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Innovative Preservation and Training Utility of Chemically Preserved Swine Viscera for Veterinary Surgical Education

Guaraná JB[1]*, Müller AF[1], Pieritz SO[1], Rihs PGM[1], Silva JM[1], Oliveira FS[2], and Freitas SH[1]

[1]Department of Veterinary Medicine, Faculty of Animal Science and Food Engineering, University of São Paulo (USP), Brazil

[2]Department of Animal Morphology and Physiology, School of Agrarian and Veterinary Sciences, São Paulo State University (Unesp), Brazil

Research Article

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***Corresponding author:** Julia Belotto Guaraná, Department of Veterinary Medicine, Faculty of Animal Science and Food Engineering, University of São Paulo, Av. Duque de Caxias Norte, 225, 13.635-900 Pirassununga, São Paulo, Brazil, Email: julia.guarana@usp.br

Abstract

The use of live animals during surgical practical classes exposes students to stressful situations that are often common in the operating room. However, this practice can generate a negative emotional state and hinder the student's cognitive mechanisms, causing different degrees of dissatisfaction and being detrimental to the learning process. Research involving alternative methods for diaeresis and synthesis training has already been carried out with promising outcomes, providing low-cost preserved anatomical pieces. Thus, the aim of the present study was to analyze the anatomical viability of chemically preserved swine viscera for operative techniques training and to evaluate the acceptance of students facing this educational approach, using viscera subjected to plastic bag vacuum conditioning in two different ways of storage: room temperature (20 to 25ºC) and refrigerated (0 to 6ºC). Thirty-six jejunum segments and 36 urinary bladders from swine were fixed in absolute ethanol (AE) for 30 days and subsequently preserved in sodium chloride hypersaturated solution (SCHS) for seven days. At the end of the fixation and preservation periods, the samples were placed in vacuum plastic bags and divided into two groups containing 18 urinary bladders and 18 jejunum segments each. After 60 days of storage, the samples were used for surgical technique classes. The results showed good acceptance by the participating students; the technique proved to be safe, has low cost, and has an easy implementation in terms of both preservation solutions and storage.

Keywords: Alternative Methods; Learning Process; Surgical Skills; Swine Viscera

Abbreviations

AE: Absolute Ethanol; SCHS: Sodium Chloride Hypersaturated Solution; RT: Room Temperature; USP: University of São Paulo.

Introduction

Surgical practical classes with live animals aim to expose the student to stressful situations. They are in direct contact with the patient and exposed to organs,

hemorrhages, and distinct technical procedure difficulties. This teaching method was used for a long time in many universities worldwide. However, this practice can generate a negative emotional state and hinder the student's cognitive mechanisms, causing different degrees of dissatisfaction and being detrimental to the learning process [1]. These methods can also be considered unhealthy due to the risk of infections involving biological materials, such as secretions and/or blood. In addition, they can lead to accidents related to inexperience or even recklessness of the student regarding the inappropriate use of sharp objects like needles and scalpels [2,3]. Currently, with the intellectual and technological advances in the development and application of alternative methods to vivisection, educational institutions have been able to adapt to the principles of ethics and animal welfare [4-6]. According to the Normative Resolution No. 17 of July 3, 2014, from CONCEA (National Council for Animal Experimentation Control, Brazil) [7], an alternative method is understood as any method that can be used to replace, reduce or refine the use of animals in research activities and by substitutive methods, educational resources or educational approaches that replace the use of animals or complement humanitarian teaching practices. In Veterinary Medicine, substitutive methods such as videos, anatomical models, computer programs, prototyping, and carcass preservation using formaldehyde, freezing, glycerin, and the Laskowski technique, among others, are often employed [8]. Teaching methods using cadavers for surgical practices can improve learning and provide exercise repetition, increasing students' confidence and satisfaction when compared to the use of live animals [6]. Several authors have demonstrated the effectiveness of using chemically prepared cadavers for Surgery and Anatomy classes [9-15]. Research involving different animals fixed in absolute ethanol and preserved in a sodium chloride aqueous solution as an alternative method for diaeresis and synthesis training has already been carried out with promising outcomes, providing low-cost preserved anatomical pieces that keep satisfactory organoleptic morphological characteristics for surgical training, such as good malleability and resistance to incisions and sutures [16-19]. Furthermore, Rocha [17] observed that 81.08% of the students are favorable to initial training in surgical practices using chemically preserved cadavers.

