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ANIMAL SCIENCE

Biomechanical and microbiological analysis of embalmed cats – acute effect of conservation

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Abstract: Animals corpses in teaching and research institutions could be sources of infection for students and teachers when applied for dissection and surgical practice. This research aimed to evaluate cats' corpses' conservation using a new anatomic technique and vacuum package for seven days, aiming surgical practicing. A 150 mL/kg of alcohol with 5% glycerin and 120 mL/kg of a 20% sodium chloride, 1% nitrite, and 1% sodium nitrate solution was injected on corpses sealed in vacuum packages and put on 0 to 4°C. Skin and jejunum were collected on day 0 (fresh samples/control), and traction analysis was performed for seven consecutive days. On the last day, the liquid in the plastic bags was microbiologically analyzed. There was no statistical difference between control and conservation moments (D1 and D2) in maximal rupture force of the skin, and jejunum was similar to control in D2, D4, and D6. The microbial population did not exceed $6.0x10^4$CFU/mL in total aerobics and $4.8x10^4$CFU/mL in total anaerobes. Biomechanics was not significantly affected, and the microbiological count was low during conservation, demonstrating the possible effectiveness of this anatomical technique for surgery training.

Key words: anatomy, microorganisms, acute effect, curing salt, surgery.

INTRODUCTION

Embalming is a process using chemical solutions to sanitize and preserves corpses after death. For more than 5,000 years, there has been a concern for preserving anatomical specimens, and human beings have tried to stop after-death body decay (Cury et al. 2013, Balta et al. 2015). Fresh corpses present limited handling time due to rapid putrefaction and risk of infection (Hayashi et al. 2014). It is necessary to use fixation and preservation methods to keep tissues firm, insoluble, and protected (Calamares Neto & Colombo 2015).

Embalming solutions can eliminate bacteria such as *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Animals and human corpses, used in teaching and research institutions, could be a source of infection for both students and teachers when used for surgical practicing and dissection. Therefore, embalming is used to reduce or eliminate the risks of zoonoses transmission, ethical considerations, teaching effectiveness, hygiene of the professional environment, economic pressures, and adverse publicity. For this reason, legal rules were established for the use of these animals in research and classes, thus ensuring their welfare (Arluke 2004, Balta et al. 2015).

An embalmed cadaver should present the same feature as a fresh corpse. Several fixatives and preservatives solutions can be used in

corpses to prevent decay. Studies reported that embalmed bodies changed the tissue and joints quality, which stayed rigid, decrease the range of motion (Balta et al. 2015, Hayashi et al. 2016). The most used fixatives are glycerin, ethyl alcohol, phenol, and formaldehyde (Rodrigues 2010, Balta et al. 2015, Hammer et al. 2014, Hayashi et al. 2016). At high levels, the latter causes tissue hardening (Hart 1990) and affects the cadaver quality, mainly in soft tissues (Hayashi et al. 2014).

Thiel's solution provides texture and color close to that of live animals and presents many applications in surgery, ultrasound, regional anesthesia, and airways anesthesiology research (Eisma et al. 2011, 2013). This solution contains salts, such as ammonium nitrate, potassium nitrate, and sodium sulfite, with small percentages of formaldehyde, ethylene glycol, boric acid, and p-chlorocresol (Rodrigues 2010, Hayashi et al. 2016). The intense color obtained with this solution is due to nitrites and myoglobin in muscles that form nitrosomyoglobin (Hammer et al. 2014). Food additives such as nitrite, nitrate, sodium chloride, and sugars are applied to keep the red color of cured meat and cause a bacteriostatic effect, maintaining its physical-chemical properties (Iamarino et al. 2015).

Some embalming solutions have been tested with success. The ICL-SP (Imperial College London Soft-Preservation) solution with phenol, alcohol, water, and glycerol (Barton et al. 2009) did not affect the joint flexibility, probably due to the presence of alcohol and absence of formaldehyde. Ethanol-glycerin-fixed specimens can keep the colorfastness, rigidity and joint motion better than formaldehyde-fixed tissues (Hammer et al. 2011, 2012), besides more pleasant odor (Hammer et al. 2014).

Saturated salt solutions have proven to be effective in conservation, with low cost and contributing to the extensive use of embalmed corpses in surgical training (Hayashi et al. 2014, 2016).

There is no ideal solution to embalm and preserve corpses. The solution should protect the cadavers from microorganisms that accelerate decomposition and make them bacteria-free (Balta et al. 2015). The use of corpses has been described as the best method for training medical specialties, promoting great learning, increasing efficiency and confidence, decreasing costs, and allowing repetition (Balta et al. 2018, Rocha et al. 2019).

