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Implementation of a New Solution for the Preservation of Anatomical Specimens Made of Non-Toxic Substances

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Abstract

The preservation of anatomical pieces in Veterinary Anatomy is essential since it is not possible to dissect all domestic species. Most techniques use reagents with high levels of toxicity such as formaldehyde. The objective of this work was to develop a new preservation technique that uses reagents with zero toxicity and that allows obtaining preserved pieces suitable for anatomical studies. The alcohol propylene glycol technique was developed, the method of which uses a fixation step with alcohol, sodium chloride, commercial vinegar and subsequently the impregnation of the preservation solution made from propylene glycol and commercial vinegar, which are non-toxic. As a result of this work, adequately preserved sheep hearts were obtained that preserved their morphology with slight changes in size and weight, which did not affect their external and internal anatomical structure. Its coloration was not substantially affected, remaining a little lighter. The pieces obtained showed flexibility which allowed dissections to be carried out. The time to develop the technique was 20 days. A comparative study was carried out with the phenolated glycerin technique that uses toxic reagents (formaldehyde and phenol) and the pieces obtained with the alcohol propylene glycol technique were of better quality, observing that the pieces with phenolated glycerin tend to darken and are more rigid. And the time to develop the technique is 24 days. In conclusion, a preservation technique for anatomical pieces was developed that allowed the preservation of the organs under study, which allow their use for anatomical studies, and which have been preserved without changes until the time of this publication (8 months) and there are pieces preserved with this technique for 2 years.

Keywords

Veterinary Anatomy, Heart, Preservation Techniques, Alcohol Propylene Glycol Technique, Fixation, Necropsy, Toxic Chemicals

1. Introduction

In order to achieve a solid training of professionals in veterinary medicine and zootechnics, it is essential to know the anatomy of domestic animals. In the field of veterinary anatomy, dissection is the most fundamental learning activity, but it is complex due to the difficulties accessing to cadavers of all domestic species. This problem has been mostly solved, to a large degree, by using didactic material consisting of preserved anatomical pieces [1]. The ideal preservation technique for anatomical pieces seeks to maintain biological tissues and organs in adequate conditions, that do not enter a state of decomposition, that maintain the characteristics of the postmortem tissue and that uses non-toxic reagents both for those who apply the technique and for those who use the techniques. pieces preserved by this technique within its use in areas such as medical education, scientific research and museum exhibition.

Formaldehyde has traditionally been used for organ preservation, since it offers good tissue fixation, is economical and easy to use in aqueous solutions at different percentages (aqueous formalin), nonetheless, it also possesses a considerable risk due to its toxicity. The most common symptoms of formaldehyde exposure are eye, nose and throat irritation and it is currently listed as a carcinogen in humans and animals (International Agency for Research on Cancer, IARC; National Toxicology Program of the United States, NTP).

Several techniques have proven to be effective for the preservation of anatomical specimens, such as glycerin, insufflation [2] and the modified Larssen's solution [3], however, all of these techniques use aqueous formalin during the fixation stage of the procedure. Other preservation methods seek to reduce the toxicity of the preservation processes by using aqueous formalin and phenol at low concentrations in solutions with different mixtures of alcohols, glycerin, and salts such as sodium chloride, sodium nitrate, benzalkonium chloride [3] [4] [5] [6] [7] [8]. Formaldehyde-free techniques, such as alglifen, use alcohol, glycerin, and phenol-based solutions for the preparation of preserved pieces of soft texture [9]. As for preservation techniques of rigid specimens, the plastination method described and developed by Von Hagens [10] remains the gold standard, either in the original form or one of its variants in which flexible plastinated parts are obtained [11] [12] [13] [14].

The importance of the present study was to develop a preservation method that allows obtaining anatomical pieces with characteristics similar to those of an organ recently obtained from the corpse where the shape and structure of the organ is not altered to carry out anatomical studies and that does not present

changes due to entering in a state of decomposition for a considerable time, with the use of reagents with zero toxicity, low cost, easy to apply and free of vapor emission. The objective of present work was to propose a new technique that employs several non-toxic reagents that have proven their effectiveness in obtaining anatomical pieces with a long useful life, preserving the shape and structure of the fresh organs.