Currently, research using plastic bag vacuum packaging for preserving cadavers after fixation has shown good results in inhibiting microbial growth and maintaining the biomechanical properties of the anatomical parts [19,20]. The preservative effect of vacuum packaging is due to the creation of an anaerobic environment, which inhibits the growth of microorganisms responsible for organic materials deterioration, especially those of animal origin [21,22]. Thus, the aim of this study was to analyze the anatomical viability of chemically preserved swine viscera (urinary bladder and jejunum segments) subjected to plastic bag vacuum conditioning in two different ways of storage (room temperature and refrigerated) for operative techniques training and to evaluate the acceptance of students facing this educational approach.

Materials and Methods

This study was approved by the University of São Paulo Committee on Ethics in the Use of Animals (CEUA nº 9148181220) and by the Committee on Ethics in Research on Human Beings (CAAE 52551121.80000.5422) of the same institution. Thirty-six jejunum segments (20 cm in length) and 36 urinary bladders from swine were used. The anatomical pieces were obtained at the slaughterhouse school of the Faculty of Animal Science and Food Engineering (Faculdade de Zootecnia e Engenharia de Alimentos - FZEA) Campus "Fernando Costa" of the University of São Paulo. The viscera were fixed in absolute ethanol (AE, 99.8 ºGL) for 30 days and preserved in sodium chloride hypersaturated solution (SCHS, 30%) for 7 days. The parts were prepared as described by Guaraná [18], and the preservation time was based on the same study, which showed that swine viscera preserved using this methodology present favorable physical and histomorphological conditions and are efficient for training the operative technique with one week of conservation in SCHS.

At the end of the fixation and preservation periods, the urinary bladders were placed in vacuum plastic bags measuring 15 x 20 cm and 12 microns thick, and the jejunum segments were accommodated in vacuum plastic bags measuring 20 x 25 cm with the same thickness. With the aid of an EVA plate for support, the viscera were positioned and subjected to packaging using a vacuum sealer (Commercial Vacuum Sealer with Reservoir - Cetro® - Bauru/SP - Brazil) and then had their end sealed for 3 seconds using the same device (Figure 1). The packaged anatomical pieces were separated into two groups containing 18 urinary bladders and 18 jejunum segments each. The room temperature group (RT) was placed on open shelves (20 to 25°C), and the refrigerated group (R) was placed in a refrigerator (0 to 6 ºC).

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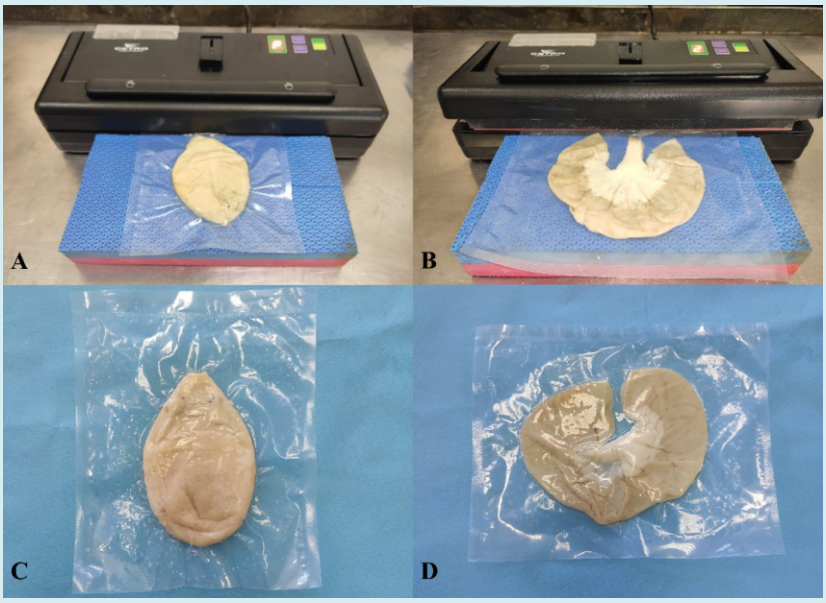

