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Conservation of Organs (Heart, Brain and Kidney) of Canine by Cold-Temperature Silicone Plastination in an Animal Anatomy Laboratory in Ecuador

Conservación de Órganos (Corazón, Encéfalo y Riñón) de Canino Mediante la Técnica de Plastinación en Silicona al Frío Realizada en Laboratorio de Anatomía Animal en Ecuador

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SUMMARY: For the purposes of teaching anatomy, the use of cadaver preparations is considered the most efficient way of ensuring that students retain knowledge. Nevertheless, in Ecuador the use of animal specimens in universities must comply with the internationally accepted principles of replacement, reduction and refinement (3Rs). Plastination is an alternative technique which allows organs to be conserved in the long term and complies with the 3Rs. The object of the present work was to use cold-temperature silicone plastination with Biodur® products to obtain long-lasting, easy-to-handle canine organs for use as tools for the teaching of animal anatomy. Six canine cadavers were obtained from local animal protection charities. The hearts, brains and kidneys of the cadavers were dissected and fixed with formaldehyde 10 %. They were then dehydrated with acetone at -20 °C. The specimens were impregnated with Biodur® S10:S3 (-20 °C) and finally cured with Biodur® S6. We plastinated six hearts, twelve kidneys, four brains and one encephalic slice of canine. The application of cold-temperature plastination to canine organs followed the parameters established for the conventional protocol, enabling us to obtain organs of brilliant appearance, free of odours, in which the anatomical form was preserved. Thus the technique helped us to comply with the 3Rs, as we obtained easy-to-handle teaching models to replace fresh or formaldehyde-fixed samples for the teaching-learning of the canine anatomy.

KEY WORDS: Plastination; Cold-Temperature; 3Rs; Teaching-learning; Anatomy.

INTRODUCTION

Nowadays, training students in medical subjects requires innocuous and durable specimens (Bicklely *et al*., 1981; Peralta *et al*., 2017; Acevedo *et al*., 2018). Plastination is a conservation technique that allows anatomical material to be conserved in the long term, and therefore complies with the objective of using appropriate specimens for teaching (Miklosˇová & Miklosˇ, 2004; Ottone *et al*., 2015). The technique was developed by von Hagens in 1977 (von Hagens, 1979; von Hagens *et al*., 1987) and consists of four phases: fixing, dehydration, impregnation and curing (Henry *et al*., 2019; Peralta *et al*., 2017). Through these processes the water, fat and biological liquids are replaced by polymers such as silicone (von Hagens, 1979; Ottone *et al*., 2015; Acevedo *et al*., 2018).

Various authors have used different silicones, or modified the exposure temperature/times, in order to reduce costs or improve the technique (Brown *et al*., 2002; Smodlaka *et al*., 2005; Henry *et al*., 2007; Ottone *et al*., 2015). However, the basic elements of the technique have been maintained, and it is considered a fundamental tool for morpho-functional study (Yu *et al*., 2015; Acevedo *et al*., 2018; Popp *et al*., 2018). There a several laboratories which use silicone plastination to create teaching material (Pashaei, 2010; Peralta *et al*., 2017). In South America, work has been done using the technique for example in Chile, Argentina and Colombia (Ottone *et al*., 2015; Acevedo *et al*., 2018; Popp *et al*., 2018). In Ecuador, the first plastination laboratory was opened in the Faculty of Veterinary Medicine and

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Zootechnology (FMVZ) of the Central University of Ecuador (UCE) in 2018. The aim of the present work was to use cold-temperature silicone plastination to conserve organs that would be useful for teaching canine anatomy, and so demonstrate work carried out in the UCE plastination laboratory.

MATERIAL AND METHOD

Six canine specimens donated by local animal protection charities were dissected to obtain the brains, hearts and kidneys. Because the study was carried out using cadavers previously subjected to euthanasia, the approval of the Faculty's Investigation Committee (COIF) was not required, while the ethical endorsement of the FMVZ committee is applicable only to investigations, either experimental or observational, in which living vertebrates are used.

This work was designed to enhance compliance with current legislation, article 81 of the Metropolitan Ordinance of the City of Quito 019 – 2020, which states that both teaching and investigation shall be carried out under the principle of the 3Rs. The cadaver specimens were used for various purposes, both in teaching canine myology and in the subjects of surgery and emergencies in small animal medicine; furthermore, osteological pieces were obtained for inclusion in the bone collection of the Animal Anatomy Laboratory of the FMVZ of UCE.

