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Research article

POLYESTER RESIN EMBEDDING FOR THE STUDY OF THE OSTEOLOGY OF THE CANINE FRONT PAW

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The creation of anatomical models of high didactic value, which are durable, simple, and inexpensive to maintain for the study of the appendicular skeleton of canines is a challenge, due to the size of the bones composing the base bones of the paw. As the topographic study progresses distally, this material's quality, integrity, and longevity becomes considerably more difficult. Osteotechnics is a tool that has made it possible to obtain the material for the study of bones that make up the skeleton of domestic animals. However, in the case of the anatomy of the canine paw, pieces of bone are easily lost. Using 10 paws from canine cadavers donated by the Canine Control Centers for use in Veterinary Anatomy dissection practices, pieces encapsulated in polyester resin were obtained, allowing anatomical models to be made in order to enable the study of bone structures and their relationships between the bones that make up the skeleton of the canine paw, providing a self-taught support material for students. Including small bones in polyester resin represents a conservation technique of high didactic value with the additional benefits of long life, easy storage, and zero toxicity, without the risk of losing small bone pieces.

Keywords: veterinary anatomy, anatomical preservation techniques, paw bones, canine, teaching.

INTRODUCTION

Anatomical knowledge of domestic animals enables the optimal development of medical, surgical and zootechnical skills and techniques. The teaching of veterinary anatomy aims to know and understand the different anatomical structures that form the body of domestic animals. Compared and integrated theoretical knowledge is

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making it possible to hightlight the similarities and differences between domestic animals, for which the study on biological material is fundamental [1-3].

Interaction with conserved material enables learning strategies with interactive 3D models for students, promoting collaborative learning, discussion, and building knowledge through social interactions [4]. Currently, the availability of biological materials is limited by the legal framework in our country, which is prompting researchers, academics, and students to look for alternatives. Thus there is a need for research and optimization in the development of techniques for the preservation of anatomical pieces that make it possible to generate highly practical, durable, and non-perishable materials, reduce the demand for cadavers and organs, eliminate health risks by obtaining quality material and favor direct contact between students and the structures to be studied [1,5,6].

The term osteotechnics refers to various techniques for preparing and preserving pieces of bone, either for didactic use in anatomy laboratories or for demonstration purposes, such as in museums. It is mainly based on maceration, cleaning, bleaching of bone material, and assembly of the skeleton [7].

Osteotechnics is a tool that has made it possible to generate the material for the study of bones that make up the skeleton of domestic animals. However, in the specific case of the anatomy of the appendicular canine skeleton, the size of the bones that form the bone base of the hand becomes a challenge. As the topographic study progresses distally, the quality, integrity, and durability of this material becomes considerably more difficult [8,9].

The bones that form the canine front paw (manus) are the carpus, where the bones that make up the carpus are grouped in a proximal row (intermediate-radial carpus, ulnar carpus, and accessory carpus) and distal row (carpus I, II, III and IV), the matacarpal bones (I, II, III, IV and V), the phalanges (proximal, middle and distal, except in finger I where the middle phalanx is absent), the proximal sesamoids (two on the palmar side of the metacarpophalangeal joint, except in finger I where 1 or 2 sesamoids may be present), the distal sesamoids (which remain cartilaginous in canines) and the dorsal sesamoids (one on the dorsal side of the metacarpophalangeal joint) [3,10-13].

The routine use of these anatomical pieces assembled by osteotechnics has the disadvantage that the bones deteriorate and are lost, making the understanding of the subject and the comparative anatomical study with other domestic animals difficult. For this reason, this work sought the implementation of a new preservation technique that allows the generation of quality pieces that maintain the totality of bones, with an excellent didactic value, long life, easy maintenance, and high aesthetics, such as the Technique of Inclusion in Polyester Resin (TIPR).

MATERIALS AND METHOD

Anatomical specimens

Ten thoracic limbs from cadavers used for anatomical dissection in veterinary anatomy courses were segmented by transverse sectioning at the level of the distal third of the forearm using a cutting saw and stored at – 20°C in plastic bags until preparation.

