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SPECIAL ARTICLE

Virtual Fetal Autopsy

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ABSTRACT

Determining the cause of stillbirth is crucial for understanding preventable factors and managing future pregnancies. Currently, many parents decline conventional autopsy due to its invasive nature. To address this, less invasive autopsy methods based on imaging technology have been introduced as more accessible and acceptable options for parents. In addition to radiographs, other traditional clinical imaging techniques such as magnetic resonance imaging (MRI), ultrasound, and computed tomography have been used in postmortem investigations, especially for fetuses over 20 weeks of gestation. Advanced techniques like high-field MRI and micro-focus computed tomography have demonstrated higher diagnostic accuracy, though they remain limited by accessibility. This article aims to provide a perspective on "virtual fetal autopsy" from a pathologist's point of view to enhance obstetricians' understanding.

Keywords: fetal autopsy, virtual autopsy, postmortem, ultrasound, MRI, CT scan, micro-CT, radiographs.

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Stillbirth is a serious pregnancy complication and can be very traumatizing for any family. For obstetricians, identifying the cause of stillbirth is crucial to understanding preventable factors and managing future pregnancies. Typically, the evaluation of stillbirth includes a clinical history, perinatal autopsy, placental examination, and genetic testing[1]. Conventional autopsy is considered the gold standard because it can reveal more information through histological findings that cannot be seen with structural examination alone. However, some families find conventional autopsy invasive because they worry about body disfigurement and the removal of tissue needed for further analysis, which can reduce consent rates. In cases of stillbirth with severe maceration, a thorough evaluation via conventional autopsy is often not possible, especially for the fetal brain, which is delicate and tends to break during skull opening. The

very small fetus also poses problems for conventional autopsy, even when performed under direct microscopy. Additionally, the accuracy of findings from a conventional autopsy depends heavily on the skill of the pathologist examining it, which can typically be done only once and is often limited to documentation via photographs or videos. Conversely, imaging data from other methods can be stored and reviewed multiple times as needed, including for consultation with experts. It can also be viewed from different angles and used to create 3-dimensional structures for further analysis.

Noninvasive perinatal virtual autopsy using imaging techniques was introduced to address the limitations of conventional autopsy. Since the primary purpose of a traditional autopsy is to visually examine organs, any imaging method that provides a clear view of internal organs can serve as an alternative procedure. Postmortem imaging options include magnetic resonance imaging (MRI), ultrasound (US), computed tomography (CT), microfocus computed tomography (micro-CT), and X-ray examination[2-5].

Postmortem magnetic resonance imaging (MRI)

Postmortem MRI has been used since 1990[6] and is very popular in developed countries because it provides the clearest images compared to other imaging techniques[2]. The procedure should be performed as soon as possible for the best image quality[7]. The magnets commonly used in routine virtual autopsy are 1.5-Tesla (T) and 3-T, but the error rate is lower with 3-T magnets, especially in very small fetuses. Ultra-high magnetic field MRI with a strength of 7-T and higher has also been studied to produce detailed images of fetal anatomy, but it is mainly used for research due to instrument limitations and long scan times[8-11].

The cutoff for performing postmortem MRI varies depending on the type of MRI and techniques used. In earlier studies of 1.5-T MRI, a cutoff of 500 grams or 20 weeks of gestational age was suggested. Today, with advanced techniques and increased availability of 3-T MRI, it can be performed on early second-trimester fetuses with a weight of 300 grams. For very small fetuses, the image signal can be enhanced by placing the fetus in a saline bag or a 60 ml syringe filled with saline[12].

There are various protocols for the examination. If an MRI cannot be performed immediately after delivery, the fetus should be stored in a refrigerator at 4°C until the procedure, for up to 6 days. The fetus should be placed supine in an anatomically neutral position. The coil used must be adapted to the body size and as small as possible. Typically, a head coil is used for the brain and spine, while a body coil is used for overall body imaging[7]. T2 sequences are generally preferred because they provide better tissue contrast than T1 sequences. However, T1 and T2 signals in a deceased fetus may change due to cell death, maceration, decreased temperature, and preservation methods, which can lead to misinterpretation as pathological conditions[13]. T1 images generally have poor contrast and low signal, although high T1 signals are observed in the thyroid and bowel, related to meconium content[14].