The organs obtained were washed under running water, weighed, and measured using a Vernier calibrator to calculate their volume. They were dissected individually along the sagittal or transverse plane to present their internal structures, and placed in formaldehyde 10 % in a proportion of 3:1. The organs (hearts, kidneys and brains) remained in the fixing solution for three months.

The next stage of plastination was dehydration, carried out using acetone 99 % as intermediary solvent (IS). The volume ratio of acetone to the organ was 10:1. The samples were submerged in acetone without previous washing and conserved at -20 °C. The concentration of the IS was measured with an acetonometer every 48 hours, and recorded. After measurement, the organs were placed in acetone at a higher percentage concentration. The process was repeated until a percentage of 99 % was obtained in two consecutive measurements. Fat was extracted from the brains by leaving them in acetone 99 % for one week at ambient temperature. Dehydration of the hearts and kidneys took four weeks. Dehydration and fat extraction from the brains took a total of five weeks.

On completion of dehydration, forced impregnation was carried out using Biodur® silicones S10 and S3. This was done by preparing a solution at 100:1 (S10:S3). Air was eliminated from the mixture using vacuum pressure for 6 hours, and then the organs were left to rest for 24 hours without pressure. The dehydrated organs were submerged completely in the silicone mixture, without touching the sides or the base of the impregnation chamber. They remained submerged for 24 hours without pressure. Impregnation was then commenced at a pressure of 50 mmHg. The pressure was gradually reduced as the bubbles were observed to disappear, until 5 mmHg was reached, with no bubbles. The impregnation process took five weeks. At the end of this period, the organs were removed from the S10:S3 mixture, drained, and placed individually in Ziploc® bags (pre-curing). We should mention here that this stage occurred at the height of the COVID 19 pandemic, and for that reason pre-curing took twenty weeks.

Curing of the organs was effected using Biodur® silicone S6. The organs were placed in the curing chamber, Biodur® model HH13A1.0, which is fitted with a fan, three 500ml Erlenmeyer flasks and three air tubes to vaporize the S6. The chamber was set to come on in four-hour cycles, and the position of the anatomical pieces was changed twice per day to ensure that all the external surfaces of the organs were polymerised. Finally, after two weeks of curing in the chamber, the organs were stored in sealed Ziploc® bags for three weeks to promote polymerisation inside the organs (post-curing).

On completion of the process, the organs were measured to calculate their volume and estimate shrinkage. The plastinated organs were weighed and then stored in plastic boxes for use as teaching material in the animal anatomy laboratory of the FMVZ.

RESULTS

We plastinated six hearts, twelve kidneys, four brains and one encephalic slice of canine. These organs obtained by cold-temperature silicone plastination were of brilliant appearance, free of odours, and their anatomical form was preserved (Fig. 1). The hearts and kidneys lost between 2 and 3 grams of their original weight; however, the brains lost between 3 and 6 grams (Table I).

The average volumetric shrinkage found for the kidneys was 16.08 %, with a range between 11.07 and 19.84 % (Table I). The hearts presented average shrinkage of 14.70 %, with a range between 17.30 and 13.03 % (Table I). In the case of the brains, only the volumetric shrinkage was estimated, and found to be 18.91 % on average.