Figure 1: Vacuum sealing process. (A) Fixed and preserved urinary bladder and (B) jejunum segment, placed in a plastic vacuum bag and supported on an EVA plate. At the end of the vacuum sealing process, the urinary bladder (C) and the jejunum segment (D) were ready to be stored.

After the fixation periods in AE for 30 days and conservation in SHCS for seven days, before vacuum conditioning (D0), two urinary bladders and two jejunum segments were used to perform surgical techniques (cystotomy and cystotomy closure; enterotomy and enterotomy closure; intestinal resection and anastomosis) by the researchers (AFM and JBG) to analyze the samples physical characteristics. The same techniques were performed in two urinary bladders and two jejunum segments randomly chosen from the RT and R groups after 30 (D30) and 60 (D60) days of vacuum conditioning. The samples also had its physical characteristics examined and subjectively evaluated in each period (D0, D30, and D60). The results were recorded in a report following a scale of 1 to 5, with 1 being "unusable" - major physical characteristics changes, improper material; 2 "poor" - altered physical characteristics, impaired use; 3 "regular" - minor physical characteristics changes, use slightly impaired; 4 "good" - minor physical characteristics changes, use not impaired; 5 "excellent" - preserved physical characteristics.

At the same time as the execution of the techniques, urinary bladder and small intestine fragments were randomly collected for histological analysis just before the vacuum conditioning (D0), and at 30 and 60 days of vacuum conditioning (D30 and D60), from both groups (RT and R). Morphological characteristics (integrity/preservation of the mucosa, submucosa, muscular and serous layers) were compared with fresh samples of the urinary bladder and small intestine fixed in 10% formalin (microscopic evaluation control) and between the experimental groups to assess the differences of vacuum conditioning time and between the groups' way of storage (RT and R).

To survey the students' impressions regarding the use of the samples (n=58), the swine's viscera fixed for 30 days in EA, preserved in SCHS for seven days, and vacuum packed for 60 days, were removed from the packaging, placed in a container with potable water for rehydration for 20 minutes (Figures 2A and 2B), accommodated in a surgical model (Figures 2C and 2D), covered with unsterilized surgical drapes (Figures 2E and 2F) and provided to students enrolled in the Surgical Technique discipline of the Veterinary Medicine course at the Faculty of Animal Science and Food Engineering (FZEA) of the University of São Paulo (USP). The practice of the operative techniques performed (cystotomy and cystotomy closure; enterotomy and enterotomy closure; intestinal resection and anastomosis) (Figures 3A-3F) occurred simultaneously with their demonstration, through TV monitors in real-time, by the professor in charge of the discipline, as described by Fossum. Subsequently, the same students voluntarily filled out a form/questionnaire with objective and subjective questions regarding the fixed and preserved swine viscera. The first six questions were about the resistance of the viscera to the incision/suture and its malleability, in which the students should assign a score from 1 (unusable) to 10 (excellent) for each characteristic. The answers referred to the personal experience of each student

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and their experiences during the discipline using these and other educational approaches available: fresh pieces and synthetic suture models.

