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Original Article

The Role of histo-taphonomy in postmortem interval estimation: A preliminary study of porcine liver and kidney

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ABSTRACT

Background and aim: Accurate estimation of the postmortem interval (PMI) remains a critical challenge in forensic investigations due to the complex interplay of decompositional processes. Histo-taphonomy, the microscopic examination of postmortem tissue changes, offers a potential approach for PMI determination. This study investigates the histological changes in porcine liver and kidney tissues over a controlled postmortem timeline to assess their forensic applicability.

Methodology: A healthy female pig (*Sus scrofa*) was euthanized, and liver and kidney tissues were harvested immediately postmortem. Samples were collected at 0, 24, 36, 48, 60, 72, 84, and 96 hours postmortem. The tissues were fixed in 10% buffered formalin, processed using standard histological techniques, and stained with hematoxylin and eosin (H&E) for microscopic examination.

Results: Histological analysis revealed progressive tissue degradation over time. Kidney tissues initially showed mild structural alterations, followed by significant nuclear changes and architectural disintegration. Liver tissues exhibited early-stage fibrosis and progressive hepatocyte necrosis. The observed cellular degeneration followed a time-dependent pattern, with both organs demonstrating severe autolysis by 96 hours postmortem.

Conclusion: The findings suggest that histo-taphonomy provides a structured framework for PMI estimation based on organ-specific decomposition patterns. Given the anatomical and physiological similarities between porcine and human tissues, this model could serve as a valuable tool in forensic investigations. Future studies should incorporate environmental variables and molecular markers to enhance the accuracy and applicability of histological PMI estimation in forensic casework.

Keywords:
Histo-taphonomy; Postmortem interval; histological analysis; porcine model

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INTRODUCTION

Accurate estimation of the postmortem interval (PMI), the time elapsed between death and the discovery of remains, remains a critical challenge in forensic investigations (Franceschetti *et al*., 2023). Determining PMI is essential for reconstructing events surrounding death, corroborating or refuting alibis, and ensuring the integrity of forensic evidence (Wang *et al*., 2018; Franceschetti *et al*., 2021). Decomposition is influenced by autolysis, putrefaction, and environmental factors, making PMI estimation complex (Howie *et al*., 2024). While conventional methods such as forensic toxicology and macroscopic observations provide useful insights, particularly within the early postmortem period (Ikpa *et al*., 2025), more objective, quantitative techniques are needed. Histological analysis offers a promising approach by identifying cellular and tissue-level changes that occur after death, potentially improving PMI estimation (Palić *et al*., 2024; Surendhar *et al*., 2023).

Studies have demonstrated that postmortem histological changes follow organ-specific decomposition patterns. Tomita *et al*. (2004) observed ultrastructural variations in rat organs, noting early mitochondrial deposits, glycogen depletion, and nuclear chromatin alterations in the kidney, pancreas, and liver. Ceciliason *et al*. (2021) further developed a hepatic decomposition scoring system that improved PMI estimation in human liver tissues. Since microbial activity is minimal in the first 24 hours postmortem, decomposition is largely driven by cellular autolysis, which progresses rapidly (Paternoster *et al*., 2019; Tozzo *et al*., 2022). Histological studies have shown that significant tissue disruption can occur within hours after death, making early fixation crucial to preserving

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tissue integrity (Wei *et al*., 2020; Miller & Zachary, 2017).

Despite its well-established role in forensic pathology for organ identification and disease diagnosis, histological analysis remains underutilized for PMI estimation. This study addresses this gap by investigating postmortem histological changes in porcine liver and kidney tissues as a model for PMI determination. Given their anatomical and physiological similarities to human tissues (Kelly *et al*., 2009; Miles *et al*., 2020), porcine models offer a controlled experimental framework for forensic taphonomic research. By examining the microanatomical changes in these organs, this study aims to enhance the accuracy and reliability of histological PMI estimation, potentially complementing existing forensic methods.

MATERIALS AND METHODS

Ethical Approval

Ethical approval was obtained from the Faculty of Basic Medical Sciences, Research and Ethics Committee (FBMSREC), University of Cross River State, Okuku Campus, Nigeria. The ethical approval certification number is FBMS/REC/2021/113 dated 4th December 2021.

Animal Model and Euthanasia

One healthy female pig (*Sus scrofa*) weighing 26 kg was obtained from a local pig farm (approximately 2 km from the UNICROSS laboratory, Okuku, Cross River State). The pig was certified healthy by a licensed veterinarian before the study. Euthanasia was performed at the Department of Anatomy Laboratory using a captive bolt to the base of the skull. This method was chosen to ensure rapid and humane euthanasia, minimizing animal suffering and preventing blood spillage.

