



Article

Development of a Post-Mortem Autolysis Scoring Scale for Bovine Perinatal Mortalities: A Cluster-Analysis-Based Approach

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Abstract

Background/Objectives: Perinatal mortality causes substantial economic losses in dairy and beef suckler industries, yet diagnosis remains challenging as post-mortem autolysis can mask lesions and hinder definitive diagnosis. To establish a standardized tool for objectively quantifying carcass decomposition and aiding in the categorization of perinatal timing of death, this study developed a simple, visual 3-point scoring scale based on the macroscopic evaluation of nine parameters. **Methods:** Retrospective scoring was performed using necropsy findings and high-resolution photography from 127 calves, including a Reference group ($n = 32$; comprising 26 perinatal mortality cases which breathed and six live-born control calves with normal vitality that were euthanized), and a test group ($n = 95$; perinatal mortality calves which did not breathe). Hierarchical clustering was performed on the total autolysis score, calculated as the sum of points across all evaluated parameters, using Ward's linkage method with Euclidean distance. **Results:** Calves in the test group were clustered into three distinct decomposition profiles, with total autolysis scores differing highly significantly across all study groups ($H(3) = 111.71, p \leq 0.0001$). Cluster 1 represented an early decomposition stage where most organs showed no macroscopically detectable autolysis; Cluster 2 exhibited advanced but variable autolysis with intermediate tissue decay and the absence of rigor mortis; and Cluster 3 was marked by advanced, uniform decomposition across all features. Neither the calving/death-to-necropsy interval ($p = 0.09$) nor season ($p = 0.43$) significantly affected the severity of autolysis. The Kruskal–Wallis test confirmed highly significant differences ($p \leq 0.001$) for all nine individual parameters, demonstrating a stepwise progression of post-mortem changes. **Conclusions:** This novel visual scoring scale provides an objective numerical proxy for carcass decomposition, offering a practical tool for categorizing general perinatal death timing rather than precise chronological dating.



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Keywords: bovine perinatal mortality; post-mortem autolysis; scoring scale; cluster analysis; death timing

1. Introduction

Perinatal mortality (PM) represents a substantial source of economic loss in the dairy industry, which exceeds the immediate value of the dead calf. Apart from the loss of genetic potential for herd improvement, producers face significant costs related to the dam's subsequent health and performance [1]. Definitions of PM across the literature

typically encompass calves born dead (at ≥ 260 days of gestation) or those born full term that die within 12 to 48 h postpartum [2,3]. Dairy bovine PM is estimated internationally to be between 2.0% for cows and heifers in Norway up to 12.1% in USA heifers [4]; however, it follows a skewed distribution; most herds have low losses, but some face high mortality rates where nearly one-third of born calves do not survive [5,6].

Diagnosing the causes of PM remains a significant challenge, with approximately one-third of cases remaining unexplained even after thorough post-mortem examinations [2,7,8]. A primary obstacle is the lack of international standardization in the criteria used to define and assign causes of death, which prevents the comparison of data across different regions and laboratories [9]. The presence of autolysis may negatively impact test results, make histologic examination of vital organs impossible, or may obscure pathological lesions during the necropsy, making it difficult to determine the primary cause of death [8,10,11]. Finally, the complexity of co-mortality (multiple causes of death) and the fact that many infections do not produce visible gross lesions make it difficult for practitioners to reach a definitive etiologic diagnosis [8,10].

Accurately establishing the timing of PM—whether it occurred before (in utero), during (intrapartum), or shortly after birth—is a critical diagnostic step for identifying the specific etiology and implementing effective preventive measures. The use of sequential autolytic changes, such as corneal opacity, subcutaneous edema, and organ softening, serves as a vital index for determining the duration of fetal retention in the uterus [12]. In the literature, investigations into the time of death in ruminants are based on references from ovine pathology papers [13–15]. However, there are few studies or gross pathology manuals focused on estimating the time of death in PM calves, and those which are available [2,16] estimate time of death in very broad timeframes. Accurate differentiation of death moment is crucial for both clinical and forensic evaluations, providing producers with a tool to audit calving supervision and helping to determine if a loss was preventable, highlighting areas where management or intervention could be improved. Currently, there are no simple methods that allow veterinarians to estimate the post-mortem interval. Standardizing pathological descriptions across diagnostic laboratories remains challenging due to the subjective evaluation and variable reporting of post-mortem autolysis. Rather than being categorized as a uniform pathological lesion, autolysis is interpreted inconsistently across institutions: some pathologists use signs of decomposition—such as subcutaneous edema, fluid accumulation, or mummification—as a descriptive indicator to estimate how long a calf has been dead in utero [2], whereas others treat autolysis strictly as a sample-quality threshold, outright excluding excessively degraded or scavenged tissues from histopathological examination [11]. Without standardized criteria defining autolysis, decisions to evaluate or reject degraded tissue rely on subjective thresholds, leading to variable reporting across laboratories. Therefore, the objective of this study was to develop a simple visual scale to objectively quantify the extent of perinatal calf carcass autolysis in the field, thereby aiding in the preliminary classification of fetal retention and possible death timing.

2. Materials and Methods

2.1. Data Collection

The study included 127 dairy calves, comprising 121 cases of bovine perinatal mortality (PM) and a group of 6 calves born alive. Detailed methodology was previously published by the authors of [8,10]. Briefly, all calves were born after a gestation of ≥ 260 days. PM cases, which included Holstein-Friesian and crossbred individuals, were defined as calves that died before, during, or within 6 h of birth. The group of Holstein-Friesian calves born via assisted, singleton calving ($n = 6$), with normal vitality were euthanized between 1.5 and 8.5 h after birth. Euthanasia was performed via intravenous administration in a two-step

procedure. Initial sedation using 20 mg xylazine (Sedazin®, Biowet Pulawy, Poland) and 100 mg ketamine (Bioketan®, Vetoquinol, Poland) was followed by a lethal dose of pentobarbital (48–96 mg/kg body weight, Morbital®, Biowet Pulawy, Poland). Standardized necropsies were performed by a research team at the university laboratory according to a standard operating procedure. This involved a thorough external examination followed by an internal evaluation of the thoracic, abdominal, and cranial cavities. Organs were excised in a predefined order and assessed for both pathological and autolytic changes. In all cases, photographic images were collected from all organs in order to retrospectively assess the degree of autolysis. The interval from birth to the start of the necropsy was recorded in each case.

