

APPLICATION OF CRYODEHYDRATION TECHNIQUE IN DOG KNEES (*CANIS LUPUS FAMILIARIS*)

Isadora Schnorr Martins

Veterinary course- Universidade Federal do
Rio Grande do Sul
Porto Alegre - RS
<http://lattes.cnpq.br/8895042265823051>

Nicolle de Azevedo Alves

Veterinary course- Universidade Federal do
Rio Grande do Sul
Porto Alegre - RS
<http://lattes.cnpq.br/1390979456292154>

Camilla Elias Bruno

Veterinary course- Universidade Federal do
Rio Grande do Sul
Porto Alegre - RS
<http://lattes.cnpq.br/5034727419513144>

Werner Krebs

Department of Morphology- Universidade
Federal do Rio Grande do Sul
Porto Alegre - RS
<http://lattes.cnpq.br/4267508064597739>

Bianca Martins Mastrantonio

Department of Morphology- Universidade
Federal do Rio Grande do Sul
Porto Alegre - RS
<http://lattes.cnpq.br/5055359115249510>

All content in this magazine is licensed under a Creative Commons Attribution License. Attribution-Non-Commercial-Non-Derivatives 4.0 International (CC BY-NC-ND 4.0).



Giovana Silveira Rocha

Veterinary course- Universidade Federal do Rio Grande do Sul
Porto Alegre - RS
<http://lattes.cnpq.br/1167511117799387>

Filipe Martins Pimentel

Veterinary course- Universidade Federal do Rio Grande do Sul
Porto Alegre - RS
<http://lattes.cnpq.br/2256860248256840>

Laura Ver Goltz

Department of Morphology – Universidade Federal do Rio Grande do Sul
Porto Alegre – RS
<http://lattes.cnpq.br/3639378385545865>

Ana Cristina Pacheco de Araújo

Department of Morphology - Universidade Federal do Rio Grande do Sul
Porto Alegre - RS
<http://lattes.cnpq.br/2599270947966957>

Abstract: The use of alternative techniques to formaldehyde in the conservation of anatomical pieces is a solution to reduce the contact of teachers and students with this carcinogenic chemical compound, which causes airway irritation. Several alternative techniques to formaldehyde are already used, however, some are still unfavorable due to their high cost. Cryo-dehydration is a cost-effective, low-maintenance, odorless conservation method with excellent results in tissue preservation. This technique is based on freezing the piece, promoting the solidification of water crystals in the fabrics and subsequently releasing this water through thawing, dehydrating and preserving the fabric. In this work, the cryodehydration procedure was performed on 16 femorotibiopatellar joints of dogs, eight on the right antimer and eight on the left antimer. Dissection and application of the cryo-dehydration technique were easy to apply to 100% of the study samples. Regarding the preservation of structures at the time of dissection, in 62.5% of the pieces it was possible to preserve all ligaments, muscle tendons and sesamoid bones. It was also possible to observe the darkening of soft tissue structures, such as ligaments and tendons, in some of the preparations. According to the results found in this study, it is possible to conclude that the cryo-dehydration technique in joints is viable, resulting in parts with little or no odor, eliminating the need for formaldehyde and being a low-cost procedure. The technique allows structures to be easily visualized, handled and stored.

Keywords: Defrosting. Freezing. Anatomical techniques. Formalin. Morphology. Articulation. Veterinary. Little animals.

INTRODUCTION

Used as a preservative, formaldehyde is widely used in academic environments in the preservation of corpses for the study of human and veterinary anatomy, in addition to having antiseptic, antifungal and bactericidal functions. It guarantees the pieces a long period of preservation, with little loss of shape and slight darkening and increased rigidity (KRUG et al., 2011). The adverse effects on humans caused by exposure to formaldehyde are directly related to its duration and frequency, the route through which it occurs, as well as the concentration of formaldehyde in the solution (PINA, 2010). Being a strong chemical component, formalin is already registered as a carcinogen by the International Agency for Research on Cancer (IARC, 1995). Faced with the harm caused by the wide use of formalin in academic environments and seeking the best preservation of tissues, taking into consideration, their morphology and future maintenance needs, combined with a favorable cost-benefit ratio, many anatomy laboratories have alternative conservation techniques applied concomitantly with the use of formaldehyde (DA SILVA et al., 2016).

