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Preparation of Sets of Large Animal Organs Using Cryodehydration

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ABSTRACT

Introduction: practical anatomical studies using cadavers of large animals, such as equines, pose difficulties due to their volume, weight and the conditions required for handling and storage of certain groups of organs. This paper describes a way to overcome these problems by using cryodehydration for the preparation of a set of organs. This description includes the metamere formed by the abdominal segment of the oesophagus, stomach, duodenum, pancreas, liver, spleen, adrenal glands and kidneys, as well as the vascular segment associated with these organs. It also describes the preparation of the organs in the thoracic cavity.

Material and Methods: after fixation, the abdominal cavity is opened to expose a wide caudal view of the organs mentioned above. A layer of polyurethane foam is applied to this surface to form a mold. When dry, this mold is removed. The organs are dissected, removed as a unit and immediately placed back on this mold to preserve their morphology and positions relative to each other. The side that was exposed undergoes cryodehydration. When completed, this surface is molded again and cryodehydration is repeated, now to the newly exposed side. For the organs in the thoracic cavity, the diaphragm is removed and the first polyurethane mold is prepared on the diaphragmatic surface of the lungs, and the second, on their dorsal edge, alternating the cryodehydration techniques at all times. Dissections to expose specific structures may be conducted during all the procedure and, at the end of this process, each organ may be painted in a characteristic colour.

Results: these units last longer and have the advantage of being lighter and easier to handle and store.

Conclusion: as teaching aids, the thoracic set may be re-connected to the abdominal set, which facilitates learning, preserving the original topography and morphology. These same sets are also excellent material for museums.

Keywords: Anatomical technique; Anatomical slices; Anatomical molds; Polyurethane foam.

Introduction

In Veterinary Medicine, the anatomical study of large animals, such as equines, requires unique strategies, as the viscera received from slaughterhouses do not maintain their morphology and natural spatial relations. Moreover, even when preserved in fixation agents, the wear resulting from handling a very heavy organ rapidly deteriorates rich resources of morphological characteristics, which have great value for research and for students in laboratories.

In addition, warnings have already been issued[1,2] about the carcinogenicity of formaldehyde, the chemical most often used for fixation, the reason why its use should be avoided.

In practical studies, the substantial contribution of a technique called cryodehydration has already been demonstrated in several publications that described the preparation of muscles[3], hollow and parenchymatous organs[4] and anatomical segments[5,6]. After these first publications, studies have added suggestions and procedures for the improvement of specimen cryodehydration[7,8].

Cryodehydration and its variations, used for the preparation of viscera in repeated freezing and thawing sessions (FTS), provide excellent material for practical anatomy studies. The focus of this study is a set of equine viscera consisting of the abdominal segment of the oesophagus, stomach, duodenum, pancreas, liver, spleen, adrenal glands and kidneys, as well as the segment of vessels that supply and drain blood from these organs. Organs in the thoracic cavity were also prepared.

Material and Methods

The two horses used for this technique were received from the Veterinary Hospital of the Federal University of Pelotas.

First, the common carotid artery was dissected and cannulated, and the jugular vein was sectioned in the same area. After that, perfusion with the 10% formalin aqueous solution began and continued until rigor mortis was complete, which confirmed that fixation agent diffusion was extensive and favourable.

Three days after the chemical was applied, the

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abdominal wall was widely opened and the segments of the large intestine were dissected carefully. The first incision completely sectioned the ileum, close to the ileocecal sphincter, and the other incision was at the end of the descending colon, close to the pelvic cavity entrance. After that, the mesentery was dissected at its origin, leaving a short segment of the cranial mesenteric artery fixed to the aorta. The ascending duodenum was then sectioned right at its end, and the portal vein was completely sectioned before the point where the splenic vein joins it, releasing the jejunum/ileum segments. Finally, the omentum (epiploon) was transected along the greater curvature of the stomach.

After the stomach surface was exposed, the duodenum, liver, pancreas, spleen, kidneys and adrenal glands were sprayed side by side with polyurethane foam (Mundial Prime - Espuma de Poliuretano, Aeroflex Indústria de Aerossol Ltda, Curitiba, Brazil), thus fabricating a mold that accurately reproduced each organ and the irregularities of the surface that the foam was covering. Pieces of twine were sparsely distributed between spray layers for reinforcement, and a flat platform for support was prepared on the free surface.

