3D capturing anatomic complex biomodel in medicine veterinary: how to prepare, digitize and replicate the male canine urogenital system

Captura 3D de biomodelos anatômico complexos na medicina veterinária: como preparar, digitalizar e replicar o sistema urogenital canino macho

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ABSTRACT
Medical images combined with 3D printing techniques have been a differential for clinical surgical and didactic approaches. The segmentation of anatomical structures in medical images for their 3D printing of biomodels has been showing promise in medical applications. The use of iodine contrast in solution on anatomical specimens promotes an increasing the density and better caption resolution of CT, expanding the range of possibilities for 3D medicine. With the capture of a complex anatomical arrangement, there is an advantage over the use of biomodels both in a digital and physical interactive way. In this article, we explain how to digitize a set of anatomical structures of the canine urogenital system in topographic position and how to create 3D digital and printed replicas. The biomodel can be applied in the most diverse areas of veterinary medicine and related fields.

Keywords: 3D printing, digital medicine, DiceCT.

RESUMO
Imagens médicas combinadas com técnicas de impressão 3D têm sido um diferencial para abordagens clínicas cirúrgicas e didáticas. A segmentação de estruturas anatômicas em imagens médicas para impressão 3D de biomodelos tem se mostrado promissora em aplicações médicas. O uso de contraste a base de iodo em solução em peças anatômicas promove aumento da densidade tecidual e melhor resolução de captação da TC, ampliando o leque de possibilidades da medicina 3D. Com a captura de um arranjo anatômico complexo, há uma vantagem sobre o uso de biomodelos tanto de forma digital quanto física interativa. Neste artigo, explicamos como digitalizar um conjunto de estruturas anatômicas do sistema urogenital canino em posição topográfica e como criar réplicas 3D digitais e impressas. O biomodelo pode ser aplicado nas mais diversas áreas da medicina veterinária assim como em ares relacionadas.

Palavras-chave: impressão 3D, medicina digital, DiceCT.
1 INTRODUCTION

3D printing has provided a revolution in the area of additive manufacturing for creating objects aimed at medical anatomical education. More precisely regarding the use of raw materials, the rapid prototyping system adds material in liquid or solid form in horizontal layers on the X and Y axes superimposed by a new layer on its Z axis in vertical lift, generating the object from the gradual union of material (BIGLINO, et al., 2011). Due to its dynamic overlapping of layers to easily generate a complex object, this technique has been opening previously unimaginable possibilities (GIANSETTO, 2015).

Veterinary Medicine has benefited greatly in the areas of creating an anatomical collection for educational purposes, surgical training, implants and prostheses for specific patients, as well as a valuable object for communication and clarification of the case between professional and tutor (LIMA et al., 2020, MIMOUNE, et al., 2023).

The use of anatomical biomodels obtained via computed tomography (CT) has provided advances and facilities for the medical routine both in educational institutions and in the labor market, being a gold standard tool for understanding orthopedic cases. However, this technique has been used for mineralised areas with more impact due limitations for capturing soft tissue data and sets of different structures due to low density of the cells and unspecified image data (GIGNAC & KLEY, 2014; BORDELO et al., 2018; LIMA et al., 2020). Making it difficult, nearly impossible, to identify and differentiate the viscera, muscles and adjacent tissues, since the tissue density is low and within the same range of radiopacity threshold.

Since the creation of diffusible iodine-based contrast-enhanced computed tomography (diceCT), new possibilities for capturing complex three-dimensional anatomic arrays using contrast agents and radiographic images have emerged for microtomography (mCT) (METSCHER., 2009; COX & JEFFREY, 2011). The combination of techniques proved to be of great value in optimizing the understanding of anatomy and generating 3D material for teaching, research and training in the medical field, both digitally and physically (METSCHER, 2009; GIGNAC & KLEY, 2014; CHEN et al, 2017). The use of very small anatomical pieces preserved and immersed in contrast allowed the penetration potential using Lugol’s solution (I2KI) and capturing by
mCT to better be identified (METSCHER., 2009). This experimental model promoted a growing development of new protocols for the improvement of the microCT techniques with contrast agents impregnated in anatomical specimens for better capturing images of soft tissues. When replicated in post-embryonated animals in microCT, the technique faced limitations of Lugol's perfusion in the innermost tissues, causing incomplete visualization of the structures (JEFFERY et al., 2011; GIGNAC & KLEY, 2014).