2.2. Autolysis Scale

The autolysis scoring was conducted retrospectively, relying on high-resolution photography and the detailed descriptions recorded in the original necropsy notes. Specific organs were selected empirically based on their well-documented, varying degrees of autolysis as observed during these examinations. Certain organs evaluated during necropsy were excluded from the final scale: the gastrointestinal tract was omitted due to the rapid progression of autolysis driven by endogenous enzymes; the pancreas was omitted because it is notoriously difficult to rapidly identify and consistently isolate during routine farm necropsies; adrenal glands, the heart, skeletal muscle, and thymus exhibited color changes but did not undergo liquefactive breakdown like other organs.

To characterize the progression of post-mortem changes, autolysis was assessed using a standardized three-point scoring system (0, 1, 2) across seven internal and two external parameters. For the lungs, score 0 represented a light pink color with >30% aeration and spongy consistency; score 1 indicated a firm, dark red structure with <30% aeration, and score 2 denoted a complete lack of aeration with a dark pink, collapsed parenchyma. Body cavity fluids (thoracic and abdomen) were scored from a straw-colored volume of approximately under 100 mL (score 0) to a serosanguinous character of approximately under 100 mL (score 1), reaching a distinctly bloody appearance and exceeding approximately 100 mL at score 2. The aortic arch endothelium was evaluated based on endothelial staining, ranging from a pale porcelain appearance (score 0) to slight pinkish discoloration (score 1) and uniform red staining due to hemoglobin imbibition (score 2). The spleen was graded by its capsule color and parenchymal consistency, progressing from a blue capsule with firm structure (score 0) to a pinkish capsule with semi-liquid tissue (score 1) and finally a red capsule with completely liquefied parenchyma (score 2). Kidney assessment focused on capsule adhesion and the corticomedullary junction, which was sharp and adherent in score 0, showed focal detachment with a blurred junction in score 1, and was fully separated with a liquefied cortex in score 2. The liver was scored based on color, varying from a red-brown or yellow hue with reversible lividity (score 0) to brown (score 1) and eventually pink coloration caused by extensive hemoglobin imbibition (score 2). The encephalon showed a transition from firm consistency with defined gyri (score 0) to a soft texture with cerebellar liquefaction beginning (score 1), culminating in advanced liquefaction of the cerebral and cerebellar cortex at score 2. Apart from internal evaluation, calves were also scored for external parameters: corneal opacity and the presence of rigor mortis. For corneal opacity, a score of 0 was assigned when the cornea was transparent, allowing unobstructed visualization of the iris and pupil. A score of 1 was given if the corneal surface appeared desiccated or irregular, and a score of 2 was assigned if the corneal surface exhibited opacification that hindered or prevented visualization of the iris and/or pupil. Rigor mortis was evaluated by flexing different body regions (neck, forelimbs, and hindlimbs); it was assigned a score of 0 if absent and 1 if present in any of these regions.

The scoring criteria for eight of the nine parameters (excluding rigor mortis) are presented in Table 1. Macroscopic necropsy evaluations were performed jointly by two evaluators to establish a final consensus score for each parameter. Scores for corneal opacity and rigor mortis were extracted from standardized post-mortem protocols that underwent dual verification. No formal testing for inter-observer reliability was performed.

2.3. Statistical Analysis

Out of 127 calves, those that were born alive with normal vitality and subsequently euthanized ($n = 6$) and those that exhibited spontaneous breathing immediately after birth based on the history ($n = 26$) were grouped together ($n = 32$). These calves were used as the reference group, since their time of death could be accurately determined. The remaining calves ($n = 95$, PM) were used for hierarchical clustering analysis.

All analyses were carried out in Python (version 3.11.14) using the SciPy (v1.17.0), scikit-learn (v1.8.0), NumPy (v2.4.2), and Matplotlib (v3.10.08) libraries, while visualizations—including plots and heatmaps—were generated using the Matplotlib. Generative AI (Gemini 2.5 Pro, Google) was used to assist in writing the code for data analysis and generation of figures. All code and outputs were independently reviewed and verified by the authors before inclusion in the manuscript. Hierarchical clustering was chosen over centroid-based (e.g., k-means) or distribution-based methods because it does not require a prior specification of the number of clusters, allowing the optimal number of groups to be determined post hoc from the dendrogram. This approach allowed the natural grouping of calves based on their cumulative autolysis scores to emerge organically from the data. Hierarchical clustering was performed on the total autolysis score, calculated as the sum of points across all evaluated parameters, using Ward's linkage method with Euclidean distance. Ward's method minimizes the increase in within-cluster variance at each merging step, producing compact and well-separated clusters. The clustering solution was visualized using a dendrogram, with individual calves labeled by identification number. To determine the optimal number of clusters, a visual inspection of the dendrogram was combined with the evaluation of two formal cluster quality indices: the Calinski–Harabasz index and the silhouette score. These metrics were evaluated by systematically varying the dendrogram linkage distance threshold (cut-off) from 5 to 29 with a step of 1, which generated solutions containing 2, 3, and 5 distinct clusters. The Calinski–Harabasz index reached its maximum for the 5-cluster solution (2011.79; silhouette score = 0.72), whereas the silhouette score peaked for the 2-cluster solution (0.82; Calinski–Harabasz index = 927.85). The 3-cluster solution (cut-off distance of 10) was selected as the optimal quantitative compromise between both validation metrics. Transitioning from 2 to 3 clusters yielded a substantial increase in the Calinski–Harabasz index (from 927.85 to 1293.55; a 39.4% improvement), while maintaining an exceptionally high silhouette score (0.80). This 3-cluster framework avoided both statistical under-segmentation (2 clusters) and over-segmentation (5 clusters), providing an optimal balance between mathematical structure and biological interpretability.

Table 1. Evaluation criteria for selected parameters in graphical version.

