

Bone marrow quality in chemically prepared dogs

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SUMMARY

Besides preventing deterioration, conservation also prevents the proliferation of pathogens that cause diseases in those who work in laboratories. This research aimed to evaluate the color of the bone marrow of chemically prepared dog corpses, seeking to determine whether long-term preservation maintains their original morphological aspect. Fifty cadavers of adult dogs were used. The control group (G0) consisted of fresh corpses; the others were fixed in curing salt solution (20% sodium chloride, 1% nitrite, and 1% sodium nitrate) (120 ml/Kg) and ethanol with 5% glycerin (120 ml/Kg). Cadavers were assessed at 30 days (G30), 60 days (G60), 90 days (G90), and 120 days (G120). In addition, two veterinary observers evaluated the long bones' bone marrow during a biomechanical compression test after a fracture. Qualitative bone marrow color variables were transformed into scores, in which 0=excellent (more vivid than fresh cadaver), 1=good (similar to G0), 2=poor (darker than G0), and 3=bad (rotten appearance, brownish). By Dunn's test, the bone marrow of the bones was better at 30 days when compared to G0 ($G0 > G30$), $G0 \cong G60$ and $G0 < G90$, and $G0 < G120$. This conservation technique was promising in orthopedic training in dogs, and

provided a better bone marrow aspect between 30 to 60 days after fixation.

Key words: Anatomy – Conservation – Orthopedics – Surgery

INTRODUCTION

Anatomical specimens are fixed so that tissues do not deteriorate, so tissues remain firm, insoluble, and protected (Rodrigues, 2010). Besides preventing deterioration, exemplary conservation also avoids the proliferation of pathogens that can cause diseases in those who live daily in laboratories (Corrêa, 2003).

For conservation, the most common substances used to avoid microorganisms' proliferation are formaldehyde, glycerin, ethanol, and phenol (Rodrigues, 2010).

Formaldehyde is the most widely used fixer and preservative, commonly in a 10% aqueous solution. Because it is not expensive and penetrates rapidly into tissues (six millimeters in twelve hours), it is widely used in anatomy laboratories (Rodrigues, 2010). In addition, several types of fixative solutions can preserve cadavers for surgical use, as Thiel's solution (Groscurth et

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al., 2001), Jores' solution (Rodrigues, 1998), and Larssen's modified solution (Silva et al., 2004), Laskowski solution (Silva et al., 2007), all of them presenting formaldehyde.

In a research using curing salt (CSS) as an alternative to formaldehyde in veterinary anatomy teaching in Germany, the solution successfully preserved dogs' corpses without environmental and health risks at a low cost (Janczyk et al., 2011). In Japan, using a salt-saturated solution in preserving human cadavers aiming for surgical education was successfully evaluated (Hayashi et al., 2016). In Hungary, a 16% curing salt solution was used as an alternative to formaldehyde for long-term preservation of anatomical specimens of dogs subjected to dissection at the University of SzentIstván Sciences (Werdelmann and Gerics, 2016).

The current model that best approaches the fresh corpse presents several problems: freezing, limited practising time due to fast putrefaction, and risk of infections. On the other hand, saturated salt solutions are a simple method with a low risk of infection and cost (Hayashi et al., 2016).

For five years, a 30% sodium chloride aqueous solution (30% SCAS) was used to conserve anatomical specimens previously fixed by formaldehyde; no visual contamination, putrefaction odors, or alteration of color and softness were noticed (Oliveira, 2014). The use of the sodium chloride hypersaturated solution on the pericardium (Brun et al., 2002) and phrenic center of dogs (Brun et al., 2004), both for surgical purposes, has also been successfully described.

The curing of meat products is a traditional practice in the food industry. The process consists of applying nitrite, nitrate, sodium chloride, and sugar to meat. By combined action, these components have a pronounced effect on the characteristics and stability of those products. For example, sodium nitrite causes stabilization of the typical red color, improves the organoleptic characteristics, and inhibits the growth of *C. botulinum* (Leitão, 1978).

Both nitrites and nitrates are curing salts widely used as additives by the food industry, mainly the meat industry. They are classified as conservative

substances. They are added to foods to prevent or delay microbial or enzymatic actions, thus protecting the food from deterioration. They are also color fixers (Iamarino et al., 2015).