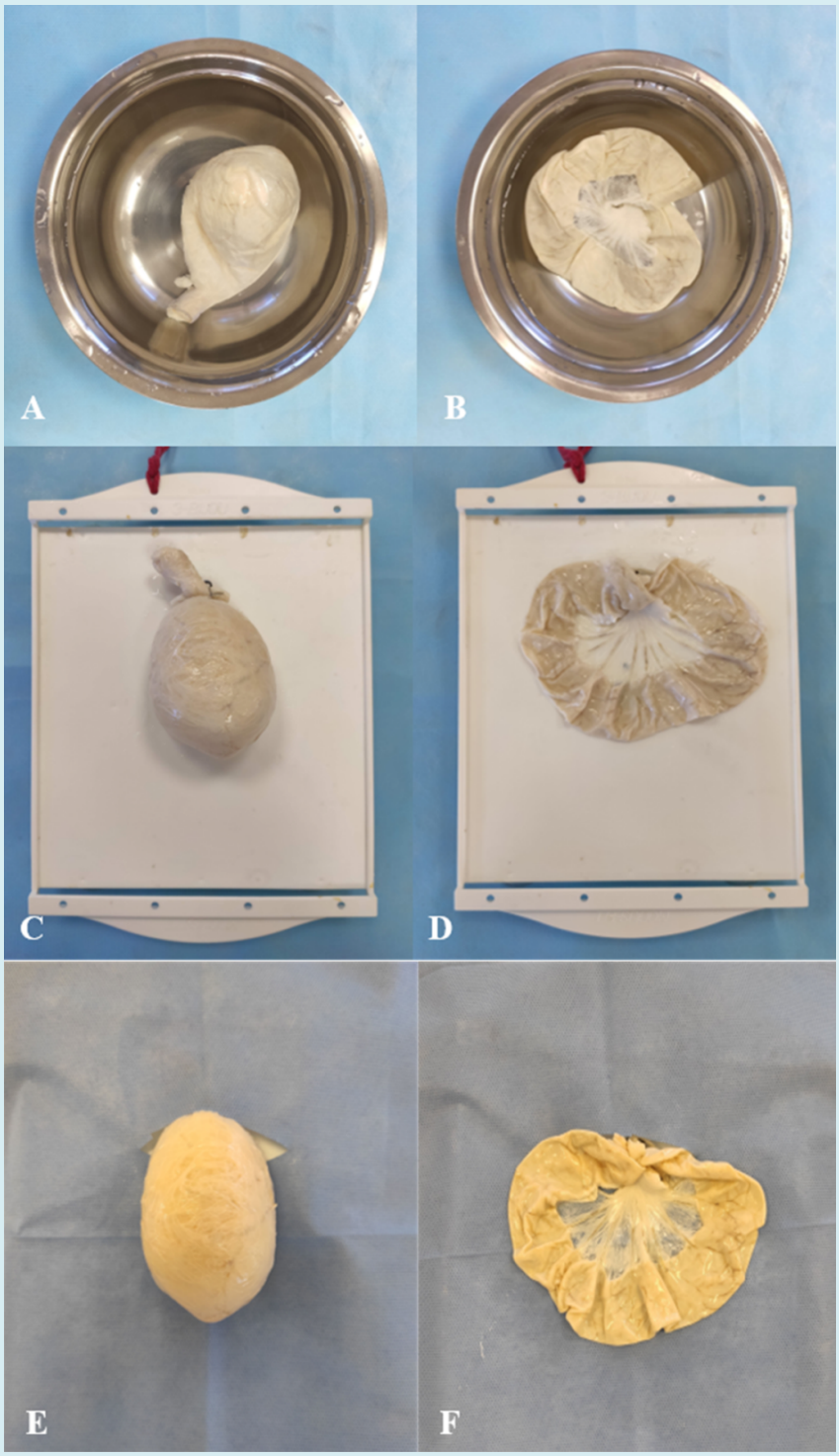

Figure 2: Urinary bladder and jejunum segment being prepared for use. The vacuum-packed samples were removed from packaging, placed in a container with potable water for rehydration for 20 minutes (2A and 2B), accommodated on a surgical model (2C and 2D), and covered with unsterilized surgical drapes (2E and 2F).