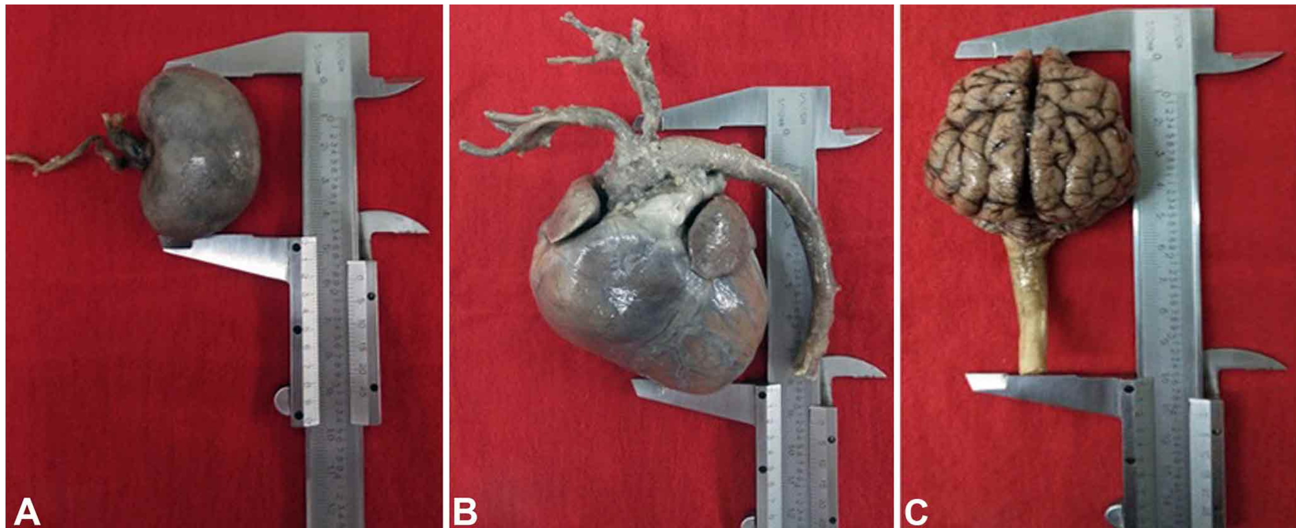

Fig. 1. Organs plastinated with cold silicone. (A) Plastinated kidney coded as 1R. (B). Plastinated heart coded as 1C. (C). Plastinated brain coded as 1E.

Table I. Weight and shrinkage percentage for hearts, kidneys and brains obtained with the cold plastination technique.

Code	Organ	Original weight (g)	Weight after plastination process (g)	Shrinkage (%)
1RD	Right kidney with vessels and ureter	12	10	19.31
1RI	Left kidney with vessels and ureter	13	11	11.07
2RD	Right kidney with vessels and ureter	5	3	14.96
2RI	Left kidney with vessels and ureter	6	4	17.11
3RD	Right kidney (midsagittal section)	12	11	11.36
3RI	Left kidney (midsagittal section)	13	11	13.26
4RD	Right kidney (midsagittal section)	16	14	17.08
4RI	Left kidney (midsagittal section)	17	16	18.91
5RD	Right kidney	19	17	19.42
5RI	Left kidney	20	18	18.43
6RD	Right kidney (midsagittal section)	16	14	12.27
6RI	Left kidney (midsagittal section)	18	16	19.84
1C	Heart with main vessels	91	88	13.94
2C	Heart with main vessels	76	73	13.03
3C	Heart with main vessels	30	26	17.30
4C	Heart (cross section, visualization atria and ventricles)	56	53	14.65
5C	Heart (midsagittal section)	75	72	13.68
6C	Heart with main vessels	32	29	15.57
1E	Whole brain	40	34	15.22*
2E	Brain (midsagittal section)	34	30	22.47*
3E	Brain with eyeballs	66	61	20.75*
4E	Brain partially covered by dura mater	46	41	17.19*
5T	Brain stem	13	10	n/c

* Retraction determined only for telencephalon volume. n/c not calculated.

DISCUSSION AND CONCLUSIONS

The organs processed by conventional cold-temperature silicone plastination maintained their form and texture. Handling was safe for the users, and there were no unpleasant odours; these characteristics have been associated with this technique since it was first developed (von Hagens, 1979; von Hagens *et al*., 1987; DeJong &

Henry, 2007; Ottone *et al*., 2015; Acevedo *et al*., 2018). Use of the plastination technique made it possible to reduce the number of animals used in the teaching-learning process, replacing cadaver preparations (Peralta *et al*., 2017; Acevedo *et al*., 2018). Use of cold-temperature silicone plastination therefore complied with the principle of the 3Rs required by current legislation in Ecuador (General Regulation of the Organic Agricultural and Livestock Health Law and Metropolitan Ordinance of the City of Quito 019 – 2020).

Fixing with formaldehyde produced a loss of colour in the specimens, but the insertion of methylene bridges between the nitrogen atoms of the proteins meant that the structure of the samples was preserved (Brenner, 2014). Formaldehyde is therefore considered the ideal fixer, although the time that the specimens must remain in formaldehyde will depend on their size (Bickley *et al*., 1987; Peralta *et al*., 2017). In general terms, a period of one to eight weeks is required for correct fixing (Peralta *et al*., 2017; Acevedo *et al*., 2018). The organs in the present work were fixed for three months, but no difficulties were experienced in carrying out the technique. Cases have been reported of specimens being plastinated successfully after preservation in formaldehyde for several years (Ottone *et al*., 2015; Acevedo *et al*., 2018).

