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REPVAS
RESEARCH & PRACTICE in VETERINARY & ANIMAL SCIENCE

Volume:3 Issue:2 Year: 2026 | Research Article | ISSN: 3023-6681

Investigation of the Effect of Seawater on the Fixation of Sheep Tissues with Formalin Fixation

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Article Info

Received: 18.03.2026
Accepted: 06.07.2026
Online first: 21.07.2026
Published:

Keywords:
Distilled water,
Ethanol,
Formalin,
Seawater,
Sheep tissue fixation

ABSTRACT

This study investigated the effect of seawater as a diluent in formalin- and ethanol-based fixation solutions on the histological preservation of sheep tissues. Liver, heart, and spleen samples obtained from 40 sheep were fixed using six different solutions prepared with distilled water, seawater, or their combinations. Tissue samples (approximately 3 mm in thickness) were fixed for 24 hours at room temperature, followed by routine histological processing, sectioning at 4 µm, and hematoxylin and eosin (H&E) staining. Histological preservation was evaluated using a semi-quantitative scoring system, and the resulting scores were analyzed using appropriate statistical methods. Formalin-based fixatives demonstrated superior structural preservation compared to ethanol-based solutions. Among the formalin groups, seawater-containing solutions yielded the highest histological scores across all tissues. In contrast, ethanol-fixed tissues exhibited reduced structural integrity and lower preservation scores. These findings suggest that seawater can enhance the efficiency of formalin-based fixation and improve histological outcomes, offering a simple and cost-effective alternative to conventional diluents.

To cite this article:

Ünsaldı, E. (2026). Investigation of the Effect of Seawater on the Fixation of Sheep Tissues with Formalin Fixation, *Research and Practice in Veterinary and Animal Science (REPVAS), 3(2), 95-107.*
https://doi.org/10.69990/REPVAS.2026.3.2.4

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INTRODUCTION

Tissue fixation is a fundamental step in histological and diagnostic studies as it preserves cellular and tissue structures in a state similar to their natural condition. This prevents autolysis and decay (Fox et al., 1985; Arnold et al., 1996; Kmieć, 2016; Karataş & Akcakavak, 2024; Kilinc & Oruc, 2024). Effective fixation maintains macroscopic tissue features and enables high-quality microscopic sectioning and staining, which are both essential for accurate histopathological evaluation. Inadequate fixation can produce artefacts that obscure cellular details, potentially leading to misinterpretation or erroneous diagnoses (Ajileye and Esan, 2022).

The efficiency of fixation is influenced by multiple factors, including pH, fixative concentration, temperature, osmolarity, exposure time, and tissue size (Freida, 2015; Singh et al., 2019). Common fixatives include aldehydes, oxidizing agents, and protein-denaturing chemicals (Thavarajah, 2012; Ekmen, 1992; Ünsaldı and Çiftçi, 2010). Formaldehyde (CH_2O), an aldehyde-based fixative, is most commonly used in a 10% formalin solution, which can preserve 2–3 mm thick tissue slices effectively within 24 hours (Bancroft and Gamble, 2008; Özdemir, 2016). Alcohol-based fixatives such as ethanol are also employed, although their slower tissue penetration may necessitate longer incubation periods (Braet and Ratinac, 2007; Rockey et al., 2009).

Although distilled water is the traditional diluent for fixatives, recent studies have suggested that seawater, due to its mineral and salt content, may improve fixation efficiency (Stowell, 1941; Titford and Horenstein, 2005; Bancroft and Gamble, 2008). However, systematic comparative studies evaluating seawater as a diluent in routine histological fixation remain scarce, particularly in formalin- and ethanol-based fixation solutions across different tissue types. Therefore, the present study aims to address this gap by systematically comparing the effects of seawater and distilled water as diluents on the histological preservation of sheep liver, heart, and spleen tissues.

MATERIAL and METHODS

Liver, heart, and spleen tissues were collected from sheep slaughtered for commercial food production at a licensed abattoir in Ankara, Türkiye. No animals were sacrificed specifically for this study, and no experimental procedures were performed on live animals. Therefore, ethical committee approval was not required in accordance with institutional and national regulations governing the use of slaughterhouse-derived tissues. The liver, heart, and spleen were selected because they represent structurally and functionally distinct parenchymal organs with different vascularity, cellular organization, and tissue architecture, allowing comparison of fixation quality across different tissue types. Fixative solutions were prepared separately for each sample using standard laboratory reagents obtained from commercial sources (Formalin: formaldehyde solution, ~37%, Germany; Alcohol: absolute ethanol, Isolab, Germany).