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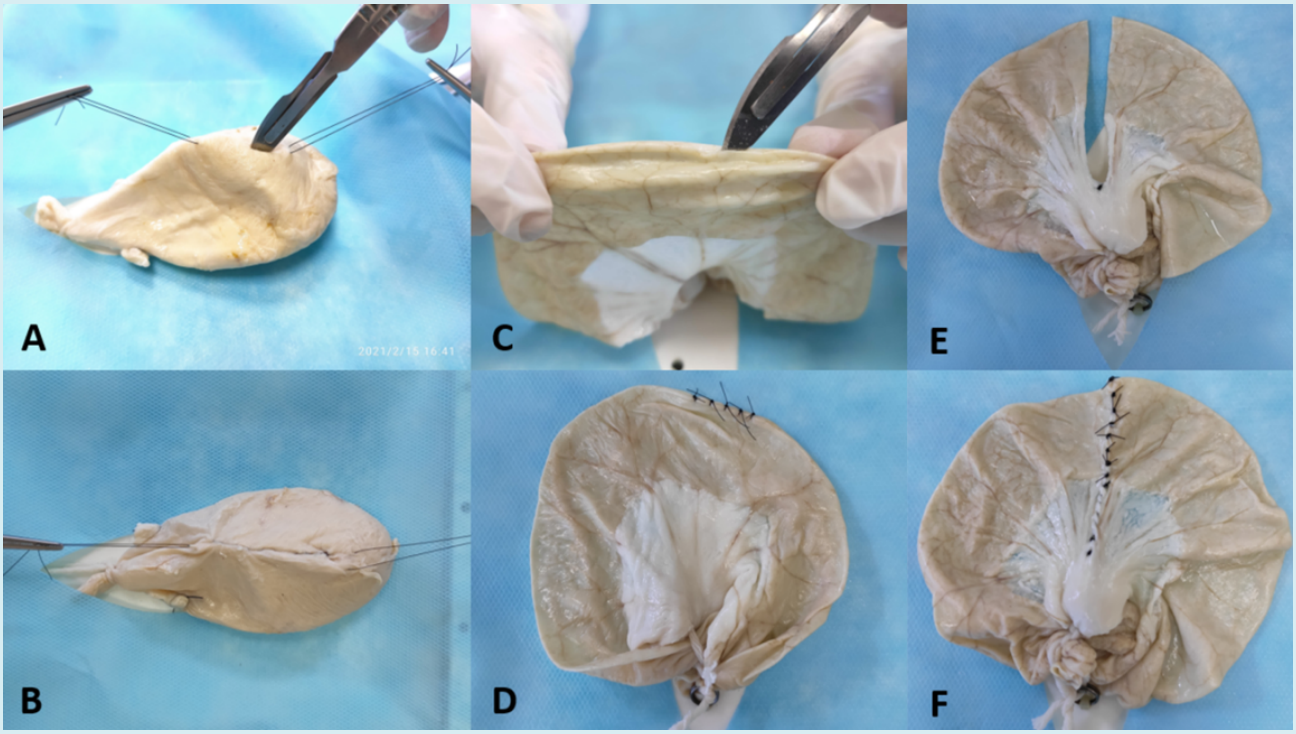

Figure 3: Operative techniques performed on the viscera. (A) Cystotomy and (B) cystotomy closure; (C) enterotomy and (D) enterotomy closure; (E) intestinal resection and (F) anastomosis.

Results

The grades attributed by the evaluators (AFM and JBG) to the physical characteristics of the viscera (appearance, texture, flexibility, odor, and color) in each period in the two different ways of storage (RT and R) are shown in the following table (Table 1).

A - Urinary bladder physical evaluation (RT Group) on each preservation period.

Features	D0	D30	D60	General Evaluation
Aspect	good	good	good	good
Texture	good	good	good	good
Flexibility	good	good	good	good
Odour	excellent	excellent	excellent	excellent
Colour	good	good	good	good

B - Jejunum physical evaluation (RT Group) on each preservation period.

Features	D0	D30	D60	General Evaluation
Aspect	good	good	good	good
Texture	good	regular	regular	regular
Flexibility	good	regular	regular	regular
Odour	excellent	excellent	excellent	excellent
Colour	good	good	good	good

C - Urinary bladder physical evaluation (R Group) on each preservation period.