Acetone was used as the intermediary solvent for dehydration because it contributes to fat extraction; it has also been reported to cause less shrinkage of the organs (Baptista *et al*., 1988; Brown *et al*., 2002; Ottone, 2021). Shrinkage is reduced by the so-called “freeze-substitution" process, generally carried out at -25 °C (Henry *et al*.,1997; Ottone, 2021). In the present work, however, dehydration was carried out at -20 °C, considering that the evaporation point of acetone starts at -18 °C, and other investigators have worked in the range -10 to -20 °C without any adverse effects being observed in the specimens (Brown *et al*., 2002; Riederer, 2014; Sora, 2016). In the present work, in fact, the mean shrinkage of the canine kidneys was 3.08 % higher than the 12.8 % reported by Brown *et al*. (2002). Likewise, the average shrinkage of the hearts was 4 % higher than that reported by Brown *et al*. (2002), but for hearts of feline (10.7 %). In general, the shrinkage observed in all the organs processed may be considered minimal, and the canine kidneys, hearts and brains obtained are of optimum quality for teaching purposes (Sora, 2016).

Brown *et al*. (2002) say that the minimum time required to dehydrate hearts and kidneys of carnivores with cold acetone is five days. However, we carried out cold-temperature plastination of hearts, kidneys and brains of canine for four weeks; Ottone (2021) indicates that this is the minimum required for freeze-substitution in acetone at cold temperatures.

With respect to the impregnation process, Ottone (2021) says that the minimum time required for forced cold impregnation is one month, although it may be longer, depending on the size and number of samples. Thus the impregnation process used here for canine brains, kidneys and hearts is within the range established by the standard protocols, since the time taken was five weeks (Henry *et al*., 2019). At this point it may be noticed that although impregnation was started with 50 mmHg, lower than the 60 mmHg suggested in the impregnation protocol published by Henry *et al*. (2019), the slow fall in the pressure justifies the time invested. It is thus considered that effective impregnation was achieved, thanks to the vacuum pressures applied progressively in accordance with the bubble velocity observed (Sora, 2016).

Finally, the pre-curing of the organs at ambient temperature was much better than the four weeks reported by Bickley *et al*. (1987). Nevertheless, considering that Henry *et al*. (2019) talk about several weeks for this slow curing phase, and given that there was no severe shrinkage of the samples, satisfactory results were obtained from polymer draining. Using an aquarium pump during curing is reported to accelerate the process, reducing it to two or three weeks (Bickley *et al*., 1981; Peralta *et al*., 2017). This time agrees with that invested in the present work to cure canine organs using vaporizing tubes in the Biodur® chamber. Although the post-curing time used in our work was shorter than the thirty days suggested by Acevedo *et al*. (2018), this did not impair the internal curing of the organs, which were firm, of brilliant appearance and free of odours.

Consequently, we can report that cold-temperature plastination with Biodur® silicones and equipment in the Animal Anatomy Laboratory of the FMVZ, following the parameters used in the conventional protocol, enabled us to produce conserved hearts, kidneys and brains with appropriate characteristics for use as easy-to-handle teaching aids. Furthermore, the organs obtained enabled us to reduce the number of specimens and replace the cadaver preparations used in the teaching-learning process.

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RESUMEN: En la enseñanza de la Anatomía, el uso de preparaciones cadavéricas se considera el método que permite a los estudiantes retener el conocimiento de una forma más eficiente. No obstante, en Ecuador, el uso de especímenes animales en las universidades se debe realizar bajo el principio internacional de reemplazo, reducción y refinamiento (3Rs). La técnica de plastinación es una técnica alternativa que permite preservar órganos a largo plazo y que se adapta al principio de las 3Rs. El objetivo del trabajo fue utilizar la técnica de plastinación en silicona al frío con productos Biodur® para obtener órganos caninos duraderos y manejables útiles como herramienta para la enseñanza de la anatomía animal. Se obtuvieron seis cadáveres de caninos de fundaciones locales para la protección animal. Se realizaron disecciones de corazones, cerebros y riñones de los cadáveres caninos. Los órganos se fijaron con formalina al 10 %. A continuación, se llevó a cabo la deshidratación con acetona a -20 °C. Los especímenes fueron impregnados con S10:S3 Biodur® (-20 °C) y al final fueron curados con Biodur® S6. Se lograron plastinar seis corazones, doce riñones, cinco encéfalos y un tallo encefálico de canino. La técnica de plastinación al frío utilizada para obtener órganos de canino conservó los parámetros empleados en el protocolo convencional y permitió obtener órganos que presentaron aspecto brillante, ausencia de olores y mantuvieron la forma anatómica. Por lo que, la técnica facilitó cumplir con el principio de las 3Rs al obtenerse modelos didácticos fáciles de manipular que pueden reemplazar muestras frescas o formolizadas en el proceso de enseñanza-aprendizaje de la anatomía del canino.

PALABRAS CLAVE: Plastinación; Temperatura fría; 3Rs; Enseñanza-aprendizaje; Anatomía.

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