In cryo-dehydration, the material is dehydrated by freezing and thawing. The technique is based on the principle that the slow freezing of intracellular water causes its expansion and crystallization, which, due to its significant size, causes the cell plasma membrane to rupture. Repeats of the procedure promote a considerable number of ruptures in the cell wall, facilitating the release of water from the tissue and enabling its conservation (KOONZ; RAMSBOTTON, 1939; HINER; HANKINS, 1947). This method has the advantages of easy packaging, low cost of the technique, durability and lightness of the pieces, as well as eliminating the use of fixatives (formaldehyde) in maintaining conservation (KREMER, 2011). Some authors

have already applied this freezing and thawing technique to equine and dog joints (MELO et al, 2009; BRAUNER, 2016), as well as to viscera, such as brains (PESSOA et al, 2017; REIS et al, 2020), kidneys and hearts (BATISTA et al, 2017; CARMO, 2017; MIRANDA E BOSSO, 2020) and also the digestive system, such as the stomachs of some animal species (KREMER; SCHUBERT; BONFÍGLIO, 2011; ARAÚJO et al, 2021).

Knowledge of the morphology and structure of the organism is the academic basis for professional training in medical sciences (DA SILVA et al., 2016). In dogs, the study of the anatomy of the femorotibiopatellar joint, or knee joint, is extremely important, as it is a joint that can be affected by various pathologies, both bone and joint, such as patellar dislocation, rupture of cruciate ligaments (cranial and caudal), meniscal injuries, among others (WOODARD; JONES; HUNT; KING, 1997).

The objective of the present work was to apply the cryodehydration technique to pieces of dog knee joints, aiming to contribute to facilitating research into comparative anatomy and the manipulation of the piece by its users when under study. The ease of handling and storage of specimens submitted to the technique will also be evaluated, as well as the durability and low cost of execution.

MATERIAL AND METHODS

To carry out this study, 16 femorotibiopatellar joints were used - eight from the right antimer and eight from the left - from formaldehyde dog cadavers from the Pathology Sector of the Veterinary Faculty of Universidade Federal do Rio Grande do Sul (FAVET/UFRGS), which were studied in the Anatomy subjects for the Veterinary Medicine course. These knees were chosen and dissected randomly, with no correspondence between the origin of the

antimeres. Approximately 7 cm of the distal end of the femur and 7 cm of the proximal end of the tibia and fibula were preserved.

When dissecting the structures that make up the dog's knee, an attempt was made to preserve the patella, the insertion tendon of the quadriceps muscle, part of the rectus femoris muscle, the patellofemoral ligaments (medial and lateral) attached to the sesamoid bones of the gastrocnemius muscle (located on the caudal aspect of the distal end of the femur, proximal to the condyles), in addition to the patellar ligament up to the crest of the tibia, cranially. Furthermore, the collateral femorotibial ligaments (medial and lateral) and cruciate ligaments (cranial and caudal) were maintained, in addition to the caudal ligament of the lateral meniscus (distal branch and proximal branch, called the meniscofemoral ligament). We sought to preserve two other structures, the tendons of origin of the extensor digitorum longus muscles (laterally, starting from the extensor fossa, close to the trochlea of the femur) and popliteus (also laterally, passing medially to the lateral collateral ligament, with its sesamoid bone). Finally, all muscle remains, periosteum, adipose tissue and bone marrow were removed from both the femur and tibia.

All pieces were washed in running water and randomly identified by placing a string (a small hole was made in the remaining portion of the femoral diaphysis) with a plate D (right) or E (left) and with a number of 1 to 8 (D1-8 and E1-8). Furthermore, each sample was photographed (cranial and caudal views) and weighed on an electronic scale (LTOMEX ALSF400) before carrying out the freezing and thawing process (cryodehydration). Then, each preparation was placed in individual plastic bags and stored in a freezer (horizontal, Nihonfreezer, -4 °C) for 18 hours. After this period, each piece was weighed again and suspended to thaw at room temperature for

six hours; At the end, they were weighed again, packed in plastic bags and placed back in the freezer. This process was carried out 13 consecutive times; In the 13th Session, the pieces remained thawed for 48 hours. The ambient temperature and relative humidity were also measured daily during the experiment period, using a digital thermo-hygrometer (model FEPRO-MUT60OS).