Interpreting postmortem MRI is also difficult and can be misinterpreted due to autolytic changes. After fetal death, fluid buildup may occur, causing subcutaneous edema, pleural or pericardial effusion, and ascites. For heart imaging, it is common to see small pericardial effusions, intracardiac air, blood clots, and fluid–fluid levels in the heart and major vessels. The cardiac ventricles may appear thickened after death, which can be mistaken for ventricular hypertrophy[15]. Increased gas in the hepatobiliary system, distended bowel loops, and enlarged appearance of the normal fetal liver may be normal postmortem changes[16]. Skull deformities; brain ischemia indicated by edema, loss of gray-white matter differentiation, and low T2 signal in the basal ganglia; tonsillar descent; and small intraventricular hemorrhages without dilation can also be seen as postmortem changes[17].

The overall diagnostic accuracy of postmortem MRI ranges from 77% to 94%, depending on factors

such as protocol, gestational age, birth weight, organ system abnormalities, the reason for fetal death (if known), circumstances of death (pharmaceutical termination of pregnancy with or without feticide, spontaneous death, live birth, or stillbirth), organ system malformations, and the interval between death and MRI[15, 18-20]. When combined with ancillary investigations, such as placental examination and postmortem blood sampling, postmortem MRI can identify the cause of fetal death or major pathological abnormalities as effectively as conventional autopsy, with an accuracy of up to 89%[21]. The concordance rate improves with higher gestational age, especially in fetuses over 24 weeks' of gestation, reaching 95.7%. In the case of organ systems, postmortem MRI shows high diagnostic accuracy in the neurological, cardiovascular, pulmonary, and renal systems. Cerebral postmortem MRI has 87.5% sensitivity and 74.1% specificity for detecting overall brain pathology, and 88.4% sensitivity with 95.2% specificity for cerebral malformations. The detection of major intracranial bleeding achieved 100% sensitivity and 99.1% specificity[22]. The overall sensitivity of 3-T postmortem MRI for diagnosing fetal congenital heart disease is 78.2%, with a specificity of 85.4%[19]. The accuracy is notably lower in fetuses under 20 weeks and those with a birth weight below 100 grams. For non-cardiac thoracic abnormalities, postmortem MRI is highly sensitive in detecting pleural effusions (100% sensitivity) and lung or thoracic hypoplasia (60% sensitivity), but less effective for pulmonary infections and hemorrhages (12.5% and 33.3% sensitivity, respectively)[15]. Regarding abdominal abnormalities, postmortem MRI demonstrates 72.5% sensitivity and 90.8% specificity for overall abdominal pathology[18]. It performs well in identifying renal abnormalities (80% sensitivity and 98.6% specificity) and splenic abnormalities (100% sensitivity and 99.6% specificity), though its accuracy remains low (sensitivity of 50-55%) for the intestinal, liver, and adrenal gland.

Besides several advantages of postmortem MRI, there are some limitations, such as limited accessibility to MRI, the time-consuming procedures (around 60 minutes), the lack of trained radiographers to perform the procedure, especially for deceased fetuses, and the shortage of trained pediatric radiologists, particularly for postmortem MRI. The last point is very important because, beyond MRI technique skills, they must also have a good understanding of fetal anatomy, including congenital anomalies, which is significantly different from general MRI.

Postmortem ultrasound (US)

Compared to MRI and CT scans, ultrasound is much cheaper and more accessible, especially in developing countries. The procedure is straightforward: placing the fetus in a supine position inside a small tub and completely covering it with a 2 cm layer of water. The ultrasound probe is partially submerged in the water, making direct contact with the fetus[23].