This paper aimed to evaluate the conservation of vacuum-packed cat corpses fixed with glycerinated ethyl alcohol and curing salt for veterinary surgical practicing throughout seven days by measuring the maximum rupture force (in Newtons - N) and the rupture elongation (mm) of the skin and jejunum, besides microbiological analysis during the conservation.

MATERIALS AND METHODS

Eight cat corpses, males and females, adults, weighing 3.76±1.27kg, and whose death did not involve morphological changes were used. Cadavers were obtained from the Zoonosis Control Center in Ribeirão Preto, SP, and the donation was approved by the Municipal Legal Department (process 02.2014.000027-1) and by the local ethics committee of UNESP - Jaboticabal (process 4593/19). The animals were frozen (freezer at -18° C) after death and then transported to the Laboratory of Animal Anatomy at UNESP Jaboticabal, SP, located 50km away.

The animals' body score was 5 (palpable ribs without excessive adipose coverage, when viewed from above; the waist is seen behind the ribs, and the abdomen can be seen retracted

from the side), on a scale of 1 to 9, considered as an ideal score body for cats by Laflamme (1997).

Anatomical technique

Corpses were thawed in tapping water for 12h, and trichotomy was performed, focusing on not causing any damage to the skin. Three skin and jejunum fragments were collected (fresh/control, before fixation). For skin collecting, corpses were initially positioned in the right lateral decubitus. With a scalpel (blade number 23), a 1x5cm stainless steel mold was contoured in three sequential skin samples, perpendicular to the tension lines of the cat's skin, on the lateral of the thorax, and 5cm away from the median plane (Figure 1), which is the direction that provides higher tensile strength (Haar et al. 2013), as demonstrated in the sheep (Jacinto et al. 2004). A median celiotomy was performed for the jejunum collecting, the intestine exteriorized, and the duodenojejunal flexure identified; the steel mold was positioned to delimit the sample, which was then sectioned longitudinally with a Metzenbaum scissor. Subsequently, a section of the mesenteric border was performed, exposing the lumen for the incision using the same stainless steel mold (three 1x5cm fragments from each animal) (Figure 2). Then, samples were immediately subjected to biomechanical analysis. After collecting the samples (D0-Fresh sample/control group), the common carotid artery was dissected, a 40x12 cannula was inserted on it, and 150ml/kg of ethyl alcohol (Usina São Martinho®, Pradópolis, SP) with 5% glycerin (Dinâmica®, Indaiatuba, SP) (EA) and 120ml/kg of curing salt solution (CSS) were injected. The CSS was prepared with 200g/L of sodium chloride (Cisne®, Cabo Frio, RJ), 10g/L of sodium nitrite (Êxodo Científica®, Sumaré, SP), and 10g/L of sodium nitrate (Êxodo Científica®, Sumaré, SP). Then, the corpses were individually vacuum-packed (Figure 3) by a professional machine (Cetro® DZ Q600 DE) and kept in a horizontal refrigerator (Fricon®, Paulista, PE) between 0 and 4°C.

Tissues samples were taken daily for seven days. The packages were opened every day, collected, and put the animals in another sterile plastic bag so the vacuum could be done again.

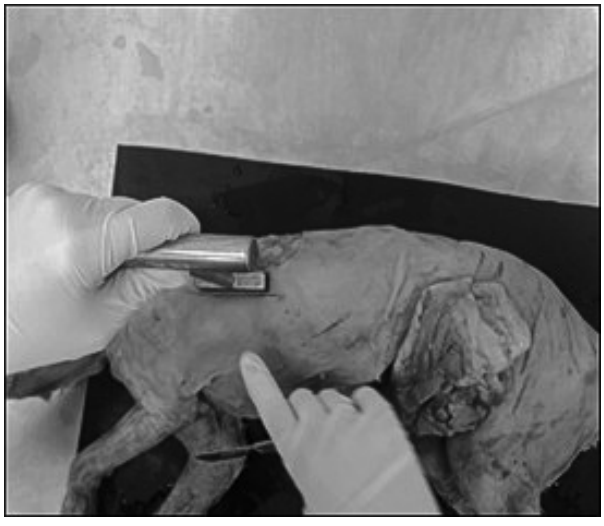

Figure 1. Cat positioned in right lateral decubitus to collect three samples of the skin parallel and 5cm from the median line on the chest.