After the freezing and thawing periods, all samples were photographed again in the cranial and caudal views. Four pieces of each antimeres (chosen according to the best preservation) had their structures identified through the application of colored paint (acrylic). Structures related to muscles were identified with red; ligaments, yellow in color; menisci, blue in color; and sesamoid bones (patella and sesamoids of the gastrocnemius and popliteus muscles) colored green. Then, all pieces received a layer of varnish, with the aim of conservation, thus preventing the growth of microorganisms - such as fungal colonies - and the accumulation of grease due to handling.

RESULTS

In 100% of the dog knee pieces used in this study, it was possible to dissect and apply the cryo-dehydration technique (freezing and thawing), as seen in figures 1 and 2, with these images and schematic drawings of the pieces not being colored, and also in figure 3, in the samples with the structures identified in color.

Regarding the preservation of structures at the time of dissection, in 10 of the 16 samples (62.5%) (D1, D2, D4, D5, D8, E3, E4, E5, E7 and E8), it was possible to preserve all ligaments, muscular tendons and sesamoid bones present. However, in six samples (37.5%) the preservation of all structures was not achieved; in three of the six preparations (D3, E1 and E6) it was not possible to maintain the popliteal muscle tendon and, consequently, its sesamoid bone; in the remaining three samples

(D6, D7 and E2) it was not possible to preserve the tendon of origin of the extensor digitorum longus muscle. In samples D7 and E2, the medial patellofemoral ligament and the distal branch of the caudal ligament of the lateral meniscus were not observed, respectively.

Another situation observed in the samples was the darkening of non-bone structures, such as ligaments, menisci and tendons, in four of the sixteen pieces (25%) - D5, D6, E2 and E5. And it was also observed that in 93.7% of the preparations they had a final appearance that was a little greasy to the touch, that is, in only one piece this fact did not occur (E7). In both situations, no losses were found in identifying the structures.

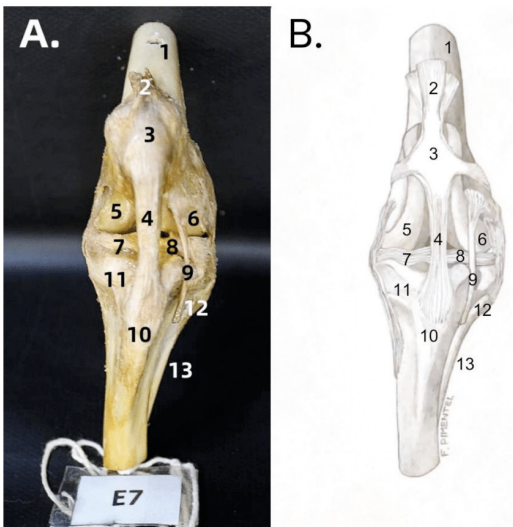


Figure 1 – Image (A) and schematic drawing (B) of the final appearance of a dog's left knee in cranial view after application of the cryo-dehydration technique. Piece already varnished (A), with all structures preserved.

A: Image of the cranial view; B Schematic drawing in cranial view: 1 femur, 2 tendon of insertion of the rectus femoris muscle, 3 patella, 4 patellar tendon, 5 medial condyle of the femur, 6 lateral condyle of the femur, 7 medial meniscus, 8 lateral meniscus, 9 origin of the muscle extensor digitorum longus, 10 crest of the tibia, 11 medial condyle of the tibia, 12 lateral condyle of the tibia, 13 fibula.

Source: the author himself.

