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Cryodehydration: Quality and Durability of Specimens Subjected to Vacuum-forced Impregnation with Wood Glue

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ABSTRACT
Introduction: This study describes a variation of the cryodehydration technique, whose first phase follows the same procedures previously described in the literature (fixation, freezing, and sectioning according to established goals (in the longitudinal, horizontal, and transversal planes) as preparation for repeated freeze-thaw cycles.
Material and Methods: In the second phase, the anatomical specimens were fully immersed in wood glue and placed in a vacuum chamber, with application of negative pressure.
Results: Thus, the once air-filled spaces were suffused with glue and the bubbling and movement of gases from tissue fluids facilitated the seepage of glue into the existing spaces.
Conclusion: The obtained material has a unique quality, no shrinkage, and high resistance associated with longer durability for use in theoretical and practical classes and also as museum specimens.
Keywords: Anatomical technique; anatomical slices; anatomy teaching; cryoconservation.

Introduction

Techniques that are paramount to the practical study of anatomy require, more often than not, high investment for their application, in addition to specific and costly preservation and storage of specimens. Several techniques require polluting reagents, whose fumes or degradation products are harmful to the environment. These reagents are not easily available because of their regulated use or because of their prohibitive costs. Of note, fixatives such as formaldehyde, which is extensively used for fixation and preservation of cadaveric specimens, despite its carcinogenic effects[1,2], cause damage to the organs. Overall, reagents irritate the eyes and mucous membranes and can also cause sudden discomfort and allergies, and they could eventually predispose to oncological diseases.

The first descriptions of the care and procedures for the preservation of animal protein were those conducted by Koonz and Ramsbotton (1939)[3] and Hiner and Hankins (1947)[4]. In their studies, the authors stated that end-product quality is more easily achieved when the material to be preserved is subjected to quick freezing, i.e., to very low temperatures. The same experiments revealed that slow freezing leads to the formation of large ice crystals within cells and in interstitial spaces, causing cell disruption, tissue disorganization, and substantial release of fluids, resulting in remarkable loss of flesh quality.

Following this principle, Teixeira, Guarenti, and Teixeira Filho (1990)[5] developed a technique known as cryodehydration, which employs freeze-thaw cycles (FTCs) that serve a dual purpose. In the first phase, in a process referred to as “cold burning,” slow freezing cycles cause cell disruption and microcracks in supporting tissues generated by large ice crystals formed from the release of tissue fluids and by the water in which the anatomical specimen is partially immersed. In the second phase, as the loss of structure of the material facilitates the release of fluids during FTCs, a cryodehydrated anatomical specimen with little or no tissue shrinkage is obtained. After establishing these basic principles, Teixeira Filho, Guarenti, and Teixeira *et al.* (1996)[6] devised a technique for the preparation of hollow and parenchymal organs, whereas Teixeira Filho and Busch (1994)[7] and Teixeira, Schäfer and Vives (2019)[8] developed a technique for the preparation of anatomical specimens.

Cryodehydration has a large number of variations, which result from some advantages of the technique such as easy application, low cost, and no need for various reagents or for reagents that cannot be easily obtained. Hence, the interest in development of this technique provides excellent outcomes, as observed and described in other studies[9,10].

The variation of the technique described herein allows obtaining dehydrated anatomical specimens using repeated FTCs, but with subsequent application of

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vacuum, when anatomical specimens are impregnated with wood glue under negative pressure, a process that is commonly observed in plastination. The resultant material allows qualified study of practical anatomy, with easy storage, longer durability, and easy handling of the specimens, in addition to being a useful teaching tool during practical classes and providing material for exhibitions at museums. Moreover, contact with toxic chemicals occurs only during fixation of the cadaveric specimens, posing no risk during the technique itself.