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0.0556 in	D0	D30	D60	General Evaluation
Aspect	good	good	good	good
Texture	good	good	good	good
Flexibility	good	good	good	good
Odour	excellent	excellent	excellent	excellent
Colour	good	good	good	good

D - Jejunum physical evaluation (R Group) on each preservation period.

Features	D0	D30	D60	General Evaluation
Aspect	good	good	good	good
Texture	good	good	good	good
Flexibility	good	good	good	good
Odour	excellent	excellent	excellent	excellent
Colour	good	good	good	good

Table 1: Subjective evaluation report of the swine viscera physical characteristics (RT and R Groups).
Note: Unusable: major physical characteristics changes, improper material; Poor: altered physical characteristics, impaired use; Regular: minor physical characteristics changes, use slightly impaired; Good: minor physical characteristics changes, use not impaired; Excellent: preserved physical characteristics.

Histomorphological analysis revealed that the cell layers of the urinary bladder (serous, muscular, and submucosa) and intestine (muscular and submucosa) were preserved after vacuum conditioning for 30 and 60 days in both groups (RT and R) when compared to the control group (Figure 4). Mucosal degradation and damage to intestinal villi were similar to control in all experimental groups. The student's responses to the form/questionnaire were collected, the arithmetic mean of these values was calculated, and the result is presented in the following figure (Figure 5). The mean grades attributed by undergraduate students about viscera malleability were 9.5 for the urinary bladder and 8.5 for the jejunum segment. Regarding the resistance of the viscera on the incision/suture, the average was 8.6 for the urinary bladder and 8.85 for the jejunum segment.

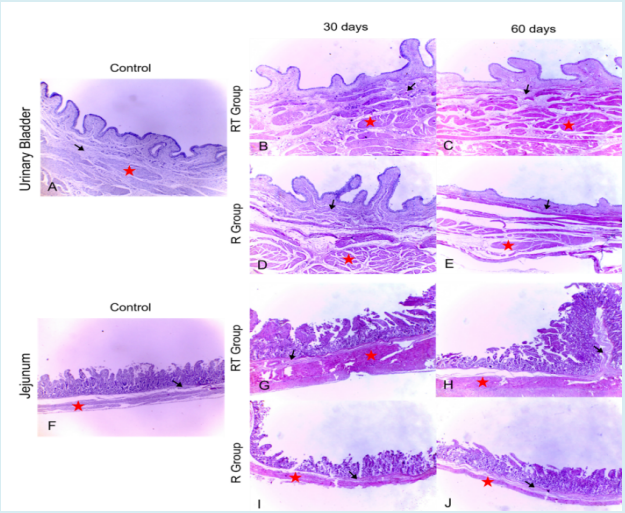

Figure 4: Histological photomicrographs of the analyzed viscera. (A) Urinary bladder and (F) jejunum control samples; Room Temperature urinary bladder experimental group after 30 (B) and 60 (C) days of storage; Refrigerated urinary bladder experimental group after 30 (D) and (E) 60 days of storage; Room Temperature jejunum experimental group after 30 (G) and (H) 60 days of storage; Refrigerated Temperature jejunum experimental group after 30 (I) and (J) 60 days of storage. Note that the cell layers (muscular – red stars and submucosa – black arrows) of the urinary bladder and intestine were preserved after conditioning for 30 and 60 days in both groups when compared to the control group.