As the material hardened, the mold was displaced, and the careful dissection of the viscera started for their en bloc removal. Beginning in the liver, the right and the left triangular, falciform, round and coronary ligaments, the last two together with the caudal vena cava, were dissected. Because of its proximity, the oesophagus had already been sectioned close to the diaphragm.

In the spleen, only the connective fibres of the splenorenal ligament that fixed it to the left crus of diaphragm were sectioned, and not those that unite it to the left kidney. The connections joining the stomach, pancreas, duodenum and kidneys were not dissected. The segments of the aorta and caudal vena cava associated with these organs were sectioned so that they could be removed with the block prepared.

Finally, once removed, these viscera were placed back on the mold previously fabricated (Fig. 1) to begin the freezing and thawing sessions (FTS) on the surface that remained exposed, as explained for the technique used for cryodehydration.

This set of structures, now called a block, was placed in the freezer for a first freezing session that lasted 48 hours because of the block's large volume. According to the protocol, the block should be removed from the freezer in the beginning of the day and returned to it right after thawing, which, in this first phase, may be carried out under slow running water.

After completing the first phase of the technique, called "cold burning", a new mold was fabricated, this time of this cryodehydrated surface. Immediately after that, FTS began for this new surface, which was now exposed.

As described for cryodehydration, the burning phase ends when the organ surfaces appeared to be all burned by the cold temperature. That means that, initially, there were only small whitish areas resulting from the action of ice, but, in the end, these areas covered the whole organ. The number of FTS depends on the size of the material, but a minimum of 25 to 30 FTS on each side is recommended.

For the thorax, the diaphragm was removed and the first mold was fabricated on the diaphragmatic surface of the lungs, while all viscera were still in place. When the mold was ready, the organs were removed using a full incision of the structures at the level of the first pair of ribs, after which all the vessels and structures that attached these set of structures to the thoracic cavity wall were dissected. This new block underwent the same procedures, and the second mold was fabricated on the dorsal border and lateral face of the lungs (Fig. 2).

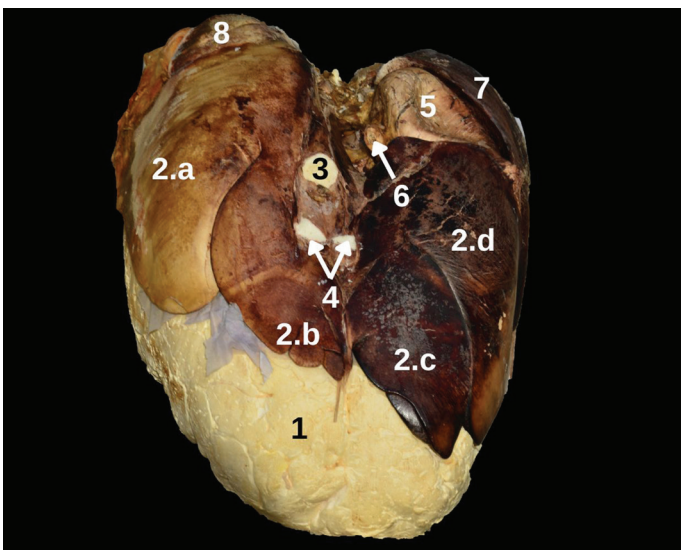

Figure 1. Craniocaudal view of block of abdominal viscera, already dehydrated, laying over polyurethane foam. 1. Polyurethane foam; 2. Liver (a. Right lobe; b. Quadrate lobe; c. Left medial lobe; d. Left lateral lobe); 3. Caudal vena cava; 4. Opening of hepatic veins; 5. Stomach; 6. Oesophagus; 7. Spleen; 8. Right kidney.