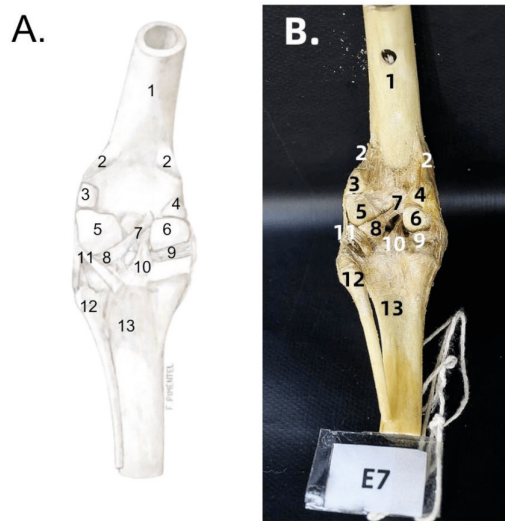


Figure 2 - Schematic drawing (A) and Image (B) of the final appearance of a dog's left knee in caudal view after applying the cryo-dehydration technique. Piece already varnished (B), with all structures preserved.

A: Schematic drawing in caudal view; B: Image of the caudal view: 1 femur, 2 origin of the gastrocnemius muscle, 3 2 lateral sesamoid, 4 medial sesamoid 2, 5 lateral condyle of the femur, 6 medial condyle of the femur, 7 meniscofemoral ligament (proximal branch) of the caudal ligament of the lateral meniscus, 8 lateral meniscus, 9 medial meniscus, 10 caudal cruciate ligament, 11 origin of the popliteal muscle (with its sesamoid), 12 fibula, 13 tibia. Source: the author himself.

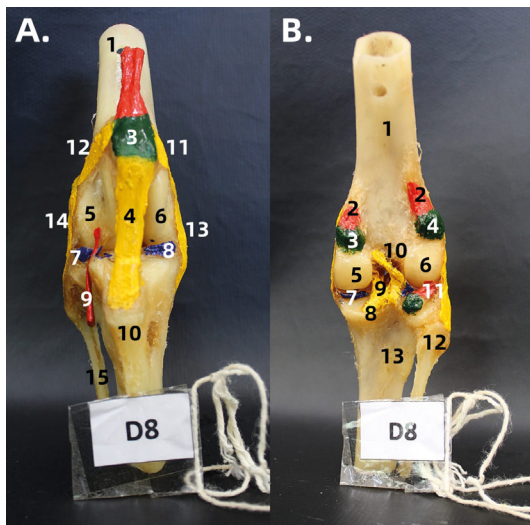


Figure 3 - Images of the final appearance of a dog's right knee in cranial (A) and caudal (B) views after applying the cryo-dehydration technique and painting (with acrylic paint) of the structures. Piece also already varnished.

A: Cranial view: 1 femur, 2 tendon of insertion of the rectus femoris muscle, 3 patella, 4 patellar tendon, 5 lateral condyle of the femur, 6 medial condyle of the femur, 7 lateral meniscus, 8 medial meniscus, 9 origin of the extensor digitorum longus muscle, 10 tibial crest, 11 medial patellofemoral ligament, 12 lateral patellofemoral ligament, 13 medial femorotibial ligament, 14 lateral femorotibial ligament, 15 fibula. B: Caudal view: 1 femur, 2 origin of the gastrocnemius muscle, 3 2 medial sesamoid, 4 2 lateral sesamoid, 5 medial condyle of the femur, 6 lateral condyle of the femur, 7 medial meniscus, 8 medial condyle of the tibia, 9 ligament caudal cruciate, 10 meniscofemoral ligament (proximal branch) of the caudal ligament of the lateral meniscus, 11 origin of the popliteal muscle (with its sesamoid), 12 fibula, 13 tibia. Source: the author himself.

At the beginning of the application of the cryo-dehydration technique, all samples were weighed (Table 1) and during the process, the weight was always measured at the time of removal from the freezer and after six hours of defrosting at room temperature. In total,

13 freezing and thawing sessions were carried out. Size, apparently, had no influence on the percentage of weight loss, as the simple average was around 22% (average of the left antimer 21.7% and 22.29% of the right antimer) on each side, regardless of the initial weight (Table 2).