Soft tissue removal

The pieces were thawed at room temperature for 12 hours, to proceed to the removal of the skin, connective, and muscular tissue, finishing with the disarticulation of the bone pieces by carefully sectioning the joint capsules.

Bone cleaning

To continue with the soft tissue cleaning, the bones were individually placed in nylon bags and subjected to continuous boiling cycles for eight hours. The bones were then brushed to remove any remaining muscle, cartilage, and ligaments and subjected to a second boiling cycle in water with 4% commercial detergent powder to remove the fat material. These cycles were repeated as many times as necessary to obtain clean pieces.

Degreasing and bleaching

To continue with the degreasing and bleaching process, the bones were immersed in hydrogen peroxide of 11 volumes for twenty-four hours, rinsed, and dried by indirect exposure to the sun for 10 days; the time depending on the ambient humidity. These steps were based on the technique described by Nieto et al. 2014 [9].

Assembly and painting

The cleaned and dried bones were placed on individual sheets to position them in the correct anatomical order and proceeded to painting with acrylic dyes using a different color code for each bone that makes up the skeleton of the paw.

Mounting and embedding

Wooden plates of 10 cm by 40 cm were used as a base for mounting the material, each bone was glued using Resistol 5000 glue and left to dry for 24 hours. The annotations and signs printed on a vinyl label were arranged; they were then covered with two coats of glazing liquid applied with a fine bristle brush and proceeded to dry for 24 hours. Finally, the material was coated with a layer of polyester resin and left to dry on a level surface for 72 hours. Each piece was placed in a polyethylene bubble plastic bag for storage at room temperature.

RESULTS

The bones that make up the osteology of the canine paw are small and are located in small anatomical spaces. The pieces were dissected through a transverse cut at the level of the distal third of the forearm (Fig 1A). The soft tissue was removed by anatomical dissection, starting with the skin, muscles, tendons, ligaments, and adipose tissue (Fig 1B). The cleaning procedure with continuous boiling cycles with detergents enabled the removal of the connective tissue and fat adhering to the bones. Degreasing and bleaching by immersion in hydrogen peroxide resulted in clean bone pieces with a color corresponding to that of the bones in their fresh state, to continue with the insertion of the skeleton of the hand, respecting the spatial allocation (Fig.1C).

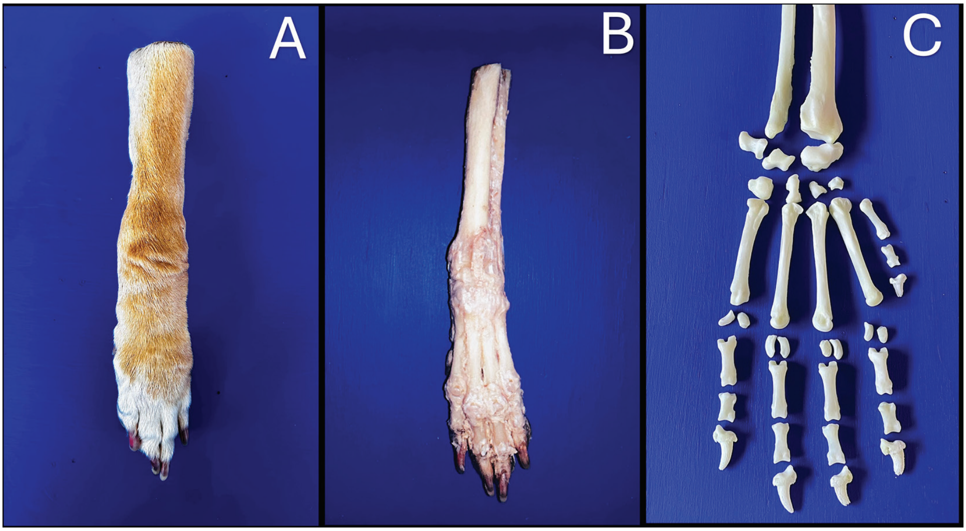

Figure 1. Bones of the canine paw. A: The paw were sectioned by a uniform transverse cut over the radius and ulna, approximately 3 cm from the carpal region. B: The skin was removed to expose the connective and muscular tissues. C: Cleaned bone pieces and spatial arrangement of the bone parts. Dorsal view. Panoramic image.

