91(1092):20180319.

45. Arthurs OJ, Guy A, Kiho L, Sebire NJ. Ventilated postmortem computed tomography in children: feasibility and initial experience. Int J Legal Med. 2015;129(5):1113-20.

46. Sarda-Quarello L, Bartoli C, Laurent PE et al. Whole body perinatal postmortem CT angiography. Diagnostic and interventional imaging. 2016;97(1):121-4.

47. Shelmerdine SC, Davendralingam N, Palm L et al. Diagnostic Accuracy of Postmortem CT of Children: A Retrospective Single-Center Study. AJR Am J Roentgenol. 2019 Mar 27:1-13.

48. Prodhomme O, Baud C, Saguintaah M et al. Principles of fetal postmortem ultrasound: A personal review. J Forensic Radiology and Imaging. 2015;3(1):12-5.

49. Shelmerdine SC, Sebire NJ, Arthurs OJ. Perinatal post mortem ultrasound (PMUS): a practical approach. Insights into imaging. 2019 Mar 18;10(1):35.

50. Kang X, Shelmerdine SC, Hurtado I et al. Postmortem examination of human fetuses: a comparison of 2-dimensional ultrasound with invasive autopsy. Ultrasound Obstet Gynecol. 2019 Feb; 53(2):229-238

51. Votino C, Cos Sanchez T, Bessieres B et al. Minimally invasive fetal autopsy using ultrasound: a feasibility study. Ultrasound in Obstetrics and Gynecology. 2018;52:776-83.

52. Tuchtan L, Lesieur E, Bartoli C et al. Diagnosis of congenital abnormalities with post-mortem ultrasound in perinatal death. Diagn Interv Imaging 2018;99(3):143-9.

53. Prodhomme O, Baud C, Saguintaah M et al. Comparison of postmortem ultrasound and X-Ray with autopsy in fetal death: Retrospective study of 169 cases. J Forensic Radiology and Imaging. 2015;3(2):120-30.

54. Kang X, Cos Sanchez T, Arthurs OJ et al. Postmortem fetal imaging: a prospective blinded comparison study of 2-dimensional ultrasound with MR imaging. Ultrasound Obstet Gynecol. 2019. Doi: 10.1002/uog.20217 [Epub ahead of print]

55. Shelmerdine SC, Hickson M, Sebire NJ, Arthurs OJ. Post-Mortem Magnetic Resonance Imaging Appearances of Feticide in Perinatal Deaths. Fetal Diagn Ther. 2019; 45(4):221-229

56. Jawad N, Sebire NJ, Wade A et al. Body weight lower limits of fetal postmortem MRI at 1.5 T. Ultrasound Obstet Gynecol. 2016;48(1):92-7.

57. Kang X, Cannie MM, Arthurs OJ et al. Post-mortem whole-body magnetic resonance imaging of human fetuses: a comparison of 3-T vs. 1.5-T MR imaging with classical autopsy. Eur Radiol. 2017;27(8):3542-53.

58. Staicu A, Albu C, Popa-Stanila R et al. Potential clinical benefits and limitations of fetal virtopsy using high-field MRI at 7 Tesla versus stereomicroscopic autopsy to assess first trimester fetuses. Prenat Diagn. 2019 Jun; 39(7):505-518

59. Thayyil S, Cleary JO, Sebire NJ et al. Post-mortem examination of human fetuses: a comparison of whole-body high-field MRI at 9·4 T with conventional MRI and invasive autopsy. The Lancet. 2009; 374(9688):467-75.

60. Hutchinson JC, Kang X, Shelmerdine SC et al. Postmortem microfocus computed tomography for early gestation fetuses: a validation study against conventional autopsy. Br J Radiol. 2018;218(4):445.e1-.e12.

61. Shelmerdine SC, Hutchinson JC, Kang X et al. Novel usage of micro-focus computed tomography (Micro-CT) for visualisation of human embryonic development - implications for future non-invasive post-mortem investigation. Prenat Diagn. 2018 Jun; 38(7):538-542.

62. Shelmerdine SC, Singh M, Simcock IC et al. Characterization of Bardet-Biedl syndrome by postmortem microfocus computed tomography (micro-CT). Ultrasound Obstet Gynecol. 2019;53(1):132-4.

63. Lombardi S, Scola E, Ippolito D et al. Micro-computed tomography: a new diagnostic tool in postmortem assessment of brain anatomy in small fetuses. Neuroradiology. 2019 Jul; 61(7):737-746

64. Hutchinson JC, Arthurs OJ, Ashworth MT et al. Clinical utility of postmortem microcomputed tomography of the fetal heart: diagnostic imaging vs macroscopic dissection. Ultrasound Obstet Gynecol. 2016;47(1):58-64.

65. Lombardi CM, Zambelli V, Botta G et al. Postmortem microcomputed tomography (micro-CT) of small fetuses and hearts. Ultrasound Obstet Gynecol. 2014;44(5):600-9.

66. Hutchinson JC, Barrett H, Ramsey AT et al. Virtual pathological examination of the human fetal kidney using micro-CT. Ultrasound Obstet Gynecol. 2016;48(5):663-5.

67. Hutchinson JC, Ashworth MT, Sebire NJ, Arthurs OJ. Multiple Cardiac Rhabdomyomas Visualised Using Micro-CT in a Case of Tuberous Sclerosis. Fetal Diagn Ther. 2017;41(2):157-60.

68. Montaldo P, Addison S, Oliveira V et al. Quantification of maceration changes using post mortem MRI in fetuses. BMC Med Imaging. 2016;16:34.

69. Barber JL, Hutchinson JC, Sebire NJ, Arthurs OJ. Pleural fluid accumulation detectable on paediatric post-mortem imaging: a possible marker of interval since death? Int J Legal Med. 2016;130(4):1003-10.

70. Shelmerdine SC, Main C, Hutchinson JC et al. The use of whole body diffusion-weighted post-mortem magnetic resonance imaging in timing of perinatal deaths. Int J Legal Med. 2018;132(6):1735-41.

71. Arthurs OJ, Price GC, Carmichael DW et al. Diffusion-weighted perinatal postmortem magnetic resonance imaging as a marker of postmortem interval. Eur Radiol. 2015;25(5):1399-406.

72. Iwamoto C, Ohuchida K, Okumura M et al. Postmortem interval estimation using the animal model of postmortem gas volume changes. Legal Med (Tokyo, Japan). 2018;32:66-70.

73. Maile AE, Inoue CG, Barksdale LE, Carter DO. Toward a universal equation to estimate postmortem interval. Forensic Sci Int. 2017;272:150-3.

74. Okumura M, Usumoto Y, Tsuji A et al. Analysis of postmortem changes in internal organs and gases using computed tomography data. Legal Med (Tokyo, Japan). 2017;25:11-5.

75. Lewis C, Latif Z, Hill M et al. "We might get a lot more families who will agree": Muslim and Jewish perspectives on less invasive perinatal and paediatric autopsy. PLoS One. 2018 Aug;13(8):e0202023.

76. Ben Taher M, Pearson J, Cohen M, Offiah AC. Acceptability of post-mortem imaging among Muslim and non-Muslim communities. Br J Radiol. 2018;91(1091):20180295.

77. The Society for Pediatric Radiology (SPR), Post-Mortem Imaging Committee. https://www.pedrad.org/Specialties/Post-Mortem-Imaging#46413095-roster. Accessed July 15, 2019

78. de Bakker BS, de Jong KH, Hagoort J et al. An interactive three-dimensional digital atlas and quantitative database of human development. Science. 2016 Nov 25;354(6315).