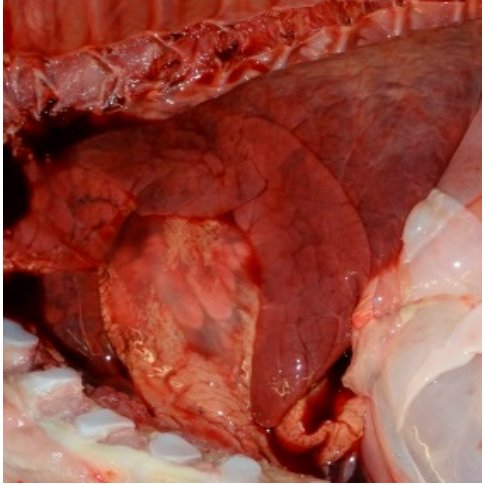
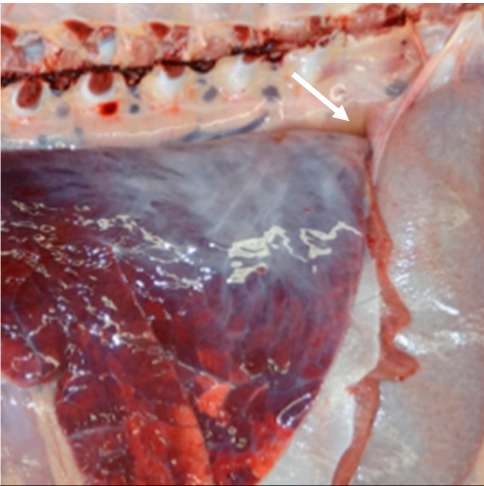
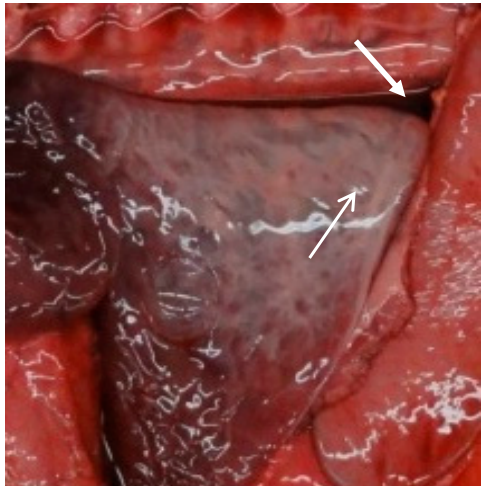
Degree of Change (Points) Parameter	No Autolysis (0)	Slight Autolysis (1)	Moderate to Severe Autolysis (2)
Lungs (aeration and appearance)			
Thoracic cavity fluid (color) (white arrows indicate the location of the fluids)			

Table 1. Cont.

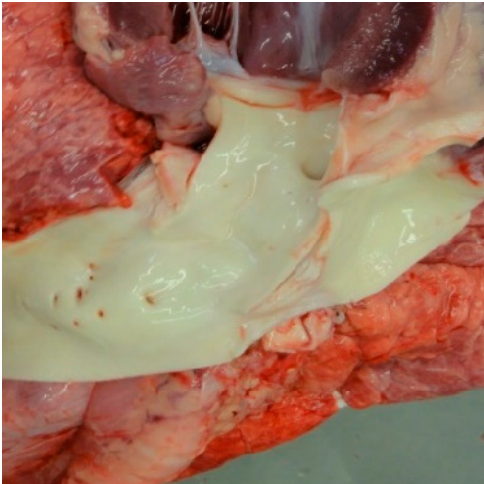
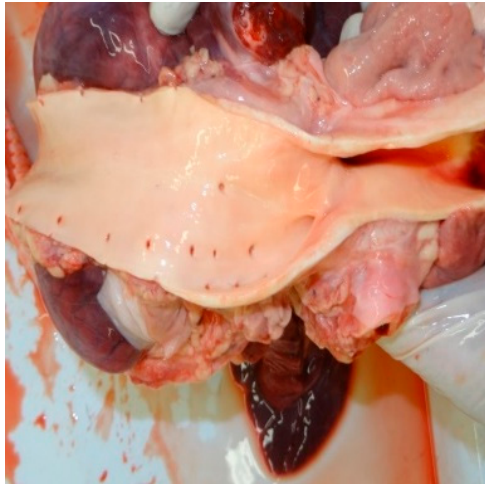
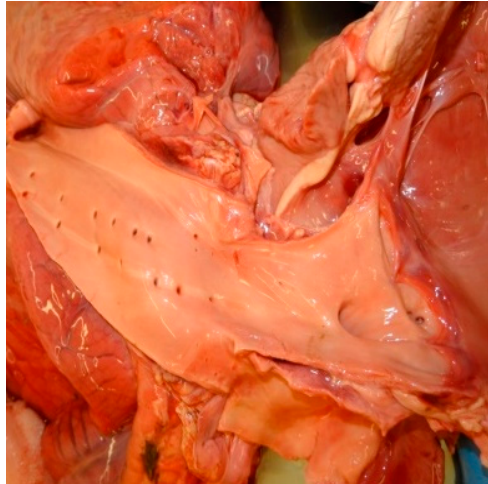
Degree of Change (Points) Parameter	No Autolysis (0)	Slight Autolysis (1)	Moderate to Severe Autolysis (2)
Aortic arch (endothelial color)			
Spleen (color of the capsule and consistency of the stroma)			

Table 1. Cont.