The overall sensitivity ranges from 67% to 77%, and the specificity ranges from 74% to 90%[24, 25] Diagnostic accuracy is notably high in neurological abnormalities (84.3% sensitivity and 96.7% specificity) and abdominal abnormalities (78.4% sensitivity and 97.3% specificity), while lower in cardiothoracic abnormalities (52.1% sensitivity and 96.6% specificity[26]. This is because the brain and abdominal organs, especially the kidneys, are easier to assess. In contrast, many postmortem artifacts occur in the heart, leading to potential misdiagnoses. Longer intrauterine retention times increase autolytic changes, making it difficult to distinguish soft tissue planes. Postmortem intracardiac blood clots have echogenicities very similar to the myocardium. Air in the cardiac chambers is common in cases of fetal intracardiac injections used for pregnancy termination[27]. For fetuses after 20 weeks of gestation, postmortem ultrasound can clearly detect major anomalies in the four-chamber view, but it is much less effective in visualizing the great vessels[24]. When comparing postmortem 1.5-T MRI and ultrasound for non-invasive perinatal autopsy, ultrasound matched the MRI diagnosis in 86.8% of cases, with the highest agreement for spine (99.3%) and cardiac findings

(97.3%), while brain concordance was lower at 85.2%[27].

It appears that postmortem US is appropriate for assessing the fetal brain and abdomen, but it has limited diagnostic value for the fetal cardiovascular system. The main limitation is that the performance of postmortem US depends on the operator and requires specialized skills. Ideally, the sonographer should be a specialist in fetal medicine with extensive knowledge of fetal normal and abnormal anatomy.

Postmortem computed tomography (CT) Scan

Postmortem CT scans are more widely available, less expensive, and quicker to perform than MRI. However, the overall rate of concordance with conventional autopsy is only 38.1% in fetuses less than 24 weeks of gestation and 71.4% in fetuses greater than 24 weeks, with sensitivities of 27.8% and 50%, respectively[29]. Based on the body system, the sensitivity is highest in the musculoskeletal system (66.7%), and relatively low in the other systems. Compared to postmortem MRI, CT scans without contrast have a low success rate in examining the brain and thoracoabdominal organs. To improve the diagnostic accuracy of CT scans for identifying congenital cardiac malformations, CT angiography can be performed using contrast injection either through the umbilical cord or directly into the heart under ultrasound guidance. Direct injection into the heart achieved a 96% success rate in demonstrating major abnormalities of the four-chamber view and the great vessels[30].

A postmortem CT scan of a small fetus shows limited results due to the small size of the specimen and poor soft tissue contrast. Although postmortem CT is considered a very effective diagnostic tool for musculoskeletal abnormalities, another limitation to consider is limited ossification during early gestation.

Postmortem microfocus computed tomography (micro-CT)

Micro-CT is an X-ray-based technology similar to conventional CT, but instead of a rotating gantry, micro-CT scanners feature a fixed radiation source while samples are mounted on a rotating platform. Radiation source-to-sample distance and the sample-to-detector distance can be adjusted to achieve much higher resolutions, up to sub-micron (<μm) levels. Compared to conventional CT, micro-CT typically involves longer scan times and higher radiation doses[31]. Staining is only required when the researcher aims to study soft tissue. Without staining, micro-CT provides excellent spatial resolution for high-density structures (e.g., orbit, humerus, femur), even if these structures are not fully ossified yet[32].

For better visualization of soft tissue, staining is necessary before scanning. The fetus must be submerged in a staining solution[33]. The most commonly used staining solution is Lugol's solution, which is a water-based mixture containing two parts potassium iodide (KI) for every one part iodine (I2) or potassium triiodide (I2KI). This solution is preferred because it penetrates quickly and deeply, provides excellent contrast in all tissues, is non-toxic, and is relatively inexpensive[34]. However, the effectiveness of staining depends on fetal size, the concentration of the staining solution, and the duration of staining. As gestational age increases, fetal skin becomes less permeable to iodine, requiring longer incubation times. Higher concentrations can reduce staining time but may cause overstaining and loss of tissue differentiation. Since staining time correlates with diffusion speed, it takes longer in larger fetuses, ranging from hours to several weeks. For fetuses under 20 weeks of gestation, adequate staining usually takes between 3 and 10 days. Additionally, Lugol's solution may cause tissue shrinkage of up to 30%, which can be prevented by using buffer-prepared Lugol's solution (B-Lugol)[35].

