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Full Length Research Paper

Suture analysis and arterial traction test in dogs fixed on alcohol and preserved on saline solution aiming surgical practice

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There are several fixative solutions to preserve corpses for surgical use and lots of them uses formalin. The objective of this research was to determine, in dog corpses, the best fixative period in ethylic alcohol (EA) and preservation in sodium chloride aqueous solution 30% (SCAS 30%), aiming the arterial micro-surgical training. Corpses of five groups (G1 to G5) were fixed with EA, and put in boxes containing EA for 30 (G2), 60 (G3), 90 (G4) or 120 days (G5). After that, each group was preserved in SCAS 30% for 120 days. The control group (G1) was composed of corpses without fixation/ preservation. At the end of each period, two fragments of common carotidartery per corpse were collected, for traction test. Immediately after the collection, the femoral arteries were evaluated (by 2 people) regarding the suture quality in binocular surgical microscope, and attributed scores from 0 (bad) to 5 (excelente), regarding the fresh samples. Although, ethylic alcohol has been used as fixative, there were few changes regarding the traction tests in the common carotid arteries. Regarding the conservation in SCAS 30%, there were changes in relation to the control group at 60 and 90 days of conservation in saline solution, but not at 30 and 120 days. As the score at the 30 days group was higher than the 120 days group, we considered G2 (30 days fixation and conservation group) the best group for arterial surgery practice.

Keywords: anatomy, biomechanics, surgery, training.

INTRODUCTION

Alternative methods that look for the animal's welfare in the veterinary surgical technique teaching are becoming more commom. They aim to substitute the use of live animals, generating similar or superior learning to the students (Silva et al., 2007). A negative emotional state may disrupt a significant learning. As the majority of the students feel uncomfortable and even chocked in classes using live animals, it can be concluded that what occurs in fact is only a visual memorization and not a significant

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learning, in many of situations (Paixão, 2008).

There are several fixative solutions to preserve corpses for surgical use, among them, the Thiel solution (Groscurth et al., 2001), the Klotz solution, the Jores solution (Rodrigues, 2010), and all have formalin, just like the modified Larssen solution (Silva et al., 2004).

The Laskowski solution contains ethanol and demands corpses preserved at 0 ºC temperature until the use in surgery classes and with this solution, the tissues became excessively dark (Silva et al., 2007). The Larssen liquid is described as a solution that keeps the original consistency, color and characteristics of the biological material (Mathews et al., 2010). At the original Larssen solution there is no liquid glycerin (Carpenter et al., 1991).

Papers have been demonstrating the efficacy of using chemically prepared corpses for use in veterinary surgery classes (Silva et al., 2004), with students' wider acceptance as well as a better learning. To perform such procedure, animals that died in shelters and clinics that would be discarded might be used (Silva et al., 2007).

There were no significant differences in the surgical performance in veterinary students that worked in both corpses and live animals (Silva et al., 2007; Goyri-O´neill et al., 2013) and 88.9% of the veterinary medicine students reported that the teaching was very satisfactory in chemically preserved corpses. And, regardless of the chemical procedure used for corpses preservation, 95.7% of the students approved the use of corpses in surgery learning (Silva et al., 2007).

The objective of this work was to determine, in dog corpses, the best fixative period in ethylic alcohol (EA) and preservation in sodium chloride aqueous solution 30% (SCAS 30%), aiming the arterial micro-surgical training.

MATERIALS AND METHODS

We investigated 40 adults, male and female dog corpses, from the Center of Zoonosis Control of Ribeirão Preto, São Paulo, Brazil, in a process previous approved by the Legal Department (process 02.2014.000027-1). The animals were frozen (freezer at -18 ºC) after death and then transported to the Laboratory of Surgical Anatomy of the UNESP Jaboticabal, SP, located 50 km away.