Antimer	Initial weight (g)	Antimer	Initial weight (g)
D1	68	E1	65
D2	64	E2	105
D3	62	E3	56
D4	59	E4	58
D5	64	E5	91
D6	112	E6	109
D7	51	E7	75
D8	94	E8	93

Table 1 - Initial weight of the pieces before the beginning of the freezing and thawing sessions, measured in grams.

Source: adapted from Camilla Elias Bruno.

Antimer	Loss (g)	Loss (%)	Antimer	Loss (g)	Loss (%)
D1	11	16,18%	E1	11	16,92%
D2	13	20,31%	E2	22	20,95%
D3	14	22,58%	E3	12	21,43%
D4	11	18,64%	E4	9	15,52%
D5	18	28,13%	E5	18	19,78%
D6	21	18,75%	E6	22	20,18%
D7	16	31,37%	E7	28	37,33%
D8	21	22,34%	E8	20	21,51%

Table 2 - Total grams lost from each piece, both right and left, during the experiment and also the percentage of weight loss compared to the initial weight

Source: adapted from Camilla Elias Bruno.

Around the 10th Session, weight loss stabilized in most of the pieces, becoming relatively constant until the 13th Session (figure 4).

Throughout the experiment, air humidity

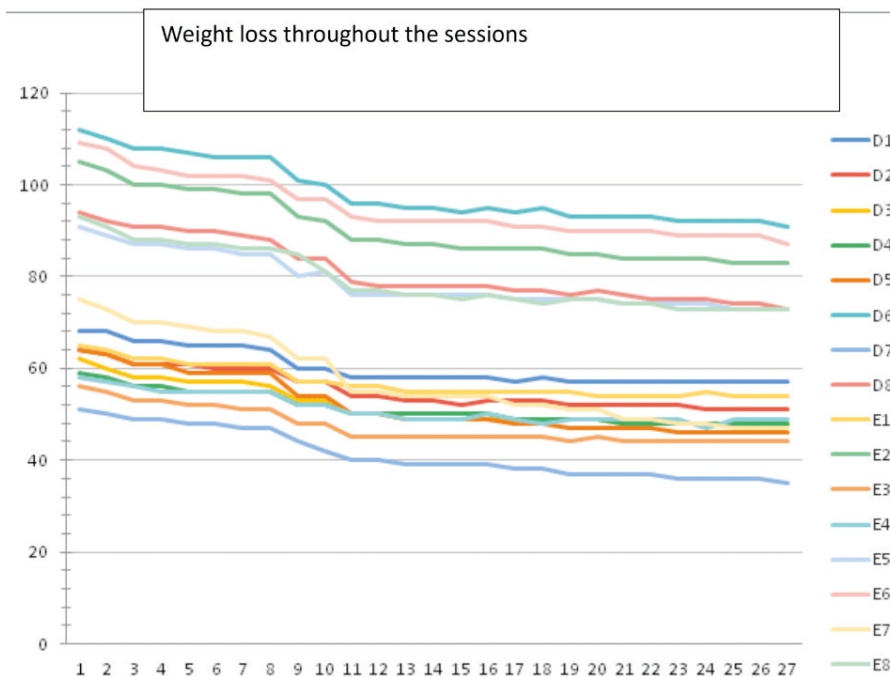


Figure 4 – Graph illustrating weight loss after each freezing and thawing session. Note that the lines remained relatively constant in all samples, regardless of the initial weight of the piece and that from the 10th session onwards, in all samples, the weight loss was no longer significant.

From 0 to 120 (grams) initial weight of the samples, and from 1 to 27 total number of freezing and thawing sessions. Source: Camilla Elias Bruno.

and ambient temperature were also observed using a digital thermo-hygrometer (Table 3). Thus, it was possible to notice that in the 3rd Session we had the highest temperature (23.1°C) and in the 4th Session the lowest relative air humidity (52%). Therefore, observing the weight loss per Session of the pieces, it was found that in both the 3rd and 4th Session we had the greatest weight loss (Table 4).