Six fixation solutions were prepared by combining formaldehyde or ethanol with varying ratios of distilled water and seawater, as described in Table 1. Seawater used in the preparation of selected fixation solutions was obtained from the Mediterranean coastline in the Hatay region of Türkiye. Following collection, the seawater was filtered to remove suspended particles and stored at room temperature until use. To reflect the typical salinity of Eastern Mediterranean seawater and to ensure consistency among all seawater-containing fixation solutions, salinity was adjusted to approximately 38‰ (38 ppt). The ionic composition was not determined analytically in the present study but was described based on the established physicochemical characteristics of Eastern Mediterranean seawater reported in the literature (Krom et al., 1991; Özsoy et al., 1993; Millero et al., 2006). The pH was measured at approximately 8.1, reflecting the typical slightly alkaline nature of Mediterranean seawater. Electrical conductivity ranged between 50 and 60 mS/cm, and density was approximately 1.025 g/cm³, both values consistent with reported characteristics of Eastern Mediterranean seawater.

Table 1. Composition and concentration ratios of fixatives

NBF	**NBF 2**	**NBF 3**
10% NBF	10% NBF	10% NBF
90% distilled water	0% distilled water	45% distilled water
0% seawater	90% seawater	45% seawater
EtOH 4	**EtOH 5**	**EtOH 6**
70% EtOH	70% EtOH	70% EtOH
30% distilled water	0% distilled water	15% distilled water
0% seawater	30% seawater	15% seawater

Note: The ratios of seawater and distilled water used in the preparation of NBF and EtOH solutions are presented (NBF= neutral buffered formalin; EtOH= ethanol).

The ionic composition was predominantly composed of sodium (Na^+) and chloride (Cl^-) ions, along with appreciable concentrations of magnesium (Mg^{2+}), calcium (Ca^{2+}), potassium (K^+), and sulfate (SO_4^{2-}). These ions play an important role in maintaining osmotic balance and may contribute to enhanced tissue preservation by reducing fixation-induced structural alterations. At the time of collection, seawater temperature ranged between 20 and 25°C, corresponding to typical environmental conditions of the Mediterranean coastal region. Overall, the physicochemical properties of the seawater used in this study were consistent with previously reported values for the Eastern Mediterranean (Krom et al., 1991; Millero et al., 2006; Özsoy et al., 1993).

The NBF 1 group (10% neutral buffered formalin diluted with 90% distilled water) was designated as the reference control, as it reflects the conventional fixation method widely accepted as the standard in routine histopathological applications.

Group classification was performed based on fixative category, tissue origin, and formulation of the fixation solutions to ensure consistency and traceability throughout the study. Formalin-containing groups were denoted as NBF (10% neutral buffered formalin), whereas ethanol-based groups were labeled as EtOH. Tissue abbreviations included L for liver, H for heart and S for spleen. Numerical codes ranging from 1 to 6 correspond to the specific solution compositions outlined in Table 1.

Tissue samples were assigned to groups according to the corresponding fixation solutions (Table 2).

Table 2. Tissue grouping and abbreviations for fixatives

Fixatives	**Liver**	**Heart**	**Spleen**
NBF 1	NBF L1	NBF H1	NBF S1
NBF 2	NBF L2	NBF H2	NBF S2
NBF 3	NBF L3	NBF H3	NBF S3
EtOH 4	EtOH L4	EtOH H4	EtOH S4
EtOH 5	EtOH L5	EtOH H5	EtOH S5
EtOH 6	EtOH L6	EtOH H6	EtOH S6

Note: *NBF = neutral buffered formalin; EtOH = ethanol; L = Liver; H = Heart; S = Spleen.*
1=10% NBF + 90% distilled water; 2=10% NBF + 90% seawater; 3=10% NBF + 45% distilled water +45% seawater; 4= 70% EtOH + 30% distilled water; 5=70% EtOH + 30% seawater; 6=70% EtOH + 15% distilled water + 15% seawater

Each sample was trimmed to a uniform thickness of approximately 3 mm, immersed in the designated fixative, and maintained at room temperature for 24 hours. Following fixation, the tissues were rinsed under running tap water for 12 hours to remove residual fixative. Subsequently, the samples underwent routine histological processing, including dehydration through a graded alcohol series and clearing with xylene.

Paraffin-embedded tissues were sectioned at a thickness of 4 μm using a microtome. Sections were stained with hematoxylin and eosin (H&E) following standard protocols (Luna, 1968; Tuzcu et al., 2022; Akcakavak et al., 2023). Histological evaluation was performed using a Leica DM 2500 LED light microscope equipped with a Leica 170 HD digital imaging system (Singapore).

Histopathological assessment was conducted using a semi-quantitative scoring system adapted from Meyerholz et al. (2019). Six parameters—cellular integrity, nuclear detail, cytoplasmic staining, tissue architecture, shrinkage artifacts, and extracellular space expansion—were evaluated using a 4-point ordinal scale (0–3). Scores ranged from 0 (poor) to 3 (excellent), and the total score for each sample was calculated by summing individual parameter values, with higher scores indicating better preservation quality. The scoring criteria are presented in Table 3.