In a broader sense and compared with other procedures used for the preservation of cadavers, plastination techniques have been standardized – specimen preparation, dehydration, vacuum resin impregnation, and curing of the material[11] – in order to obtain anatomical specimens that can be used for teaching purposes. By looking at recent studies that employ thinner serial sections[12], the present study applies the "first phase" of cryodehydration, also applying the vacuum technique in the "second phase" so as to speed up and enhance the impregnation of tissues with wood glue.

Materials and Methods

For application of the technique described herein, it is recommendable to use cadavers of lean animals, given that, after the completion of dehydration, when the specimen has already dried, the adipose tissue undergoes dispersion and negatively impregnates the other organs.

The present study is based on the preparation of a young, female, and mixed-breed dog run over by a motor vehicle.

Fixation occurs through perfusion of the vascular bed with an aqueous solution of 10% formalin, but other fixatives can be employed, enabling the use of specimens preserved with various types of chemicals that are less deleterious to human health.

By using a long needle in the shortest possible time, the fixative should be injected directly into digestive organs (stomach, intestines, rectum - especially in case of herbivores), suppressing fermentation, whose gases and compression would interfere with the morphology and the correct relationship between organs. After being subjected to the fixative for 4 days, the cadaver should be kept at an average temperature of approximately -10º to -15º C until it is completely frozen, always in the position required for the final outcome.

Once frozen, the cadaver should be sectioned using a hand or electric saw with 1.5 to 2.0 cm in thickness, according to the predetermined plan to obtain metameric), longitudinal (antimeric), and horizontal (pachymeric) sections.

After the sections, each specimen should be cleaned by gently washing it with a paintbrush under slow and thin water flow, removing tissue debris, but avoiding complete thawing, which could lead to the "breaking apart of the specimen" and wastage of body segments.

In the case of herbivores, the contents of the stomach and intestines may be kept because, from a teaching perspective, it is interesting to show the different stages of digestion foods go through in each of the compartments. In the case of exhibitions at museums, not removing the contents may be useful to demonstrate the large amount of food in the compartments.

After the body segments have been prepared, they are subjected to the "cold burning" phase, alternating it with FTCs.

For cold burning, the sections should be placed in a tray in the freezer, with no protective cover, and thawed in the shade, at room temperature, or under slow and careful water flow after each thaw cycle. To allow cold burning of both sides, the specimen should be flipped over immediately after it is taken out of the freezer and while it is still ice-hard, thus preventing the loss of small body segments.

This procedure should be repeated every 24 h and, to establish a routine, it is recommended first to remove the segments from the freezer in the morning, flip them over and, once they have been thawed, return them to the freezer until the next morning.

The number of FTCs depends on specimen size and on the performance of each body segment, considering different types of organs and reactions between them (e.g., between heart and lungs and between liver, kidneys, and intestines).

For that reason, and as a general rule, this "burn" phase comes to an end when the tissues, mainly the muscle tissue, have a similar texture to that of a sponge, releasing fluids when the tissues are compressed; however, the tissues tend to slowly return to their original shape after compression. For visualization of parenchymal organs, the "burn" phase leads to the formation of lighter-colored areas on the surfaces, indicating correct processing.

Based on the observations, at least 18 to 20 freeze-thaw cycles are recommended for each segment.

After the "burn" phase, unlike the previous procedures, now in the second phase, excess water should be eliminated by placing the specimens on absorbent material (paper or fabric) or inclining them for a short time to prevent the surface from drying up. Thereafter, each segment should be placed on a tray and completely covered with water-soluble wood glue, and then decompressed for 10 minutes in a vacuum chamber. As the negative pressure rises, the glue seeps into the air-filled spaces. Also, the extraction of gases diluted in water and tissue fluids through bubbling helps with the penetration of the wood glue.

In the present study, we used an iron chamber (4 mm in thickness; 50 x 40 x 19 cm.; lid fixed with screws and sealed with rubber) and a vacuum pump (Quimis Q355d2), with a final vacuum of 660 mmHg (Fig. 1).