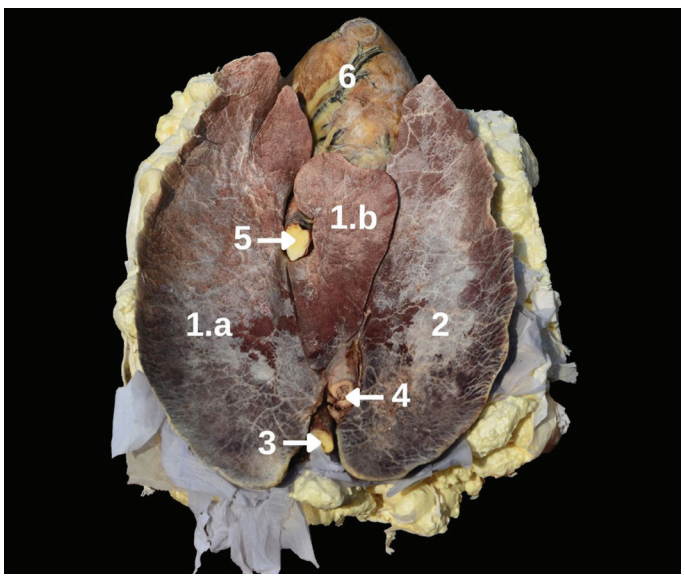

Figure 2. A caudocranial view showing position of polyurethane foam supporting organs of thoracic cavity. 1. Right lung (a. Caudal lobe; b. Accessory lobe); 2. Left lung (caudal lobe); 3. Aorta; 4. Oesophagus; 5. Caudal vena cava; 6. Heart.

The next stage was the second phase of FTS, when the blocks were thawed at room temperature, without exposure to the sunlight or use of water after the freezing stages.

The process was accelerated by alternating each surface, as the blocks were sequentially turned and moved to another mold until cryodehydration was complete. Absorbing material, such as paper or fabric, may be placed between the mold and the viscera to accelerate water removal.

During this process, several techniques may be used, preferentially in the first days. For example, vessels, such as the aorta, the caudal vena cava and the portal vein, may be injected with the same foam used to build the molds. Another option is the dissection of different tissues during the whole process, exposing structures of interest, such as arteries, veins, ureters, perirenal fat and others.

Once dried, each organ may be painted in colours that indicate their natural state (Fig. 3), and then several layers of wood glue (carpenter's glue) should be applied as a protection against powder and as an element to protect and hold together all the anatomic structures. Total weight may be reduced by 70% to 85% of baseline weight, making easy the handling during explanation in classes or exposition (Fig. 4).

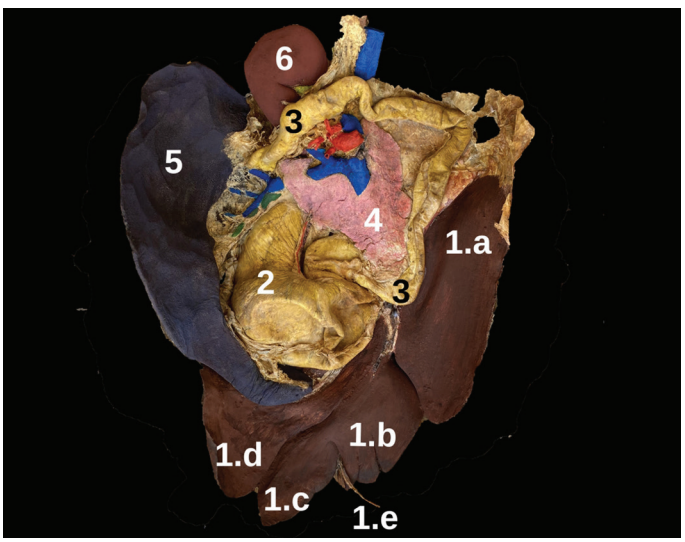

Figure 3. Caudocranial view of organs of abdominal cavity after use of cryodehydration technique. Each organ was painted in characteristic colour. 1. Liver (a. Right lobe; b. Quadrate lobe; c. Left medial lobe; d. Left lateral lobe; e. Falciform ligament with round ligament); 2. Stomach; 3. Duodenum; 4. Pancreas; 5. Spleen; 6. Left kidney.

Results

The results of the procedure described here - the dissection and en bloc removal of the organs of the thoracic and abdominal cavities for later application of cryodehydration – have been satisfactory.