It has been a global goal to implement the 3Rs (reduce, recycle and reuse) in the research and teaching field, directly collaborating with the preservation of the environment and minimizing the use of animals in scientific academic routine. Anatomical biomodels can be applied by researchers, educators and professionals to clarify different treatment options, explain the case to tutors and analyze the best approach, promoting clinical-surgical planning with residents team (SINGHAL et al., 2016; REIS et al., 2017; BORDELO et al., 2018, LIMA et al., 2020). The urogenital system of male dogs is one of the first challenges faced by students and young professionals, whether for surgical training in castration or bladder obstructions in the clinical routine. In this article, we describe the protocol for obtaining 3D anatomic replicas of the urogenital system of male dogs in its topographic position and replicating with 3D printing by fused deposition modeling (FDM) technique.

2 ANIMAL MODEL

This project used as model cadavers of ten male adult dogs without definite race, without anatomical variations, belonging to the sector of Veterinary Pathology of the HOVET-UFMT. Ten CT images belonging to the database of the Image Diagnosis Sector - HOVET that contained the pelvic region of random male dogs in previous routine examinations were also used as controls for the technique.

3 MATERIAL AND METHODS

3.1 ANATOMICAL PREPARATION

The specimens were dissected as fresh as possible, no more than 24h after death hour, in the meantime the corpses were maintained inside a cold chamber in the Pathology
department of the Hospital. The specimens were positioned in the dorsoventral decubitus, the region of interest was traditionally dissected in a block, using scalpel n23, tweezers and scissors. Delimiting the abdominal area from the navel to the scrotum, to preserve the interconnected structures; bladder, prostate, vas deferens, scrotum, testicles, penile body and glans. Soon after the dissection a probe was inserted into the urethra to keep the channel open, the fresh anatomical pieces were positioned topographically for fixation in formalin. The anatomic system was located inside a plastic box with a lid, its interior was lined with an adapted soft support, creating scaffold levels to position the set of dissected structures in their topographical position, by inserting soft towel papers between all sides of the anatomical differs structures. With the piece in place, 10% formalin was carefully added until it was completely covered, keeping it in the fixation process for 7 days (Figure 1).

Figure 1. Preparation of the complex anatomical arrangement. (A) lateral view of male canine urogenital tract dissected en bloc. (B) Dorsal view of anatomic piece positioned above soft support scaffolds. (C) Piece being carefully immersed and 10% formalin, maintaining its morphological topography. (D) Anatomical piece completely fixed in formalin and accommodated to be immersed in the contrast solution for DiceCT.

Source: Data from the survey itself.
All anatomical pieces went through the same four process steps (desiccation, fixation, CT scans, storage in contrast) and were used as their own image control during the process. After fixation, the models were submitted to traditional tomographic image capture, then put stored back in the plastic box filled with 10% formalin now with 5% Iohexol contrast solution in it. Every seven days, the pieces were removed from the boxes and new tomographic captures were taken during the following three weeks.

3.2 IMAGE CAPTURE

For tomography scans, the urogenital system was removed from the solution, washed in running water to remove the excess of contrast, dried with paper and placed on soft adapted support to keep in morphological position during scans. For image capture, a SOMATOM® Spirit® CT was used, following the protocol; bone and soft tissue window capture, Fov 512mm, Kv 130, mAs 80, Slice 1.5mm, Pitch 1.5, helical capture. The images were then saved and exported in Digital Imaging and Communications in Medicine (DICOM) format (SCHWARZ & O’ BRIEN, 2011)

3.3 EDITING AND 3D PRINTING

Image segmentation was performed via free software, inVesalius®, using automatic segmentation of the structure, performing adjusting the threshold density inside the desired range to select the entire set of structures as one piece, using the range of -700/+225 Hounsfield Unit (HU). The created models were then saved and exported as .obj format. The files were opened in an educational version of Meshmixer® software to smooth out capture artifacts, fix holes in the object, make it solid for manufacture compatibility and isolate different structures (SILVA E GAMARRA ROSADO, 2014). Part of the bladder was reconstructed using the "Inflate" tool to recreate the incomplete area. The urethra was digitally constructed using the "add tubes" tool. Models were fragmented into different parts using the "plane cut" tool to facilitate the 3D printing process (Figure 2) and to create new perspectives for its use. The Simplify3D® software was used to establish the printing protocol for each part, using as protocol: 0.15mm layers height, 3 outside shells, 25% filling, automatic support, table temperature at 60°C and
extruder nozzle at 210°C, speed 40mm/s. With the setup defined, the files were sent to a 3D printer Ender® 5 pro, using a 1.75mm Polylactic Acid (PLA) filament of the 3DLAB® brand.