79. Yamada S, Uwabe C, Nakatsu-Komatsu T et al. Graphic and movie illustrations of human prenatal development and their application to embryological education based on the human embryo specimens in the Kyoto collection. Dev Dyn. 2006 Feb;235(2):468-77.

80. Smith CM, Shelmerdine S, Arthurs OJ et al. HoloLens for medical imaging using post-mortem fetal micro-CT data. European Congress of Radiology; 2019; Vienna, Austria. Doi: 10.26044/ecr2019/C-0153

81. Shelmerdine SC, Simcock IC, Hutchinson JC et al. 3D printing from microfocus computed tomography (micro-CT) in human specimens: education and future implications. Br J Radiol. 2018 Jul; 91(1088):20180306.

82. Biglino G, Layton S, Lee M et al. 'Making the Invisible Visible': an audience response to an art installation representing the complexity of congenital heart disease and heart transplantation. Med Humanit. 2018 Oct 18. Doi:10.1136/medhum-2018-011466.

Table 1. Various terminologies used in the discussion of post-mortem imaging and autopsy

Terminologies used	**Type of Autopsy**
Standard autopsy; Conventional autopsy; Invasive autopsy	Terms that include the 'traditional' method of performing large midline or 'Y' shaped incisions across a body, with the removal and inspection of organs and acquisition of tissue for histology.
Less invasive autopsy (LIA); Virtopsy;	An umbrella term to describe an autopsy that primarily uses cross-sectional post-mortem imaging techniques, which may include organ tissue sampling, but without large incisions made to the body.
Non-invasive autopsy (NIA) Imaging only autopsy	An autopsy utilising only post-mortem cross sectional imaging with ancillary testing. No incisions or tissue sampling is obtained from the body.
Minimally invasive autopsy (MIA)	An autopsy utilising both post-mortem cross sectional imaging with organ tissue sampling by either needle biopsies or laparoscopic guided biopsies. Small incisions may be made to the body to allow needle access.
MINIMAL procedure (Minimally Invasive Autopsy with Laparoscopic assisted sampling)	A type of MIA, using laparoscopic assisted methods to visualise internal organs and acquire organ tissue sampling. A single small incision (approximately 1cm) is made in the left upper quadrant of the abdomen or epigastric region to allow insertion of the laparoscope.
INTACT procedure (INcision-less TArgeted Core Tissue)	A type of MIA, involving ultrasound guided organ biopsies of fetuses via the umbilicus so that no incisions to the body are made.

Table 2. Sequence parameters for full post-mortem MRI protocol in perinatal deaths (adapted with permission from Norman W et al[30]). Two sequences below followed by '*' denote an abbreviated PMMR protocol we locally follow to reduce scanning time, the only difference being that coverage for both includes the head to pelvis (not neck to pelvis as stated below for full protocol).

Sequence	FOV (mm)	Slice thickness (mm)	Matrix	Voxel size (mm)	TR (ms)	TE (ms)	Averages (NEX/NSA)	Number slices and gap	Length of sequence (min)
BRAIN IMAGING									
3D FLASH T1-w (sag)									
	256	1	256/256	1.0 x 1.0 x 1.0	11	4.9	3	60 per slab	5.44
2D DESTIR T2-w (axial and coronal)									
	100	2	172/256	0.4 x 0.4 x 2.0	5460	16 and 115	6	18 (1mm)	13.46
2D GRE T1 HEME (axial)									
	100	4	120/256	0.5 x 0.4 x 4.0	800	26	4	18 (0mm)	6.26
DWI (b-values 0, 500, 1000)									
	230	5	128/128	1.8 x 1.8 x 5.0	2700	96	3	19 (0mm)	1.06
SPINE IMAGING									
2D T2-w TSE (sag)									
	150	1.5	128/256	0.6 x 0.6 x 1.5	9.1	4.5	8	12 per slab	4.24
3D FLASH T1-w (sag)									
	150	1.25	128/256	0.6 x 0.6 x 1.3	11	5.3	10	16 per slab	3.19
BODY IMAGING (NECK TO PELVIS)									
3D T2-w TSE (cor)*									
	200	0.8	160/256	0.8 x 0.8 x 0.8	3500	275	2	72 per slab	6.20
3D T1-w VIBE (cor)*									
	200	0.8	160/256	0.8 x 0.8 x 0.8	5.9	2.4	8	72 per slab	5.52
3D CISS T2-w (axial) (thoracic coverage for cardiac assessment)									
	150	0.6	192/256	0.6 x 0.6 x 0.6	5.6	2.5	10	Cover heart and lungs	29.26
2D T2-w tirm (axial) (Ti = 150) (to cover body and pelvis, not head)									
	180	5	160/256	0.7 x 0.7 x 5.0	5080	109	5	Cover body and pelvis	6.58
DWI	As for head with greater number of slices to cover chest, abdomen and pelvis								1.06