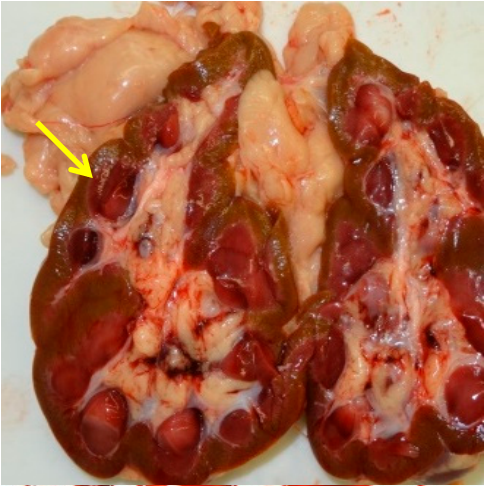
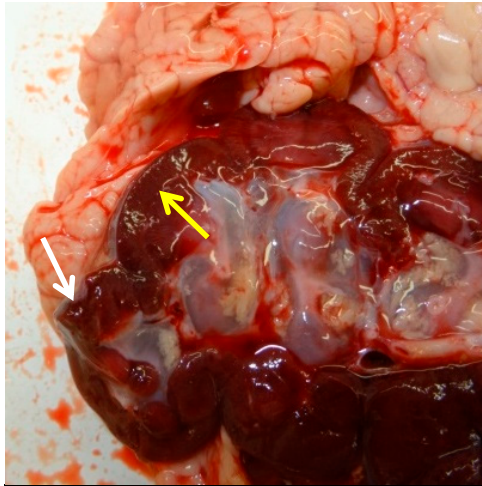
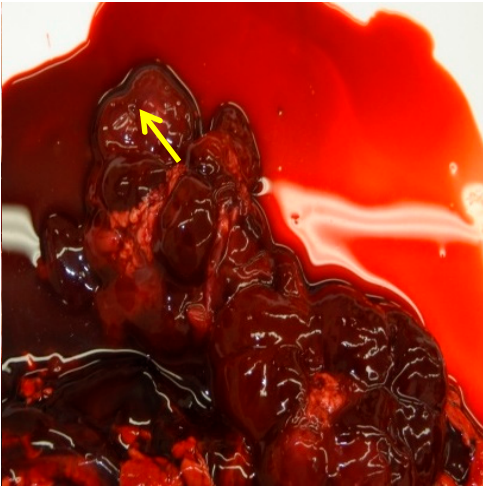
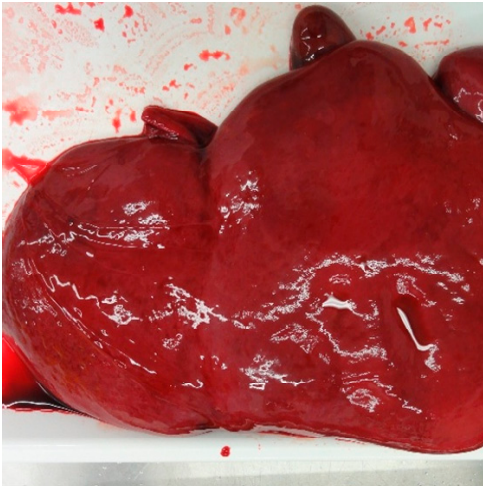
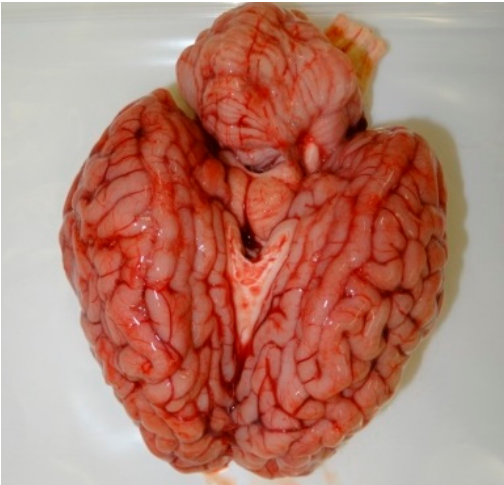
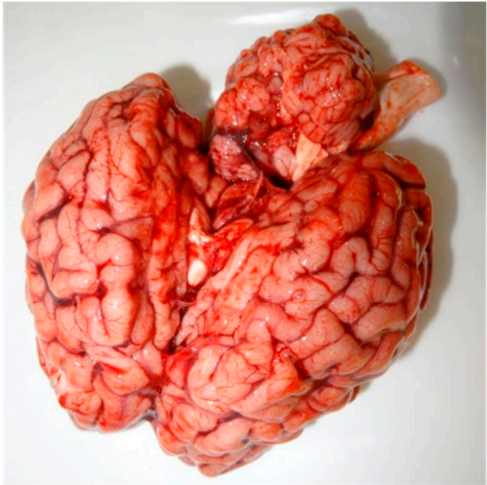
Degree of Change (Points)			
Parameter	No Autolysis (0)	Slight Autolysis (1)	Moderate to Severe Autolysis (2)
Left kidney (adhesion of the capsule to the cortex and its consistency; white arrow indicates separation of cortex and kidney, yellow arrow corticomedullary junction)			
Liver (color, presence of hypostatic spots, rib impressions)			

Table 1. Cont.

Degree of Change (Points) Parameter	No Autolysis (0)	Slight Autolysis (1)	Moderate to Severe Autolysis (2)
Encephalon			
Eye (corneal opacity)			

For details, see description in the text.

Differences in the interval from death (or calving where time of death was unknown) to necropsy among clusters were evaluated in order to assess whether this interval could impact autolysis score. As the assumptions for analysis of variance (ANOVA) were not met (the residuals deviated from normality according to the Shapiro–Wilk test), a non-parametric Kruskal–Wallis test was applied to compare the clusters. After that step, the reference group was added to the data set. The total autolysis scores, as well as calving/death-to-necropsy interval, were compared across all four groups (three clusters and reference). For the same reason as above, a Kruskal–Wallis test with Dunn’s post hoc tests with Bonferroni correction (for total autolysis score) was used. The differences in the degree of autolysis for each parameter on the scale were also analyzed between groups using the Kruskal–Wallis test with Dunn’s post hoc tests with Bonferroni correction.

Thresholds for the total autolysis score were defined as the midpoints between the medians of adjacent groups, thereby accounting for intra-group variability while providing objective cut-off points between consecutive autolysis stages.

To investigate whether the occurrence of autolysis in calves was associated with season, the calves were categorized into four distinct seasons based on the month of necropsy: Winter (December, January, February), Spring (March, April, May), Summer (June, July, August), and Autumn (September, October, November). Due to low and uneven monthly sample sizes, this seasonal aggregation was necessary to ensure statistical validity and reduce data noise. A chi-squared test of independence with Monte Carlo simulation (10,000 resamples) was conducted to examine the relationship between season and the four groups. The Monte Carlo method was selected over the standard asymptotic chi-squared test due to small expected cell frequencies (minimum expected count = 2.72), which violated the assumptions of the classical approach.

Effect sizes were calculated to complement significance testing: rank eta-squared for Kruskal–Wallis tests and Cramér’s V for the chi-squared test of independence. For both measures, 95% confidence intervals (CI) were estimated using a non-parametric bootstrap procedure with 2000 resamples (percentile method).

Throughout the text, “Cluster” refers to cohorts identified via clustering analysis, whereas “Group” includes these cohorts alongside the Reference group.

3. Results

The hierarchical clustering and cut-off points are presented in Figure 1. At a dendrogram cut-off level of 10, three clusters were identified with 45, 15, and 35 individuals.

The calving/death-to-necropsy interval did not differ significantly among the three clusters ($H(2) = 0.98$, $p = 0.613$, rank eta square = 0.0, 95% CI 0.00–0.09), nor when the reference group was included in the analysis ($H(3) = 6.5$, $p = 0.09$, rank eta square = 0.03, 95% CI 0.00–0.16) (Figure 2).

The reference group (Ref, $n = 32$) had a median interval of 384 min (IQR: 315.0–522.0 min). Among the clustered groups of PM calves, Group 1 ($n = 45$) exhibited a median time of 462 min (IQR: 395.0–530.0 min), Group 2 ($n = 15$) showed a median time of 429 min (IQR: 367.5–562.5 min), and Group 3 ($n = 35$) had a median interval of 470 min (IQR: 408.5–688.5 min).