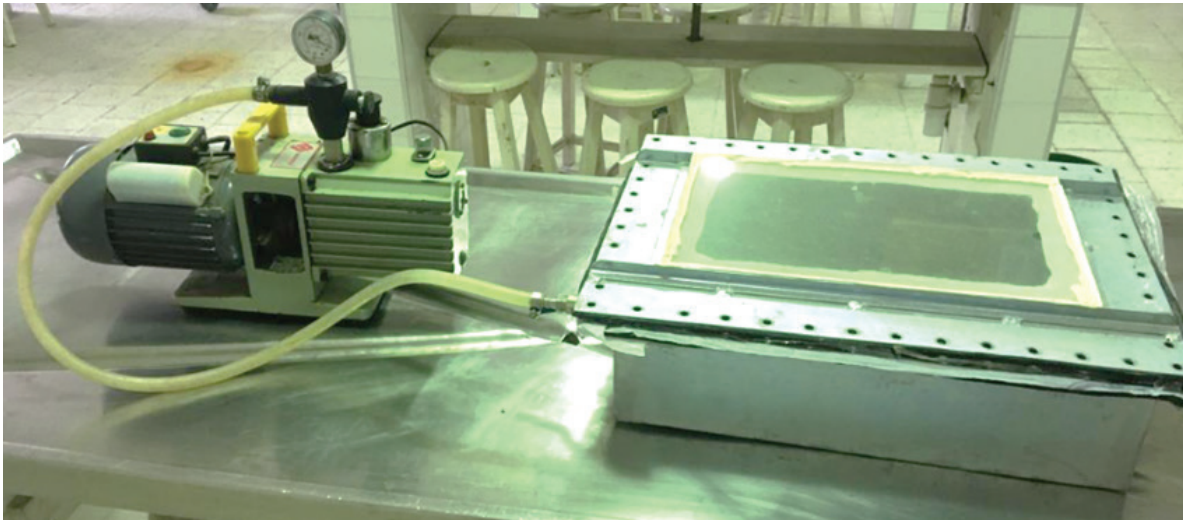

Figure 1. Iron vacuum chamber (4 mm in thickness; 50 x 40 x 19 cm; lid fixed with screws and sealed with rubber), coupled to the vacuum pump (Quimis Q355d2), with a final vacuum of 660 mmHg.

Cascola-Cascorez, a polyvinyl acetate (PVA)-based glue (Henkel Ltda), was diluted in water at 60%, 70%, 80%, 90%, and 100%.

The whole process was performed at room temperature.

Immediately after removal from the vacuum chamber, excess glue was removed, taking care not to break the specimen apart or waste small pieces of organs. The sections had to be kept on a flat surface at room temperature until they were totally dry. In case of twisting or bending after drying, the anatomical specimen had to be pressed until correction (pressing between wooden surfaces should be avoided to prevent the specimen and surfaces from being glued together). These negative changes occur if the first phase is not conducted properly or if drying is too quick or is carried out in the presence of a heat source.

After having been dried completely, both surfaces were polished to obtain a better finish (Fig. 2) and the organs in each section could or could not be painted to resemble their natural color and hue (Fig. 3).

Finally, one or more coats of glue, applied with a paintbrush as if it were paint should be used for finishing, thus enhancing the appearance of the specimen, improving adhesiveness between the organs, and protecting the material against dust penetration.

Some precautions should be taken throughout the process:

In the first phase, as soon as thawing occurs, the anatomical specimen should be placed back in the freezer. If it is not possible to do that immediately, it is important that it be made within 24 or 48 hours, not allowing the material to dry up.

In any of the phases, the surfaces can not be allowed to dry, thus preventing shrinkage and darkening of the organs.

The slower the freezing processes, the better the final outcome (the temperature should range between -10 °C and -15 °C).