During assembly, the separation of the pieces using polyester resin embedding technique allows the study of the articular surfaces to be examined, unlike skeletoraphy, where these surfaces are not perceptible as they are in direct contact. The coating of the bone pieces with acrylic paint makes it possible to create a visual aid that associates the type, number, and spatial location of the bones with a color code that, together with the relevant information about the bone, represents a self-learning resource (Fig. 2A). Once the spatial and color pattern is established. The bones are adhered to a wooden base to finish the encapsulation process by coating them with polyester resin (Fig. 2B).

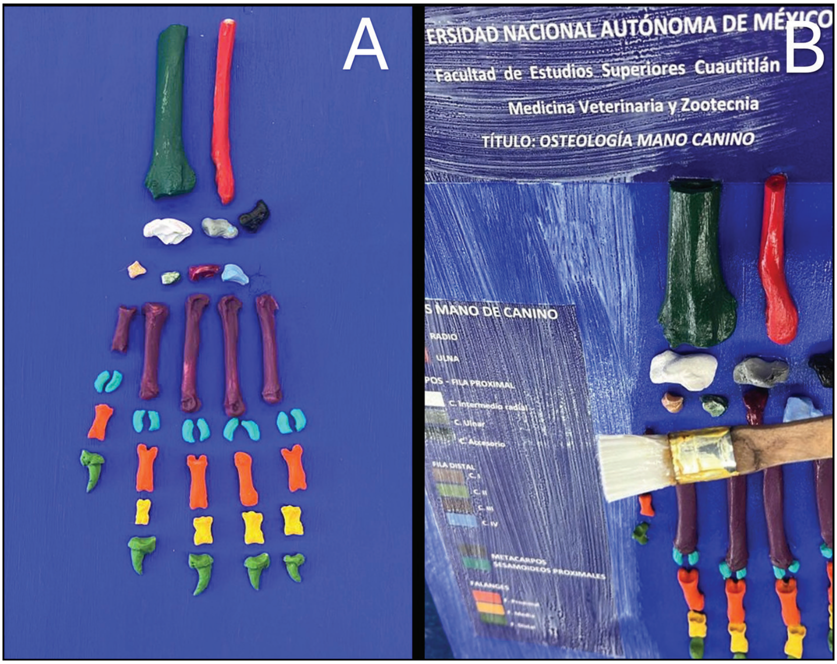

Figure 2. Assembly with bone pieces. The identification of the different structures allows the assembly of the skeleton of the paw by studying the individual bones and in association. **A:** Bone pieces were coated with colored paint and arranged in anatomical positions. B: The pieces and information labels of color codes were covered with glue on the base.

In invasive osteotechnics or nylon fixation skeletorapy, small components tend to rotate (Fig 3A, yellow arrows) and get lost, leading to incomplete skeletal structures with inaccurate spatial alignments (Fig. 3A, white arrows). Canine paw bones preserved by TIPR show the exact location of the carpal bones in their proximal and distal rows, the metacarpal bones, phalanges, and the proximal sesamoid bones (Fig. 3B). The dorsal sesamoids and distal sesamoids were not preserved as they are cartilaginous and are lost in the boiling process.