As the assumptions of parametric tests were not met, non-parametric methods were applied. Statistical analysis was performed using the Kruskal–Wallis test, followed by Dunn's post hoc test for multiple comparisons. Histological scores are presented as mean ± standard error (SE) for descriptive purposes, in accordance with recommendations for statistical reporting in veterinary sciences (Selvi, 2024). The Kruskal–Wallis test compares groups based on mean ranks rather than arithmetic means; therefore, all statistical comparisons and significance levels were determined using rank-based analyses. All statistical analyses were performed using GraphPad Prism version 9 (GraphPad Software, USA). Statistical significance was set at *P < 0.05, **P < 0.01 and ***P < 0.001.

Table 3. Criteria used for semi-quantitative histopathological evaluation

Parameter	Score 0	Score 1	Score 2	Score 3
Cellular preservation	Extensive cellular disruption	Marked cellular alterations	Slight cellular changes	Intact cellular morphology
Nuclear clarity	Nuclei not discernible	Poor nuclear definition	Moderately distinguishable nuclei	Clearly defined nuclear structures
Cytoplasmic staining	Minimal or absent staining	Irregular staining pattern	Adequate staining intensity	Homogeneous and intense staining
Structural organization	Complete loss of tissue architecture	Partial disorganization	Largely preserved structure	Fully preserved architecture
Tissue shrinkage	Pronounced shrinkage artifacts	Moderate shrinkage	Minor shrinkage	No shrinkage observed
Interstitial space changes	Marked expansion	Moderate widening	Slight enlargement	No detectable change

Scoring definition:
0 = severe deterioration of tissue morphology
1 = evident structural alterations
2 = mild changes with largely preserved features
3 = optimal preservation with minimal or no artifacts

RESULTS

In this study, tissues from the liver, heart, and spleen tissues obtained from 40 sheep were immersed in different fixation solutions for 24 hours to evaluate the effectiveness of the fixatives. Four hours after immersion, during the trimming procedure, it was observed that the liver tissues in the NBF L1, NBF L2 and NBF L3 groups were easier to section than those in the EtOH L4, EtOH L5 and EtOH L6 groups. Four-micrometre-thick tissue sections were prepared and stained using the haematoxylin and eosin (H&E) method.

Liver Tissue

During trimming, liver tissues fixed in ethanol-based solutions were more difficult to section than those fixed in formalin-based solutions. Histological examination of haematoxylin and eosin

(H&E)-stained sections revealed clearer cellular morphology, as well as better preservation of hepatic cords and sinusoidal structures, in formalin-treated tissues than in ethanol-fixed samples (Figure 1).

Statistical evaluation demonstrated that the fixation efficiency of the NBF L1 group was significantly higher than that of the EtOH L6 group ($P < 0.01$). The NBF L2 group showed a highly significant increase in histological scores compared with the EtOH L6 group ($P < 0.001$). Additionally, the NBF L2 group demonstrated significantly higher scores than the EtOH L5 group ($P < 0.01$). Ethanol groups containing seawater (EtOH L5 and EtOH L6) showed lower histological scores compared to formalin-based fixatives (Table 4).

Figure 1. H&E staining appearance of sheep liver tissue in different fixation solutions (20× magnification). A. NBF L1; B. EtOH L4; C. NBF L2; D. EtOH L5; E. NBF L3; F. EtOH L6 (NBF: neutral buffered formalin, L: liver; 1: 10% formalin solution prepared with distilled water; 2: 10% formalin solution prepared with seawater; 3: 10% formalin solution prepared with a mixture of distilled water and seawater; EtOH: ethanol; 4: 70% EtOH with 30% distilled water; 5: 70% EtOH with 30% seawater; 6: 70% EtOH with 15% distilled water and 15% seawater).