Experimental Design

The pig was transported to the laboratory at approximately 6:30 am. Initial assessments, including body weight (using a calibrated digital scale) and core body temperature (measured rectally using a Taylor digital thermometer, with readings taken after approximately five minutes when stable), were performed immediately before and after confirmation of death. Ambient temperature was recorded concurrently using an atmospheric thermometer.

Following confirmation of death, the pig was immediately eviscerated, and the liver and kidneys were harvested. These organs were placed separately in covered bowls on a laboratory shelf. The bowls were covered with netting to prevent insect access while allowing for air circulation. This controlled environment minimized temperature fluctuations that could influence the decomposition process.

Tissue Sampling

Tissue samples were collected from both the liver and kidneys at specific postmortem intervals. The first samples were collected immediately following evisceration (approximately 7:00 am). Subsequent samples were collected at 12-hour intervals after the first 24 hours (at 0, 24th, 36th, 48th, 60th, 72nd, 84th, and 96th hour) until 96 hours postmortem.

Tissue preparation and histological analysis

Following collection, tissues were immediately fixed in 10% buffered formalin. Subsequent processing involved dehydration through a graded series of alcohols, clearing with xylene, and infiltration with paraffin wax. Tissues were then embedded in paraffin blocks, sectioned using a microtome, and mounted onto albumin-coated slides. Slides were stained with hematoxylin and eosin using standard protocols, involving dewaxing, rehydration, staining, dehydration, and clearing steps. Finally, the stained tissue sections were examined under a light microscope, and digital images were captured for analysis.

Photomicrographs were interpreted by an experienced histopathologist and the corresponding results were used to explain the postmortem cellular degeneration in liver and kidney tissues.

RESULTS

Histological Observations of the Kidney

The result showed that the initial tissues showed a mild fatty change otherwise normal with glomeruli (G), bowman space (SB), renal tubules (R) and tubular cell (TC). The tissues were shown to be normal as demonstrated in Figure 1.

By the second day, approximately 24 hours postmortem, the kidneys showed mild deterioration with mild fatty necrosis (FN) as seen in Figure 2. The deterioration continued till the 36th hour and by the 48th hour, the fatty necrosis (FN) became more noticeable with the renal cells becoming indistinguishable (Figure 3). Between the 60th and 72nd hours, pyknotic glomeruli (PG) could be seen with renal tissues rapidly degenerating until the fifth day postmortem (Figure 4). By the 96th hour postmortem when the kidney samples were collected, more fatty necrosis (FN) had engulfed the cell membrane which became indistinct in the final tissue section (Figure 5).

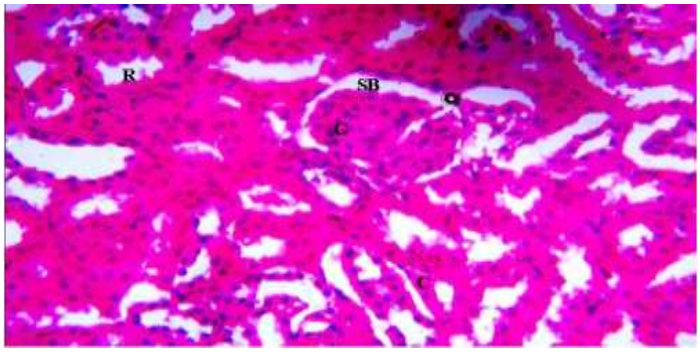

Figure 1: A section of the kidney of the carrion 0 hour postmortem showing normal renal tissues with glomeruli (G), space of bowman (SB), renal tubules (R) and tubular cell (C) very visible. H&E; 100×.

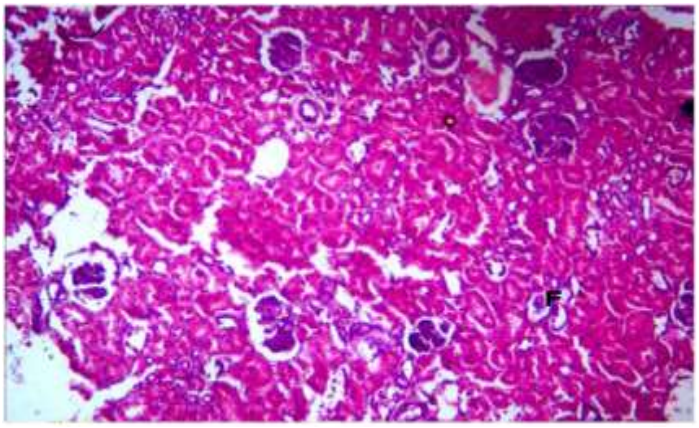

Figure 2: Photomicrograph of a section of the kidney of carrions at 24hrs post-mortem, showing mild deterioration in the renal tissues with Fatty Necrosis (FN) in E2. H&E; 100×.