Session of defrosting	Air humidity (%)	Temperature (°C)
Session 1	67	22,9
Session 2	69	22,7
Session 3*	55	23,1
Session 4*	52	21,3
Session 5	55	20,9
Session 6	58	20,2
Session 7	57	22,1
Session 8	59	22,9
Session 9	61	22,2
Session 10	68	22,2
Session 11	62	21,8
Session 12	65	22,4
Session 13	58	21,2

Table 3 - Ambient temperature and air humidity for the days on which the defrosting sessions were carried out.

* Highlighted are sessions with higher ambient temperature and lower humidity. Source: the author himself.

Piece	Frozen	Session 3	Frozen	Session 4
D1	64	60	60	58
D2	60	57	57	54
D3	56	53	53	50
D4	55	52	52	50
D5	59	54	54	50
D6	106	101	100	96
D7	47	44	42	40
D8	88	84	84	79
E1	61	57	57	56
E2	98	93	92	88
E3	51	48	48	45
E4	55	52	52	50
E5	85	80	81	76
E6	101	97	97	93
E7	67	62	62	55
E8	86	85	81	77

Table 4 - Pieces of the right and left antimeres with the weight at the time of removal from the freezer, in sessions 3 (highest temperature, 23.1°C) and 4 (lowest humidity, 52%), and after 6 hours of defrosting.

Source: the author himself.

DISCUSSION

We observed that the cryo-dehydration technique on dog knees was a viable method for their conservation, easy to apply and with a very satisfactory final result. This corroborates the observations of Brauner (2016), in his work using two dog knees, as well as in another article that described the use of cryo-dehydration in six equine synovial joints (MELO et al, 2009). Other articles also stated that it is possible to perform the cryodehydration technique on viscera such as brains (PESSOA et al, 2017; REIS; BOSSI; MARTINS; MAZZUCATTO, 2020), kidneys and hearts (BATISTA et al, 2017; CARMO, 2017; MIRANDA E BOSSO, 2020) and also the digestive system, such as the stomachs of some animal species (KREMER; SCHUBERT; BONFÍGLIO, 2011; ARAÚJO et al, 2021).

In the current research, before applying the

freezing and thawing technique, the pieces were removed from corpses that had already been formalized, which was also observed in the overwhelming majority of articles researched (regardless of the viscera or joint), that is, in some way (injection or immersion) the samples were always fixed in formalin and then cryo-dried. Only in one article found (FONTANA et al, 2019) was another dehydration technique applied to parts of an equine pelvic limb. In this work, the authors state that they carried out only one freezing and that after thawing, the piece dehydrated due to repeated applications of thinner, on the internal/medial side of the sample, and sodium chloride on the other side, this being done daily for 30 days. The structures that were not identified in the current research occurred due to an error in dissection and not due to a failure of the cryo-dehydration technique. What made it easier, in the current research, to better visualize the structures was that, in half of the samples, colored acrylic paint was used, each portion that was preserved in the dissected knees being painted (BRAUNER, 2016).

Another situation observed in the current work was that in 25% of the samples, structures that were not bony were darkened, which did not affect their visualization in any way. In the literature, only Fontana et al (2019) observed any change in the color of certain structures, but these authors did not use the freezing and thawing technique described here to dehydrate the piece. Another point described in the current research was the greasy appearance of the vast majority of samples. This fact also did not affect the identification of the structures and, possibly, occurred due to the fact that, together with the formaldehyde, a small amount of glycerin (around 1 liter for every 20 liters of formaldehyde) was applied at the time of embalming the corpse. Only one article was found in the literature (RECKZIEGEL;

CULAU; ARAÚJO; VOLL, 2015) which observed that pieces of dog intestine with excess fat took longer to dehydrate than other less fatty pieces, and that this could favor the proliferation of microorganisms over time. This has not been observed, so far, in the pieces of the current research.