The nomenclature used in this work is in accordance with the Nomina Anatomica Veterinaria [15].

2. Materials and Methods

2.1. Biological Material

Seven sheep fresh hearts from animals destined for slaughter were washed with water to remove the remains of blood and to dissect the structures that make up their external anatomy, including the aorta artery, pulmonary trunk, caval veins (cranial and caudal) and pulmonary veins. The sheep heart is a good anatomical model due to its anatomical similarity to the heart of other animal species. Due to its size, it is easy to manipulate and obtain from animals for slaughter, thus avoiding bioethical problems, which makes them attractive for scientific research and medical education.

2.2. Characterization

The hearts were weighed, and their measures taken at 2 sites: at the circumference of the coronary sulcus and at the junction of the paraconal sulcus with the coronary sulcus down to the apex of the heart on the atrial surface, to determine the preservation of the initial conditions of weight in grams and dimensions in centimeters, prior to processing of the pieces.

2.3. Preservation Techniques

The phenolated glycerin technique was used as reference, for which three hearts were fixed in a 7% aqueous formalin solution for 72 h. After fixation, the hearts were removed from the solution and washed with running water for 24 hours. Subsequently, they were impregnated with a 10% phenol solution in glycerin (phenolated glycerin). During the processing time the pieces were covered with cotton cloths saturated with the phenolated glycerin solution for 21 days and massaged daily for 20 min for one week. At the end of the processing time, each piece was removed, drained, and kept covered in dry, clean cotton cloths in closed plastic bags at room temperature, until they were used [2].

For the technique developed and evaluated in this study, named alcohol propylene glycol technique (developed in Facultad de Estudios Superiores Cuautitlán UNAM), four hearts were fixed in a solution of 50% alcohol, 10% sodium chloride and 10% commercial vinegar for 10 days at room temperature. Subsequently, the pieces were removed, and excess solution was eliminated using cotton cloths and were then immersed in a solution of 90% propylene glycol and

10% commercial vinegar for 10 days at room temperature for impregnation. At the end, the pieces were removed, drained, and dried with cotton cloths and subsequently kept inside zip-lock bags until they were used.

To compare the characteristics of the pieces processed by the techniques used during the present study, the following aspects were evaluated: color, weight, size, structural change, odor, texture (flexibility: hardness, adhesiveness, and deformability) as well as presence or absence of signs of decomposition/putrefaction. Color was determined by evaluating the color space L, a and b on the CIELAB scale (Commission Internationale de L'Éclairage, CIE) using a Minolta CR-400 colorimeter, while a Brookfield texturometer, CT3 texture analyzer, was used for texture evaluation.

3. Results

Table 1 shows the weight and measurements of the base and length of the hearts treated with the glycerin technique.

Table 2 shows the weight and measurements of the base and length of the hearts treated with the alcohol propylene glycol technique. The time allocated for each technique was of 24 days for the phenolated glycerin technique and of 20 days for the alcohol propylene glycol technique.

Table 1. Weight, base and length measurements of the hearts preserved with the phenolated glycerin technique.

Heart	Before technique			After technique		
	Weight (g*)	Base (cm*)	Length (cm)	Weight (g)	Base (cm)	Length (cm)
1	218	22	11	226	21.5	10.5
2	240	22	11	247	20.5	10.5
3	190	20	11	193	20.5	10
Average	216	21.33	11	222	20.83	10.33

*g: gram; cm: centimeter.

Table 2. Weight, base, and length measurements of the hearts preserved with the alcohol propylene glycol technique.

Heart	Before technique			After technique		
	Weight (g)	Base (cm)	Length (cm)	Weight (g)	Base (cm)	Length (cm)
1	223	18	12	215	22	11.5
2	220	21	11	216	19	10.5
3	180	17.5	11	169	17	10.5
4	214	21	10.5	205	20.5	9.5
Average	209.3	19.38	11.13	201.3	19.63	10.5

The sheep hearts preserved using either of the two techniques maintained their morphological characteristics. Figure 1 shows a photograph of 3 sheep hearts, prior to the application of the preservation techniques, Figure 1(A) preserved with the phenolated glycerin technique, Figure 1(B) and preserved with the alcohol propylene glycol technique, Figure 1(C). As can be seen, with both preservation techniques, the general morphology of the organ was maintained; the overall color of the organ was darker with the phenolated glycerin technique and lighter with the alcohol propylene glycol technique. Using the phenolated glycerin technique, with the passage of time the organ becomes darker, while the specimens treated with the alcohol propylene glycol technique maintain their original coloration since their preparation.