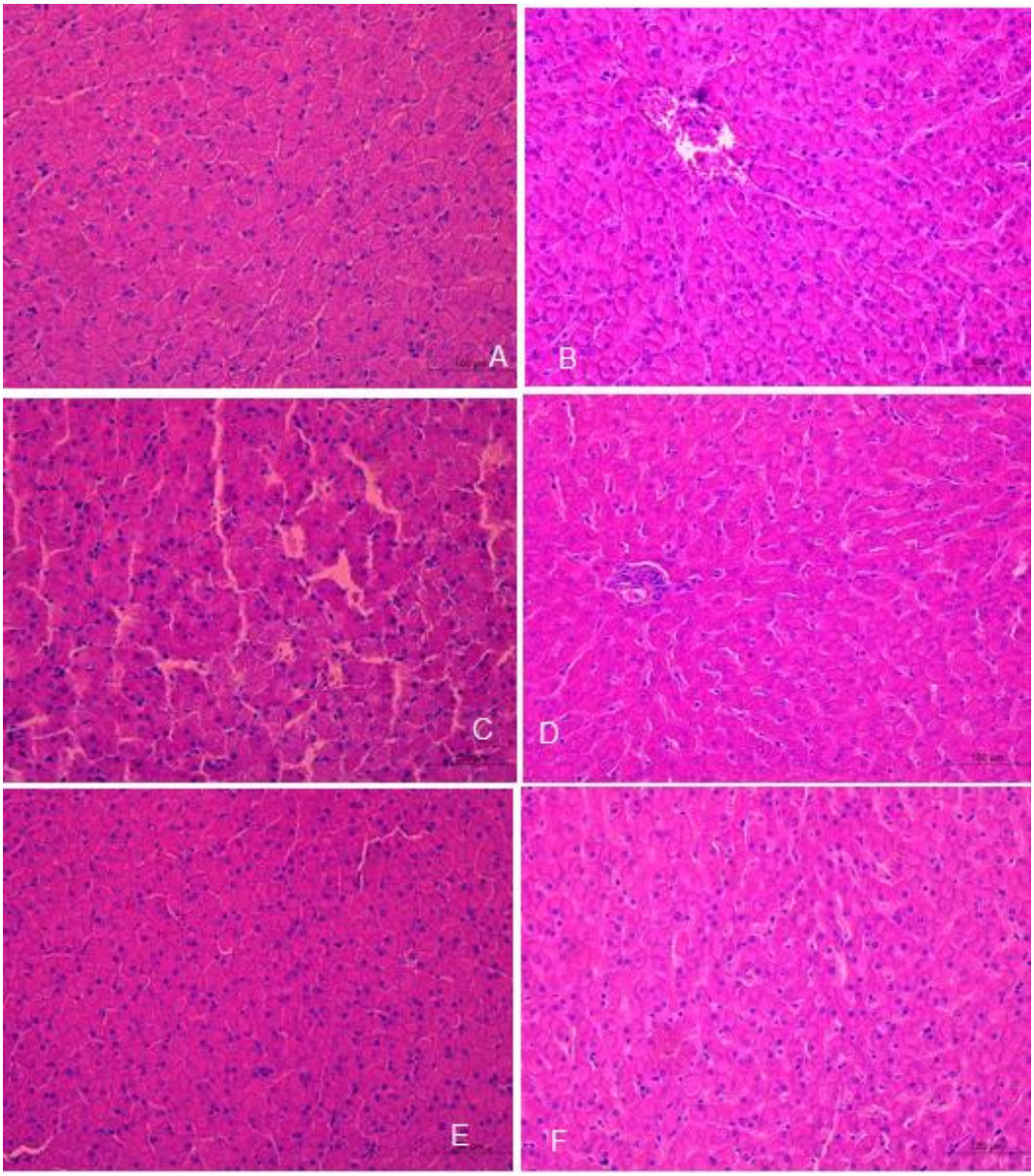


Table 4. Histological scores and statistical comparisons for liver, heart, and spleen tissues

Tissue	Fixative	Mean Histological Score ± SE	Statistical Significance vs. EtOH
Liver	NBF L1	8.2 ± 0.13	P < 0.01 vs. EtOH L6
	NBF L2	8.8 ± 0.10	P < 0.001 vs. EtOH L6; P < 0.01 vs. EtOH L5
	NBF L3	8.0 ± 0.17	NS
	EtOH L4	6.1 ± 0.20	–
	EtOH L5	6.3 ± 0.17	–
	EtOH L6	5.8 ± 0.13	–
Heart	NBF H1	8.0 ± 0.13	P < 0.05 vs. EtOH H6
	NBF H2	8.5 ± 0.10	P < 0.01 vs. EtOH H4/H5
	NBF H3	8.1 ± 0.17	NS
	EtOH H4	6.2 ± 0.13	–
	EtOH H5	6.4 ± 0.10	–
	EtOH H6	5.9 ± 0.17	–
Spleen	NBF S1	8.0 ± 0.13	P < 0.05 vs. EtOH S6
	NBF S2	8.6 ± 0.10	P < 0.001 vs. EtOH S6; P < 0.01 vs. EtOH S5; P < 0.05 vs. EtOH S4
	NBF S3	8.1 ± 0.17	P < 0.05 vs. EtOH S6
	EtOH S4	6.0 ± 0.13	–
	EtOH S5	6.3 ± 0.17	–
	EtOH S6	5.7 ± 0.10	–