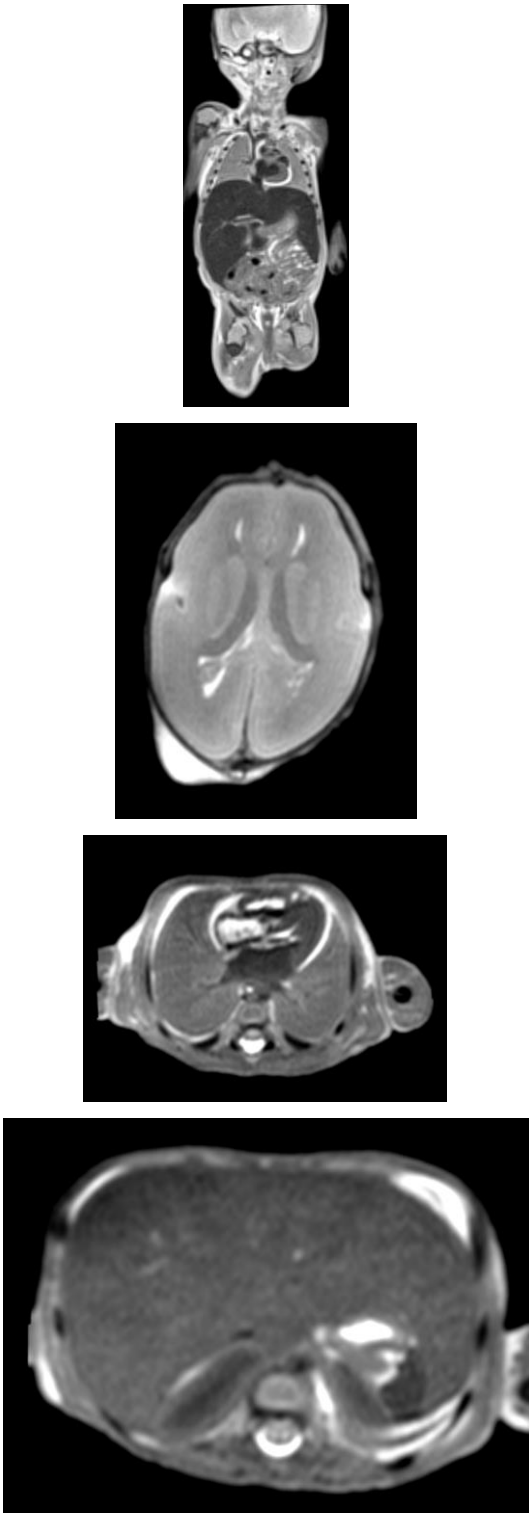
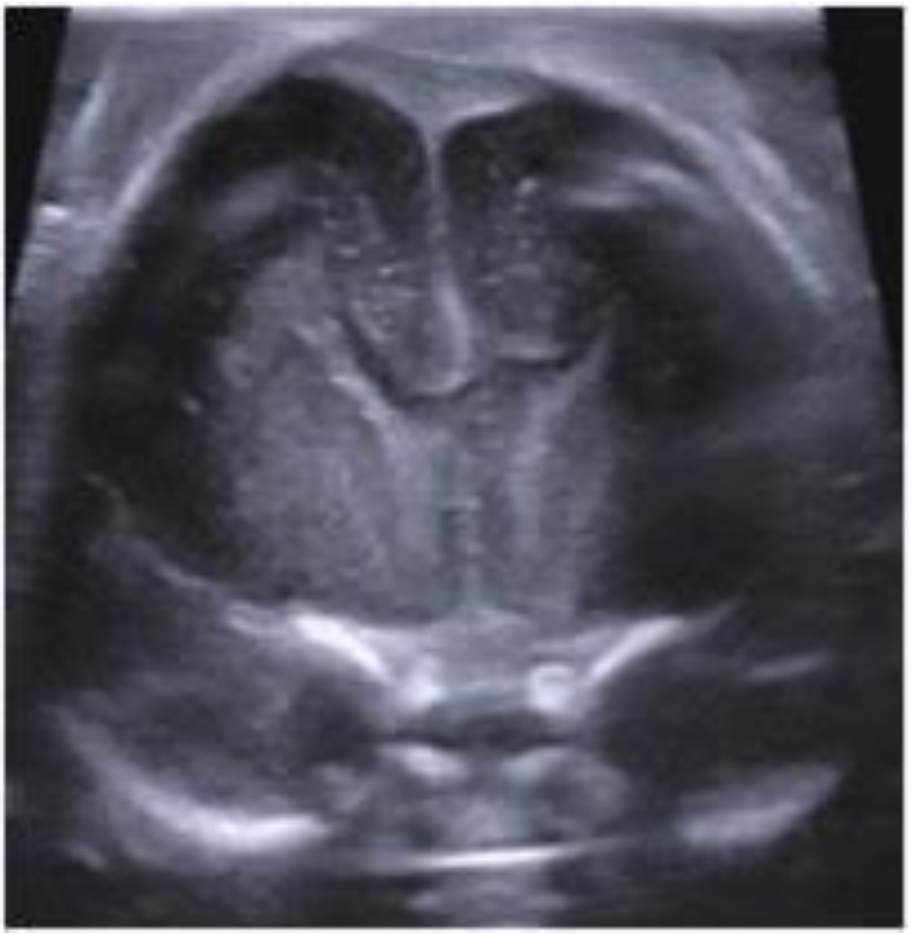