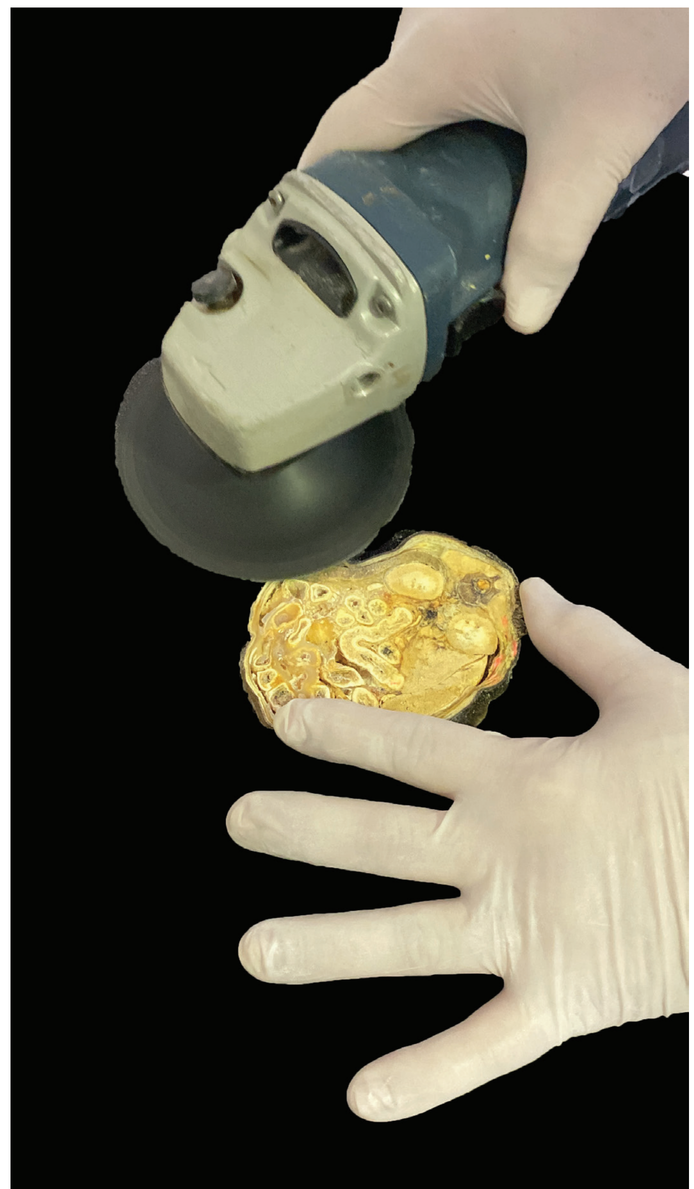

Figure 2. Careful and delicate polishing of the anatomical specimen, indicating the use of high-speed instrument for a better finish (grit size 80)