The animals were between 5 and 12 Kg of body weight and presented body score 4 (easily palpable ribs, with minimum fat cover) or 5 (palpable ribs and without fat cover excess; abdominal waist observed caudally to the ribs when dorsally seen; evident abdominal fold when seen laterally), in a scale from 1 to 9, considered as an ideal body score (LaFlamme, 1997). They were thawed in horizontal refrigerators at 4-6 ºC, weighted and then randomly divided in groups for the fixation with ethylic alcohol 96 ºGL; for each kilogram of weight, the animals received 120 mL of fixative solution (95% of alcohol and 5% of glycerin), via common carotid artery. The glycerin in low quantity was used only for obtaining a higher visceral malleability. After that, they were divided in groups that remained in different times under fixation (except the group 1) before being kept preserved in sodium chloride aqueous solution (SCAS) 30%:

- Group 1: control group – 8 corpses, from which tissue samples were taken out for analysis and that were ***not*** submitted to fixation – fresh corpses;
- Group 2: 8 corpses that remained under fixation for 30 days in the alcohol solution. After this period, they remained for 30 days in the SCAS 30%;
- Group 3: 8 corpses that remained under fixation for 60 days in the alcohol solution. After this period, they remained for 60 days in the SCAS 30%;
- Group 4: 8 corpses that remained under fixation for 90 days in the alcohol solution. After this period, they remained for 90 days in the SCAS 30%;
- Group 5: 8 corpses that remained under fixation for 120 days in the alcohol solution. After this period, they remained for 120 days in the SCAS 30%;

Eight plastic boxes, with lids, 310 liters, were used for storing the animals, both during the fixation procedure in alcohol and during storage in sodium chloride aqueous solution 30%. For each group, one box was used during the fixation phase and during the conservation, which were kept in covered environment, but opened in the sides and with abundant ventilation, without igneous sources nearby, avoiding any kind of local accident.

For evaluating the tissue resistance, an Assay Universal Machine (EMIC® DL-2000, Brazil) was used. A 50N-charge cell was used and the charge application speed of 10 mm/min, with 10 mm clearance between the material gripping claws.

Because of the common carotid artery length of the corpses used was small, making it difficult the traction analysis in the assay machine, vascular analysis (2 fragments of 4 cm per animal/moment) were performed in the:

- Moment Zero: in corpses from the control-group, without any fixative – fresh animals;
- Fixation Moment: after the fixation period of each group (1 month to 4 months);
- Conservation Moment: fragments collection was made in sodium chloride aqueous solution in period equivalent to the same fixation period in alcohol. Thus, corpses fixed for 1 month had their common carotid arteries evaluated after 1 month in the sodium chloride aqueous solution; animals fixed for 2 months had their common carotid arteries evaluated after 2 months in the sodium chloride aqueous solution, and so on. The evaluated blood vessel fragment was longitudinally opened to look like a rectangular tape at the biomechanical evaluation.

Table 1. Averages of maximum rupturestrength, in N, of the traction tests of the dogs' common carotid arteries from the groups fixed with alcohol and conserved in sodium chloride aqueous solution 30%.

	G1	G2	G3	G4	G5
Final Fixation	25.77 ± 15.43	28.19 ± 8.34 (p= 0.9991)	16.44 ±5.56 (p= 0.2169)	31.79 ±10.35 (p= 0.776)	24.26 ±7.25 (p>0.9999)
Final conservation		29.13 ±12.75 (p= 0.9915)	13.46 ±5.02 (p= 0.0267)*	13.11±7.82 (p= 0.0199)*	22.78±14.62 (p= 0.9962)

G1: the control group maximum rupture strength, without fixative or preservative substances; G2: alcoholic fixation and saline preservation for 30 days; G3: alcoholic fixation and saline preservation for 60 days; G4: alcoholic fixation and saline preservation for 90 days; G5: alcoholic fixation and saline preservation for 120 days.

Table 2. Score average (1 – very bad, 2 – bad, 3 – moderate, 4 – good, 5 – excellent) attributed to the femoral artery suture in dog corpses fixed in alcohol and preserved in sodium chloride aqueous solution for different periods.

	G1	G2	G3	G4	G5
Final fixation	5.0	4	4	4.5	4
Final conservation		3.5	3.5	3.5	3

The group G1 was formed by fresh samples, with no fixative/conservative substances; G2: 30 days' fixation and preservation; G3: 60 days' fixation and preservation; G4: 90 days' fixation and preservation; G5: 120 days' fixation and preservation.