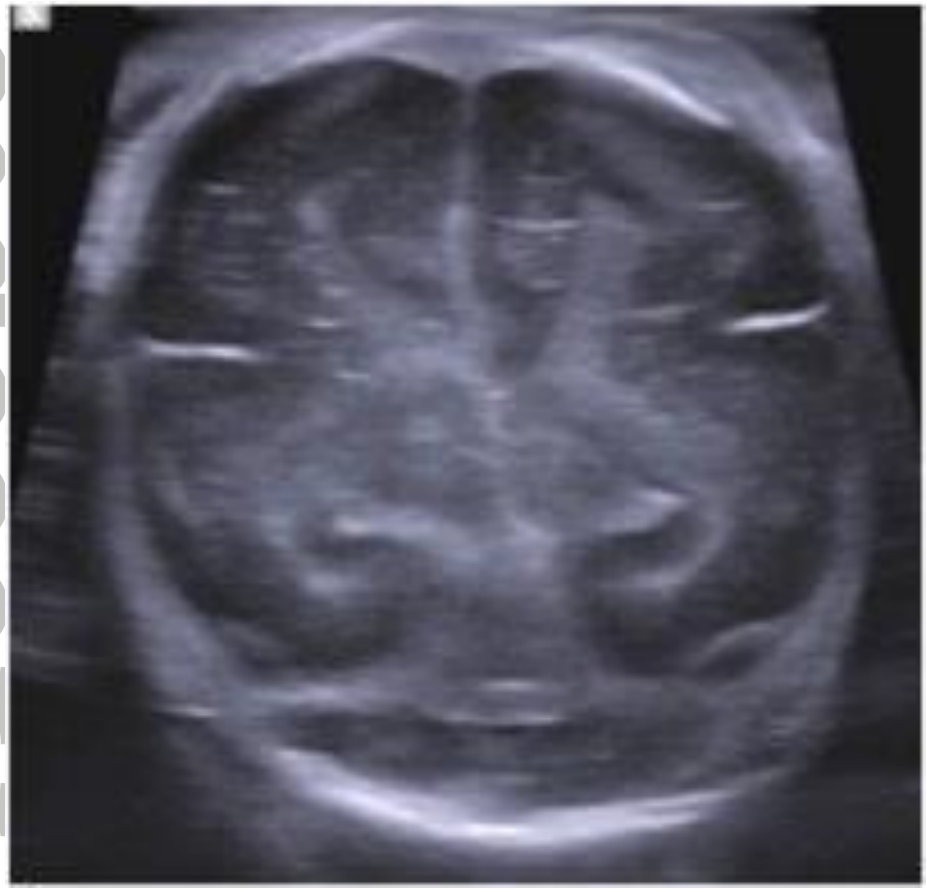
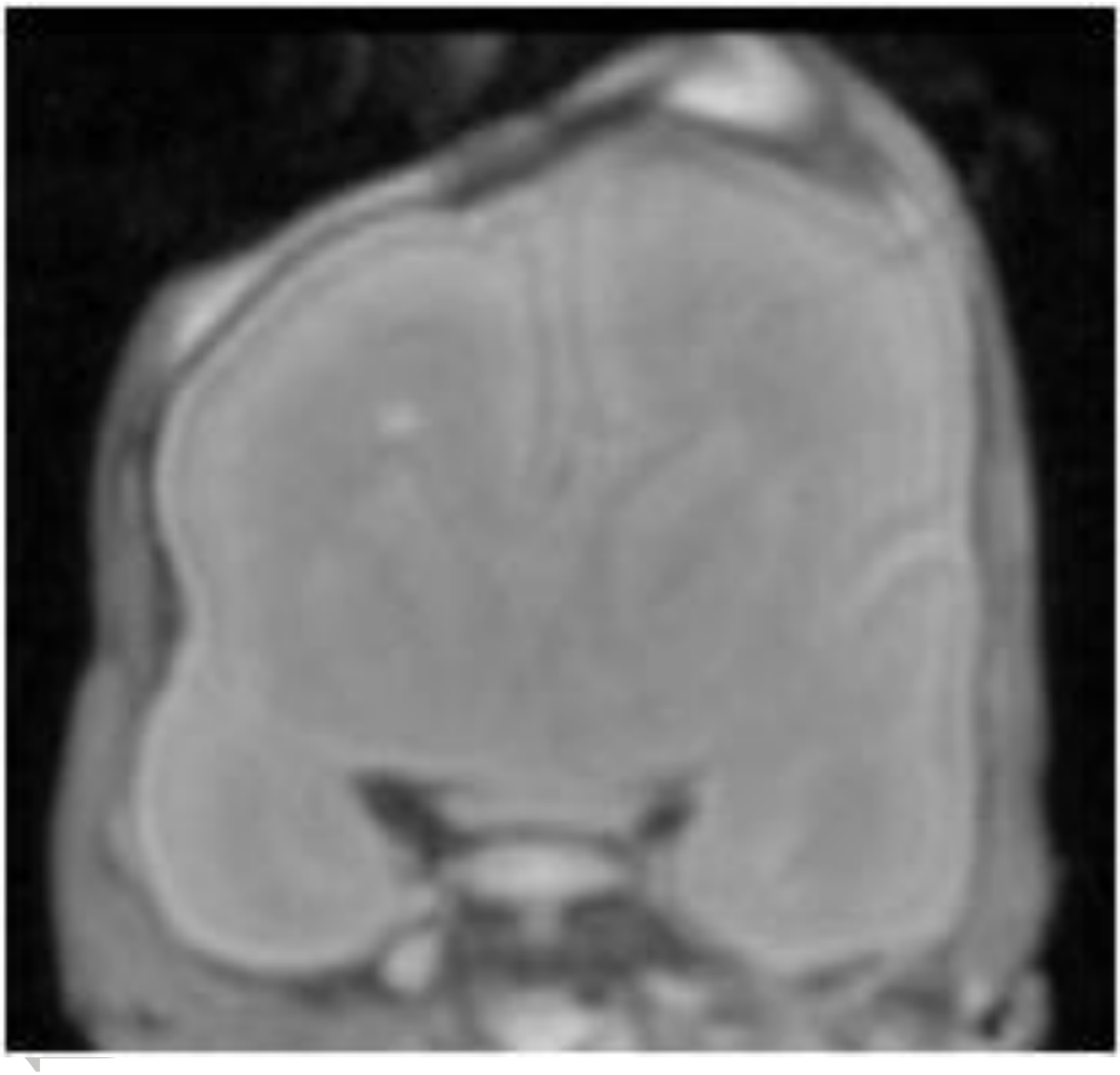