Figure 2 shows a heart (atrial surface) preserved with the phenolated glycerin

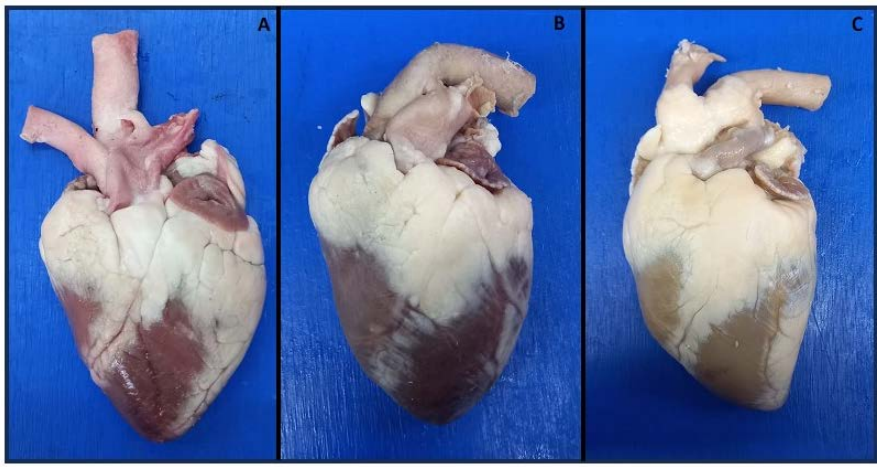

Figure 1. Sheep hearts, auricular surface. (A): prior to the application of preservation techniques; (B): preserved with the phenolated glycerin technique; (C): preserved with the alcohol propylene glycol technique.

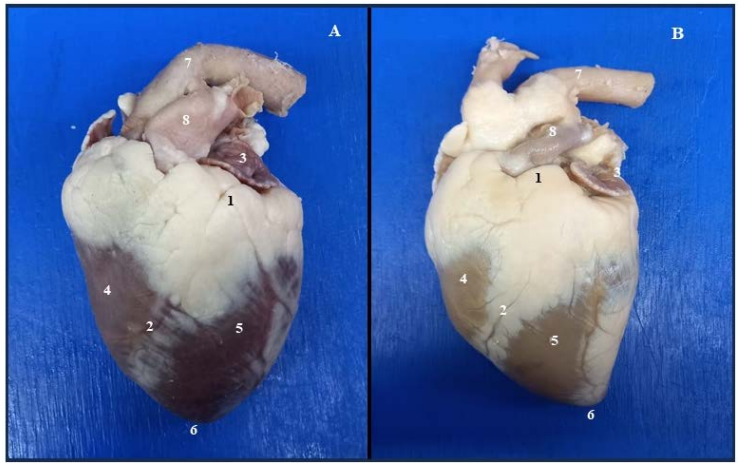

Figure 2. Sheep hearts preserved with the phenolated glycerin technique (A) and with the alcohol propylene glycol technique (B). Auricular surface. Coronary sulcus (1), paraconal interventricular sulcus (2), auricle of left atrium (3), right ventricle (4), left ventricle (5), apex (6), aorta artery (7), pulmonary trunk artery (8) are indicated.

technique, Figure 2(A) and another preserved with the alcohol propylene glycol technique, Figure 2(B). In both hearts the coronary and paraconal sulci can be observed, as well as the left atrium, the ventricles (right and left) and the apex. The aorta and pulmonary trunk arteries can also be seen.

Figure 3 shows a heart (atrial surface) preserved with the phenolated glycerin technique, Figure 3(A) and another preserved with the alcohol propylene glycol technique, Figure 3(B). In both hearts the coronary and subsinusal sulci can be observed, as well as the atria (right and left), the ventricles (right and left) and the apex. The aorta artery, the cranial cava, the caudal cava, and the pulmonary veins are also visible.