The freezing temperature applied in the current research was -4°C , which is sufficient to achieve the objective of crystallizing water particles from dog knee samples (REIS; BOSSI; MARTINS; MAZZUCATTO, 2020). But for other authors, the ideal temperature for freezing would be -17°C or -18°C in their respective studies of dog and ruminant stomachs and pig kidneys and hearts (KREMER, SCHUBERT AND BONFIGLIO, 2011; CARMO, 2017). The freezing and thawing period was one of the factors that differed most between that applied in the current work and that of another authors' research. In the present study, 18 hours of freezing and six hours of thawing for each piece at room temperature and suspended was enough for the experiment to be successful. For Reis et al (2020), in their research with pig and dog brains, the ideal period was 12 hours freezing and 12 hours thawing; for Araújo et al (2021), in a study on cryo-dehydration of sheep stomachs, 22 hours of freezing and two hours of thawing in running water was enough to achieve the expected result; for Batista et al (2017) 48 hours of freezing and the same amount of time thawing was ideal for a piece of a sheep's urinary system; and also for Carmo (2017), in his study with 24 kidneys and 24 hearts of pigs, the time of 12 hours freezing and seven hours thawing in a forced air oven was the sufficient interval for applying the cryo-dehydration technique.

Another very controversial point was the number of freezing and thawing sessions applied to the different pieces studied by various authors. For some researchers, the

number of sessions was determined by the percentage of weight loss in the sample compared to their initial weight. This was described by Carmo (2017) who used as a parameter the loss of 60% to 70% of the sample's initial weight, varying from nine to 16 days for kidneys and 28 to 39 days for pig hearts. In the current study, 13 cycles of freezing and thawing were enough for each piece of dog knee to lose an average of 22% of its weight, with weight loss stabilizing in the 10th session. One of the hypotheses behind this discrepancy in weight loss percentages between the articles found and the current research was that bone structures have a smaller amount of water than viscera such as kidneys, hearts and brains (CARMO, 2017; PESSOA et al, 2017; MIRANDA E BOSSO, 2020). In the study by Batista et al (2017), weight loss was similar to that found in our research, around 26.3%, even when studying the viscera of the urinary system.

Weight loss, in the research carried out, was not related to the initial weight of the sample, as the heavier pieces did not lose more water than those with a lower weight. This corroborates the study by Miranda and Bosso (2020) on hearts from different animal species and with very different weights. For them, a bovine heart had a loss of 73% of its initial weight, an equine heart 60%, a feline heart 71% and one of the dog heart samples had a loss of 76%. For us, what was most decisive in weight loss was the increase in ambient temperature (23.1°C was the highest recorded) and the low relative humidity (52% the lowest recorded), as in the sessions in which this occurred we observed- if a significant difference. In the literature, few studies comment on ambient temperature. In Reis et al (2020) the average temperature was 27°C and for Carmo (2017) it was 25°C , but controlled in a greenhouse. As for relative humidity, no article was found talking about it.

CONCLUSION

According to the results obtained in this study, it is possible to conclude that the cryo-dehydration technique in dog knee joints is

viable, easy to apply and with good results. The technique eliminates the use of formaldehyde for maintenance and has a low execution cost, in addition to allowing visualization of all desired structures.