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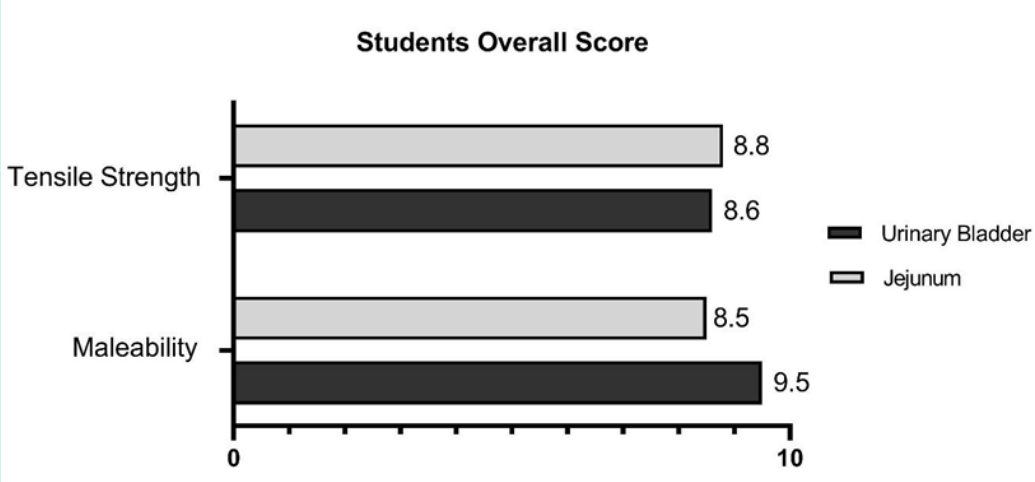

Figure 5: Average grades (from 0 to 10) attributed by the students to the viscera characteristics concerning tensile strength and malleability.

The subsequent questions aimed to investigate the student's acceptance (in favor of or against it) of the use of preserved viscera in surgical technique classes. It was also questioned whether the students had changed their opinion after their experience. The results showed that 100% of the students were in favor of using preserved viscera for teaching surgical techniques, and none of them changed their opinion after the study.

The last alternative questioned which kind of teaching method is preferred by the students for practicing surgical techniques among the options available throughout the discipline: training in synthetic models, training in small fresh anatomical parts, such as beef tongue and chicken thigh, or practices using chemically preserved viscera. The results showed that 3.45% (2/58) of the students prefer classes that provide training in synthetic models, such as plastic or silicone/foam models, 29.3% (17/58) prefer classes that provide training in small fresh anatomical parts, such as beef tongue and chicken thigh, and 67.25% (39/58) prefer classes that provide training with chemically preserved viscera (Figure 6).

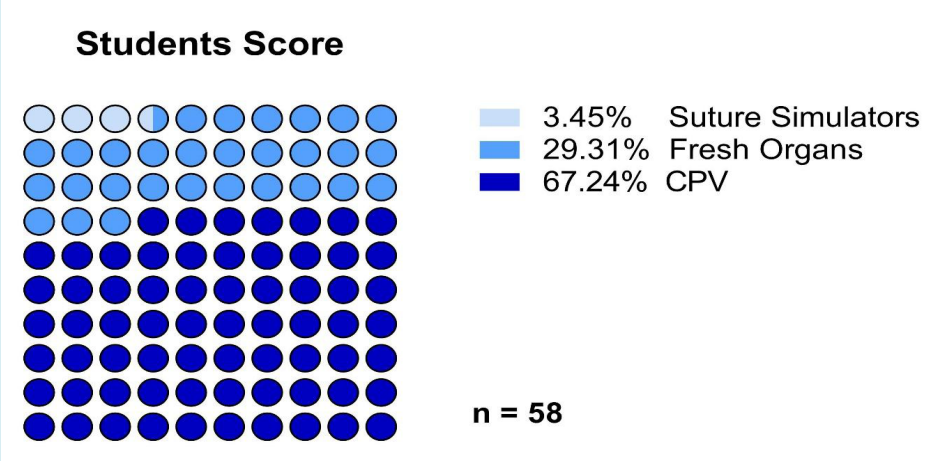

Figure 6: Students' preference in relation to the teaching method used during the course. CPV = Chemically Preserved Viscera.