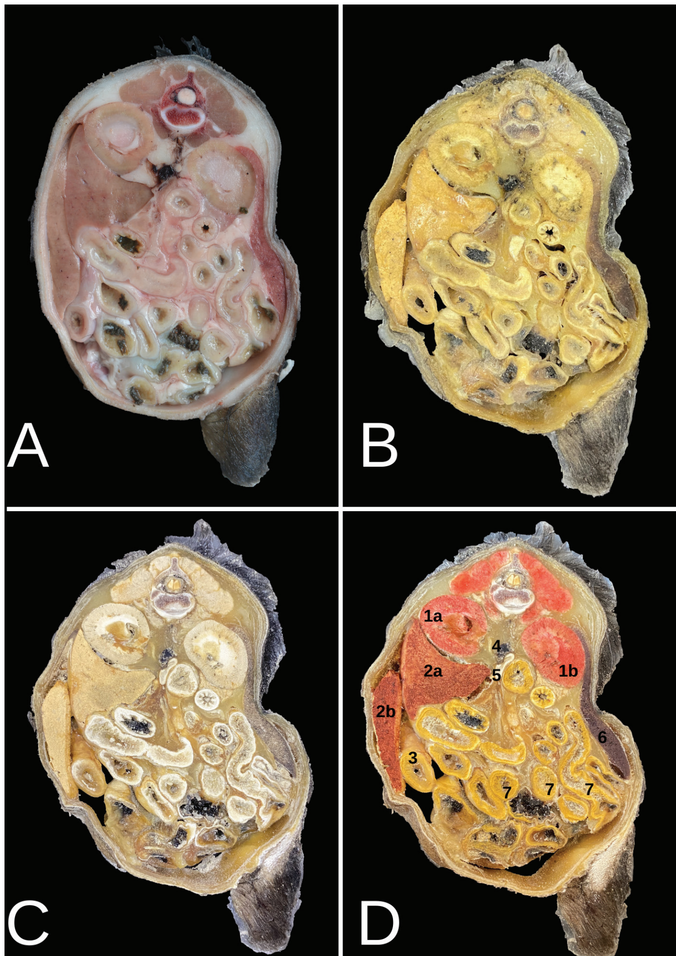

Figure 3. Cranial-caudal view of the same transversal section of a young female dog run over by a motor vehicle (organs with altered position due to the trauma), in the region of the second lumbar vertebra. A – Abdominal segment immediately after section; B – Dry section immediately after completion of the technique; C – Section immediately after polishing; D – Section after painting and artistic finish. 1.a. Right kidney; 1.b. Left kidney; 2a. Liver – right medial lobe; 2b. Liver – right lateral lobe; 3. Descending duodenum; 4. Caudal vena cava; 5. Aorta; 6. Spleen; 7. Jejunal loops.

If significant shrinkage occurs, the section should be placed back on a tray with water, where it will be kept fully immersed until the original shape is restored, prior to the first phase.

Water-soluble wood glues are recommended because they cause no shrinkage and have a better appearance after drying.

Radiographs should be taken before sectioning the body parts of the animals, thus allowing students to establish correlations between the radiographic image and the sections.

Results

Cryodehydration is a simple technique, but it may consist of several different procedures and modes of application.

The present study describes an alternative cryodehydration technique previously described by Teixeira Filho and Busch (1994)[7] and by Teixeira, Schäfer and Vives (2019)[8] for anatomical sections. The technique used herein allows enhancing the outcomes by using vacuum, as occurs with plastination, and impregnation with wood glue. Moreover, large weight loss is observed (in some cases, 70% or higher), in addition to excellent resistance to the mechanical actions performed in the final preparations.

The technique has some advantages such as low cost; ready availability of the material; strong resistance of the material to handling, mechanical action, and occasional falls to the floor; longer durability (the course on Anatomy of the Domestic Animals taught at the Universidade Federal de Pelotas has muscle specimen preparations that have been used for 40 years in a row); no need for fixatives for their preservation and elimination of odor emissions; protection for students and professors; easy storage of anatomical material; "reconstruction" of the specimen by stacking the sections atop each other, providing excellent practical and topographical anatomy studies, in addition to good-quality syntopia and skeletopia analyses; interdisciplinary approach in areas that use radiographs, tomographic scans, resonance imaging, and clinical medicine; stratigraphic approach to surgical studies and procedures; in the present case, it is possible to verify the pathological impact on the patient (Fig. 4).

Regarding the dilution of wood glue, no significant differences were observed between proportions; only higher "softness" on the section surface at 60%, whereas the undiluted glue showed higher "hardness." All the outcomes were satisfactory in terms of final outcomes.

Therefore, it is possible to conduct an extremely dynamic, illustrative, fruitful, and fascinating anatomic study, with clearly established interdisciplinary aspects, as mentioned earlier.