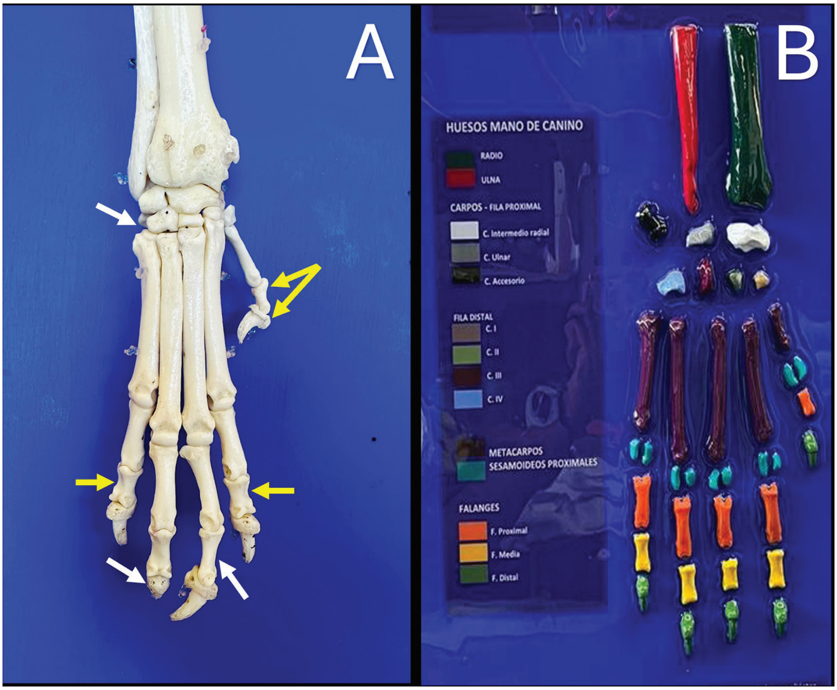

Figure 3. Osteotechnics and bone embedding in polyester resin. A: osteotechnics of the canine paw, small components tend to rotate (yellow arrows) or get lost, resulting in incomplete skeletal structures with inaccurate spatial orientations (white arrows). B: bone embedding in polyester resin. Bone embedding facilitates the study individually and in association, making it possible to distinguish articular surfaces not visible in the osteotechnics, allowing the preservation of the proximal sesamoids not present in the fixation with thread.

DISCUSSION

Osteotechnics is an anatomical preservation technique that allows the preparation and exhibition of vertebrate skeletal parts by assembling their bone pieces [8,9,14,15].

Bone materials have three main physical properties that determine the alterations caused by environmental factors: porosity, hygroscopicity, and anisotropy. Bones can undergo alterations such as decohesion, erosion, pulverization, and changes due to chemical reactions during decomposition. From our experience, small bones are more sensitive to undergo these alterations, so the TIPR protects bones from these variations and allows longer preservation [16].

Osteotomy involves four main stages: defleshing, cleaning, bleaching, and assembly. Defleshing is performed by separating the soft tissue using knives and scalpels. Different methods have been reported for cleaning bones: boiling, bacteriological, maceration (by insects or inorganic or organic chemicals) [7], burying, putrefaction [16]; scraping, with dermestids, caustic soda, with predatory insects [17], defleshing with potassium hydroxide 0.5% has also been reported [18]. Boiling with calcium oxide, 50g/L [19], calcium carbonate [7], detergent [20,21], or sodium borate has been used [16].

Bleaching and degreasing with hydrogen peroxide [16-19,21], with 10% sulphuric acid [7], and degreasing with white benzine before treatment with hydrogen peroxide has also been reported [21]. In our laboratory, we use the cleaning method of boiling with detergent, bleaching, and degreasing with hydrogen peroxide which has provided us with satisfactory results. It is also an accessible and inexpensive technique that allows us to obtain clean and unaltered artefacts. Cleaning with sodium hypochlorite (commercially available) diluted between three to six parts with water makes it possible to obtain articulated bone pieces [22], and could be useful in the case of small bones such as those of the canine paw; however, this technique does not allow the observation of the complete anatomy of each bone, apart from the decalcifying effect, which can cause erosion and pulverization.

For assembly, the use of glue, silicone, wires, or nylon is reported. Invasive osteotechnics or skeletoraphy with the use of nylon to assemble the bone pieces [18,19] are not very applicable to pieces smaller than 3 cm in any of their dimensions, since drilling of the bones for the introduction of fixing material jeopardizes the piece by causing structural damage ranging from weakening or fractures, to decay disintegration, the latter undesirable feature being of greater relevance in pieces of young animals or small birds [23-25].