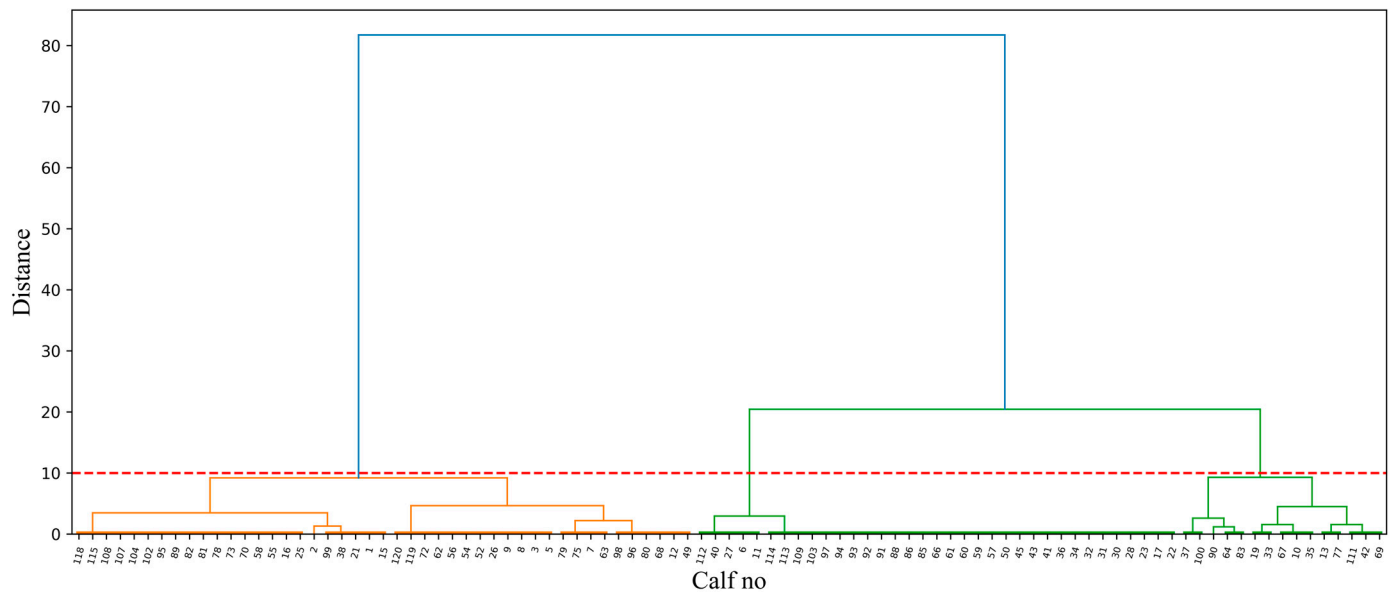


Figure 1. Hierarchical clustering of dead calves. The horizontal dashed red line indicates the chosen cut-off threshold at a linkage distance of 10, which successfully identifies the distinct clusters. The different colors (orange and green) represent sub-clusters of calves with highly similar autolytic profiles, while the blue lines represent higher-level linkages between distinct major groups.

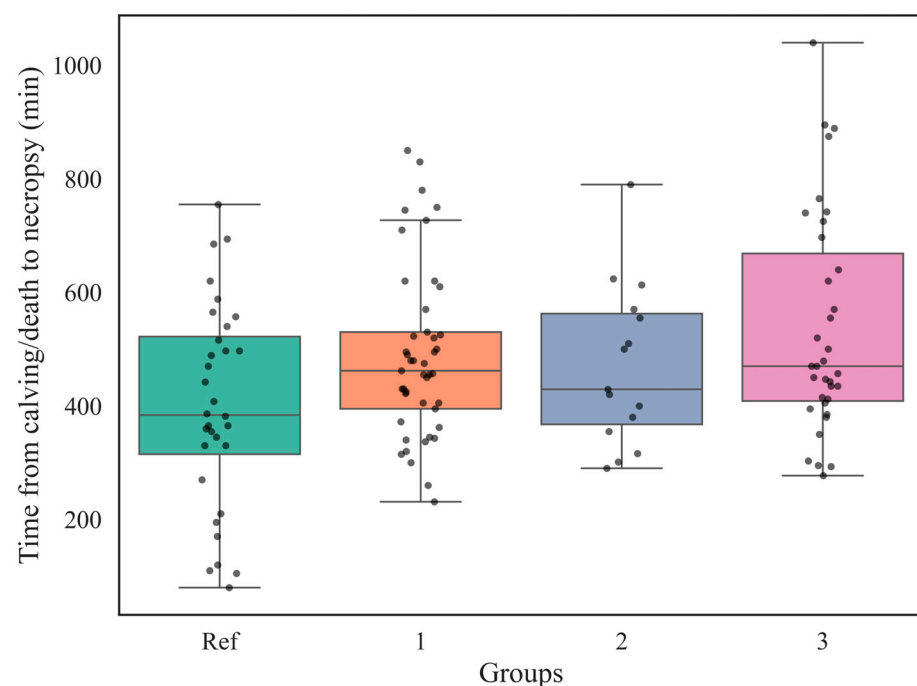


Figure 2. Interval from death (reference group) or calving (non-reference groups) to necropsy, across all groups. The figure shows box plots with individual data points for death (or calving where the time of death was unknown) to necropsy interval in minutes across different groups. Each box represents the distribution of values within a group: the central line indicates the median, the edges of the box correspond to the first and third quartiles (Q1 and Q3), and the whiskers extend to the smallest and largest values within $1.5 \times \text{IQR}$ (interquartile range). The reference group (Ref) consists of calves exhibiting spontaneous breathing immediately after birth, while Groups 1, 2, and 3 represent the three distinct clusters derived from hierarchical clustering based on autolysis severity (mild, moderate, and severe, respectively).

Kruskal–Wallis analysis ($H(3) = 111.71$, $p \leq 0.0001$, rank eta square = 0.88, 95% CI 0.85–0.92) and Dunn’s post hoc tests showed significant pairwise differences in autolysis scores among the four groups of calves (Figure 3). The total autolysis scores in Groups 1, 2, and 3 were significantly higher than in the Reference group ($p = 0.0011$ for Group 1, and $p \leq 0.001$ for Groups 2 and 3). Additionally, the score in Group 1 was significantly lower compared to Group 2 ($p = 0.014$) and Group 3 ($p \leq 0.001$).

