

Original Article

Production and use of plastinated anatomical specimens as teaching and learning tools in veterinary gross anatomy in the Caribbean

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ABSTRACT

Objective: Veterinary Anatomy is considered as the backbone subject in the veterinary medicine program. Formalized specimens were not very much accepted by the veterinary students due to their wetness, bad smell and potential harmful effects. Plastination has evolved as a new technique for prolonged preservation of specimens by replacing the water and fat by a curable plastic polymer.

Material and methods: The process of plastination involved fixation, dehydration, impregnation and curing. The plastinated specimens were used for teaching and learning of veterinary gross anatomy courses for veterinary students.

Results: Plastinated specimens were appreciated by the staff and students as they were odorless, non-toxic and durable, needed less space for storage and they were used in teaching the veterinary anatomy courses. Plastinated specimens were used for studying not only in the dissection hall but also in the lecture room, both individually and in groups.

Conclusion: The plastinated specimens were excellent for demonstration but they could not completely replace the traditional dissection because students learn best by exploratory and hands-on dissection. Moreover, the wet specimens provided a more accurate visual representation of the body parts and skills to aid in dealing with actual clinical cases in the future. Therefore, the plastinated and wet specimens were used simultaneously depending on the topic being taught at the time.

KEYWORDS

Plastinated specimens; Production; Teaching; Veterinary anatomy

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INTRODUCTION

The animal cadaver and its organs are an integral part of learning veterinary anatomy in the DVM programme. The specimens used in teaching may be fresh or preserved ([Ameko et al., 2012](#)), however decaying of the fresh specimens in a short time is one disadvantage. Specimens can be preserved to stay for a prolonged period by both traditional methods such as mummification and embalming or by modern methods such as plastination ([Amin and Yesmin, 2015](#); [Menaka and Chaurasia, 2015](#))

Non-plastinated specimens fixed with formaldehyde are toxic, allergenic and possibly carcinogenic. Concentrations of the formaldehyde above 0.1 ppm in air can irritate the mucous membrane and eye causing watery eye. Moreover, inhalation of formaldehyde at the same concentration may cause difficult breathing, burning sensation in the throat, headache and may even lead to asthma. Also, non-plastinated specimens do not facilitate close interaction and manipulation of specimens and there is deterioration over time ([De Jong and Henry, 2007](#)).

There were problems that occurred for both instructors and students during the teaching of practical gross anatomy such as handling of the gastrointestinal tracts of equines and ruminants due to the size and flaccidity of the walls of the stomachs and intestines. In addition, these tracts were difficult to be demonstrated topographically and their ingesta needed to be evacuated in order to study the internal structures. Preservation of the specimens and tissue by plastination technique was first made by Dr. Gunther von Hagens in Heidelberg, Germany, in 1977. The process was based on replacement the fat and water by polymeric materials to produce hard, dry and odourless specimens ([Weiglein, 1997](#)). The aim of the study was to give detailed information about the production of plastinated specimens and to highlight the advantages of their use in combination with the traditional wet specimens in the teaching and learning of veterinary gross anatomy.

MATERIALS AND METHODS

Ethical statement: This study has been conducted after Ethical approval from the ethics committee.

Materials: The specimens such as whole dogs and their fresh organs were removed from euthanatized dogs from the Trinidad and Tobago society for the prevention of

cruelty to animals (TTSPCA). The organs of sheep, pig and ox were obtained from the U.W.I field station and abattoirs in various locations in Trinidad

Dissection: The whole animals were dissected by making a median incision from the mandibular space and caudally to the anus. The digestive and urogenital organs as well as brain were collected. The limbs were dissected. The domestic birds were slaughtered in a humane method and their abdominal cavities were opened to show the viscera in situ. The digestive tract was washed carefully of all its contents by making small incisions at various points and flushing with a steady stream of water from a small pipe. Hollow areas were dilated to maintain shape and assure proper dilation during curing

Plastination method: The S10/S15 Plastination Technique ([Henry, 2004](#)) was used in the present study for the plastination of the organs, chicken and limbs.

Fixation: All the specimens were placed in 5% formalin solution for three (3) weeks to fix properly.

Dehydration: The digestive and urogenital tracts specimens as well as heart and brain were then washed for 24–48 h under tap water. Excess water was drained from the specimens and the hollow spaces in the digestive tract were filled with cold acetone and arranged in an anatomically correct position in the specimen basket. Specimens were then submerged into an aliquot of -25°C acetone (90–100%) and placed in the freezer at -25°C . After six days the purity of the acetone was checked and recorded. This checking was done by filling a two (2) litre measuring cylinder with the used acetone. The temperature was monitored and an acetometer inserted and read. On day seven the purity of the acetone was again checked and recorded. Specimens were then placed in a new aliquot of -25°C acetone and placed back into the freezer. This procedure was repeated weekly two more times.