The TIPR is one of the non-invasive osteotechnics that are tools that aim to reassemble the bone pieces, without the need for drilling or generate structural damage [23,24]. Other non-invasive techniques involve the use of glues or silicones applied to the articular surfaces. The assembly must be performed by highly qualified personnel with knowledge of animal anatomy, to respect the angles of union and the alignment of the structure, which is important as this technique does not allow correction of direction of the assembly or the study of the articular surfaces to be corrected.

In the preservation of anatomical pieces, polyester resin has been given different uses, such as the preservation of soft tissues, in which it is recognized as a simple, versatile, highly durable, and cost-effective technique, which reduces exposure times to toxins [26-30] compared to the plastination technique of Von Hagens (1987) [31-35] and in the diaphanization technique [36]. However, this has not yet been reported in osteotechnics such as the one presented in this paper.

The TIPR described in this article represents an excellent option for the preservation of bone pieces. The encapsulation prevents small pieces from rotating or being misplaced, affecting the quality of the preserved piece, as occurs in specimens assembled by osteotechnics in regions where the bones are tiny and where pieces are frequently lost, misplaced or fractured because their structure is damaged when they are perforated to introduce the fastening materials. In addition, TIPR, by encapsulating the anatomical pieces in resin reduces the biological risks associated with the direct handling of biological material without the need for additional biosafety measures.

TIPR generates a protective barrier that prevents degradation of the bone tissue by microorganisms or external agents, increasing its durability [32]. The assembly on a solid base increases the strength of the preparations and reduces the risk of damage due to improper handling by users. Finally, TIPR has a high value as a didactic tool by allowing the addition of diagrams, drawings, and information that also represents the added value of an autodidactic resource.

CONCLUSIONS

TIPR is an ideal technique for the preservation of the bones of a canine paw. This method allows us to obtain high-quality, visually appealing, and educationally valuable specimens at an affordable production and maintenance cost for teaching and museum display. TIPR preserves the three-dimensional anatomical relationship by displaying the bone pieces in their dorsal and palmar views and avoiding the misplacement of bone pieces, without compromising the integrity of the bone at the time of assembly as is the case with skeletography.

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Data availability statement

The data presented in this study are available on request from the corresponding author.

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Authors' contributions

RPSD participated in anatomical methodology, formal analysis, investigation and data curation. BZH participated in the conceptualization, visualization, supervision and analysis and review of the final version. PMMD participated in the methodology, data curation, review of the final version and resources. GTCG made supervision, writing-review and editing of final version and resources. JXS participated in the methodology, data curation, formal analysis and writing the original draft, and review and editing of the final version. All authors read and approved the final manuscript.

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Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Statement of Informed Consent

The owner understood procedure and agrees that results related to investigation or treatment of their companion animals, could be published in Scientific Journal Acta Veterinaria-Beograd.