The preparation of cryodehydrated hollow and parenchymatous organs has already been described[4], but here this technique is associated with a special preparation procedure to maintain their natural morphological characteristics: use of polyurethane foam molds as a support for the preparation of large blocks of cryodehydrated organs.

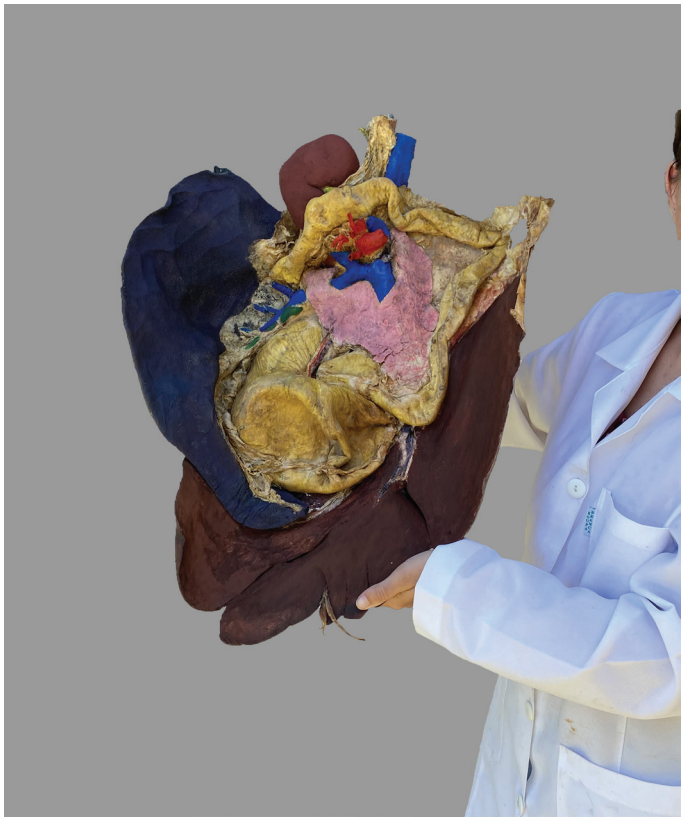

Figure 4. Photo demonstrates advantages of association of reduced weight, preservation of connections and relationships between organs and perfect morphology of each organ.

The viscera prepared using the procedure described here maintain their individual morphological characteristics at a high level of precision, an advantage of using cryodehydration. The details of the syntopic relations between organs are also maintained (Fig. 5).

In addition, these two sets (thoracic and abdominal), when placed in apposition (Fig. 6), may also clearly demonstrate the topographic relation of the organs and structures in the two cavities, such as the course of the oesophagus and the aorta from the thoracic to the abdominal cavity. Several details, which are thus

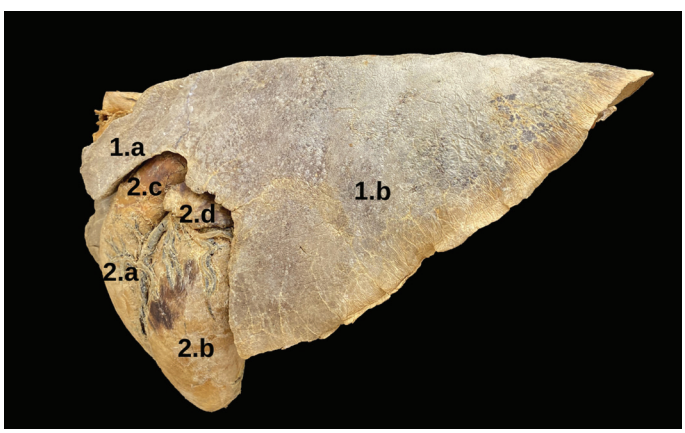

Figure 5. View of heart and left lung (facies costalis) after cryodehydration. 1. Left lung (a. Cranial lobe; b. Caudal lobe); 2. Heart (a. Right ventricle; b. Left ventricle; c. Pulmonary artery; d. Left auricle).