Biomechanical analysis

To assess tissue resistance, an EMIC® Universal Testing Machine - model DL-2000 was used. A

Figure 2. Jejunum sample collected in the longitudinal direction in an embalmed cats' corpse.

500N load cell, a 100mm/min displacement speed, and a 20mm gap space between the grips were applied. The equipment belongs to the Laboratory of Surgical Anatomy of the Department of Animal Morphology and Physiology at São Paulo State University in Jaboticabal.

A traction test was conducted up to the point of rupture of the skin and jejunum.

Microbiological analysis

Microbiological analyses were performed on the last day of conservation (D7) in 3 corpses/packages, randomly chosen. As a large amount of solutions was injected, extrapolating the blood volume of the animal, there was always some liquid on the plastic bag. For each analysis, 10mL were collected in previously sterilized bottles and sent to the Microbiology Laboratory of the Institution.

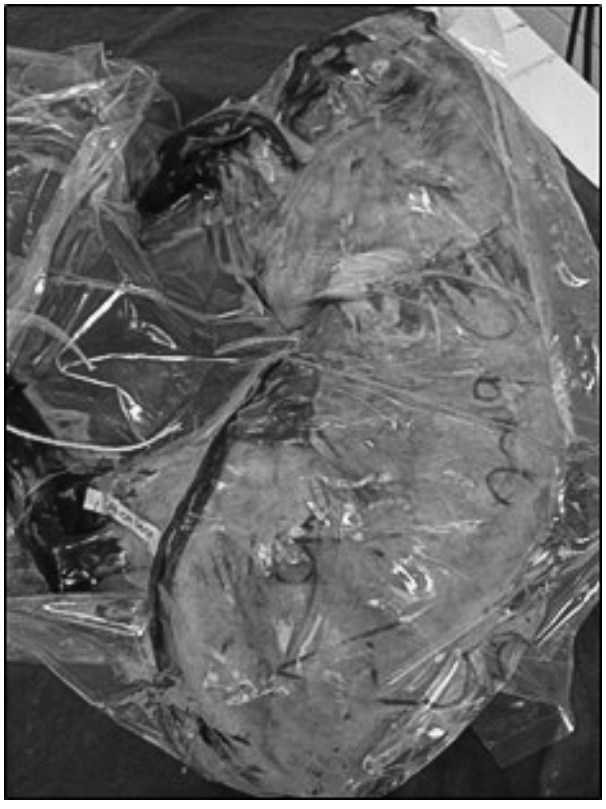

Figure 3. A vacuum-packed cat after fixation with ethyl alcohol and curing salt.

The surface plating technique was used to quantify viable facultative aerobic and anaerobic bacteria. In this technique, samples are diluted five times, 100µl as an inoculum, and distributed on agar surface plates, spreading with the Drigalski loop. The plates for counting aerobic microorganisms were stored directly in a bacteriological incubator. While the plates for counting anaerobic microorganisms were stored in anaerobic jars using Anaeroback (Probac), both incubated at 37°C for 24h. After this period, the colony-forming unit (CFU) was counted per mL using a magnifying glass (Vanderzant & Splittstoesser 1992, Jay 2005). Five colonies isolated from each plate on BHI agar (brain heart infusion), with different phenotypic characteristics, incubated at 37°C for 24 hours for later identification of the genera *Streptococcus* sp., *Bacillus* sp, *Pseudomonas* sp., *Escherichia coli* species, and *Clostridium* sp. Selective culture media sown for the genera analyzes SPS agar (sulfite-polymyxin-sulfadiazine) to identify the genus *Clostridium*, Mac Conkey agar, to evaluate *Pseudomonas* and *Escherichia coli*; and MYP base agar (mannitol-yolk-polymyxin) for isolation of *Bacillus*. Cultures were assessed for cell morphology, presence of spores, Gram classification after 24 hours of incubation at 37°C (Barrow & Feltham 1993).

RESULTS

All analyses were performed using the R 3.6.1 software for Windows. Box cox transformation of the data to guarantee homoscedasticity, when necessary. Cramer-Von Mises normality test (p=0.1894) of the residues were taken, Analysis of Variance (ANOVA) (5% and 1%) were performed, and then the Tukey test (5%).

The mean and standard deviation of the MRF and RE for skin and jejunum rupture are shown in Tables I and II.

In MRF data, the outliers were removed, and data subjected to box cox transformation (λ=0.6) using the Cramer-Von Mises test and p=0.5421. According to ANOVA (p=0.06), there is no significant difference between times (Table III).