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REFERENCES

1. Bond AP, Kelsey A, Aitken G: The future of anatomy teaching post-pandemic: An academic viewpoint. Anat Sci Educ 2023, 16(6):1121-1133.
2. Kapoor K, Singh A: Veterinary anatomy teaching from real to virtual reality: An unprecedented shift during COVID-19 in socially distant era. Anat Histol Embryol 2022, 51(2):163-169.
3. Getty R: Sisson and Grossman´s The anatomy of the domestic animals Grossman. Volume 1. W.B. Saunders Company, 5th Ed. USA. 1975.
4. Gummery E, Cobb KA, Mossop LH, Cobb MA: Student perceptions of veterinary anatomy practical classes: a longitudinal study. J Vet Med Educ 2018, 45(2):163-176.
5. Sánchez CC, Andromaco M, Páez R, Barello MR, Pedernera G: Estudio de nuevas técnicas para conservación de piezas anatómicas. Plastinación. Revista de Salud Pública 2012, (14)3:27-32.
6. Silva RM, Matera JM, Ribeiro AA: New alternative methods to teach surgical techniques for veterinary medicine students despite the absence of living animals. Is that an academic paradox? Anatomia Histolologia Embryologia 2007, 36(3):220-224.
7. Cañete BG, Sánchez PJM, Noda CL: Ensamblaje artesanal de un esqueleto canino mediantes variantes de la osteotecnia. Rev Electron Vet 2014, 15(9):1-15.
8. Allouch G: Scientific technique for skeletons preservation and preparation of anatomical models to promote veterinary anatomy. J Vet Anat 2014, 7(2):133-139.
9. Nieto BJL, García TCG, Pichardo MMR, Reyes SAL, Soto ZCI: Técnicas para preservar piezas anatómicas. México: FES – Cuautitlán UNAM 1ª edición. ISBN 978-607-02-5097-2. 2014.
10. Hermanson JW, de Lahunta A, Evans HE: Miller and Evans´ anatomy of the dog. Elsevier, 5th Ed. USA. 2018.

11. Gasse H: Nomina Anatómica Veterinaria. International Committee on Veterinary Gross Anatomic Nomenclature. Hannover Germany: Chairman Committee. 6th ed. 2017. https://www.wava-amav.org/wava-documents.html
12. Dyce KM, Sack WO, Wensing CJG: Textbook of veterinary anatomy. Elsevier, 5th ed. USA. 2017.
13. 13. Evans H: Disección del perro. 5ta edición. MCGraw-Hill / Interamericana De Mexico. 2002.
14. Jamain A: Tratado elemental de anatomía descriptiva y de preparaciones anatómicas. Editorial MAXTOR. 2011.
15. Acosta W: Cuando los huesos hablan. Museo. 2010.
16. Padilla CM: Introducción a la conservación y mantenimiento de los materiales óseos del Museo de Anatomía Comparada de Vertebrados de la Facultad de Ciencias Biológicas de la Universidad Complutense de Madrid. Reduca (Biología). Serie Técnicas y Métodos. 2015, 8(3):1-21.
17. Morales C, León E: La osteotecnia como estrategia para la enseñanza de la anatomía comparada. Educación en Contexto 2020, 6(11):123-150.
18. Riquelme L, Abarzúa R, Leichtle J: Adaptación de osteotecnia para el montaje de un ejemplar de Delfín de Risso Grampus griseus (Cuvier, 1812). Red Med Vet Invest 2018, 1(1):102-108.
19. Villarroel GMA, Troncoso FNA: Combinación de Osteotecnia más Conservación de Músculos en Montaje Único de Canis lupus familiaris. Int J Morphol 2017, 35(1):351-356.
20. Guzmán PRA: Recuperación de osamentas de mamíferos marinos otaria byronia (de blainville, 1820): osteotecnia. Sagasteguiana 2020, 8(1):1-16.
21. Cadegan SK: Anatomía comparada del esqueleto apendicular de dos especies de otaridos, *Otaria flavescens* (Shaw, 1800) *Arctophoca australis gracilis* (Zimmerman, 1783). Tesis de Licenciatura, Universidad Austral de Chile. 2013.
22. Scanferla CA: Técnicas para la preparación de esqueletos secos de lepidosaurios. Revista del Museo de La Plata, Publicación Técnica y Didáctica 2010, 49:1-6.
23. Bravo G, Gallardo D, Bulfon M: Aplicación de osteotécnicas no invasivas en la preparación esquelética del pijui cola parda. Synallaxis albesces 2016.
24. Gallardo AD, Bravo GE, Bulfon ME: Preparación del esqueleto del Pijui de Cola Parda (*Synallaxis albescens*) (AVES: *Furnariide*), utilizando osteotécnicas no invasivas. Universidad de Córdiba. 2015 http://hdl.handle.net/11086/550672
25. Betancourt GC, Pellitero JMS, Cuellar LN: Ensamblaje artesanal de un esqueleto canino mediantes variantes de la osteotecnia. REDVET. Revista Electrónica de Veterinaria 2014, 15(9):1-15.
26. Rojas Huayapa RE: Aplicación de resina en tejidos blandos para ser utilizados en la enseñanza y aprendizaje en anatomía comparada – Arequipa 2019. https://repositorio.ucsm.edu.pe/handle/20.500.12920/9582
27. Ortloff Trautmann A, Riquelme Gatica M, Morán Ruz G: Dos métodos de inclusión de biopsias de neoplasias en resina epóxica para la enseñanza de anatomía patológica veterinaria. Revista de Investigaciones Veterinarias del Perú 2019, 30(4):1796 – 1804.
28. Weber W, Weiglein A, Latorre R, Henry RW: Polyester plastination of biological tissue: P35 technique. J Int Soc Plastination 2007, 22:50-58.
29. Valenzuela M, Catoia B, Munjin A, Valdés F: Nuevo método de inclusión en resina poliéster P-4 para cortes anatómicos. Int J Morphol 2015, 33(1):275-278.