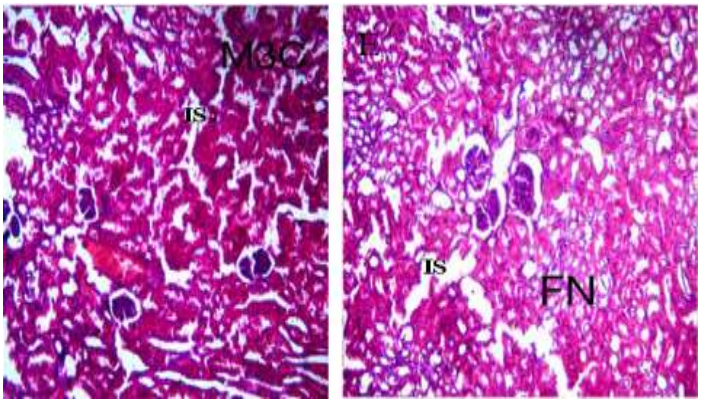

Figure 3: Photomicrograph of a section of the kidney at 36hrs after death (M3C) and 48hrs (E) showing increased pronounced fatty necrosis (FN) rapid degeneration of cells and increased interstitial spaces (IS). H&E; 100×.

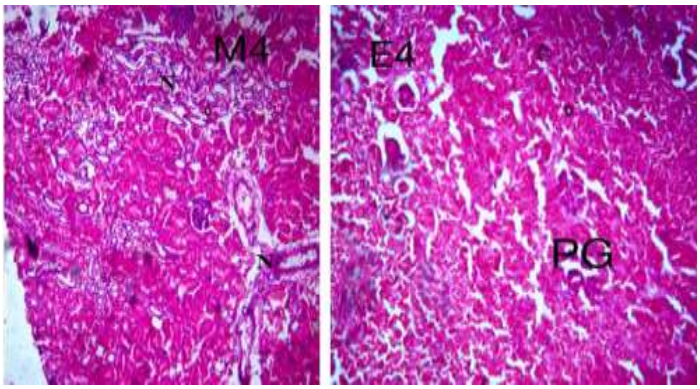

Figure 4: Photomicrograph of kidney section at 60hrs after death (M4) and 72hrs (E4) showing further degeneration and marked loss of nuclei (N) in M4 and E4 with Pyknotic glomeruli (PG) visible in E4. H&E; 100×.

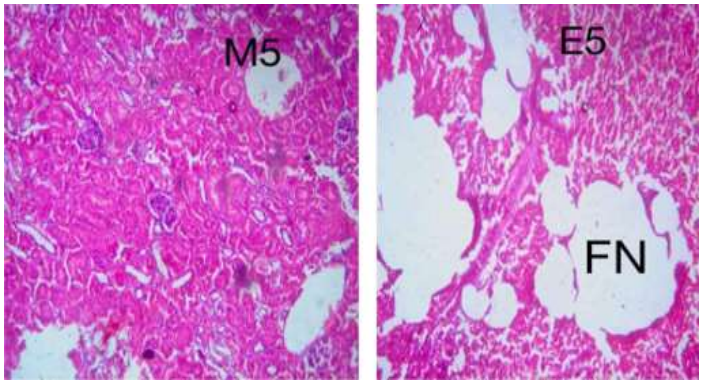

Figure 5: Photomicrograph of kidney section at 84hrs after death (M5) and 96hrs (E5) post-mortem, showing massive degeneration of renal tissues with complete breakdown of the renal architecture and extremely fatty necrosis (FN) H&E; 100×.

Histological Observations of the Liver

Liver tissues were harvested at the same 12-hourly interval as with the other organs and the result showed normal tissue features of the liver when the tissues were obtained and processed for H & E immediately after death. The results from the micrographs (Figures 6-11) demonstrated the extent of the postmortem changes in the hepatic tissues. The results showed normal tissue as that of the control (Figure 33) which showed normal hepatic architecture with Portal Triad (PT) and active Hepatocyte (H) with the tissues appearing relatively normal.