Autolysis score ranges for each group are presented in Table 2. The upper threshold for each group was determined using midpoint of the medians of neighboring groups, with the exception of Group 3, whose upper bound was defined by the maximum score achieved within that group. For each group, the range extends from its minimum value (the lowest autolysis score among individual calves) to the corresponding upper cut-off.

Table 2. Summary of autolysis scoring parameters for Clusters 1–3 and the Reference (Ref) group.

Group (n)	Min–Max	Median	Q1–Q3	Lower–Upper Bound
Ref (32)	0–2	1	0.75–1	0–1.5
1 (45)	0–5	2	2–3	0–7
2 (15)	8–14	12	10–13	8–14
3 (35)	15–16	16	16–16	15–16

Lower bound: the lowest autolysis score of an individual calf. Upper bound: the midpoint between the medians of neighboring groups (with the exception of Group 3, where the upper bound was defined as the maximum score observed in this group).

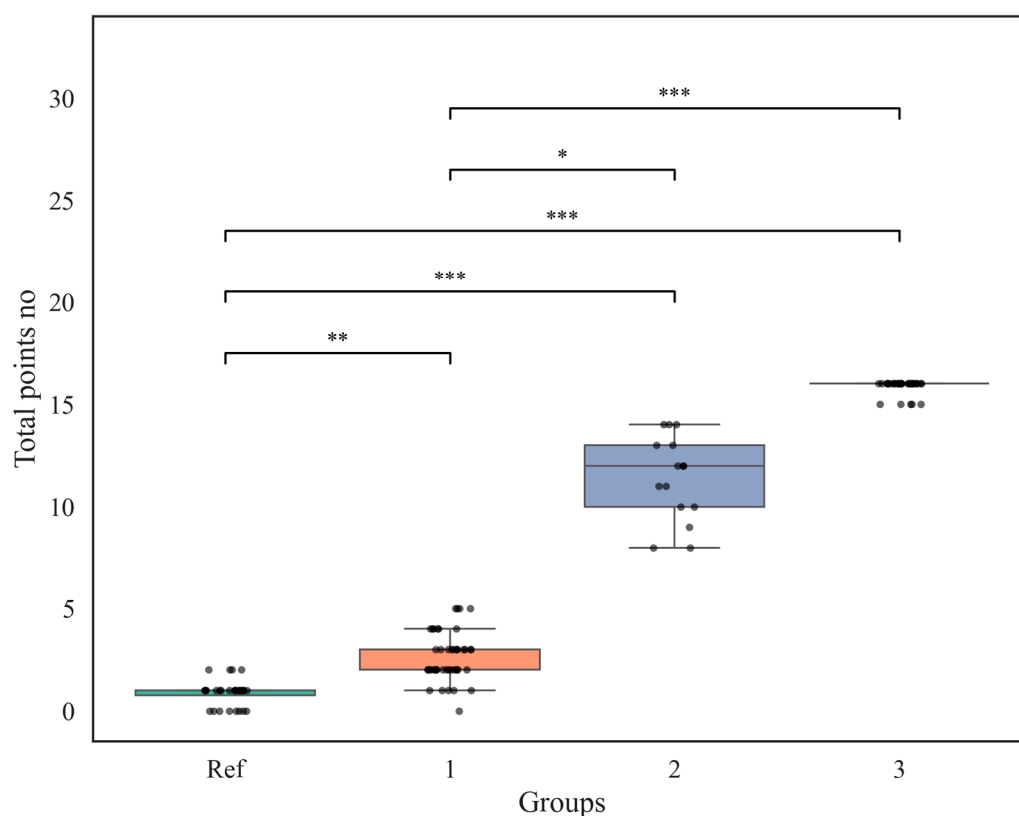


Figure 3. Total autolysis score number in examined groups. The figure shows boxplots with individual data points for the total number of autolysis points across different groups. Each box represents the distribution of values within a group: the central line indicates the median, the edges of the box correspond to the first and third quartiles (Q1 and Q3), and the whiskers extend to the smallest and largest values within $1.5 \times$ IQR (interquartile range). Significant differences are indicated by: * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$.

While the absolute ranges (Min–Max) of the autolysis scores partially overlapped between the Reference (Ref) group (0–2) and Group 1 (0–5), their core distributions remained distinct (Table 2, Figure 3); the interquartile range (IQR) for the Ref group was tightly concentrated between 0.75 and 1 (median = 1), whereas Group 1 exhibited an IQR of 2–3 (median = 2).

To visualize the extent of autolysis across different parameters within each group, a heatmap was generated (Figure 4). The heatmap presents the mean autolysis scores for each scored parameter, with statistically significant post hoc differences between groups indicated, allowing for a clear comparison of the intensity of post-mortem changes between the identified groups.