30. Valenzuela OM, Azocar SC, Werner FK, Vega PE, Valdés GF: Plastination experience in polyester resin (P-4). Int J Morphol 2012, 30(3):810-813.
31. Von Hagens G, Tiedemann K, Kriz W: The current potential of plastination. Anatomy and embryology 1987, 175:411-421.
32. Barrientos Peñaloza JM, Mendoza Lopez Videla JN, Azcárraga Hurtado M, Menduiña Pardo E, Salguedo Siles SF: Conservación de piezas cadavéricas del sistema nervioso central con resina poliéster. Revista Médica La Paz 2014, 20(1):34-39.
33. Baptista CAC, DeJong K, Latorre R, Bittencourt AS: P40 polyester sheet plastination technique for brain and body slices: The vertical and horizontal flat chamber methods. Anat Histol Embryol 2019, 48(6):572-576.
34. Bernaba EPR, Pizzo R: Preservación de Material cadavérico con resina Poliéster previa inclusión en Resina. In Libro de Ponencias: 47 Congreso Argentino de Anatomía 2012. Rio Negro Argentina; Cipolleti (pp. 13-14).
35. Latorre R, Henry RW: Polyester plastination of biological tissue: P40 technique for body slices. J. Int. Soc. Plastination 2007, 22:69-77.
36. Matamala VF, López QP, Iturriaga BP, Chávez IR: Contribucion al estudio de la resina poliester para su uso en docencia e investigacion anatomica. An anat Norm 1984, 138-42.

UKLAPANJE U POLIESTERSKE SMOLE U CILJU PROUČAVANJA OSTEOLOGIJE PREDNJE ŠAPE PSA

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Stvaranje anatomskih modela visoke didaktičke vrednosti, koji su izdržljivi, jednostavni i jeftini za održavanje, a za proučavanje skeleta ekstremiteta pasa je izazov, zbog veličine kostiju koje čine osnovne kosti šape. Kako topografska studija napreduje distalno duž ekstremiteta, očuvanje kvaliteta, integriteta i dugovečnosti ovog materijala postaje otežano. Osteotehnika je alat koji je omogućio dobijanje materijala za proučavanje kostiju koje čine skelet domaćih životinja. Međutim, u slučaju anatomije pseće šape, komadići kosti se lako gube. Koristeći 10 šapa od psećih leševa koje je donirao „Centar za kontrolu pasa za upotrebu u praksi disekcije veterinarske anatomije“, dobijeni su preparati inkapsulirani u poliestersku smolu, što je omogućilo izradu anatomskih modela kako bi se omogućilo proučavanje koštanih struktura i odnosa između kostiju koje čine skelet pseće šape, pružajući samouki pomoćni materijal za učenike. Uključivanje malih kostiju u poliestersku smolu predstavlja tehniku konzervacije visoke didaktičke vrednosti sa dodatnim prednostima dugog veka trajanja, lakog skladištenja i nulte toksičnosti, bez rizika od gubitka malih komada kostiju.