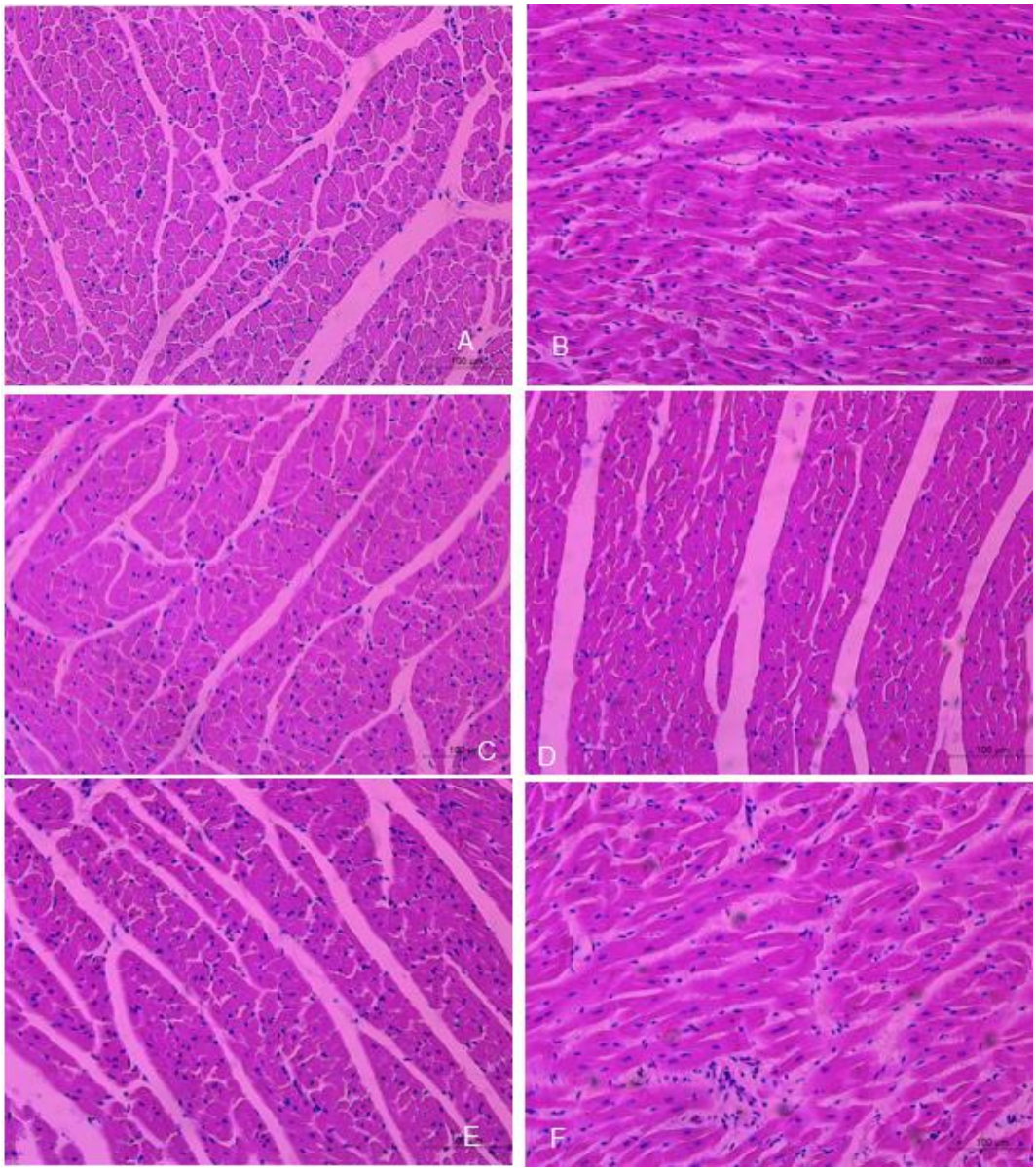
Note: Histological scores were obtained using the semi-quantitative scoring system described by Meyerholz et al. (2009). Data are presented as mean ± standard error (SE) for descriptive purposes. As the assumptions of parametric tests were not met, statistical comparisons were performed using the Kruskal–Wallis test followed by Dunn's post hoc test. Because the Kruskal–Wallis test is based on rank comparisons, all statistical inferences and reported P values were derived from rank-based analyses rather than arithmetic means. NBF = neutral buffered formalin; EtOH = ethanol; L = liver; H = heart; S = spleen.

Heart Tissue

Compared with ethanol-based fixatives, heart tissues fixed with NBF-based solutions demonstrated improved handling characteristics during trimming and superior preservation of myocardial architecture. Histological evaluation confirmed greater tissue integrity and improved staining quality in formalin-treated samples (Figure 2).

Statistical analysis revealed a significant difference between the NBF H1 and EtOH H6 groups ($P < 0.05$). Furthermore, the NBF H2 group demonstrated significantly higher preservation scores than the EtOH H4 and EtOH H5 groups ($P < 0.01$) (Table 4).