Figure 1

A, Coronal post-mortem 1.5T MRI (PMMR) image acquired using a T2-weighted sequence, of a 21 week gestational age fetus demonstrating normal anatomy of the heart, lungs and abdomen. **B,** Axial T2 weighted PMMR of the same fetus through the lateral ventricles, **C,** through the heart and **D,** through the liver all also show normal anatomy. These images serve to demonstrate how adequate image quality can be achieved, allowing for identification of structural anomalies.







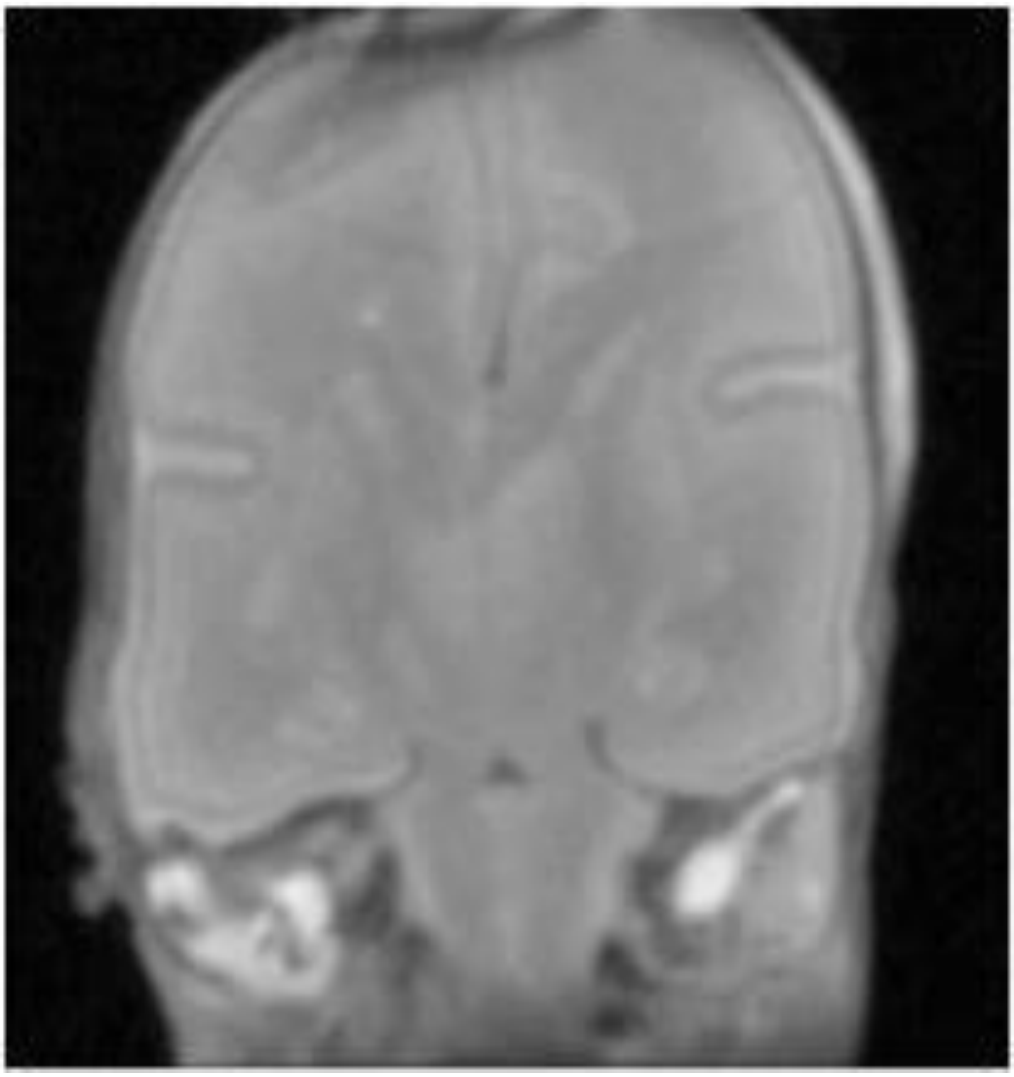
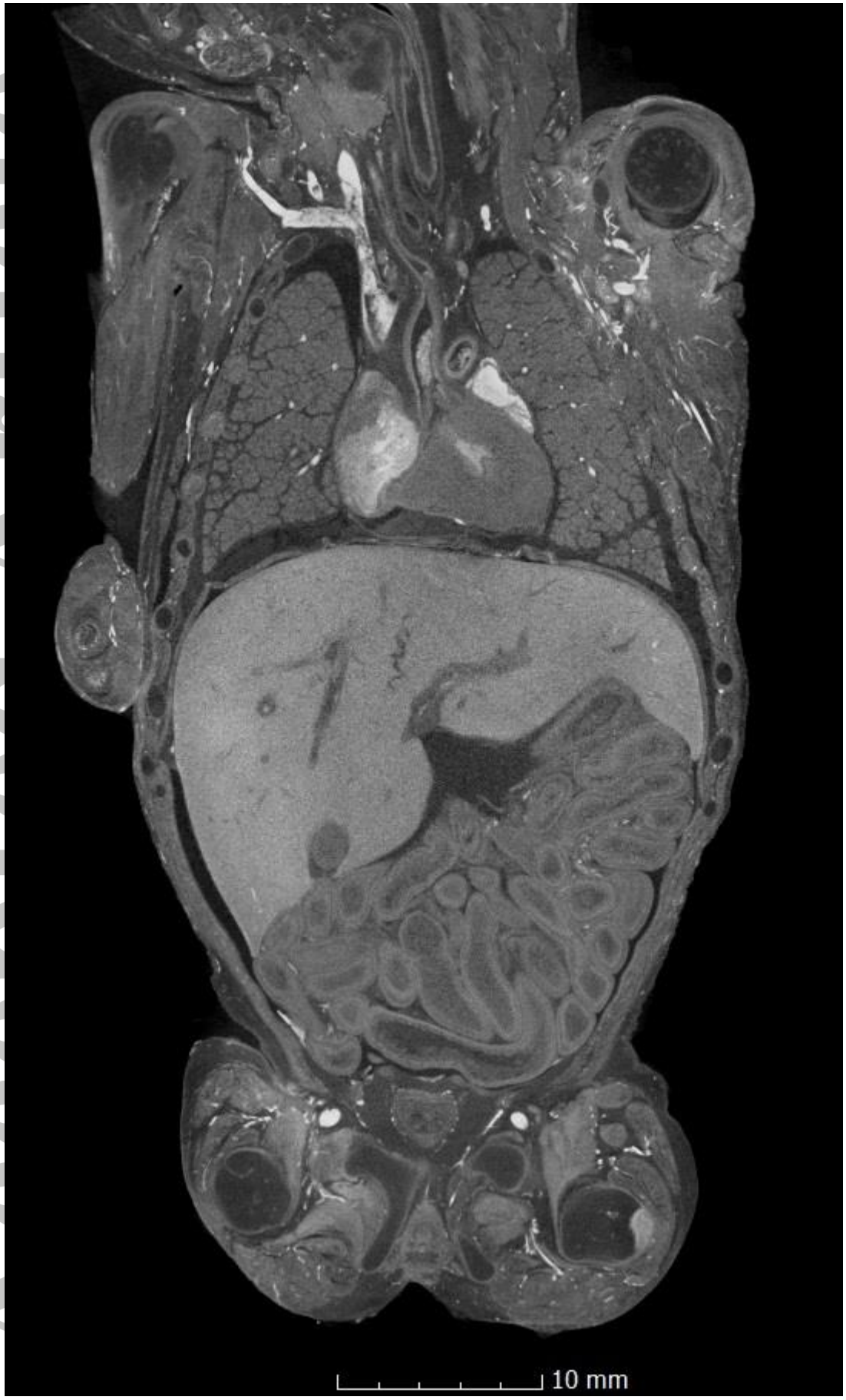
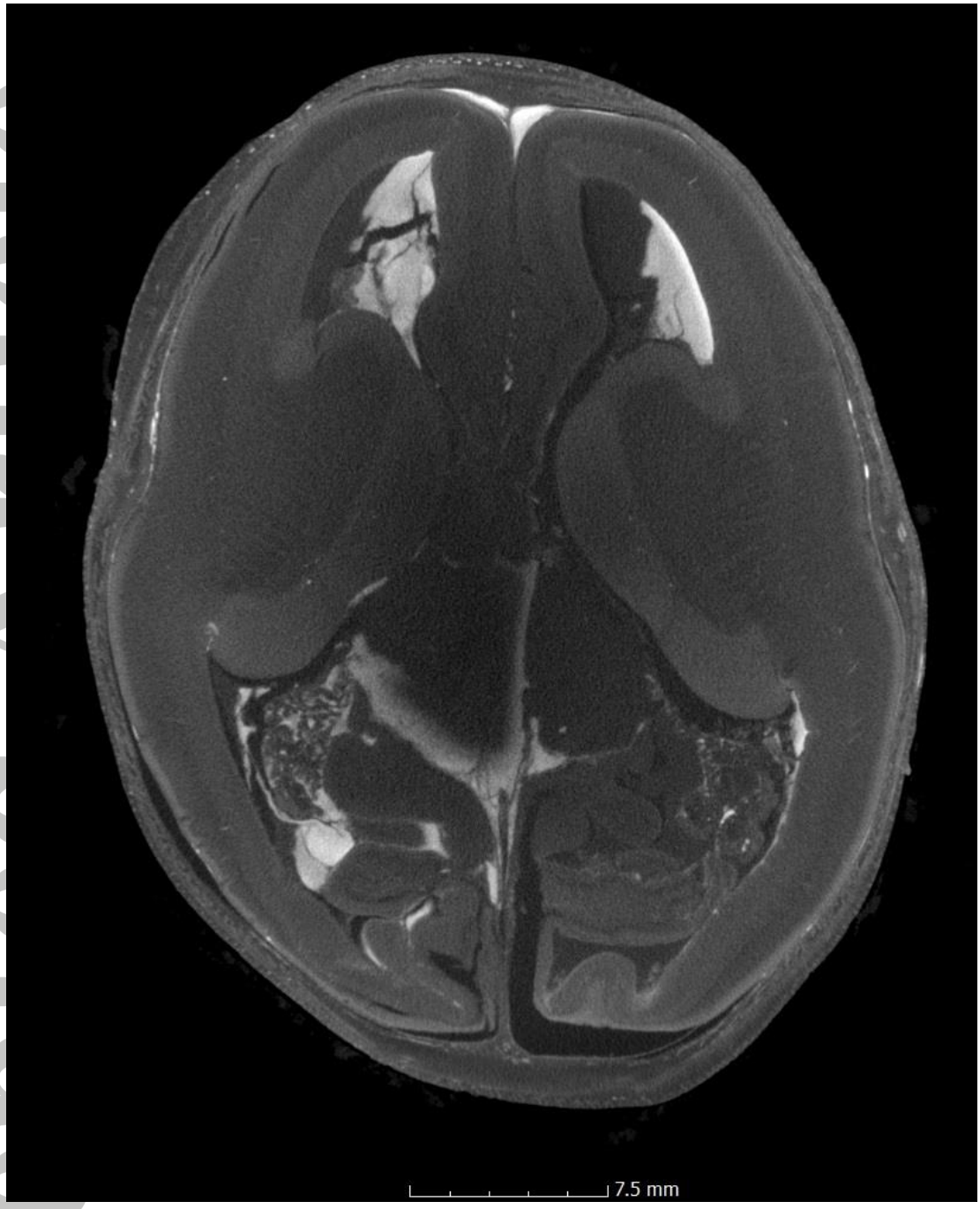
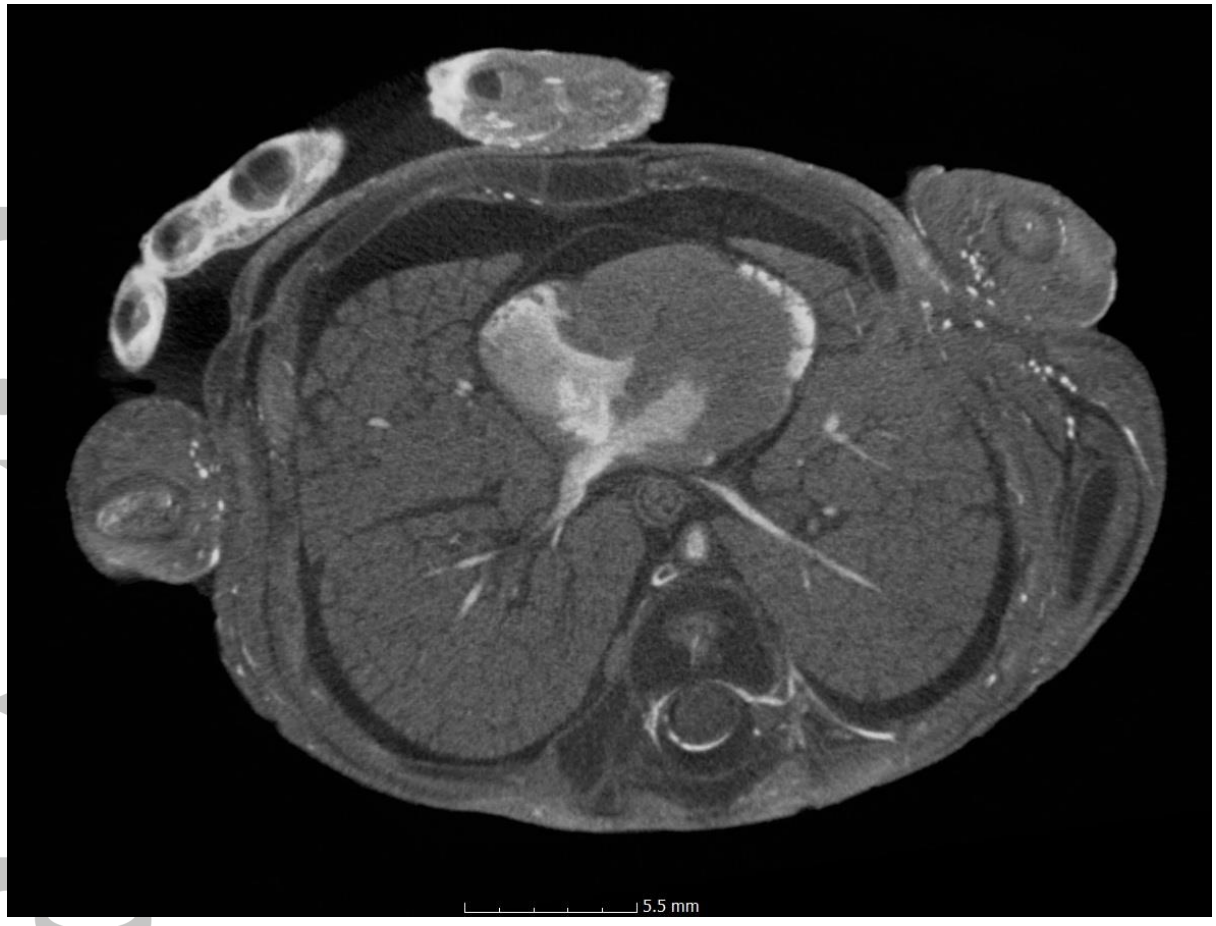