Figure 2. Screenshots of 3D image editing procedures. (A) Lateral tomographic image of the urogenital tract stained with iohexol, displayed in the inVesalius software, with visible bladder, prostate, testicles and penis. (B) Cross-sectional tomographic image of the stained anatomical complex, with visible bladder and penis body. (C) Reconstruction of the polygon mesh obtained via digital segmentation. (D) Edited anatomical biomodel with smoothed textures, digitally reconstructed bladder and urethra. (E) Image of the plane cuts made on the piece to facilitate printing and study. (F) Image of the interior of the biomodel, showing the plane sections, the urethra and the bladder lumen.

Source: Data from the survey itself.

3.4 BIOMODELS

Different pieces were printed, pieces with componentes isolated and others models with all parts connected as complex arrangements. The biomodels were inserted in the theoretical and practical teaching methodology of the residents of HOVET-UFMT surgical center by their responsible teachers, allowing the team to discuss and simulate various techniques such as; urinary catheterization, collection of biological material, urinary clearance, understanding of topography and prostatic pathologies, clinical palpation, castration approaches. Both the physical and digital biomodels were made
available to the entire surgical team and kept available for daily uses (Figure 3). For undergraduate students, only digital data as education support material was used with.

Figure 3. Process for creating and expanding physical-digital educational libraries. (A) Capture of medical images via CT, SOMATOM Spirit® equipment. (B) Rapid prototyping process of digitized and edited anatomical structures. (C) Didactic biomodel of parts of the urogenital tract printed in PLA and connected by a urethral probe. (D) Use of the digital model in an interactive way in accessibility multiplatforms, in order to optimize teaching and access to information.

Source: Data from the survey itself.

4 RESULTS AND DISCUSSION

We affirm that the traditional dissection and fixation in formalin of complex arrangements of soft tissues allows the penetration of the dye iohexol by diffusion in the tissues fixed in its topographical pattern, providing a better definition captured by tomography. Making the identification of structural components clearer due to their whiter color in medical images, the stained pieces had greater accuracy of captured structures, even the most delicate ones such as the vas deferens. Being the best level of
image quality when the pieces reached 2 weeks immersed in a 5% solution, not generating better images after this time and concentration. Parts without exposure to contrast, when dissected, fixed and submitted to CT, present lower image quality and present more image artifacts in the final 3D model. The quality of the biomodel is associated with the amount of pixels captured in the tomographic examination that will be converted into polygons creating a digital mesh, these values are expressed in the morphological refinement that the biomodel will present when segmented. A biomodel with a more developed polygon mesh presents greater morphological fidelity than a piece with lower image capture definition (MITSOURAS et al., 2015). Automatic digital segmentation in routine medical tomographic images is unsatisfactory for its use as a tool for obtaining complex anatomical arrangements (Figure 4). Due to the limitations of the CT technique, soft tissues have a low degree of distinction and identification quality, making it impossible to carry out the complete segmentation of the desired structures (JEFFERY et al., 2011; NGUYEN et al., 2023).

Biomodels can be applied by educators and professionals to clarify different treatment options, explain the case to tutors and analyze the best approach, promoting clinical-surgical planning by simulating the structures involved, reducing surgery time, reducing time under anesthesia and improving the wellbeing of paciente. In classroom, the use of this tool promotes good assimilation and reflects positively on the learning curve, as it allows students to move between the physical and virtual world in a dynamic and interactive way (SINGHAL et al., 2016; REIS et al., 2017; BORDELO et al., 2018). 3D printed anatomical biomodels provide the advantage of training cases even in non-mineralized tissues with accuracy. Since the dimensions of the part captured by tomography are the same as the printed biomodels, having less than ± 2% of general dimensional variation (LIMA et al., 2020; NGUYEN et al., 2023).

Even with the probe introduced into the urethra, due to the liquid solution, a residual part remained in the duct lumen, generating solid images, as if the channel had collapsed. Such an occurrence can be resolved in the process of editing the digital model in the Meshmixer software, with the “add tubes” tool. Allowing the tube to be created between two pre-designated points, later its positioning allowed the size of the tube light
to be regulated in millimeters on both sides, facilitating uniformity in the channel and guaranteeing success in the total probing of the urethral canal to the lumen of the urinary bladder. It is a milestone for medicine and related fields, the possibility of creating specific physical biomodels that would be difficult to find commercially. Advances in medical imaging techniques in anatomical models enable greater breadth for digital medicine, dramatically improving our ability to digitally visualize, understand, study and share complex anatomical arrangements of mineralised and soft tissue, often difficult to access (Figure 3) (GIGNAC & KLEY, 2014; LI et al., 2016; CHEN et al., 2017).