Recent studies present biomechanical analyses of tissues of dogs and cats during conservation (Cerqueira et al., 2017; Pelogia et al., 2018; Rocha et al., 2018; Fração et al., 2019; Zero et al., 2020), as well as microbiological analyses of fixative/conservative solutions (Pereira et al., 2019) and evaluation of students on surgical practice (Rocha et al., 2019).

This paper's objective was to evaluate bone-marrow aspect (staining), using glycerinated ethyl alcohol (GEA) as a fixative and curing salt as preservative solutions in dogs, with the aim of teaching veterinary orthopedics.

MATERIALS AND METHODS

Twenty-one male and twenty-nine female adult dogs whose death did not involve evident morphological alterations, such as large tumor masses, extensive lacerations, or bone fractures, were used. They came from the Zoonosis Center Control at Ribeirão Preto, São Paulo, Brazil (process 02.2014/000027-1 approved by the Municipal Legal Department and the University Ethical Committee (process 4593/19).

Shortly after death, the corpses were frozen (freezer at -18°C) and transported to the Laboratory of Animal Anatomy at UNESP (São Paulo State University), Jaboticabal, São Paulo, Brazil, located 50km away.

In this research, corpses weighing 9.46 ± 2.80 Kg and body score 4 or 5 (on a scale from 1 to 9) were used (Laflamme, 1997). The control group consisted of fresh cadavers; all the others were fixed. The fixation was performed with a CSS (20% sodium chloride, 1% nitrite, and 1% sodium nitrate) injected via the common carotid artery (120 ml/Kg). Subsequently, 120 ml/Kg of ethyl alcohol associated with 5% glycerin (GEA) was infused. Dogs were randomly divided into four groups and remained at different times, except the control group, in a horizontal refrigerator at 4-6°C after fixation:

- Group 1: refrigerated for 30 days;

- Group 2: refrigerated for 60 days;
- Group 3: refrigerated for 90 days;
- Group 4: refrigerated for 120 days.

The long bones of the control group were collected and subjected to biomechanical tests immediately after thawing. After the final refrigeration time, each group was exposed to room temperature (24°C). The bones were collected using surgical instrumentation (scalpel, Mayo and Metzenbaum scissors, and rat tooth forceps) for biomechanical assay (humerus, radius, ulna, femur, and tibia).

The bones were measured to determine the midline of the diaphysis, where the fracture was performed using a compression test by the EMIC® Universal Testing Machine – (model DL-2000). The load cell was 20 kN, and the speed was 500 mm/min., the free range of 25 mm from the shear and 60 mm between the columns. The equipment belongs to the Laboratory of Surgical Anatomy

of the Department of Animal Morphology and Physiology at São Paulo State University in Jaboticabal, Brazil.

Two veterinarians with at least 15 years of experience in anatomy and surgery, and who already had training in bone marrow observation in biomechanical assays, were chosen as the project performers and evaluators. Observers evaluated 100 analyses of the bone marrow (BM) staining during each group samples mechanical. All samples were placed in 0.9% saline solution at room temperature (24°C) to avoid tissue dehydration. To prevent more than one group from being analyzed on the same day, there was no way to carry out a blind test due to the extensive work in each collection. However, the same trained professionals performed all the analyses to minimize errors. The same professional made all the analyses. As the control group was the first