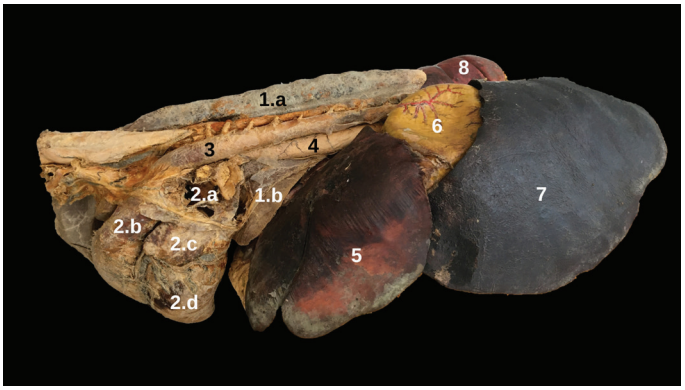

Figure 6. Image shows proximity and "coupling" of two segments, thoracic and abdominal cavity, obtained by means of cryodehydration variations and special procedures. 1. Right lung (a. Caudal lobe; b. Accessory lobe); 2. Heart (a. Left atrium – open due to lung dissection; b. Pulmonary artery; c. Left auricle; d. Left ventricle); 3. Aorta; 4. Oesophagus; 5. Liver; 6. Stomach; 7. Spleen; 8. Right kidney.

made clearer, are important for the interpretation of radiographs and for clinical and surgical procedures.

Moreover, viscera may be subsequently separated in the blocks, one of the other great advantages of cryodehydration. For example, a lung may be dissected and carefully removed because, as it is dehydrated and does not lose its original form, it may be inserted back in its right place, which provides visualization of the structures in the mediastinal space.

Therefore, as reported in other publications[3,4,5,7,9], cryodehydration is easy to apply, ready to use, has great durability and does not require the use of preservatives, characteristics of great value in the practical study of anatomy.

As reported in other publications, weight loss in cryodehydrated organs may reach about 80%[5], or about 60% to 70%[8], a highly positive factor, particularly because of the volume and size of this type of material prepared for equine specimens. Finally, the possibility of readily using the material and its easy conditioning, as it requires no use of fixatives or tanks, all contribute to the choice of this procedure.

Discussion

The practical study of anatomy of large animals poses great difficulties, such as the large volume and weight of some of the organs and their handling, as well as the complexities of the conditioning processes. At the same time, viscera currently used in animal anatomy laboratories are usually preserved by means of fixation using chemicals, such as formaldehyde, which may aggressively affect the mucosas or cause severe damage to human health.

The preparation of large blocks of equine viscera using the polyurethane foam molds preserves the unique morphology of each organ and, at the same time, their syntopy with the other organs, with great gains for practical anatomy studies.

These polyurethane foam molds, prepared before the organs of interest are removed, form a block for these viscera and provide a perfect support for their submission to controlled drying by repeated FTS, as described for cryodehydration.

The results provide a high-quality reproduction of the actual morphology and spatial relations of anatomic structures. In addition, handling and conditioning are easy, durability is long and production costs are substantially reduced. Finally, these teaching resources are substantially important as learning aids, as well as material for museum exhibits.

The procedures recommended by Kremer, Schubert and Bonfíglio (2011)[7] – to protect the organs using plastic bags during first-phase freezing – should not be followed when the volume of the material prepared is large. In fact, such recommendations not only make the process take longer, but also make it difficult to achieve the objective of burning tissues using cold temperatures. Moreover, thawing in ovens, which Martins and Sakalem (2022)[8] have suggested as indicative of the transition from the first to the second phase based on weight loss, is compromised by, first, the size of the material prepared and, second, by the use of running water at a low flow velocity.

The possibility of visualizing a set of viscera, both in the thoracic and abdominal cavity, while preserving the precision of their individual morphological characteristics and their collective topographic features, is undeniably useful as a study method.

Conclusion

The use of polyurethane foam, applied as auxiliaries molds in the development of the cryodehydration technique, facilitate and promote the final qualification of anatomical pieces of great volume and weight. Thus, the material obtained is much lighter, easier to handle and perfectly showing syntopy relationships. By maintaining the morphology and association between different organs, it offers a better experience so that students can more objectively and clearly visualize how organs are positioned, related within body cavities and preserving the original topography and morphology. These same sets are also excellent material for museums.

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Mini Curriculum and Author's Contribution

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