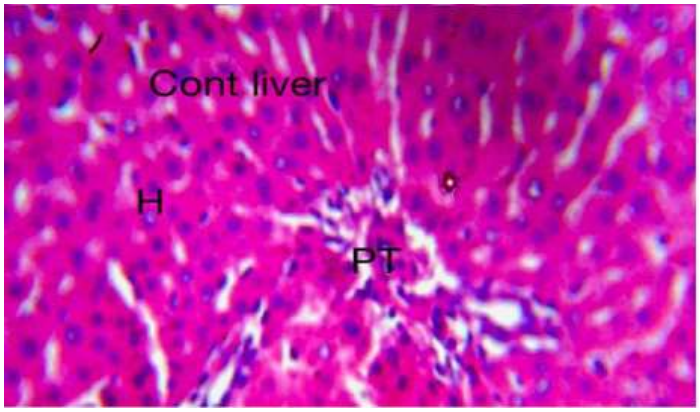

Figure 6: Photomicrograph of a section of the Liver obtained immediately after death showing a normal hepatic Cytoarchitecture with portal triad (PT) and active Hepatocytes (H) and tissues with no obvious alterations. (H&E; 100×).

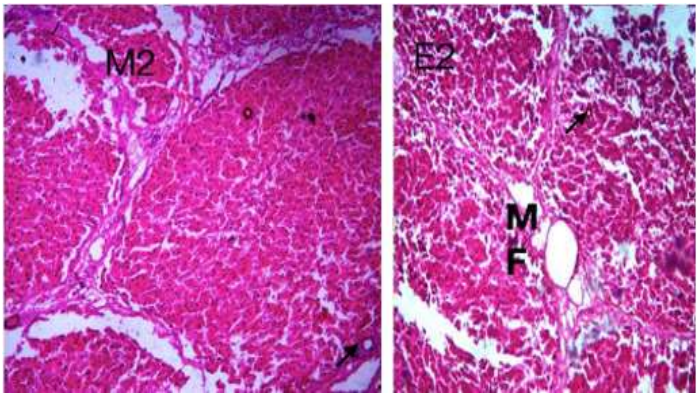

Figure 7: Photomicrograph of a section of the Liver at 12hrs after death (M2) and 24hrs (E2) showing mild deterioration of the hepatic tissues in M2 and moderate fibrosis (MF) was observed along the Portal Triad (arrows) H&E; 100×.

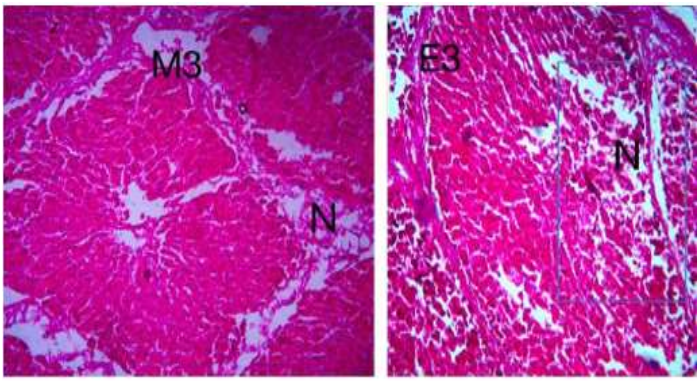

Figure 8: Photomicrograph of a section of the Liver at 36 hrs after death (M3) and 48hrs (E3) showing progressive loss of hepatocytes and focal areas of Necrosis (N) With no distinct cell outline. H&E; 100×.

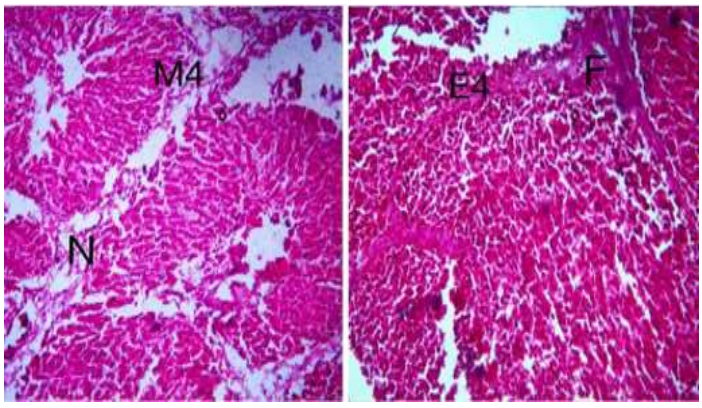

Figure 9: Photomicrograph of a Liver section at 60hrs after death (M4) and 72hrs (E4) showing progressive loss of hepatocytes and a focal area (F) of Necrosis (N) With no distinct cell outline. H&E 100x