A heart preserved with each technique was selected and dissected so as to observe the internal structure. Figure 4 shows the internal structure of a sheep heart preserved with the alcohol propylene glycol technique. The epicardium, myocardium and endocardium can be observed, and it is also possible to clearly identify the following structures: right atrioventricular valve (tricuspid, right ventricle) and left atrioventricular valve (bicuspid, left ventricle), the pectinate muscles (atria), the chordae tendineae, papillary muscles, septomarginal trabecula, and the trabeculae carneae (ventricles). In the right ventricle, the opening of the pulmonary trunk along with the pulmonary valve can also be identified, as are the aortic opening along with the aortic valve in the left ventricle. As with the phenolated glycerin technique, the morphology and structure of all parts of the heart are adequately preserved by the alcohol propylene glycol technique.

There was an increase in the average weight of the hearts treated with the phenolated glycerin technique (the weights before and after being 216 g vs 222 g

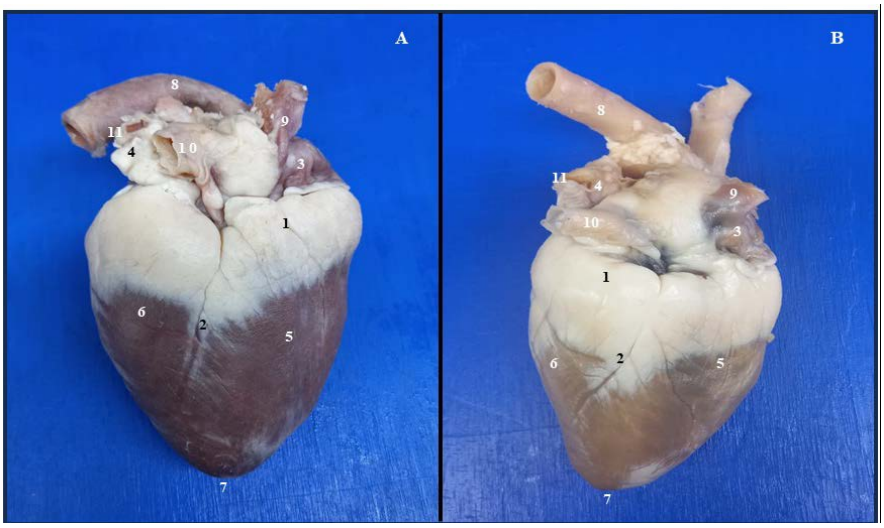

Figure 3. Sheep hearts preserved with the phenolated glycerin technique (A) and with the alcohol propylene glycol technique (B). Atrial surface. Coronary sulcus (1), subsinusal interventricular sulcus (2), right atrium (3), left atrium (4), right ventricle (5), left ventricle (6), apex (7), aorta artery (8), cranial vena cava (9), caudal vena cava (10), pulmonary veins (11) are indicated.

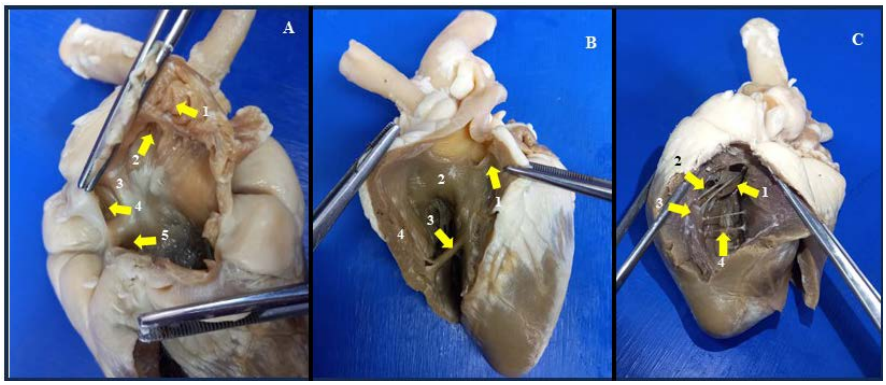

Figure 4. Internal anatomy of the ovine heart preserved with the alcohol propylene glycol technique showing the preservation of various internal structures. Right atrium (A): 1. pectinate muscles; 2. opening of cranial vena cava; 3. intervenous tubercle; 4. opening of caudal vena cava; 5. coronary sinus. Right ventricle (B): 1. pulmonary valve; 2. supraventricular crest; 3. right septomarginal trabecula; 4. myocardium. Left ventricle (C): 1. left atrioventricular (bicuspid) valve; 2. chordae tendineae; 3. papillary muscle; 4. left septomarginal trabeculae.