REFERENCES

- ARAÚJO, A.C.P.; RECKZIEGEL, S.H.; VOLL, J.; BRAUNER, R.K.; ALVES, N, A. **Descrição da técnica de criodesidratação aplicada em estômago de ovelha (*Ovis aries*)**. A face transdisciplinar das ciências agrárias 2. Editora Atena. Ponta Grossa, PR. 2021.
- BATISTA, G. R. dos S., BUENO, S. D., ROCHA, A. M. da L., RODRIGUES, M. S., MACHADO, S. V., CORADINI, M G. L., SANTOS, A. de S., LINDEN, L. de S. V. D., TEIXEIRA, B. B. M. **Aplicação da técnica de criodesidratação no sistema urinário de um ovino**. Faculdade Ideau de Bagé. 2017
- BRAUNER, R. K. **Anatomia comparada dos animais domésticos e silvestres**. UFPEL, 2016.
- CARMO, L. G. do. **Criodesidratação de rins e corações de suínos utilizando estufa de ar com ventilação forçada**. Universidade Estadual de Maringá, 2017.
- DA SILVA, G. R.; CORTEZ, P. O. B. C.; LOPES, I. S. L.; TEIXEIRA, B. A. C. B.; LEAL, N. M. S. **Métodos de conservação de cadáveres humanos utilizados nas faculdades de medicina do Brasil**. Revista De Medicina, v. 95, n. 4, p. 156-161, 2016.
- FONTANA, C. A. P., HELRIGLE, C., FERRAZ, H. T., REZENDE, P. F. Z., LOPES, D. T., SILVA, L. F. da, SATURNINO, K. C., BORGES, E. M. **Uso da técnica de desidratação para o preparo de articulações**. O estudo da anatomia simples e dinâmico, 2019, V3, 182 - 197.
- HINER, R. L.; HANKINS, O. L. **Temperatures of freezing affects tenderness of beef**. Food Industry, v. 19, p.1078, 1947.
- INTERNATIONAL AGENCY FOR RESEARCH ON CANCER. **Monographs on the evaluation of carcinogenic risks to humans: Wood dust and formaldehyde**. IARC, v. 62, p. 217-375, 1995.
- KOONZ, C. H.; ROMSBOTTON, J. M. **A method for studying the histological structure of frozen products**. I. Poultry. Journal of Food Science, v. 04, n. 02, p.117-128, 1939.
- KREMER, R.; SCHUBERT, J. M.; BONFÍGLIO, N. S. **Criodesidratação de vísceras do canal alimentar no preparo de peças anatômicas para estudo veterinário**. PUBVET, v. 05, n. 13, 2011.
- KRUG, L.; PAPPEN, F.; ZIMMERMANN, F.; DEZEN, D.; RAUBER, L.; SEMMELMANN, C.; ROMAN, L. I.; BARRETA, M. H. **Conservação de peças anatômicas com glicerina loira**. In: MOSTRA DE INICIAÇÃO CIENTÍFICA, 1, 2011, Concórdia. Resumos: Concórdia: Instituto Federal Catarinense, 2011. p. 1-6.
- MELO, L. C. S. de, SILVA, S. F. de P. da S., MONTE, G. M. S. do, LIMA, R. M. de, SILVA, J. H. C. da, XAVIER, G. A. A. **Articulações desidratadas para aulas de anatomia animal**. Universidade Federal Rural de Pernambuco, 2009.
- MIRANDA, R. M. S.; BOSSO, A. C. S. **Montagem de coleção de corações criodesidratados de animais domésticos**. Revista Desafios. V 7. Tocantins, 2020
- PESSOA, G. H., CESARI, E., ANTONIOLLI, E. C., PINTO, B. A., WEBBER, A. R., DEJAN, W. da S., MAHL, D. L., OLIVEIRA, F., OLIVEIRA, D. dos S. **Criodesidratação do sistema nervoso de um bovino**. Rev. Acad. Ciênc. Anim. 2017;15(Supl.2):S127-128.
- PINA, C. D. S. **Avaliação da exposição profissional ao formaldeído: Efeito Genotóxico**. 2010. 77 f. Dissertação (Mestrado em Contaminação e Toxicologia Ambientais) - Instituto de Ciências Biomédicas Abel Salazar, Faculdade de Ciências, Universidade do Porto, Porto, 2010.
- RECKZIEGEL, S. H.; CULAU, P. O. V.; ARAÚJO, A. C. P.; VOLL, J. **Técnicas anatômicas de preparação de vísceras secas e de angioarquitetura para visualização da ramificação da artéria mesentérica cranial em cães e gatos**. Acta Scientiae Veterinariae, v. 43, p. 1287-1291, 2015.
- REIS, N. M., BOSSI, A. G., MARTINS, L. L., & MAZZUCATTO, B. C. (2020). **Técnica de criodesidratação comparada entre encéfalos de suínos e caninos para estudo da anatomia animal**. Medicina Veterinária (UFRPE), 14(3), 193–196.
- WOODARD, J. C.; JONES, T. C.; HUNT, R. D.; KING, N. W. **Patologia Veterinária**. 6. ed. Barueri: Manole, 1997. p. 913-961.