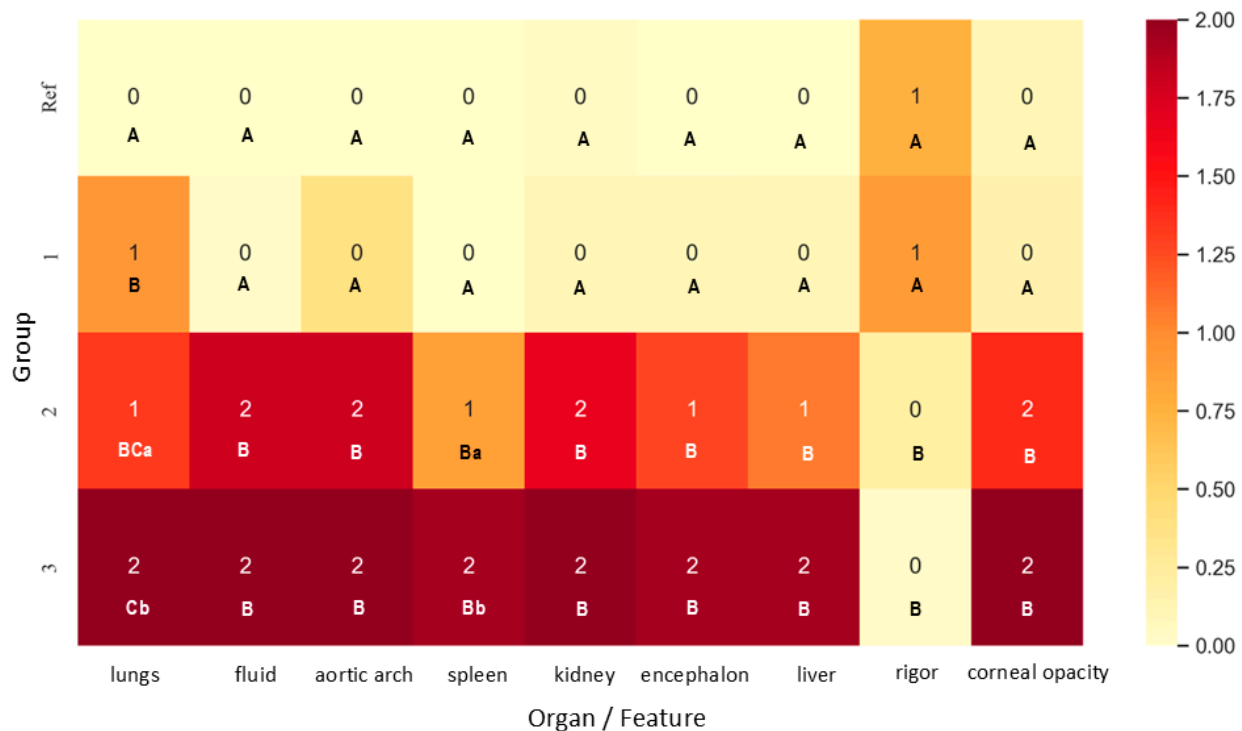


Figure 4. Heatmap of parameter-specific autolysis patterns across the four groups based on mean values (cell color) with median annotations (numbers). Different uppercase and lowercase letters indicate significant differences at $p \leq 0.01$ and $p \leq 0.05$, respectively, within organ/feature, between groups.

Organ-specific autolysis patterns varied substantially across the four groups, as visualized in the heatmap (Figure 4). The Kruskal–Wallis test revealed highly significant differences ($p \leq 0.001$) across all examined organs and features among the four groups. The $H(3)$ statistic was 122.86 for pleural fluid, 121.09 for spleen, 116.19 for kidneys, 115.09 for liver, 114.44 for lungs, 113.11 for encephalon, 108.76 for aorta, 101.54 for corneal opacity, and 70.90 for rigor mortis. Analysis of post-mortem autolysis patterns revealed a clear progression of decomposition changes across the four studied groups, allowing Group 1 to be classified as an “early-stage cluster” where most organs do not yet exhibit measurable alterations (median score of 0) and present a nearly identical profile to the Reference group, with the only statistically significant exception being the presence of lung atelectasis in Group 1 ($p \leq 0.01$). A distinct step change in the decomposition process is represented by Group 2, where autolysis is advanced yet variable across organs; the highest level of decay (median score of 2) is confined to the fluids, aorta, kidneys, and corneal opacity, while the lungs, spleen, encephalon, and liver reach an intermediate level (median score of 1), and rigor mortis completely disappears (0), resulting in significantly higher scores than the Reference and Group 1 ($p \leq 0.01$), except for lung scores when compared to Group 1.

Group 3 reached the most advanced and uniform stage of decomposition, characterized by a median score of 2 across all evaluated parameters and the absence of rigor mortis (0), which represents highly statistically significant changes compared to both the Reference group and Group 1 ($p \leq 0.01$), whereas in direct comparison to Group 2, further significant autolytic progression ($p \leq 0.05$) was observed only within the lungs and spleen.

The seasonal breakdown across the study groups showed that Group 1 comprised 15 cases in winter, 12 in summer, 10 in autumn, and eight in spring. Group 2 had the lowest overall counts, with five winter cases, four spring cases, and three cases in both autumn and summer. In Group 3, the highest frequency was observed during spring ($n = 13$), with fewer cases recorded in winter ($n = 9$), autumn ($n = 8$), and summer ($n = 5$). The Reference group consisted of 12 spring cases, nine cases each in summer and winter, and two in autumn. The analysis revealed no statistically significant association between season and group ($\chi^2(9) = 9.26$, $p = 0.43$). Cramér's V indicated a small-to-moderate effect size ($V = 0.156$, 95% CI [0.137, 0.287]). These results suggest that the degree of autolysis among calves in this study does not differ significantly across seasons.

4. Discussion

Determining the approximate time of death is a critical step for identifying whether a fetus/calf died pre-, intra-, or postpartum [17]. In the available literature, no comprehensive time of death diagnostic protocol based on a scoring system for selected parameters was found. Only one publication was found—a guide describing characteristic features of carcasses depending on the time of death, but it focused on only a few parameters [18]. Most studies do not establish the time of death, and those that do include it do not utilize a scoring scale; instead, they rely on observations of autolytic effusions and the progress of mummification [2], or a subjective assessment of the degree of organ autolysis and lung aeration [19]. In the present study, nine parameters were selected to determine the approximate time of death, and a gradation system was developed, linked to the degree of parenchymal organ autolysis, the state of lung aeration, the presence and nature of fluids in body cavities, organ coloration, and calf visual appearance (corneal opacity and rigor mortis). The selection of parameters for the study was based on literature data (the rate of post-mortem changes) and personal observations made during necropsies.

Autolytic lesions develop within approximately 12 h of death in utero and become more advanced over time [12]. Fetal tissue autolysis manifests as a series of post-mortem changes in the carcass. Following death, the cessation of tissue oxygenation and a drop in pH lead to the release of lysosomal enzymes, triggering autolysis that progresses most rapidly in enzyme-rich organs like the pancreas, kidney, spleen, and lungs. This degenerative process is significantly accelerated by high ambient or body temperatures at the time of death, while it is slower under cool or cold conditions. A key manifestation is the breakdown of erythrocytes, leading to hemolysis and the subsequent reddish-brown “imbibition” of the vessel intima. This characteristic muddy discoloration of the inner vascular layers serves as a distinct marker of post-mortem hemolytic changes [20]. Due to the normal dam body temperature, fetuses retained in utero may exhibit more rapid changes compared to those in an extra-uterine environment, which significantly complicates the identification of the time of death in utero. The analysis of the results in this study demonstrates that neither the time elapsed from birth or death to the post-mortem examination (post-mortem interval) nor the season of birth significantly influenced the degree of autolysis observed in the fetuses. However, this result should not be extrapolated to calves exposed to prolonged extra-uterine environmental conditions, in which ambient temperature may have a greater influence on decomposition.