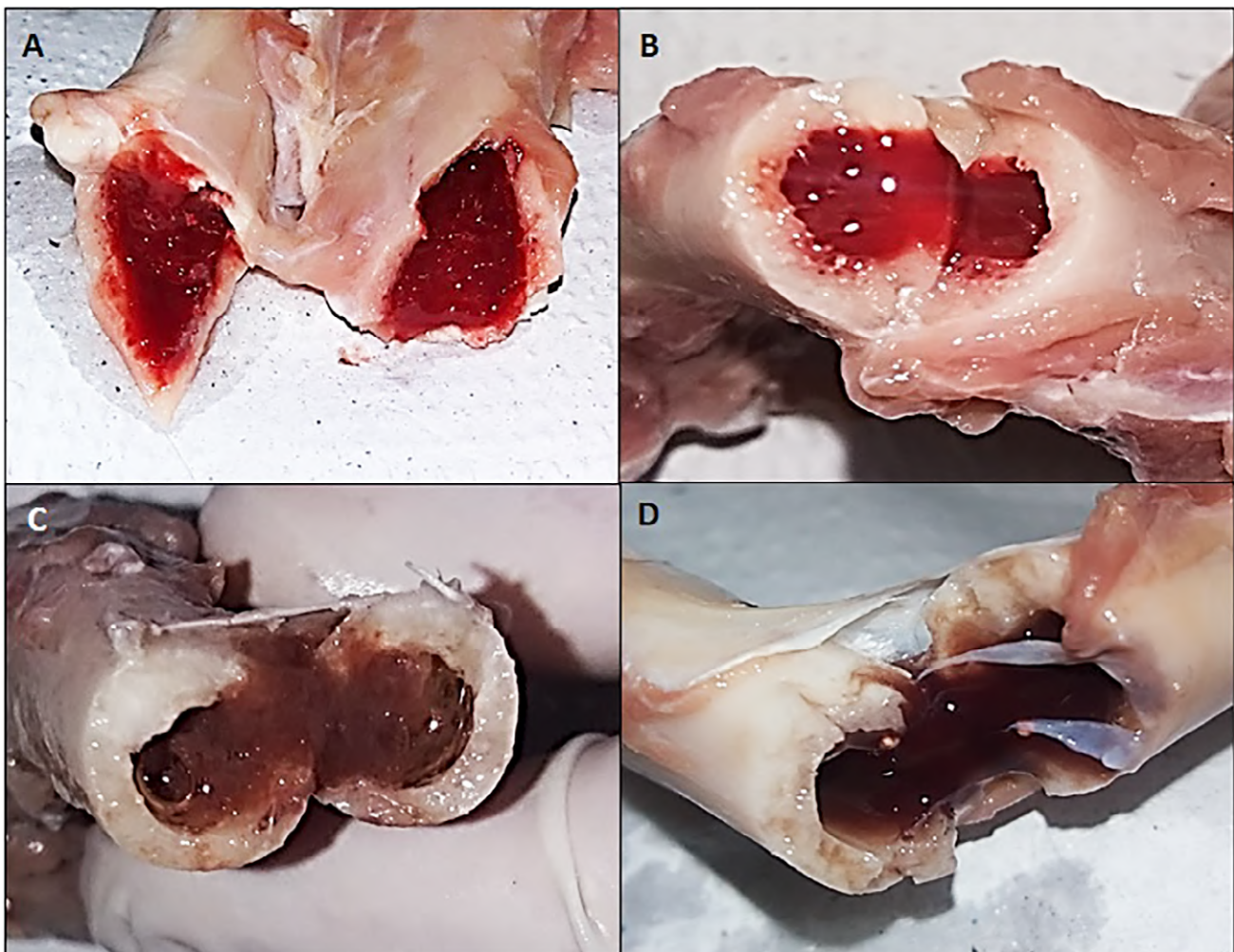


Fig. 1.- Photographic image of bone marrow stains from cadavers of dogs submitted to the fixation process with glycerinated ethyl alcohol and preserved with curing salt. **A:** excellent (more vivid coloring than fresh corpse); **B:** good (fresh corpse-like color); **C:** poor (darkened color about fresh corpse); **D:** bad (brown, putrid color).

to be evaluated (fresh corpses), the marrow's observation of its long bones is considered a parameter for the classification. Thus, the BM staining of the fresh animal was considered a reference and classified as "good." After the fracture of bone samples, they were classified as excellent (color considered more vivid than fresh bone), good (color similar to the fresh bone), poor (color darker than the fresh bone), or bad (brown color, rotten appearance) (Fig. 1).

Qualitative variables of the BM were transformed into scores, in which 0 = excellent (BM staining more vivid than in the one in fresh cadaver), 1 = good (similar to the one from the fresh carcass), 2 =

poor (darker than the one from a fresh corpse) and 3 = bad (rotten appearance, brownish), the lower the value, the better the appearance (Table 1).

After the BM scores, the genders were analyzed to search if there were differences.

The significance analysis (p-value) of the BM aspect regarding the right and left antimers was used to search for differences. There was no statistical difference between the right and left antimeres in relation to length and craniocaudal and lateral lateral diameters (Table 2). The measurements are shown in table 3. In addition, Dunn's test was performed to determine when the BM presented the best visual aspect.

Table 1. Analysis of the qualitative variables of the aspect of the bone marrow staining, generated by the analysis of two observers subjected to Dunn's test to determine the moment related to the aspect of the conservation time of dog corpses fixed with glycerinated ethyl alcohol and preserved with curing salt, subjected to biomechanical testing.