In RE data, outliers were removed, and data submitted to box cox transformation (λ=0.25) with Cramer-Von Mises test (p=0.4593). According to ANOVA, there was no significant difference between different times (Table IV).

In the Tukey test of skin MRF, the moments D1 and D2 were similar to the control group; the same occurred for RE (Table I). The jejunum MRF was similar to the control group on D2, D4, and D6. At the same time, the RE was similar in moments D2 and D6 (Table II).

Microbiological analysis was performed on three of the eight vacuum corpses packs (Table V).

DISCUSSION

Anatomical technique

The use of EA as a fixative agent proved to be efficient for cats' corpses, demonstrating adequate conservation and avoiding deterioration, similarly to the reported in dogs' (Rocha et al. 2018) and cat (Fração et al. 2019) cadavers in surgery training. Also, alcohols used as a fixative in human cadavers from 6 months to 1 year kept tissue quality similar to fresh tissue (Goyri-O'Neill et al. 2013). The solution maintained the color, rigidity, and joint motion better than formaldehyde-fixed tissues (Hammer et al. 2011, 2012).

The use of 30% sodium chloride aqueous solution (30% SCAS) was evaluated in a 5-year study and proved efficient in conserving fixed tissues, with no visual contamination, unwanted odors, and loss of softness and color (Oliveira 2014). This solution proved to be effective in the conservation of dogs previously fixed with EA for up to 120 days (Rocha et al. 2018) and in cats fixed with EA and CSS for up to 90 days (Fração et al. 2019), similar to the findings of this research. In all studies, the salt solution

Table I. Mean and standard deviation of the maximum rupture force (MRF) and rupture elongation (RE) of the skin samples from cat cadavers chemically prepared and vacuum packaging for up to 7 days.

	MRF (N)	RE (mm)
D0	344.27±102.23 a	7.87±3.05 a
D1	266.95±134.39 a	5.04±2.08 ab
D2	269.48±066.39 ab	5.66±1.26 ab
D3	237.48±087.50 b	4.46±0.99 bc
D4	337.77±083.49 b	6.22±2.66 bc
D5	241.78±086.99 b	5.15±2.47 c
D6	292.18±068.87 b	6.25±1.57 c
D7	249.70±063.80 b	5.41±1.49 c

D0: fresh samples / without preservatives; D1 to D7: 1 to 7 days of storage. Means followed by the same letter in the same column do not differ by Tukey's test 5%.

Table II. Mean and standard deviation of the maximum rupture force (MRF) and rupture elongation (RE) of the jejunum samples from cat cadavers chemically prepared and vacuum packaging for up to 7 days.

	MRF (N)	RE (mm)
D0	23.39±14.29 a	3.98±1.72 a
D1	24.31±11.69 b	4.64±2.11 c
D2	24.56±11.44 ab	4.92±2.01 ab
D3	27.60±07.70 b	3.95±0.90 c
D4	20.08±08.81 a	3.81±1.28 bc
D5	23.35±10.76 b	4.11±1.50 c
D6	19.67±10.02 ab	4.43±1.73 ab
D7	21.08±07.71 b	4.04±2.07 bc

D0: fresh samples / without preservatives; D1 to D7: 1 to 7 days of storage. Means followed by the same letter in the same column do not differ by Tukey's test 5%.

Table III. Analysis of variance of the MRF of the jejunum of cadavers of cats chemically preserved under the acute effect of conservation for seven days.

	DF	SS	MS	F value	P value
Treatments	7	38.61	5.5153	1.971	0.0610
Residues	188	526.06	2.7982		

DF: degrees of freedom; SS: sum of squares; MS: medium square

was higher than 20%, as recommended for the efficient conservation of corpses (Friker et al. 2007).

High concentration salt solution's success may be due to the difficulty of microorganisms to survive in a medium that requires an enormous capacity of osmoregulation, as occurs in the dead sea (Nissenbaum 1975).

The use of this technique with CSS and EA did not generate contamination on effluents and the odor is better than formaldehyde or Thiel embalming solution. It is a low-cost alternative to the methods that use formaldehyde (WHO 1991, Janczyk et al. 2011, Cury et al. 2013), and an option to fix the corpses to student dissection courses and surgical practicing (Hammer et al. 2014, Rocha et al. 2019).

The use of formaldehyde to fix corpses causes changes in the color, resistance, and fragility of organs and tissues, making its use limited for surgery training (Groscurth et al. 2001). This research provided, without formaldehyde, cat corpses used for one week without significant changes in tissue biomechanics, in addition to the corpses' softness and tissue malleability.