Fetuses in Group 1, as they were not significantly different from the Reference group, showed no macroscopically detectable tissue autolysis. Based on similar criteria evaluated in the study by Dillman and Dennis [14], autolysis appears several hours post-mortem in lambs (e.g., renal cortex softening as early as 4 h, distinct changes in both the renal cortex and medulla after 16 h, and corneal opacity after 12 h). Therefore, it can be assumed that the calves in this group were in the early stage of autolysis and had not been dead prior to birth for more than 12 h, although these estimations are approximate since tissue decomposition rates can vary across different species. For instance, in lambs, the earliest changes were detected in the brain [14], whereas macroscopically, based on the median in the Group 1, the brain showed no signs of autolysis. In cattle the brain is relatively resistant to autolysis when compared directly with the organs of the abdominal cavity [21]. A time of death no longer than 12 h prior to delivery in Group 1 was also indicated by the absence of hemoglobin imbibition, which is evident from approximately 12 h after death in calves [16].

The cessation of circulation and hemolysis are accompanied by the transudation of fluids into surrounding tissues, and subsequently into the body cavities and subcutaneous tissues. This process begins approximately 16–24 h post-mortem and intensifies, reaching its peak at about 96 h post-mortem. This fluid migration is accompanied by a color change from serous, through serosanguinous, to sanguinous. After this period, at approximately 6 days, a reverse process occurs, whereby fluids leave the body due to the onset of dehydration as a component of mummification [14]. Group 2 showed marked changes—a score of 2 for aortic arch endothelial color, blood-tinged fluids in the body cavities, renal autolysis, and corneal opacification—together with less pronounced changes (score 1) in lung appearance, splenic condition, encephalon, and liver. These findings suggest that the fetuses in this group had been dead for at least 12 h before birth, but no longer than 36 h. It is difficult to define this boundary precisely, since autolysis is a continuous process. In the study by Dillman and Dennis [14], a pinkish discoloration of the lungs was observed 16 h after death. Group 3 showed pronounced and statistically significant autolytic changes across nearly all examined parameters, including larger volumes of bloody fluids in the body cavities and a high level of autolysis in the liver, kidney, and spleen (intensification of splenic autolysis was reported after >36 h in the study by Dillman and Dennis [14]). These findings point towards a prolonged retention of the fetuses within the uterus after death. A high number of points (15–16) on this developed scale suggests at least 36 h; however, the fetal retention time could be even longer (72–168 h). Because autolysis is a continuous process, the boundaries may be blurred.

The uneven distribution of calves within Groups 1–3 could be attributed both to the non-linear nature of autolysis, which accelerates significantly over time and drives more cases into Cluster 3, and to the fact that the majority of mortalities occur around stage II of parturition, resulting in a highly populated Cluster 1.

Based on the results, rigor mortis exhibits the lowest H-statistic value (70.90) among the investigated parameters and disappears in Group 2. Rigor serves as a useful marker only during the early stage (differentiating the Ref/Group 1 from the others), losing its discriminatory power afterward, which accounts for the lower H value. While one may assume the dynamics of rigor mortis have already been documented in bovine perinates, this is not the case. Systematic searching of the literature failed to unearth a single paper detailing this process in this species at this time. Currently we use extrapolations from lamb muscle [22], beef muscle [23] or human babies [24]—none of which may accurately replicate a bovine perinate. In these species there are no recent experimental studies confirming extremely rare reports in ancient texts in this field, e.g., [24]. Even in standard veterinary pathology textbooks [25,26], the authors do not cite primary source data when specifying

the timeframe for rigor mortis development and they emphasize the considerable variation in these timeframes. Our test statistics indicate the limited utility of rigor mortis. This is most likely because rigor mortis disappears in calves that died long before parturition, whereas it remains present in those that died just a few hours prior, as is possible in human fetuses [24]. Consequently, while the presence of rigor mortis may indicate that a maximum of a dozen or so hours has elapsed since death, its absence is highly ambiguous—it can be associated either with a fetus that died very recently (before rigor sets in) or one that died a long time ago (after its resolution). So, rigor mortis is a moderately sensitive but poorly specific parameter to indicate time of death in bovine perinates.

This study had some limitations. While macroscopic assessment of different parameters carries inherent subjectivity—meaning different evaluators may interpret changes differently—and autolytic changes do not always fit discrete categories perfectly, high-resolution reference photographs and explicit visual criteria were incorporated to minimize bias and standardize field application. A notable consideration in the macroscopic evaluation of the lungs is the influence of physiological atelectasis. Unlike pure autolytic tissue decay, lung collapse/non-aeration is primarily a physiological marker reflecting whether a calf took a breath (or was resuscitated), thereby serving as a critical indicator to differentiate pre- and intrapartum mortality from postpartum deaths. When integrated into a composite autolysis score, baseline atelectasis in non-breathing calves may artificially elevate the initial score in fresh carcasses. However, from a practical diagnostic standpoint, combining aeration status with progressive autolytic changes (such as loss of parenchymal structure and progressive softening) provides dual utility: it simultaneously informs on breathing status and carcass degradation. It is possible that in some cases, bacterial involvement may have accelerated autolysis. However, the number of significant bacterial infections in this material was limited to two positive cultures out of 121 PM cases [10]. Although bacterial putrefaction represents a major modifier of post-mortem changes, primary bacterial infections account for only a small proportion of full-term perinatal calf mortalities, affecting up to 10% of cases [11]. Nevertheless, in the presence of lesions suggestive of bacterial infection or a positive culture result, the rate of tissue autolysis may be significantly accelerated. Since the interval from birth to autopsy was similar across all groups, the variation in the severity of autolytic changes primarily reflects differences in the duration of intrauterine retention of the fetuses after their death. This scale should be applied with caution when postpartum calves have spent an extended period in the external environment, as autolysis would then be driven by extra-uterine changes rather than internal processes. In this study, the highest group median was less than 8 h. Nevertheless, due to the lack of independent clinical data regarding the exact time of death of individual fetuses, this novel scoring system should at this stage be treated as a relative indicator of tissue decomposition (a proxy) rather than a tool for precise chronological dating of time of death. Although this study introduces the first standardized macroscopic scoring scale for assessing perinatal autolysis in cattle, further validation across larger, independent datasets under diverse field conditions remains necessary to confirm the diagnostic robustness of the proposed cut-off values. Because our study evaluated dairy calves and dairy-beef crosses, the scale should be validated in pure beef breeds; nevertheless, given similar autolytic progression reported in other ruminants like sheep, substantial differences in beef breed full-term calves are unlikely. In contrast, for aborted fetuses which are frequently severely autolyzed [7], this scoring system may have limited applicability.

5. Conclusions

The developed scoring scale can be used by veterinarians performing necropsies on farm for the preliminary classification of the approximate death timing. In addition, the

numerical scores enable an objective comparison of calf carcass conditions across scientific literature, replacing subjective terms such as “mild”, “moderate” and “advanced” autolysis.

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