respectively); also, a small decrease in heart size (base circumference and length) was observed, the measurements before and after processing being 21.33 cm vs 20.83 cm in base diameter and 11 cm vs 10.33 cm in length. In the case of the alcohol propylene glycol technique a decrease in heart weight was observed, from 209.3 g to 201.2 g, before and after respectively. Regarding heart size, an increase in base circumference was noted, from 19.38 cm to 19.63 cm, as well as a decrease heart length, from 11.13 cm to 10.5 cm.

There were no noteworthy changes in the coloration of the hearts that could affect the study of the anatomy of this organ (both before and after applying the technique and comparatively between the two techniques) this characteristic was evaluated mainly at the level of the myocardium. The presence of odors indicating that the organs entering a state of decomposition were not detected, nor were irritating or unpleasant odors related to the reagents used.

The color space on the CIELAB scale was evaluated, which allows to consistently correlate numerical color values with human visual perception by colorimetry for comparison of preservation techniques and fresh organ. L. luminosity, a. geographic coordinate difference in red and green, b. coordinate difference in yellow and blue ΔE. total color difference. The specimens processed using the alcohol propylene glycol technique maintained the parameters of luminosity and total color difference with a smaller difference than those processed using the phenolated glycerin technique, which exhibited greater opacity and darkening (**Table 3**).

The textural properties as determined by the hardness characteristics of the samples after the application of the techniques shows the hearts preserved with the alcohol propylene glycol technique are less hard and more flexible than those prepared by the phenolated glycerin technique, which presented had a higher percentage of hardness (**Table 4**).

Table 3. Color determination*.

	L	a	b	ΔE
Phenolated glycerin technique	37.74 ± 0.1	5.20 ± 0.01	6.36 ± 0.11	42.13 ± 0.12
Alcohol propylene glycol technique	41.46 ± 0.16	3.13 ± 0.04	14.38 ± 0.11	37.03 ± 0.17
Natural organ	41.27 ± 1.5	11.85 ± 0.71	7.75 ± 0.46	39.7 ± 1.51

*Left ventricle atrial surface.

Table 4. Textural properties.

	Hardness	Deformation	Adhesive strenght
	N	%	N
Phenolated glycerin technique	14.02 ± 2.56	31.97 ± 0.06	0.71 ± 0.14
Alcohol propylene glycol technique	10.20 ± 1.63	31.97 ± 0.06	0.25 ± 0.07

The pieces preserved by both techniques have remained until the time of publication of this manuscript without showing changes due to decomposition or alterations in the shape and anatomical structure (8 months).

4. Discussion

While the course of the present study, both the phenolated glycerin technique and the alcohol propylene glycol technique were applied to anatomical specimens, obtaining satisfactory results with either technique, the difference being that with the alcohol propylene glycol technique non-toxic reagents were used, contrary to the phenolated glycerin technique, in which formaldehyde and phenol are used, both of which have been reported to have toxic effects in humans.

The resulting sheep heart specimens obtained by either technique maintained the shape and structure seen in the fresh specimens, as was determined by the study of the external and internal organ anatomy, **Figures 2-4** changes in coloration are frequent when implementing techniques for anatomical preservation, the phenolated glycerin technique being the one that presented the lowest luminosity, as evidenced by a darker and lighter tone obtained using the alcohol propylene glycol technique, it should be noted that, in our experience, the anatomical pieces preserved using the phenolated glycerin technique tend to darken over time due to the use of aqueous formalin as a fixing agent, an effect also reported by Cutipa [16].