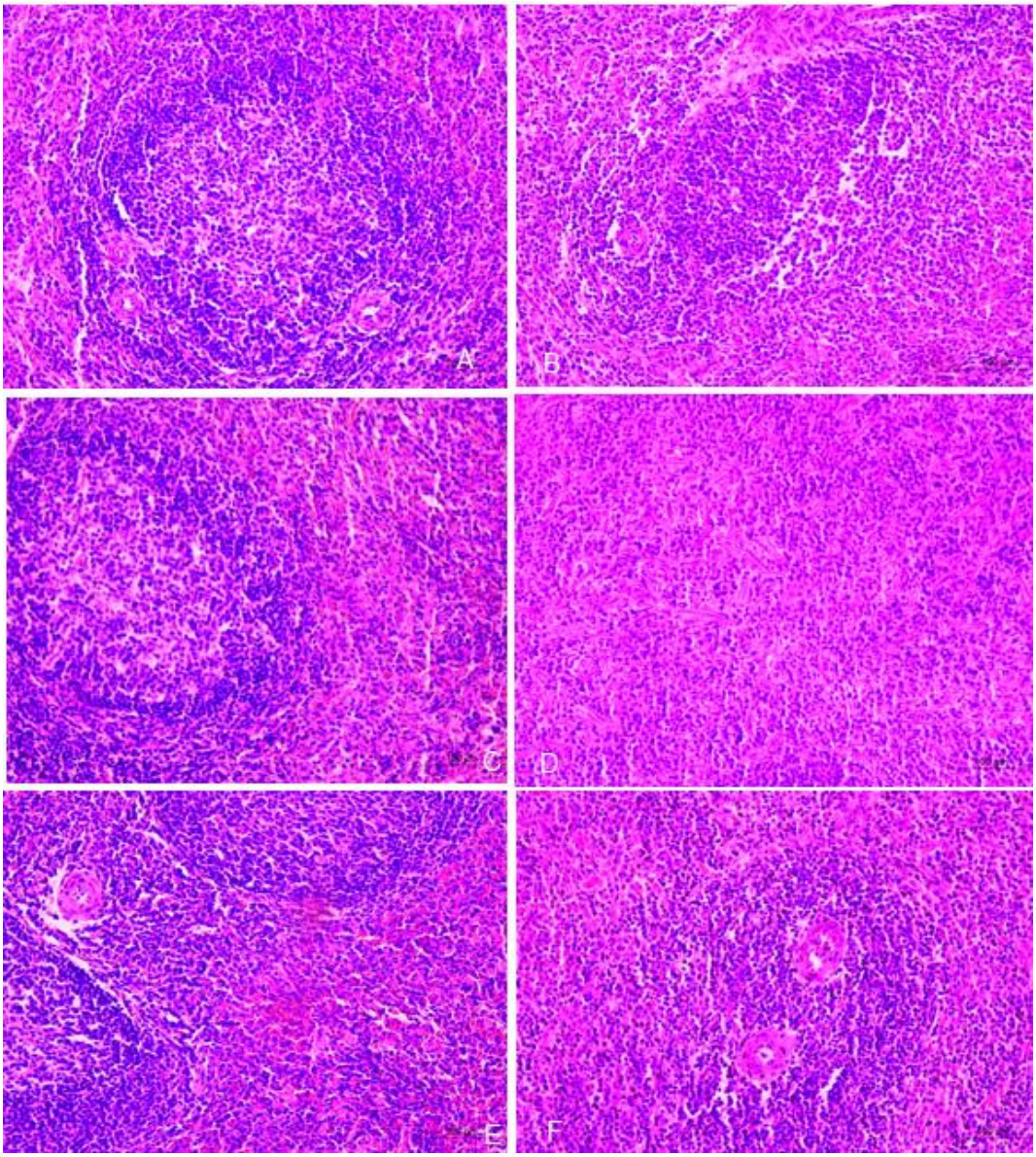
Figure 2. H&E staining appearance of sheep heart tissue in different fixation solutions (20× magnification). A. NBF H1; B. EtOH H4; C. NBF H2; D. EtOH H5; E. NBF H3; F. EtOH H6. (NBF: neutral buffered formalin; H: heart; 1: 10% formalin solution prepared with distilled water; 2: 10% formalin solution prepared with seawater; 3: 10% formalin solution prepared with a mixture of distilled water and seawater; EtOH: ethanol; 4: 70% EtOH with 30% distilled water; 5: 70% EtOH with 30% seawater; 6: 70% EtOH with 15% distilled water and 15% seawater).



Spleen Tissue

Spleen tissues fixed in formalin solutions were firmer during trimming and showed better preservation of splenic architecture, including clearly distinguishable red and white pulp regions, than ethanol-fixed tissues (Figure 3).

Figure 3. H&E staining appearance of sheep spleen tissue in different fixation solutions (20× magnification). A. NBF S1, B. EtOH S4; C. NBF S2, D. EtOH S5; E. NBF S3; F. EtOH S6. (NBF: neutral buffered formalin; S: spleen; 1: 10% formalin solution prepared with distilled water; 2: 10% formalin solution prepared with seawater; 3: 10% formalin solution prepared with a mixture of distilled water and seawater; EtOH: ethanol; 4: 70% EtOH with 30% distilled water; 5: 70% EtOH with 30% seawater; 6: 70% EtOH with 15% distilled water and 15% seawater).



Statistical evaluation revealed that the NBF S1 group had significantly higher scores than the EtOH S6 group ($P<0.05$). The NBF S2 group showed highly significant differences compared to the EtOH S6 group ($P<0.001$), as well as significant differences compared to the EtOH S5 and EtOH S4 groups ($P<0.01$ and $P<0.05$, respectively). Furthermore, the NBF S3 group demonstrated improved fixation quality compared to the EtOH S6 group ($P<0.05$) (Table 4).