	G0		G30		G60		G90		G120		
BM	Median	AQI	Median	AQI	Median	AQI	Median	AQI	Median	AQI	P Value
HUMERUS AD	1,00	0	0,00	0	1,00	1	1,00	2	2,00	0	0,0019
Dunn	b		a		ab		b		c		
HUMERUS AE	1,00	0,00	0,00	0,00	0,50	1,00	2,00	1,00	2,00	0,75	<0,0001
Dunn	bc		a		ab		c		d		
RADIUS AD	1,00	0,00	0,50	1,00	1,00	0,75	2,00	1,00	2,00	0,00	<0,0001
Dunn	ab		a		b		c		c		
RADIUS AE	1,00	0,00	0,50	1,00	1,00	0,75	1,00	1,00	2,00	0,00	<0,0001
Dunn	b		a		b		b		c		
ULNA AD	1,00	0,00	0,50	1,00	1,00	0,00	1,00	1,00	2,00	0,00	<0,0001
Dunn	b		a		b		b		c		
ULNA AE	1,00	0,00	0,00	0,75	1,00	0,00	1,00	0,00	2,00	0,00	<0,0001
Dunn	b		a		b		b		c		
FEMUR AD	1,00	0,00	0,00	0,00	1,00	1,75	1,00	1,00	2,00	1,50	<0,0001
Dunn	b		a		b		b		c		
FEMUR AE	1,00	0,75	0,00	0,00	0,50	1,75	1,00	2,00	1,50	1,00	0,0009
Dunn	bc		a		b		bc		c		
TIBIA AD	1,00	0,00	0,00	0,75	1,00	0,75	1,00	1,00	2,00	0,75	0,0002
Dunn	b		a		b		bc		c		
TIBIA AE	1,00	0,00	0,50	1,00	1,00	1,75	1,00	1,00	2,00	0,75	0,0011
Dunn	ab		a		ab		b		c		

* The bone marrow aspect, qualitative variables, were transformed into scores where 0 = excellent, 1 = good, 2 = poor, and 3 = very bad; so the lower the value, the better the appearance. AQI: interquartile range. AD: right antimer, AE: left antimer.

Table 2. ANOVA ($p < 0.05$) between right (RA) and left (LA) antimeres regarding bone midline measurements of dog cadavers subjected to chemical preparation with ethyl alcohol and curing salt.

Comparison between antimeres	
Variables	P value
UMERUS RA Length	0.92
UMERUS LA Length	
UMERUS RA Diam CCd	0.88
UMERUS LA Diam CCd	
UMERUS RA Diam LL	0.66
UMERUS LA Diam LL	
RADIUS RA Length	0.89
RADIUS LA Length	
RADIUS RA Diam CCd	0.42
RADIUS LA Diam CCd	
RADIUS RA Diam LL	0.76
RADIUS LA Diam LL	
ULNA RA Length	0.84
ULNA LA Length	
ULNA RA Diam CCd	0.97
ULNA LA Diam CCd	
ULNA RA Diam LL	0.81
ULNA LA Diam LL	
FEMUR RA Length	0.67
FEMUR LA Length	
FEMUR RA Diam CCd	0.90
FEMUR LA Diam CCd	
FEMUR RA Diam LL	0.94
FEMUR LA Diam LL	
TIBIA RA Length	0.62
TIBIA LA Length	
TIBIA RA Diam CCd	0.62
TIBIA LA Diam CCd	
TIBIA RA Diam LL	0.98
TIBIA LA Diam LL	

*Length: length em milimeters, Diam: diameter em milimeters, CCd: cranio-caudal, LL: laterolateral.

RESULTS

There were no significant differences between cadaver genders and BM appearance for all measured variables.

The p-value analysis of the BM aspect regarding the right and left antimers presented no significant differences, which increased the number of samples (n) by summing the values related to antimers. The confidence interval was 5%.

After running the Dunn's test, it showed that the humerus bone marrow aspect was better at 30 days of conservation when compared to the control group ($G_0 > G_{30}$), similar at 60 days ($G_0 \cong G_{60}$), and worse at 120 days ($G_0 < G_{120}$). The radius presented a better appearance of BM at 30 days when compared to the control group ($G_0 > G_{30}$), similar at 60 days ($G_0 \cong G_{60}$), and worse appearance at 90 ($G_0 < G_{90}$) and 120 days ($G_0 < G_{120}$). The ulna presented the best aspect of BM at 30 days ($G_0 > G_{30}$),

Table 3. Means of the long bones measurements in dog cadavers subjected to chemical preparation with ethylic alcohol and curing salt.