Discussion

The desired characteristics for anatomical pieces used in surgical skills training include flexibility (elasticity) and malleability, which allow for a more reliable simulation of living tissues [18]. The numerous substances used for its chemical preservation can alter their properties, which may or may not make it unfeasible for operative techniques

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training. Furthermore, the different ways of packaging, depending on the choice of chemical substances used to preserve the tissues, influence its quality over time, the availability of space, and the cost of storing the pieces. All these factors make it possible or not to use these materials for specific purposes, such as surgical technique classes. Fresh anatomical parts, for example, require freezing, making it necessary to have available freezers for storage [23], besides presenting a potential biological risk. Freezers are also necessary for cadavers preserved in Larssen's solution [24]. In literature, cadavers preserved in different compositions of saline solutions are kept refrigerated [25-27] or even in solution tanks [16,25,28] which requires abundant availability of material and room for storage.

Vacuum packaging of anatomical pieces after preservation in supersaturated saline solution, as described in this study, proved to be effective in maintaining physical characteristics favorable for operative techniques practices in swine viscera, allowing tissues division (diaeresis), identification and ligation of vessels, anastomosis, and different suture patterns application, among others, as shown in the swine viscera physical characteristics subjective evaluation report, in which the score attributed to nearly all parameters of the evaluated groups was between 4 and 5 in a scale from 1 to 5. Despite the regular evaluation of two parameters of the jejunum segment (RT) group, these pieces still allowed satisfactory execution of the proposed techniques. The histomorphological evaluation of the preserved viscera of different groups (RT and R) at different times (D0, D30, D60) demonstrated the integrity of the cell layers (except the mucosa), with few significant differences when compared to the control group. These results show that there are few significant differences in the material storage, making refrigeration/refrigerators unnecessary in this case.

The student's acceptance of the use of preserved samples was promising. All declared themselves in favor of using this anatomical preservation technique for training surgical skills and kept their position at the end of the study. Furthermore, their form answers about the anatomical material show high value in the grades attributed to the quality of the viscera, all scoring above 8.0 on a scale of 0 to 10, in terms of both malleability and the sample's resistance to suture/incision. Finally, the answers to the final question showed the students' preference for chemically preserved viscera (67.25%) when compared to the other options for training operative techniques, such as synthetic models (3.45%) or fresh samples (29.3%). Thus, it is concluded that this approach had the students' acceptance, and its implementation is feasible for surgical training classes.

Limitation of this Study

A limited sample group (n= 58 students) belonging to a single institution and monitored during only one semester did not allow a more accurate evaluation of the students' surgical skills evolution. Furthermore, students in that stage of the program does not have clinical practice experience with surgery on live animals, which may influence the results obtained. Only two evaluators (AFM and JBG) with previous surgical skills rated the viscera for malleability and incision/suture resistance.

Cost

The costs involved in the viscera's preparation and storage are interesting factors to highlight. Considering that many places, mainly developing countries, do not have the financial resources to acquire advanced surgical models that faithfully simulate animal anatomy, a less expensive alternative that maintains the effective teaching and learning process is warranted. Preparation and storage of the viscera requires inexpensive, readily and widely available materials in most locations. The estimated amounts for preparing the anatomical pieces, already vaccum-packaged, are about US$1 per viscera (Table 2).

	Price in US$
Packages (un.)	0.5
Salt (kg)	0.4
Absolute ethanol 99,8% (L)	2
Visceras (un.)	0.02
Vacuum sealer (un.)	172.8

Table 2: Average prices of the required materials for viscera preservation and storage in US dollars (US$).
Abbreviation: un., unit; kg, kilogram; L, liter.

Conclusion

Chemically preserved and vaccum-packed viscera using supersaturated sodium chloride solution are a viable option for storing anatomical material at room temperature, outside of solution tanks, exempting the use of refrigerators or freezers and occupying minor space in anatomy labs. Besides being well accepted by the participating students, the technique proved to be safe, low cost and easy to implement, in terms of both preservation solutions and storage. Vacuum packaging allows the preparation of didactic material in any period of the school year, depending only on the availability of materials and staff. Therefore, this may favor institutions

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that have fewer resources for the production and storage of anatomical pieces for practical classes

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