FDM 3D printing proved to be very satisfactory results replicating biomodels, with a high degree of morphological realism and resistance to the handling of those involved. Due to the use of thermoplastic PLA, the models are biosafe and biodegradable, supporting the preservation of the environment, renewable sources and reducing the use of animals in teaching and research. It is noteworthy that it is necessary to adapt the protocol for each complex model, when applied for other areas of interest. The skill and malice of the 3D editing and printing professional are essential to optimize the prototyping process, minimize errors and trials throughout the process (CHEN et al, 2017; BORDELO et al., 2018; LIMA et al, 2019; LIMA et al, 2020). The process for manufactured production of the biomodels is economically advantageous, in addition to the educational value, it was possible to demonstrate its profitability for production, being possible to replicate models in a short printing time, using a low amount of PLA material and reflected in low individual value for production. In this experiment the printing time, weight and average cost was; 18H 04min, R$18.32 and 150.66g (Table 1).

Table 1. Individual values and average printing time, value per gram of PLA filament, weight in grams of PLA and dimensions.

<table>
<thead>
<tr>
<th></th>
<th>3D Print time</th>
<th>Filament Cost/R$</th>
<th>PLA filament weight/g</th>
<th>Dimension X-Y-Z mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>17h 23min</td>
<td>17,05</td>
<td>110,25</td>
<td>75,39 / 84,91 / 175,20</td>
</tr>
<tr>
<td>Model 2</td>
<td>12h 04min</td>
<td>12,94</td>
<td>107,08</td>
<td>86,78 / 88,99 / 166,01</td>
</tr>
<tr>
<td>Model 3</td>
<td>14h 15min</td>
<td>15,15</td>
<td>126,21</td>
<td>75,45 / 82,33 / 159,64</td>
</tr>
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</table>
The application in the daily routine of the clinical surgical center promoted cases discussions and simulations in a dynamic way, generating a lot of enthusiasm in the team and providing a clear and dynamic understanding of the topics covered. Some residents used both physical and digital material to exemplify the area of surgery and the postsurgical care needed by tutors. The urethral probe technique was the most performed by those involved in the training and the one that generated the most tension in the team before classes. After free training, residents felt able to perform the technique with precision in their clinic routine when needed (BORDELO et al., 2018; REIS et al., 2017).

Undergraduate students showed great interest in classes that used the digital biomodel, and also approved the use of the app as a support material for remote study. We recommend that in future studies the use of questionnaires to collect focal data must be used when applied in teaching and training for a better understanding of the impact in the learning process.

The production of the biomodel allows institutions to create and expand didactic libraries, without worrying about creating new facilities for the storage and disposal of biological material. In the surgical center, some models as bones, malformation structures and urogenital systems were on shelves along the common area, facilitating the dynamics of their use in the daily routine (LIMA et al., 2019; LIMA et al., 2020). Models in digital

<table>
<thead>
<tr>
<th>Model</th>
<th>Time (min)</th>
<th>Volume (mL)</th>
<th>Weight (g)</th>
<th>Volume 1 (mL) / Volume 2 (mL) / Volume 3 (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 4</td>
<td>20h 25min</td>
<td>26.89</td>
<td>224.12</td>
<td>78.99 / 101.72 / 183.01</td>
</tr>
<tr>
<td>Model 5</td>
<td>17h 31min</td>
<td>17.42</td>
<td>145.18</td>
<td>76.33 / 85.97 / 177.37</td>
</tr>
<tr>
<td>Model 6</td>
<td>27h 41min</td>
<td>29.09</td>
<td>242.42</td>
<td>95.13 / 107.14 / 221.04</td>
</tr>
<tr>
<td>Model 7</td>
<td>14h 14min</td>
<td>13.15</td>
<td>109.55</td>
<td>70.51 / 79.41 / 163.85</td>
</tr>
<tr>
<td>Model 8</td>
<td>15h 49min</td>
<td>15.07</td>
<td>120.60</td>
<td>76.61 / 82.90 / 171.05</td>
</tr>
<tr>
<td>Model 9</td>
<td>19h 49min</td>
<td>19.39</td>
<td>161.55</td>
<td>82.66 / 93.10 / 192.09</td>
</tr>
<tr>
<td>Model 10</td>
<td>17h16min</td>
<td>17.05</td>
<td>159.64</td>
<td>75.39 / 84.91 / 175.20</td>
</tr>
<tr>
<td><strong>Average data</strong></td>
<td><strong>18h 04min</strong></td>
<td><strong>18.32</strong></td>
<td><strong>150.66</strong></td>
<td><strong>79.32 / 89.14 / 178.44</strong></td>
</tr>
</tbody>
</table>

Source: Data from the survey itself.
format (.obj and .stl) are compatible with the free apps (EMB3D, Exocad) to visualize and interact with the 3D structures, available for computers, tablets and smartphones. Being a powerful tool with easy usage compatibility, portability and personal storage.