Overall, seawater-containing formalin solutions, particularly the NBF 2 groups, showed higher histological preservation scores across liver, heart, and spleen tissues than ethanol-based fixation solutions, indicating a generally favorable effect of seawater on tissue fixation quality.

DISCUSSION

Effective tissue fixation is essential for preserving structural integrity and preventing autolysis, thereby ensuring a reliable histological evaluation. Previous studies have highlighted differences in the efficiency of fixation between formalin- and alcohol-based fixatives. For example, Haque et al. (2020) compared the fixation of liver, brain, and spleen tissues for 24 hours using alcohol-based fixatives and formalin. They found that alcohol fixation preserved cytoplasmic details and overall tissue morphology better than formalin fixation, which occasionally resulted in structural alterations. Similarly, Rahman et al. (2022) concluded that, for certain tissue types, alcohol-based fixatives could serve as a superior alternative to formalin, particularly for preserving delicate structures and minimising fixation artefacts. In contrast to these reports, the present study demonstrated superior histological preservation with formalin-based fixatives after 24 hours of fixation. Formalin-treated tissues exhibited faster and more complete fixation, maintained structural details more effectively, and were easier to trim, whereas tissues fixed with 70% ethanol were softer and showed minor microvoid formation, likely due to lipid extraction. This discrepancy may be explained by differences in tissue type, fixation duration, ethanol concentration, and experimental protocols. Although ethanol fixation did not leave dye residues in the tissues, a longer exposure time was required to achieve comparable preservation. These findings are consistent with previous reports indicating that alcohol-based fixation generally requires longer incubation periods to achieve fixation quality comparable to that of formalin (Fox et al., 1985; Harper et al., 1985).

It has been reported that the high ionic strength of seawater, mainly due to sodium (Na^+) and chloride (Cl^-) ions, contributes to maintaining osmotic balance between extracellular and intracellular compartments, thus limiting cellular swelling or shrinkage during fixation (Kmieć, 2016; Bancroft & Gamble, 2008). In this study, the use of seawater as a diluent for formalin significantly improved tissue preservation. The improved fixation observed in seawater-based formalin solutions can be explained by the physicochemical properties of seawater.

In contrast, distilled water lacks ionic content and can create a hypotonic environment, potentially leading to osmotic stress and structural degradation. Furthermore, the presence of divalent ions such as calcium (Ca^{2+}) and magnesium (Mg^{2+}) can further stabilize cellular membranes and protein structures by regulating electrostatic interactions and reducing excessive protein denaturation. These ions can also enhance tissue integrity by promoting smoother cross-linking during formaldehyde fixation (Fox et al., 1985; Titford, 2009). Consistent with the literature, the results of the present study demonstrated that seawater-based dilutions may facilitate improved penetration and fixation efficiency compared with distilled water.

It has been reported that pH is an important factor influencing fixation efficiency, formaldehyde stability, protein cross-linking reactions, and staining quality (Kmieć, 2016; Bancroft and Gamble, 2008). Seawater typically has a slightly alkaline pH, whereas distilled water is closer to neutral. In the present study, the improved fixation quality observed in seawater-based formalin solutions may therefore be partly associated with the slightly alkaline nature of seawater. This pH-related effect, together with the ionic composition of seawater, may have contributed to better tissue preservation and improved histological appearance compared with distilled water-based solutions.

In addition to these physicochemical effects, the tissue-specific differences observed in the present study may also be related to the structural characteristics of each organ. The liver has a highly vascular and parenchymatous architecture, the heart contains dense and regularly arranged muscle fibers, and the spleen has a delicate lymphoid and vascular organization. Therefore, the response of these tissues to different fixative solutions may vary depending on cellular density, extracellular matrix composition, and vascular spaces, as previously reported in the literature (Bancroft and

Gamble, 2008; Freida, 2015; Kmieć, 2016). In this context, the better preservation observed in seawater-containing formalin groups suggests that seawater may contribute not only to osmotic stabilization but also to more uniform fixation across tissues with different histological architectures. This may be particularly useful in routine anatomical and histological studies where multiple tissue types are processed under the same fixation conditions. Solutions of NBF prepared with seawater outperformed those diluted with distilled water, suggesting that the salt and mineral content of seawater may enhance the penetration of formaldehyde or influence the osmotic balance, thereby facilitating more efficient fixation (Stowell, 1941; Titford and Horenstein, 2005; Bancroft and Gamble, 2008). This is consistent with the findings of Stowell (1941), who reported that isotonic saline improved the dilution of formalin for rabbit kidney tissues and reduced tissue swelling. By systematically comparing distilled water and seawater dilutions, this study provides evidence to support the potential benefits of using seawater as an alternative fixative diluent, a topic that has received limited attention in the existing literature.