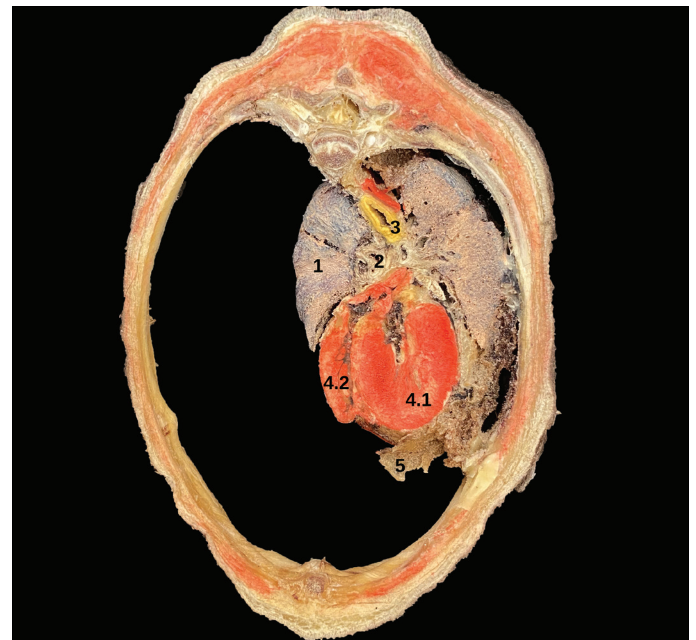

Figure 4. Cranial-caudal view of metamere in the thoracic region of a mixed-breed female puppy showing the presence of pneumothorax caused by the impact against a motor vehicle. 1. Collapsed right lung; 2. Bronchi; 3. Esophagus; 4.1. Heart, left ventricle; 4.2. Heart, right ventricle; 5. Pleura.

Discussion

The ideas about the first cryodehydration procedures arose approximately 50 years ago after observing that repeated freeze-thaw cycles caused dehydration in cadavers stored in freezers. The outstanding results obtained for the preparation of muscles[5] were later adapted for the preparation of hollow and parenchymal organs[6] and then modified for visualization on the transversal, longitudinal, and horizontal planes[7,8].

Owing to the simplicity and low cost of cryodehydration, several contributions have been made to this technique over the years. Different procedures, however, can be used, as that proposed by MARTINS and SAKALEM (2022)[10], which suggests thawing in heating chambers, as a way to speed up the process, and that proposed by KREMER (2011)[9], in which the biological material was protected with plastic bags during freezing cycles in the first phase. Nevertheless, based on the experience acquired over a 32-year period of tests, the best outcomes are achieved without such "protection," considering that "burning" is the primary goal of this phase, i.e., tissue disorganization by the formation of large ice crystals. Also, it is recommended that thawing should be performed with the specimens immersed in water or under a slow water flow in order to speed up the process.

The recommendations made by MARTINS and SAKALEM (2022)[10] for thawing in heating chambers also speed up the process. However, it should be underscored that the aim of the first phase is the use of "cold burn" instead of dehydration.

Importantly, there exists no "most appropriate time for the transition" between the first and second phases. The recommendation is for the "burn" of the organ and the "sponge-like appearance" of the muscles. Conversely, MARTINS and SAKALEM (2022)[10] suggest monitoring weight loss, recommended for either parenchymal or hollow organs separately, but not for the present case, considering the presence of numerous anatomical structures in a single section, including bones (small loss of weight) and lungs (remarkable loss of weight). In other words, when a section has apparently reached the end of the process, it does not necessarily mean that other sections have as well.

Finally, the ideas about the application of vacuum arose from the use of plastination[11, 12], with the aim of enhancing the impregnation of bodily tissues with wood glue.

During theoretical or practical classes, the sections may be stacked on top of each other in order to "reconstruct" certain regions of interest (Fig. 5.A), which is a very good and illustrative way to identify the position, size, depth, and relationship among anatomical structures (Fig. 5.B).