The immersion technique of specimens fixed in contrast has been applied to embryos, invertebrates and small post-embryonated vertebrates captured in mCT, progressively increasing the physical dimension of the specimens. Its success is linked to the correct degree of penetration of iodine trimers and their binding to soft tissues, differentiating them from each other (GIGNAC & KLEY, 2014). It is an expensive protocol to replicate it in whole adult dog specimens due to the total volume required for immersion in contrast solution. The use of the dissected and immersed part is an option to be considered for capturing the arrangements in a faster and more economical way due to the physical dimensions of the structures.

Routine medical imaging used to obtain the dog’s male urogenital system proved to be ineffective in the segmentation process. This process depends on the delimitation of pixels from the same set to create an area in the tomographic projections within the same color scale (density), thus forming a three-dimensional object. Being easily identified and executed with mineralized tissue due to the visual contrast of the white pixels with the other gray and black ones. When the technique is applied in routine exams for extraction of the urogenital system, the software produces nonspecific anatomical models, interconnecting skeletal muscle structures and the viscera of the digestive system with the urogenital system. The software's native predefined density thresholds were applied with unsatisfactory results for the proposed objective, with the applied filters; soft, muscular, adipose, epithelial and bone tissues (Figure 4). The manual segmentation technique was not applied in this project due to its high level of complexity and required skills.
Figure 4. Images from the HOVET-UFMT medical routine database, showing the pelvic region in cross-sections of a male dog submitted to automatic segmentation. (A) Tomography image without application of segmentation, control. (B) Segmentation with muscle tissue threshold, ranging from -5 to +135 HU. (C) Segmentation with soft tissue threshold, ranging from -700 to +225 HU. Segmentation with adipose tissue threshold, ranging between -205 and -51 HU. (D) Segmentation with bone tissue threshold, ranging from +226 to +1931 HU. (E) Segmentation with epithelial tissue threshold, ranging between -718 and -177 HU.

The process of dissecting the structures, fixing them and sequentially staining with a contrast agent so that they can be scanned proves to be laborious, yet extremely effective in generating digital models analogous to biological ones. This technique allows a basic science team to autonomously produce a digital complex anatomical arrangement without the need for virtual modeling knowledge. Both the dissection technique and the use of contrast in the medical routine are well-known processes, as well as the capture of radiographic images, being the technical differential the fixation step in a topographic position using individually molded structural supports, as well as of the piece fixed in iohexol contrast solution, ensuring that all structures were wrapped and stained, facilitating the identification of the complete morphology in the segmentation process. Studies on 3D printing of anatomical structures obtained from CT are a milestone for surgical anatomy, and the use of contrast agents in anatomical arrangements of fixed soft tissues is a way to expand the possibilities of digitizing complex structures. Thus creating physical and digital morphometric data with high precision, biosafety, capable of being...
recycled, with great versatility of applications in higher education and research institutions.

5 CONCLUSIONS

We affirm that the use of fresh anatomical specimens, fixed in 10% formalin and subsequently submerged in a concentrated iohexol solution of 5% for capture via CT is a combination of a technique with great value for the anatomical area to capture and create models of complete urogenital system of male dogs. Enabling the creation of interactive digital biomodels with high resolution and prototype facsimiles using sustainable and recyclable material. The use of contrast agent in fixed anatomical parts of domestic animals, associated with CT and 3D printing techniques, has much to contribute in several areas of veterinary medicine, both in theory and in practice. Driving the emergence of more digital libraries, collections of routine cases, expanding the theoretical-practical approaches to procedures and facilitating the sharing of data between professionals. The cost of the filament and the equipment associated with the time for producing replicas of the biomodels proved to be a very advantageous point for the veterinary team, making it possible to obtain printed models within 24 hours after the tomographic capture. This study came to corroborate the expansion of diceCT applications aimed at complete replication of the process of obtaining biomodels, being an innovative and bold way to capture complex anatomical arrangements of companion animals fixed in formalin impregnated with iohexol contrast. More studies in this area are needed so that there is a refinement of diceCT techniques for complex systems, with other agents and anatomical areas of interest. It is a goal to achieve the development of low-cost and ecological friendly medical teaching materials, with realistic, morphologically reliable and easily accessible worldwide, reducing the use of animals in classes, research and helping the application of the 3Rs.
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