The postmortem whole-body fetal micro-CT has high diagnostic accuracy in fetuses under 22 weeks of gestation, with 93.8% sensitivity and 100% specificity[36]. The performance of micro-CT can even be applied to embryos, which is an important advantage over conventional autopsy.

The limitation of postmortem micro-CT is dealing with large-sized fetuses (more than 20 weeks of gestation) that have sufficient iodine staining, as well as figuring out how to properly immobilize the fetal body during scanning. Since micro-CT scanners use fixed radiation sources and the specimen must be mounted vertically on an adjustable rotating platform, even though the fetus has been previously fixed in formalin, the fetal body's vertical axis can still collapse during the 20- to 30-minute scanning period[2].

The immersion of the fetus in iodine contrast can cause brown discoloration of the skin. Although there are techniques to reverse the staining, such as additional immersion in a sodium thiosulfate solution, this process requires an extra 1-2 days, which may be distressing for the parents. Additionally, this reversal may not be entirely effective[31]. These concerns should be discussed and the doctor should seek additional consent from the family before proceeding with the procedure.

Radiograph (X-ray examination)

A whole-body radiograph of the fetus, known as a babygram or skeletal survey, is usually performed in some centers as part of the routine examination of stillbirths. The main goal is to provide a general overview of fetal skeletal maturity, estimate gestational age, and diagnose genetic bone disorders. However, routine postmortem fetal radiography has been reported to be neither cost-effective nor enhances diagnostic value, except in specific cases of prenatally suspected skeletal abnormalities[37, 38]. It should only be done when a pathologist determines that it is necessary after external examinations.

A summary of postmortem imaging modalities is shown in Table 1.

Table 1. 1 Postmortem imaging modalities[39].

	Radiographs	Ultrasound	CT	MRI (3-T or 1.5-T)	Micro-CT	High-field MRI (7-T or more)
Availability	Easily available	Easily available	Easily available	Moderate	Limited	Limited
Cost	Cheap	Cheap	Moderate	Expensive	Same cost as CT scanner	Very expensive
Size of fetus	Any size	Any size	Any size	Better for larger fetuses (weight >300 or 500 g)	Small fetuses (<20 weeks of gestation) Up to 30 cm in length	Similar to micro-CT
Advantages	Easy to perform	Facilitates image-guided biopsies	High accuracy for musculoskeletal abnormalities	Multiple sequences, multiplanar reconstructions	Excellent resolution and soft tissue detail	Excellent resolution and soft tissue detail
Limitation	No internal soft tissue detail	• Operator dependent • Maceration may affect image quality	• Poor diagnostic accuracy • Poor soft tissue detail due to a lack of internal body fat	Poorer resolution in smaller fetuses	• Iodine contrast is required for soft tissue detail, which can cause tissue discoloration • Longer period of turnaround time due to pre-imaging staining process and removal	Long scanning times (hours)
Indication:	Consider musculoskeletal abnormalities	Assessment of soft tissue and internal organ detail	Consider musculoskeletal abnormalities	Assessment of soft tissue and internal organ detail	Small fetuses (<20 weeks gestation) where ultrasound and 1.5-T/3-T MRI non-diagnostic	Currently research tool only

Additional tissue sampling

There are four main types of postmortem investigation methods: conventional autopsy, less invasive autopsy (LIA), non-invasive autopsy (NIA), and minimally invasive autopsy (MIA) (Table 2)[40, 41].

Table 2. 1 Types of postmortem investigation methods[40, 41].