Tissue shrinkage events induced by formalin fixation have been reported by Boonstra [17], to current research evaluating intestinal tissue [18], renal tissue [19] and skin [20]. Another factor associated with tissue contraction that should

be considered is the relationship of fixation time and subsequent tissue contraction, as noted by Kansu [21] and fixation protocol (2%, 4% and 10% aqueous formalin or alcohol formalin) [22].

In the case of the alcohol propylene glycol technique the decrease in weight and size could be due to shrinkage as a result of tissue dehydration caused by the alcohol used in the fixation stage, nonetheless, such decrease in weight, 4%, and in length, 6%, did not affect the study of heart anatomy in relation to a fresh organ, an effect similar to that reported by Cabrera [23], who used ethanol-based fixatives with low shrinkage rates and morphological preservation of bovine testicular tissue.

Phenol is commonly used for its effective antimicrobial and antifungal properties, both of which help to preserve the organs, but due to its toxicity index its use is no longer recommended. In the alcohol propylene glycol technique this reagent was replaced by vinegar, which also exhibits antimicrobial and antimycotic activities, but zero toxicity given its low concentration of acetic acid (3% to 5%) but preventing the entry into a state of tissue decomposition, hence its use in the food industry as a preservative.

The hearts obtained by the alcohol propylene glycol technique can be subjected to dissection without any problem, the texture is not altered, a situation that is difficult when using the phenolated glycerin technique, due to the resulting rigidity in the specimens.

In terms of preservation time, we have preserved pieces with the alcohol propylene glycol technique that have remained unchanged for more than 2 years (canine and ovine stomachs) which is comparable to the results seen when using the phenolated glycerin technique. In a later work we will present our results from applying the alcohol propylene glycol technique in hollow and parenchymal organs, as well as its use in the insufflation technique, including lungs, which is comparable to the use of other techniques such as the phenolated glycerin technique.

There are other techniques that are mentioned as free of toxicity, such is the case of plastination; this technique allows to obtain high quality spacimens, with no toxicity once the silicone or epoxy resin has set in, but it has the disadvantage that during its process it uses reagents that are toxic for those who carry out the technique, such as aqueous formalin (fixation), acetone (dehydration), epoxy resins and silicones (forced impregnation), which in their liquid form emit toxic vapors, and the curing agent, which also emits toxic vapors. On the other hand, compared to the alcohol propylene glycol technique, its cost is significantly higher [8] and, due to the rigidity of the plastinated pieces obtained, it is not possible to dissect them, so their use is limited to being used as anatomical models or pieces for exhibition in museums.

Other techniques such as Thiel-Soft Fix method, although they do not use formalin, their composition is highly complex, and their development is laborious compared to the alcohol propylene glycol technique reported here [5].

Among the safe techniques that report good results with flexible anatomical pieces are the Prives technique [7] and the one reported by Muñetón [8]. The Prives technique produces discoloration of the pieces and the technique results in pieces with a greenish color and possess the difficulty that the pieces must be preserved in wells [8].

5. Conclusion

The alcohol propylene glycol technique was applied, which uses reagents with zero toxicity both for the developer of the technique and in the final piece obtained for handling by students and academics, unlike the glycerin technique. The hearts preserved with this technique maintain their dimensions with slight changes in length and weight, the time allocated for their creation is short (20 days), the durability of the preserved pieces is long (8 months at the time of this publication), without presenting changes due to decomposition, odor foul or fungal growth. They maintain their general morphology, slightly lighter in color, but without losing their external and internal anatomical structure. They maintain a texture with adequate flexibility, which allows dissections to be carried out even after applying the technique. Unlike the phenolated glycerin technique, which it was compared, where a darkening of the organs is observed as time passes, which can lead to problems when identifying the structures that make up the organ and due to the rigidity of the pieces, the dissection is difficult once the technique is applied. The quality of the pieces obtained with the proposed technique, in addition to educational use, also allows them to be used in museums, as in the case of techniques as plastination.

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Ethics Statement

The authors confirm that the ethical policies of the journal, as noted on the author guidelines page, have been adhered to. No ethical approval was required as the work conducted is original research on fresh hearts from animals destined for slaughter.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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