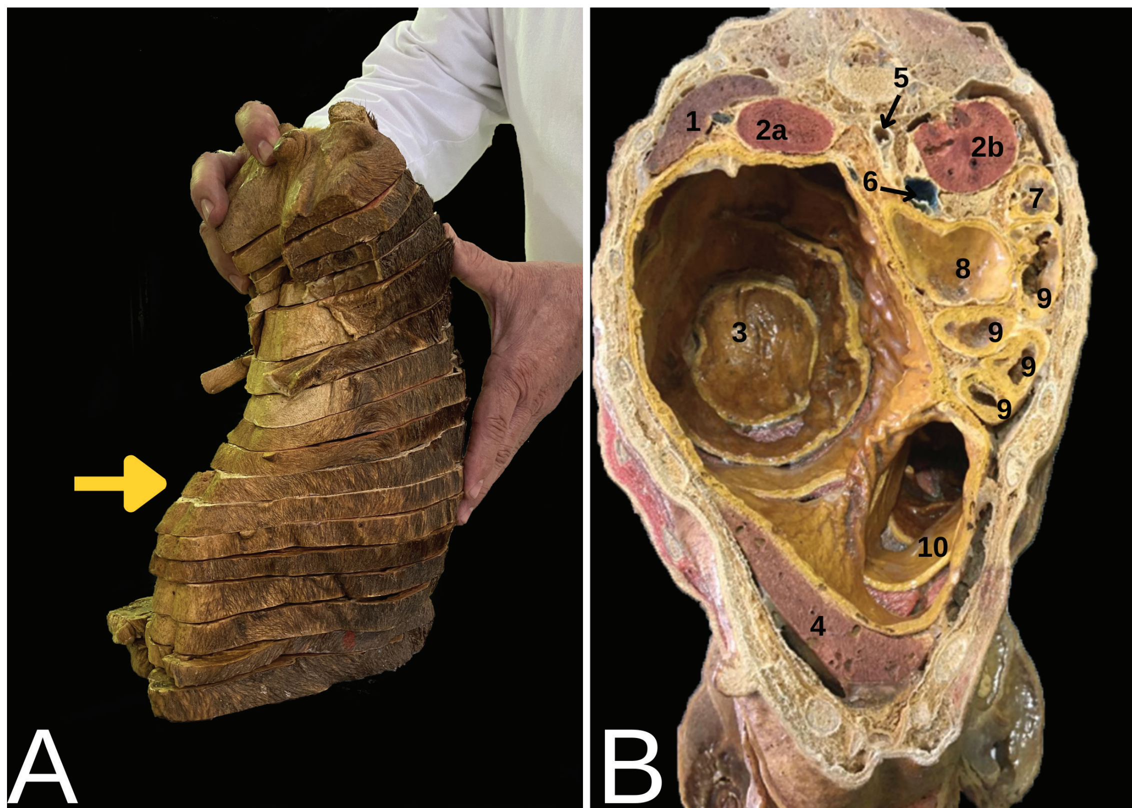

Figure 5. A – Right lateral view of the thoracic, abdominal, and pelvic regions of a mixed-breed female dog subjected to transversal sections after cryodehydration. The arrow indicates the section in the subsequent figure; B – Section indicated by the yellow arrow in the caudal-cranial view showing the three-dimensional positioning of the organs in the region, such as the stomach. 1. Spleen; 2a. Left kidney; 2b. Right kidney; 3. Stomach; 4. Liver; 5. Aorta; 6; Caudal vena cava; 7. Duodenum; 8. Transverse colon; 9. Jejunal sections; 10 Pyloric antrum.

Conclusion

At the end of the process, the obtained material shows high resistance, which improves the useful life of the segments, considering that the ultimate goal is to allow their manipulation in anatomy classes and museum exhibitions. The low cost associated with this technique is also an extremely positive aspect because the procedures can also be performed in underfunded laboratories.

As a matter of fact, the low cost, the maintenance, and the storage of the material are the major advantages of this technique.

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It is with genuine gratitude and warm regard that we dedicate this work to Prof. Dr. Antonio Fernandes Filho, retired professor/researcher from FMVZ/USP, taking into account his vast knowledge of, devotion to, and love for the study of anatomy.

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Mini Curriculum and Author's Contribution

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