Term	Components	Placental examination	Histology	Genetics
Conventional autopsy or invasive autopsy	Review of the clinical history, external examination with photographs, and internal macroscopic examination	Yes	Yes, all target organs	Tissue samples from target organs for genetic testing
Less invasive autopsy (LIA) or virtopsy	Any autopsy procedure (including imaging) that is performed with smaller, less, or no incisions than conventional autopsy	Yes	Yes, most organs with focal biopsy via MIA approach	Tissue samples from target organs for genetic testing
Non-invasive autopsy (NIA) or imaging-only autopsy	External examination with postmortem cross-sectional imaging and ancillary testing	Yes	No	No
Minimally invasive autopsy (MIA)	Combination of imaging investigations and laparoscopic or image-guided needle-biopsy approach	Yes	Yes, most organs with focal biopsy	Tissue samples from target organs for genetic testing
MinImAL procedure (Minimally Invasive Autopsy with Laparoscopic-assisted sampling)	A type of MIA, using laparoscopic-assisted methods to visualize internal organs and acquire organ tissue sampling A single small incision (1 cm) at the left upper quadrant of the abdomen or epigastric region	Yes	Yes, most organs with focal biopsy	Tissue samples from target organs for genetic testing
INTACT procedure (INcision-less TArgeted Core Tissue)	A type of MIA, involving ultrasound-guided organ biopsies of fetuses via the umbilicus	Yes	Yes, most organs with focal biopsy	Tissue samples from target organs for genetic testing

In a traditional autopsy, which is considered the gold standard and involves routine tissue sampling from internal organs, the question arises whether this sampling should also be performed in virtual autopsy. Evidence shows that histological tissue can determine the cause of perinatal death in less than 1% of cases where death is unexplained after placental, clinical, or imaging examinations[42]. This suggests that when postmortem imaging shows no abnormalities, microscopic tissue sampling is unlikely to be beneficial. Centers with access to postmortem imaging may use it to guide the autopsy process. Conventional autopsy should be reserved only for cases where postmortem imaging uncovers unexpected abnormalities, non-diagnostic findings, or findings discordant with antenatal imaging[41].

If tissue sampling is necessary, whether for histology, additional genetics, or molecular studies, minimally invasive procedures with image guidance are currently recommended. Blind percutaneous

needle biopsies are not preferred due to their relatively low success rates in obtaining the targeted tissue, especially in deeply located organs that are small in size, such as the spleen, pancreas, kidney, adrenal glands, and heart[43]. Ultrasound-guided biopsies have a higher overall success rate (76.1%), with the highest success rates for the heart (93%) and lungs (91%) by individual organs, while the lowest success rate is for the spleen (11%)[44]. The biopsy can be performed via the umbilical vein, avoiding any body incisions (known as the 'INTACT' biopsy procedure). Laparoscopically guided tissue sampling (referred to as the "MinImAL" procedure) achieves the highest success rate in obtaining adequate histological samples in most major organs, such as 100% in the heart, lung, and kidney; 96.7% in the liver; 94.5% in the spleen; 89% in the adrenal glands; and 82.4% in the pancreas[43]. However, there are many limitations, including difficulty performing the procedure in small fetuses, the high costs of laparoscopic equipment, and the need for a specially trained operator.

Conclusion

Postmortem imaging has become a useful alternative to traditional autopsy. MRI appears to be the best in terms of image quality, but it is limited in availability, expensive, and not suitable for small fetuses. Postmortem ultrasound is helpful when MRI is not accessible, but it faces limitations with severe maceration and still requires further development of training programs. Radiographs and CT scans offer limited benefits in cases of suspected musculoskeletal issues. Micro-CT is a new postmortem imaging technique for small fetuses that provides excellent image quality, but it requires pre-scanning iodine staining and removal, which delays results and is not feasible for larger fetuses. Availability is also restricted, similar to high-field MRI, which is mainly used for research.

Although there are many limitations to noninvasive perinatal virtual autopsy by imaging technique, we are now in a new era of developing technology. It may be time to move from traditional invasive autopsy, which is gradually declining among parents, to these new postmortem investigative methods.

Potential conflicts of interest

The authors declare no competing interests.

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