	G0 Control	G30	G60	G90	G120	MEAN (mm)
UMERUS Length	123.35	118	107.10	117.72	98.55	113.00
UMERUS Diam CCd	13.76	12.49	11.21	11.5	11.87	12.10
UMERUS Diam LL	10.18	9.28	9.21	9.33	9.96	9.60
RADIUS Length	118.95	113.05	99.15	114.83	92.20	107.60
RADIUS Diam CCd	6.41	6.22	7.03	6.43	6.90	6.60
RADIUS Diam LL	9.85	9.52	9.95	9.6	10.77	9.90
ULNA Length	140.60	134.50	118	139.27	111.30	108.70
ULNA Diam CCd	8.20	6.72	7.31	7.41	7.89	7.50
ULNA Diam LL	7.27	7.68	7.86	6.03	8.68	7.50
FEMUR Length	133.15	126.95	114	125.83	107.20	121.40
FEMUR Diam CCd	10.50	9.75	10.13	9.81	10.53	10.10
FEMUR Diam LL	10.82	10.12	9.86	10.03	10.75	10.30
TIBIA Length	132.95	128.50	108.26	129.38	105.30	121
TIBIA Diam CCd	10.66	9.94	9.32	9.61	10.01	9.9
TIBIA Diam LL	10.71	10.08	10.24	10.04	10.78	10.30

* Length: length em milimeters, Diam: diameter em milimeters, CCd: cranio-caudal, LL: latero-lateral. G0: fresh samples; G30: 30 days of conservation; G60: 60 days of conservation; G90: 90 days of conservation; G120: 120 days of conservation.

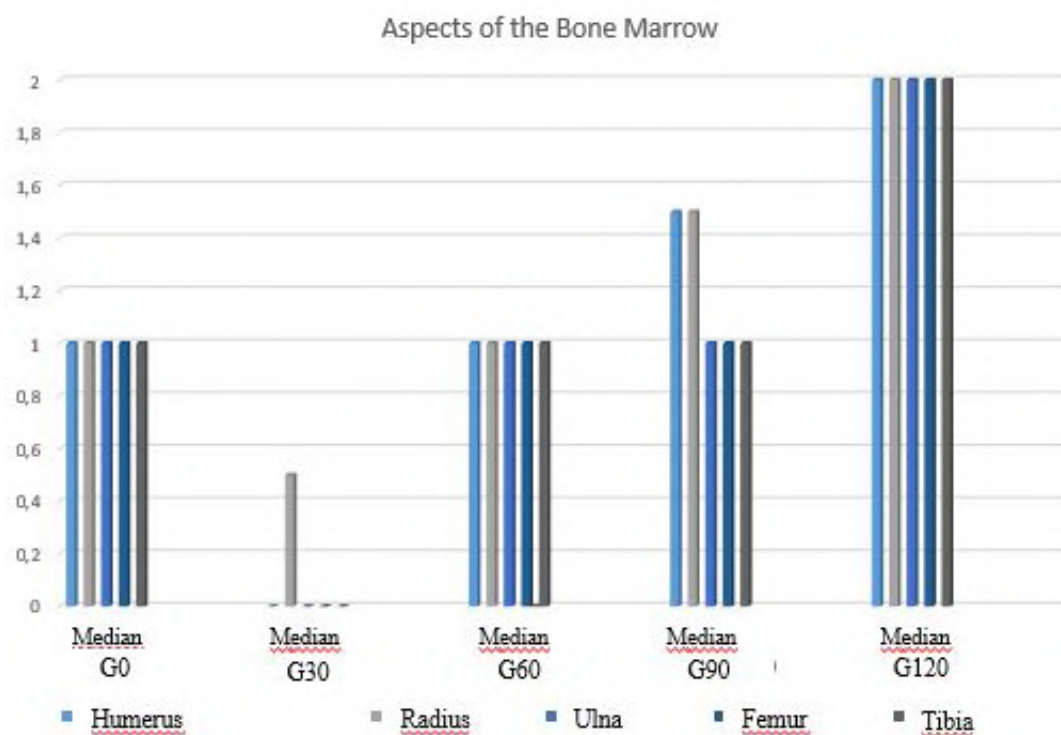


Fig. 2.- Illustrative image of the bone marrow's qualitative aspects fixed with glycerin ethyl alcohol and curing salt. Note that the medians in the 30-day conservation group (G30) are lower than those in the control group (G0) and that the medians in the 60-day conservation group are similar to G0. The other medians (G90 and G120) greater than G0.