Figure 2

A, Coronal postmortem ultrasound image through the frontal horns (using a linear 7-16Mhz probe) and **B,** through the posterior horns of the lateral ventricles in a normal 20 week gestational aged fetus. The corresponding post-mortem 1.5T MRI brain imaging in **C** and **D** demonstrate how key features of brain sulcation and ventricular appearances can already be well seen on the ultrasound and where diagnostic, further cross-sectional imaging is not always necessary. *(Reproduced with permission from Shelmerdine SC et al, Insights Imaging 2019[49]).*







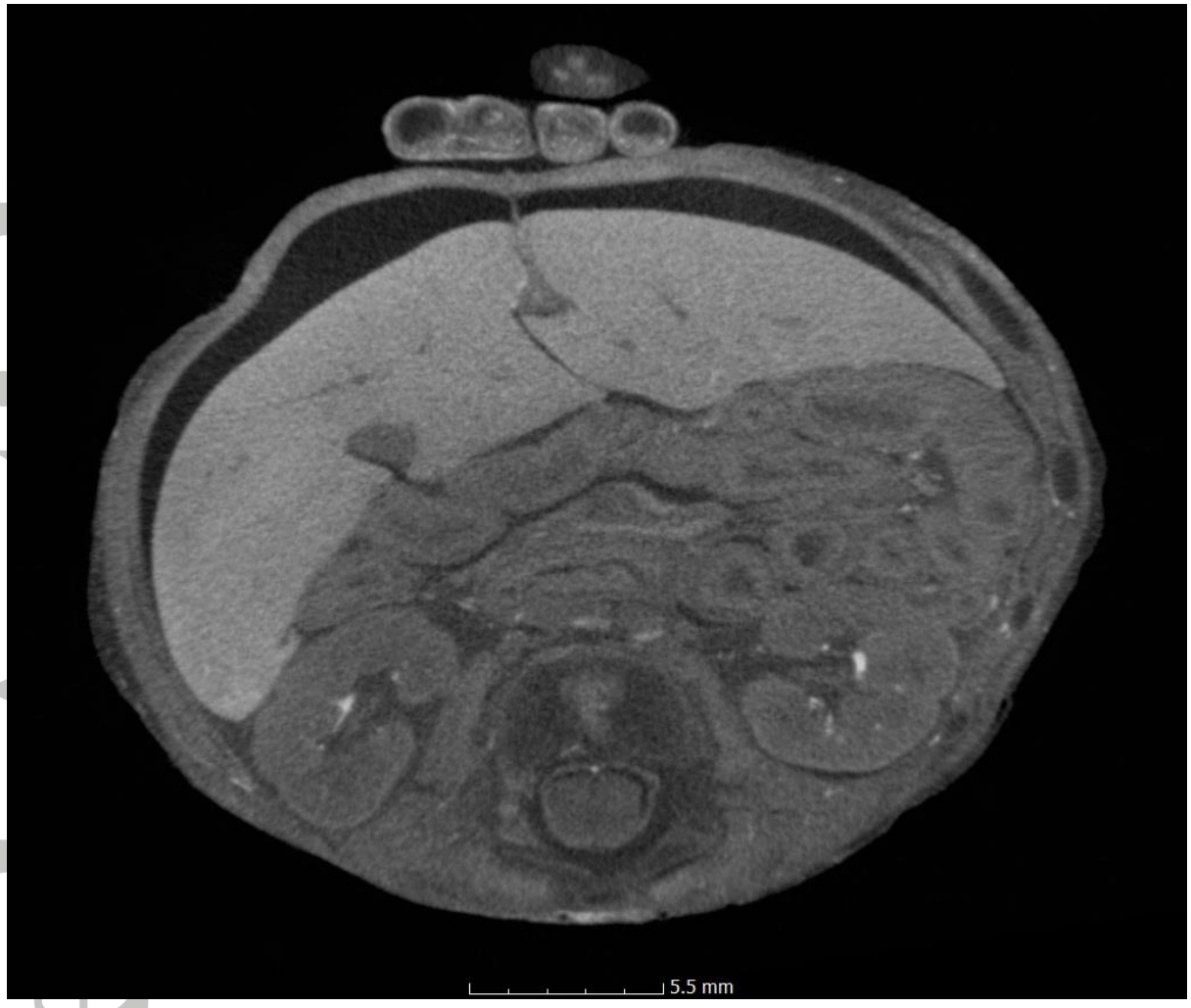
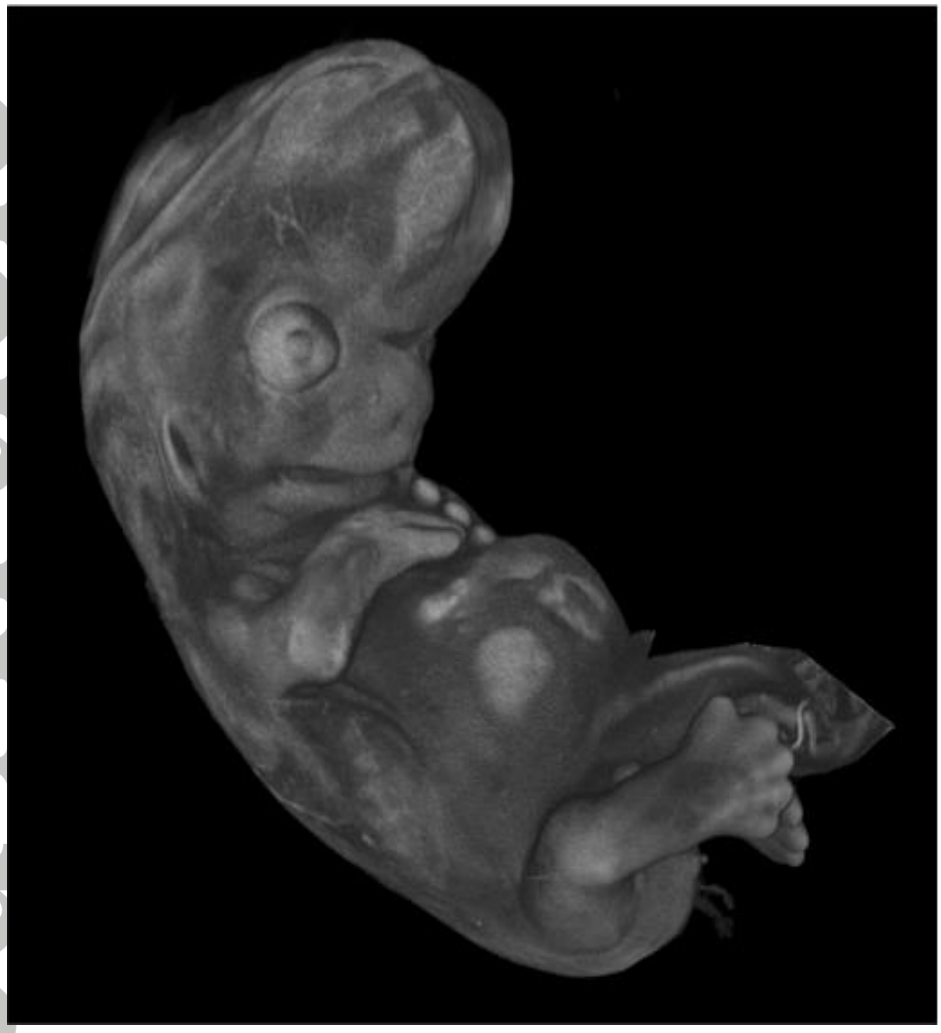
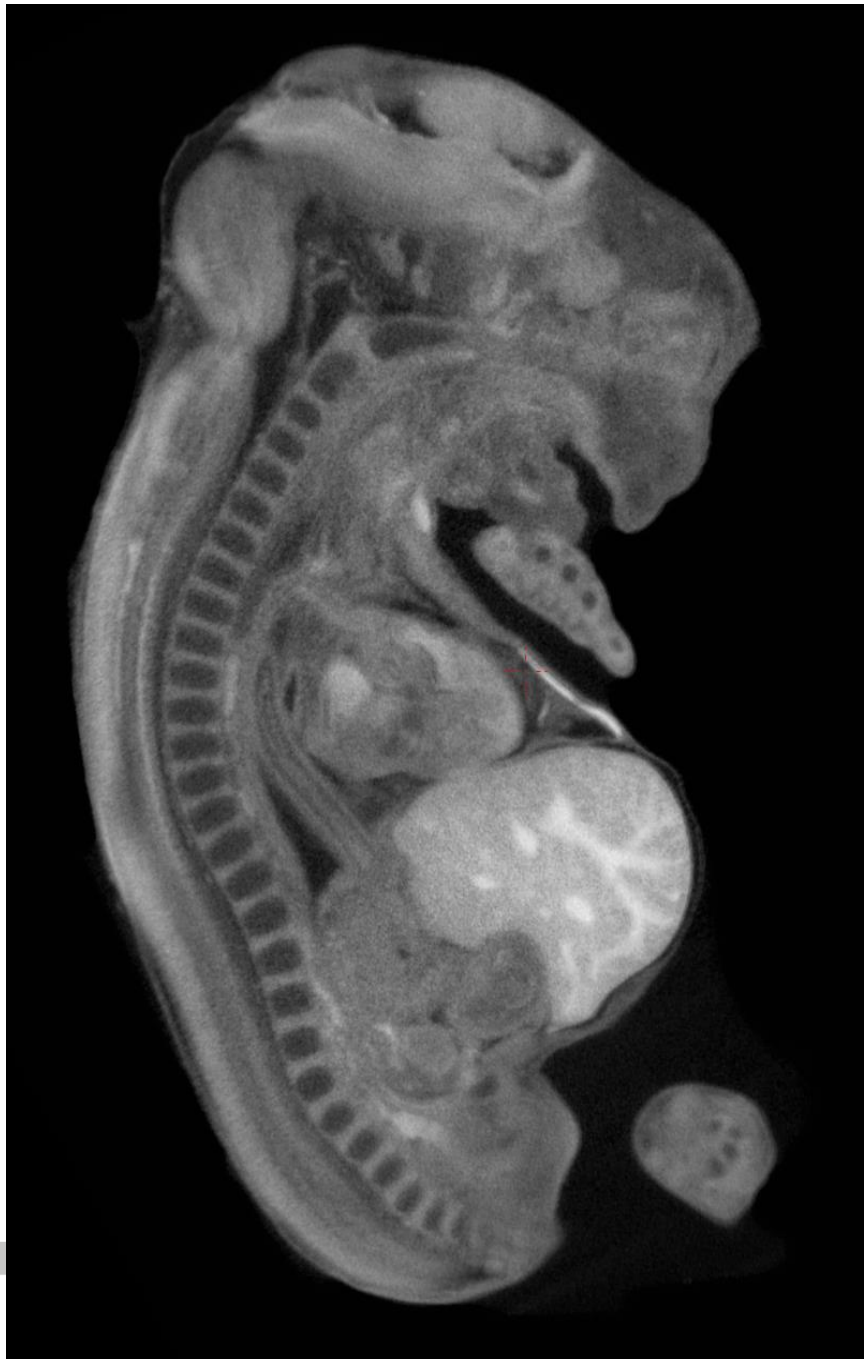


Figure 3

A, Coronal post-mortem iodinated micro-CT image of a normal 15 week gestational aged fetus, acquired at a resolution of 60um. **B,** Axial micro-CT images through the lateral ventricles of the brain, **C** the heart and **D,** the liver also show normal anatomy. The detail that can be obtained in such small fetuses allows for a thorough review of the internal structures, in some cases highlighting abnormalities that would not otherwise be visualised on other imaging modalities and which would also be challenging to identify at autopsy.