The statistics analysis where made in Graph Pad Prism 6 software. A comparison between the groups was made concerning the time in the fixative solution using ANOVA and Tukey test ($p<0.05$) between the groups.

The femoral arteries were analyzed regarding the suture in the same moments predicted to the biomechanical analysis of the common carotid arteries. It was chosen another vein for the vascular analysis regarding the suture, because there was no viable length to proceed it in the same artery from the biomechanical analysis. For this, the pelvic limbs were disarticulated from the thigh and taken to the Training Sector in Ophthalmology from the Department of Veterinary Clinics and Surgery of the University. A Desk Binocular Surgical Microscope (UM-M4, DF Vasconcelos®, Brazil) and needle-suture thread type Nylon 8-0 (Mononylon Ethicon Inc®, USA) were used. For the suture, single separated stitches were used and the femoral arteries were evaluated regarding the malleability and resistance for the suture procedure. Scores were attributed as 1 (very bad), 2 (bad), 3 (moderate), 4 (good), 5 (excellent).

In the arterial test at the surgical microscope, the evaluation was always carried out by an under graduating student and a professional, after 3 training sessions of 2 hours, in different days, for adapting to the equipment and to the suture technique, aiming to minimize interpretation mistakes of the evaluated vascular material quality. Always when it came to the evaluation day, a fragment preserved in alcoholic or saline solution, a similar fragment to a fresh corpse and without any fixative, was used for comparing the sutures quality.

RESULTS

All the results regarding the average maximum rupture strength of the carotid arteries, as well as the vascular suture scores in surgical microscope, are presented hereupon (table 1). The values of the traction test in G2 and G5, after fixation or conservation, were closer to the ones of the control group (G1 – fresh samples).

The ANOVA test showed significative difference among the treatments means at the 5% level ($p<0,005$) and the data passed through Shapiro-Wilk test ($p>0,05$) so it can be considered parametric.

Also, the Tukey test pointed out where the differences occurred, among the Control Group and the Group 3 and, 4 kept in SCAS 30% for 60 and 90 days, respectively.

The sum of suture scores in surgical microscope varied from 7.0 to 8.0 among G2 to G5, and all groups differed from G1, considered as ideal (sum = 10) (table 2).

DISCUSSION

The ethylic alcohol has shown to be efficient as dog corpses fixative, allowing good preservation and avoiding material deterioration, as described in the literarure (Groscurth et al., 2001). Still, alcohols used in human corpses fixation for 6 months to 1-year period maintain the tissue quality similar to the fresh tissue (Goyri-O´neill et al., 2013), similarly to what was observed in our results, especially in alcoholic fixation and conservation in SCAS for 30 days in dog corpses, through arterial

traction test and body score analysis.

At the end of the conservation time, the skeletal muscles that were protected by skin were still reddish similarly to the description about corpses embalmed by Larssen solution (Goyri-O´neill et al., 2013), and differently to the Laskowski´s solution that causes dark color to the corpses (Silva et al., 2007).

Biomechanical effects in animal tissues were already observed when the fixative agent was the formaldehyde, which caused a big material stiffening in analysis of 45 days (7 fold) (Guastalli et al., 2007) or up to one year (4.4 to 5fold) (Guastalli et al., 2012). When the ethylic alcohol is used as fixative, tissue stiffening increase also occurs, making it almost five fold stiffer at cutting during the first six months, and three fold stiffer after one year of immersion in the preservative agent (Nunes et al., 2011). However, in this project, although ethylic alcohol has been used as fixative, there were few changes regarding the traction tests in the common carotid arteries. Regarding the conservation in SCAS 30%, there were changes in relation to the control group at 60 and 90 days of conservation in saline solution, but not at 30 and 120 days. As the score at the 30 days group was higher than the 120 days group, we considered G2 (30 days fixation and conservation group) the best group for arterial surgery practice.

The SCAS 30% showed to be extremely efficient in preserving the fixed tissues, not observing apparent contamination during the analyzed period (Oliveira, 2014).

The use of chemically prepared corpses for students' surgical training from the Veterinary Medicine course fits perfectly in a new tendency and involves alternative and ethic methods for teaching, and avoids thousands of dogs´eutanásia (Balcombe, 2000).

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