similar at 60 days ($G0 \cong G60$), and worse aspect at 90 and 120 days ($G0 < 90$ and $G120$). The femur presented a better aspect of BM at 30 days when compared to the control group ($G0 > 30$), similar at 60 days ($G0 \cong G60$), and worse aspect at 90 ($G0 < 90$) and 120 days ($G0 < G120$). The tibia presented a better aspect of BM at 30 days when compared to the control group ($G0 > G30$), similar to 60 days ($G0 \cong G60$) and worse aspect at 90 ($G0 < 90$) and 120 days ($G0 < G120$) as seen on Fig. 2.

DISCUSSION

The GEA was efficient as a fixative, with CSS as a preservative solution, allowing good conservation and avoiding deterioration, maintaining the characteristics and the color of the dogs' corpses, similar to corpses fixed with GEA and preserved 30% SCAS (Cerqueira et al., 2017; Pelógia et al., 2018; Rocha et al., 2018). The CSS also presented similar aspects in cats fixed with GEA, with no skin hardening or color change during the conservation (Fração et al., 2019; Zero et al., 2020).

The excellent results on BM of corpses fixed with GEA and CSS are promising as for their use in orthopedic surgery, as it occurred in surgery training in chemically prepared dogs, with more than 81% (Rocha et al., 2018) or 93% (Silva et al., 2003) of students' approval in Brazil. Also, there were no differences among groups through different times, similar to the scores of incision or suture in chemically prepared cats (with GEA and CSS) compared to fresh corpses (Fração et al., 2019).

Conventional procedures for fixation of corpses using formaldehyde for conservation are limited in surgical practice due to the significant change of color, resistance, and fragility of organs and tissues (Groscurth et al., 2001). In addition, this substance also causes stiffness of corpses, coagulation of blood and presents an unpleasant odor (Hayashi et al., 2014).

Differently, the conservation solution applied in this research demonstrated that the color of the long bones' BM was markable after 30 days, with a more vivid appearance than a fresh corpse, and similar to the color of a fresh cadaver in 60 days. However, after 90 days of conservation, the BM

began to decay, and the color became progressively worse until 120 days. The better color of the BM on the 30 and 60 days of conservation could be explained using the curing salt solution, which is widely utilized in food factory industries to fix the meat's color (Iamarino et al., 2015).

Toxic preservative solutions generate contaminated effluents (Who, 1991) and dangerous vapors, such as those released by formaldehyde (Cury et al., 2013). In addition, these harmful products increase the institution's financial and environmental costs making it necessary to look for lower risky alternatives (Janczyk et al., 2011), such as the GEA and CSS solutions applied in this research.

The use of embalmed corpses in veterinary surgery practice is an alternative to using live animals. It reduces costs and increases exercise repeatability (Oliveira, 2008), and it is a trend in universities worldwide to avoid euthanasia of thousands of specimens for this purpose (Balcombe, 2000). In this research, the BM was conserved for 120 days without bad odor or visual contamination, like soft tissues fixed with the same anatomical technique (Fração et al., 2019; Pereira et al., 2019; Zero et al., 2020).

The CSS is effective for the preparation of anatomical species in Veterinary and Human Medicine in several countries (Janczyk et al., 2011; Hayashi et al., 2016; Fração et al., 2019) and provided, together with the GEA, the maintenance of corpses without visual contamination for 120 days in refrigeration temperature only.

The high concentration of sodium chloride in the CSS complicates microorganisms' survival because it requires an enormous capacity for osmoregulation, similar to what occurs in the oceans (Munro et al., 1989) and the Dead Sea (Nissenbaum, 1975). In addition, several concentrations of fixative substances in the preservation of anatomical specimens have already been evaluated and, the use of sodium chloride solution in a concentration below 20% fail to preserve specimens for tissue dissection (Friker et al., 2007), differently from what was observed in our study, in which 20% sodium chloride, associated with 1% nitrite and sodium

nitrate, is effective for the preservation of dog corpses for four months, in addition to providing good malleability to tissue handling.

Teaching models using human and animal corpses are a historical part of the surgery. Their use is secular and remains the most respected training method due to exposure to real anatomy and allowing the surgeon in training to appreciate the dissection and practice the actual handling of tissues (Silva et al., 2007; Stirling et al., 2014).

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