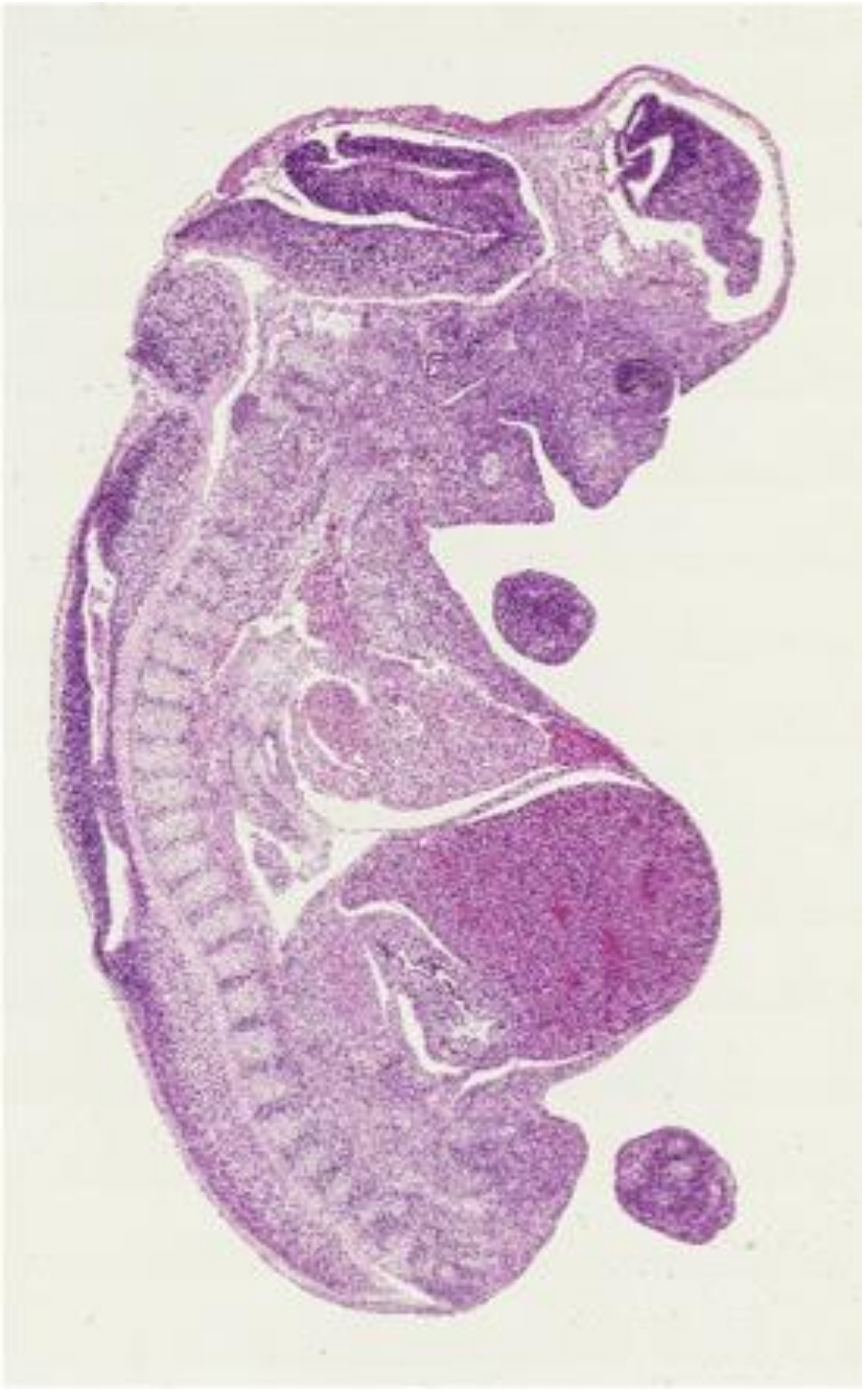


Figure 4

A, Volume rendered 3-dimensional image reconstructed from a micro-CT image of a 7-week gestation embryo. The image demonstrates early eyelid and external ear development, appearance of individual digits at the hand with toe notches at the feet. **B,** Sagittal post-mortem micro-CT appearances of the same embryo imaged at a resolution of 9.7 µm, with **C,** corresponding sagittal histopathology section after hematoxylin and eosin staining. The similarity of internal detail obtained by micro-CT imaging versus histopathology is clearly visible. *(Reproduced with permission from Shelmerdine SC et al, Prenatal Diagnosis 2018[61])*

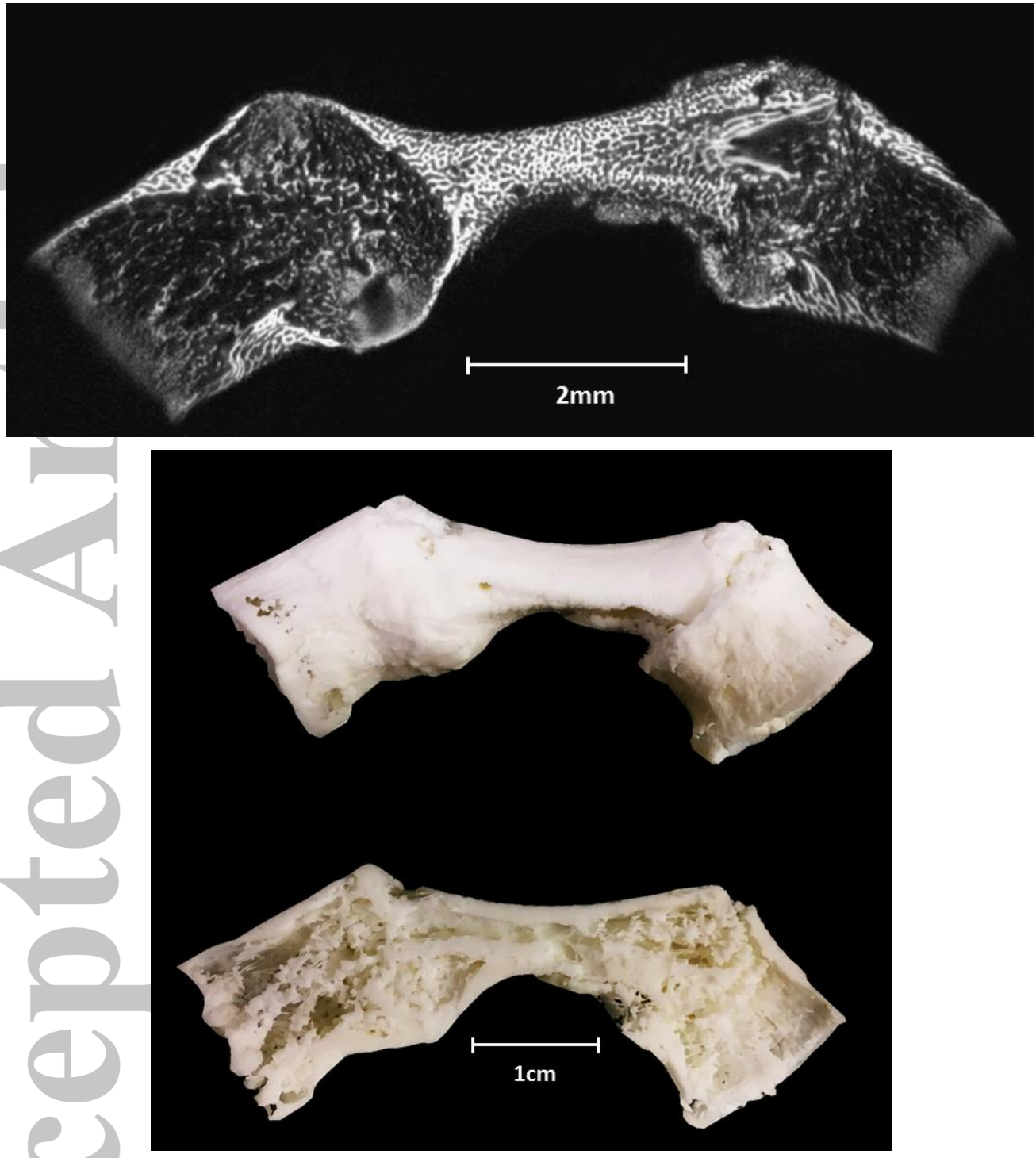


Figure 5

A, Micro-CT imaging of a femur in a 19 week gestation fetus with osteogenesis imperfect type 2a at a resolution of 18um. The image demonstrates a fracture with callus formation at both ends of the bone. **B,** A 3D printed model of the whole femur (top) and longitudinally sectioned femur (bottom) was reproduced, four times life size. A model such as this one could be helpful in counselling and explaining to parents the underlying pathology detected in their child. *(Reproduced with permission from Shelmerdine SC et al, Br J Radiol 2018[81])*