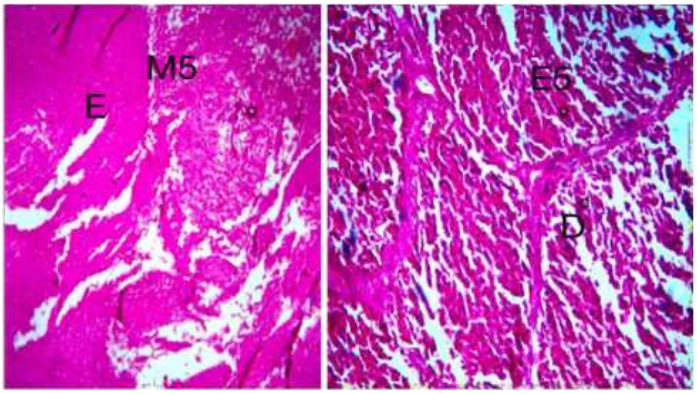

Figure 10: Photomicrograph of a Liver section at 84hrs after death (M5) and 96hrs (E5) showing severe deterioration (D) of hepatic tissues with pronounced eosinophilic (E) features. H&E; 100×.

DISCUSSION

The examination of soft tissue's decomposition rate has been subject to limited research, hence posing challenges in its use within forensic techniques. To accurately diagnose gross and microscopic pathology during an autopsy, it is crucial to possess a comprehensive understanding of the physiological and chemical alterations that take place in the body following death. Hence, the objective of this study was to record the microscopic techniques employed in the examination of certain soft tissues derived from domestic pigs (*Sus scrofa*). A more thorough understanding of the organ decompositions in this environment would enable better comprehension of the micro-anatomical components of the kidney and the liver to determine the PMI of the region under investigation.

While the assessment of the PMI is crucial in many human and animal death investigations, forensic pathology focuses on identifying the causes of death as well as the postmortem interval of the death through the analysis of the body tissues (Campobasso *et al.*, 2001). Anyone attempting to assess the microscopic or gross pathological changes at autopsy must be aware of postmortem changes in the soft tissues to avoid misinterpreting the expected postmortem changes as lesions and to avoid misidentifying the actual lesions that may be concealed or distorted by postmortem changes. In some jurisdictions, a postmortem examination is carried out by a medical or forensic pathologist, generally during the investigation of criminal law proceedings and civil law concerns (Choo & Choi, 2012). To determine the presence or absence of natural diseases and other microscopic findings, such as asbestos bodies in the lungs or gunpowder, the forensic pathologist examines tissue samples under the microscope.

The Kidneys in this showed no obvious changes immediately after animal death with the photomicrographs showing a normal cytoarchitecture with glomeruli, bowman space, renal tubules and tubular cells. This is consistent with similar research findings by Ragab *et al*. (2022). The liver tissue sections showed mild deterioration of the hepatic tissues with moderate fibrosis along the portal triad in about twenty-four hours postmortem. These tissue changes were also observed by Guan *et al*. (2022), who

concluded that the severely altered microstructure with limited recognisable histological features was due to cellular autolysis.

On day three with a PMI of thirty-six hours, the kidneys showed mild deterioration of the renal tissues, while the liver tissues within the same time exhibited considerable hepatic tissue degenerations, necrosis and breakdown of cellular outlines. Carlisle *et al*. (2021) Were able to establish that cellular alterations are important in explaining the chronology of tissue degradations as seen in the present study while supporting the findings of Ozerov *et al*. (2006), Wang *et al*. (2008); Pang *et al*. (2013) and Pittner *et al*., (2020).

At about the sixtieth-hour post-mortem, it showed signs of severe necrosis with pyknotic glomeruli which were visible in the kidneys. The liver tissue sections at the same stage showed severe deterioration of the hepatic tissues with no distinct cellular outlines. Both tissues degenerated further until most cellular structures became indistinguishable under the microscope at the end of the observations after ninety-six hours. This has demonstrated that tissues undergo acute histo-morphological changes following PMI progression. This is in agreement with the studies carried out by Bhetariya *et al*. (2016) and Geissenberger *et al*. (2024). The histological changes observed throughout the trajectory of the decomposition in the selected animal organs were important in the quest to improve the methods for estimating and establishing the PMI.

CONCLUSION

This study highlights the potential of histological analysis as a valuable tool in forensic pathology, particularly for estimating postmortem intervals. By examining progressive cellular degradation, we demonstrate the viability of histo-taphonomy in quantifying tissue decomposition over time. The findings reinforce the organ-specific nature of postmortem changes and underscore the forensic relevance of microscopic tissue evaluation. Given its applicability in controlled experimental models, this approach could complement existing PMI estimation methods, contributing to more precise and reliable forensic investigations.

Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Conflict of Interest Disclosure: The authors declare that there are no conflicts of interest related to this study.

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