Biomechanical analysis

In cats weighing 3.58±0.63kg, prepared with the same anatomical technique without vacuum packaging (EA and CSS), and preserved for 90 days, the MRF of skin samples ranged from 254.19±183.25N (fresh/control samples) to 234.68±108.17N (Fração et al. 2019). In another study with dogs weighing 7.6±2.7kg, preserved with 30% SCAS and fixed with EA up to four months, the maximum rupture force was 106.7N to 177.5N (mean 142.1N) (Rocha et al. 2018). Our study with cats prepared with CSS and EA, weighing 3.76±1.27kg, the MRF ranged from 344.27±102.23N to 249.70±63.80N after seven days, demonstrating a higher resistance and elasticity of cats' skin when compared to dogs'. The skin's flexibility and resistance are mainly dictated by the properties of the corneal layer of the epidermis, and the tensile strength of the skin is considered to be due to type I collagen fibers in the deeper layer of the dermis (Bismuth et al. 2014). The jejunum is composed of smooth musculature that is poor in collagen fibers, becoming less elastic and resistant (Bacha & Bacha 2012).

The MRF of chemically prepared cats' jejunum was 23.39±14.29N, which was similar to the 23.30±9.92N of the same intestine portion, 21.04±9.49N in the duodenum and, 23.82±7.74N in the colon of fresh dog corpses weighting 20.07±8.00kg (Queiroz et al. 2019).

In a study using cats prepared with the same anatomical technique used in our research (EA and CSS) and preserved for 90 days, the MRF of skin samples ranged from 254.19±183.25N (fresh/control samples) to 234.68±108.17N (Fração et al. 2019). The MRF reached from 344.27±102.23N to 249.70±63.80N after seven days. The difference between research in the MRF must be due to the vacuum packaging presence in our cat corpses. In the vacuum packaging, the air is removed, inactivating the aerobic bacteria, preventing deterioration, leading to a longer storage time and higher product quality. The lack of oxygen causes lactic acid bacteria's predominance, keeping tissues' good quality and flexibility (Mantilla et al. 2010).

Table IV. Analysis of variance of the RE of the jejunum of cadavers of cats chemically preserved under the acute effect of conservation for seven days.

	DF	SS	MS	F value	P value
Treatments	7	0.2620	0.0374	1.8841	0.0746
Residues	177	3.5157	0.0199		

DF: degrees of freedom; SS: sum of squares; MS: medium square.

Table V. Microbiological analysis of the liquid contained in the vacuum packaging from three randomly chosen cadavers.

Samples	Total aerobes (CFU/mL)	Total anaerobes (CFU/mL)	Microorganism
A4	5.3×10^4	1.4×10^4	*Micrococcus* sp. and *Bacillus* sp.
A5	6.0×10^4	4.8×10^4	*Bacillus* sp. and *Klebsiella* sp.
A7	1.0×10^3	2.2×10^3	*Staphylococcus* sp. and *Bacillus* sp.

Microbiological analysis

During the fixation with EA and conservation of cat cadavers with a 30% SCAS, microbiological contamination had remained low (Pereira et al. 2019), similar to the described in this research. The microbial counting of the liquid in packages reached 10^4 CFU/mL at maximum, which provides adequate health security (BRASIL 2001). In research carried out with pathogen inoculum, the bacteriae concentration that causes disease in the host was 10^9 CFU/mL (Rigobelo et al. 2016). When using probiotics, the concentration for bacteria to generate the expected benefits should be over 10^8 UFC/g (Kuru et al. 2017).

This research's limitation was not to know the precise time corpses were frozen after death so that tissues could be well preserved. However, we did not observe any cadaver with decay signs after thawing, and all of them could be used for injection.

CONCLUSION

This tested anatomical technique proved to be efficient in keeping the biomechanical characteristics of the embalmed cat cadavers. Microbiological analyses were low, demonstrating the possible effectiveness of corpses for surgery training.

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All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Andréa Barros Piazzon de Souza Queiroz, Alessandra Rodrigues, Natália Teresina Brandão Costa, Laura Gusman Soares, Alisson Denis Senna Fechis and Fabrício Singaretti de Oliveira. Marita Vedoveli Cardozo made the microbiological analysis. Andréa Barros Piazzon de Souza Queiroz wrote the first draft of the manuscript, and all authors commented and read on previous versions of the document and approved the final manuscript.