Previous studies have reported that appropriate fixative composition and ionic conditions play an important role in preserving tissue architecture and minimizing fixation-related artifacts (Stowell, 1941; Titford and Horenstein, 2005; Bancroft and Gamble, 2008). Consistent with these reports, histological scoring in the present study demonstrated that seawater-containing neutral buffered formalin consistently produced higher preservation scores than formalin diluted with distilled water or ethanol-based solutions. Red and white pulp structures in the spleen, myocardial architecture in the heart, and hepatocyte morphology in the liver were all better preserved in the seawater-containing formalin groups. Statistical analysis confirmed these observations, revealing significant differences ($P<0.05$ to $P<0.001$) between the seawater-enhanced formalin groups and their counterparts.

The slower fixation rate observed in alcohol in this study is consistent with previous reports indicating that alcohol attracts intracellular water, resulting in tissue shrinkage and potential microstructural alterations (Harper et al., 1985). Xu and Rogers (1993) demonstrated the presence of residual formaldehyde in fish tissues up to 72 hours after treatment. In contrast, ethanol fixation leaves no chemical residues, which emphasises alcohol's suitability in contexts where residual aldehydes must be minimised. In this study, however, ethanol fixation at a concentration of 70% was insufficient to fully stabilise the tissues within 24 hours, suggesting that longer incubation periods are necessary to achieve comparable histological quality.

The relatively lower fixation performance observed in ethanol-based groups may be attributed to its mechanism of action. Ethanol induces protein coagulation primarily through dehydration, which can result in tissue shrinkage and the formation of microstructural artifacts (Bancroft & Gamble, 2008; Kmieć, 2016). In addition, its penetration characteristics differ from those of formaldehyde, potentially leading to less uniform fixation within the tissue matrix (Fox et al., 1985). Previous studies have reported that alcohol-based fixatives may require longer exposure times to achieve comparable structural preservation, particularly in thicker tissue samples (Titford, 2009). These findings are consistent with the present study, in which 70% ethanol was insufficient to ensure optimal fixation within 24 hours.

Overall, the present findings have several practical implications for routine histological practice. Formalin remains the faster and more robust fixative for routine histological studies, particularly when rapid processing is required. Incorporating seawater into formalin solutions could improve fixation efficiency and tissue preservation, offering a cost-effective and easily accessible alternative to standard distilled water dilutions. Although slower, alcohol-based fixatives offer the advantage of leaving no reactive residues and may be preferable in situations where chemical interference must be minimised.

To our knowledge, this is one of the first studies to systematically evaluate the use of seawater as a diluent in the formalin fixation of sheep tissues. Direct comparisons between the present findings

and previous studies remain limited because experimental investigations specifically evaluating seawater as a formalin diluent are scarce. Therefore, the discussion of the present findings was primarily based on established physicochemical principles of tissue fixation and on studies addressing formalin fixation, saline-based diluents, and alcohol-based fixatives. Future studies should investigate different seawater concentrations, additional tissue types, longer fixation periods, and the chemical interactions between seawater ions and formaldehyde molecules to enable more comprehensive comparisons and to clarify the mechanisms underlying improved fixation efficiency.

One limitation of this study is that it only evaluated the effects of fixation at the 24-hour time point. Future studies including longer fixation durations and additional tissue types would clarify the applicability of seawater-based dilutions further.

CONCLUSION

In conclusion, formalin-based fixatives remain superior in terms of fixation speed and structural preservation. The incorporation of seawater as a diluent improved histological preservation and fixation quality in formalin-based fixation groups compared with distilled water and ethanol-based fixation solutions. Although ethanol-based solutions require longer incubation periods to achieve comparable results, they offer the advantage of minimizing chemical residues. These findings provide practical insights for routine histological applications and suggest that the use of seawater represents a simple, cost-effective strategy to improve fixation quality in laboratory settings. This approach may be particularly useful in resource-limited laboratories and during field necropsies where access to conventional laboratory-grade diluents may be limited.

Acknowledgements

The author sincerely thanks everyone who contributed to the successful completion of this study for their valuable support and assistance.

Ethics Approval

Liver, heart, and spleen tissues were collected from sheep slaughtered for commercial food production at a licensed abattoir in Ankara, Türkiye. No animals were sacrificed specifically for this study, and no experimental procedures were performed on live animals. Therefore, ethical committee approval was not required in accordance with institutional and national regulations governing the use of slaughterhouse-derived tissues.

Funding

There is not any funding in this study

Conflict of Interest

Authors declare that there is no conflict of interest.

Author Contributions

Research Design (CRediT 1) Author (%100)
Data Collection (CRediT 2) Author (%100)
Research - Data analysis - Validation (CRediT 3-4-6-11) Author (%100)
Writing the Article (CRediT 12-13) Author (%100)
Revision and Improvement of the Text (CRediT 14) Author (%100)

Sustainable Development Goals (SDG)

3 Good Health and Well-Being

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