Impregnation: For impregnating the specimens the instruments used were deep freezer, vacuum chamber, vacuum pump, vacuum gauge, tubing, fine adjustment needle-valve and Bennert mercury manometer. The impregnation reaction-mixture Biodur S10/S3 mixture was prepared.

Impregnation procedure:

Day 1: The dehydrated specimens were taken from the acetone and excess solvent drained. The dehydrated solvent filled specimens were then placed in the cold

polymer reaction-mixture. The specimens were submerged immediately to prevent solvent evaporation from the specimens and drying of their surface. The hollow parts of the digestive tract were filled with the cold reaction mixture. A glass lid was then placed on the vacuum chamber and left to equilibrate overnight.

Day 2: The vacuum pump was then turned on and run for 10 minutes to warm to operational temperature. The vacuum chamber was then sealed by closing the needle valves and applying the vacuum. When the seal was accomplished the pressure was allowed to lower slowly to 22 mm Hg. The Bennett mercury manometer was now ready to commence reading at this pressure level. The pressure was then stabilized by opening the needle valves incrementally and slowly. Small bubbles began to rise through the polymer-mix at this point.

Day 3: Pressure was slowly decreased by 1/3 of the current daily value 22 mm to 14 mm Hg.

Day 4: Pressure was slowly decreased by 1/3 the current daily value to 9 mm Hg.

Day 5: Pressure was decreased slowly by 1/3 the current daily value to 6 mm Hg.

Daily decrease of pressure was continued until 1cm bubbles continually rise to the polymer surface and burst. This process was continued for approximately four weeks. Impregnation was completed when no more bubbles appeared at the surface of the reaction-mixture for several hours and near zero pressure was maintained for a few days. This phase took approximately four weeks.

Specimen removal: Vacuum pump was now turned off. Valves were opened and vacuum chamber and specimens were allowed to return to atmosphere pressure slowly. Specimens were left for 24 h in the reaction-mixture to equilibrate.

Day 1: Specimens were removed from the reaction-mixture and excess polymer-mix was allowed to drain into the vacuum chamber. Specimens were turned a few times to allow more polymer-mix from the crevices and hollow parts of the specimen.

Day 2: Specimens were removed from the freezer and allowed to continue draining at room temperature.

Day 3: Specimens were placed on paper towels and continued to drain.

Day 4/x: Once the specimen no longer oozed silicone it was placed for gas curing for two weeks.

Curing: The equipment used for curing included the curing chamber, aquarium pump and desiccant.

Curing procedure: Specimens were placed into the curing chamber together with a desiccant (CaSO₄).

Normal anatomical shape and position of the specimens were assumed. A small container of S6 gas cure was also placed into the curing chamber. The chamber was closed and S6 was vaporized using an aquarium pump. Specimens were wiped every 2–3 h and turned on the first day. The specimens were removed after six days and placed in an air tight bag for further curing.

Teaching: Cadaveric and produced plastinated specimens of the same specimens were used in teaching veterinary gross anatomy courses for year II veterinary students, School of Veterinary Medicine, Faculty of Medical Sciences, The University of the West Indies, St. Augustine, Trinidad and Tobago.

RESULTS

The plastination unit at the School of Veterinary Medicine, University of West Indies, Trinidad and Tobago has produced good quality plastinated specimens such as gastrointestinal and reproductive organs of dog and pig, limbs of horse and dog, heart of ox and dog, brain of ox and sheep, spleen of dog and pig and chicken (**Figure 1-3**) since 2008. The thoracic and abdominal cavities and limbs of the dog were demonstrated in relation to their various structures of muscles, organs, nerves and blood vessels. These specimens were used as new anatomical models for teaching and studying veterinary gross anatomy courses. The plastinated specimens were stored occupying less space in the anatomy laboratory than the formalin preserved specimens which needed more space, containers and formalin. Also the formalin needed to be changed regularly to prevent mold growth.

Plastinated specimens have replaced some of the formalin preserved specimens. The use of plastinated organs decreased the euthanization of dogs and sheep which are usually required in each academic year since the plastinated specimens will last for a longer time. Many students expressed relief that the use of the plastinated specimens allowed them to study veterinary gross anatomy without exposure to the formalin hazard also enhanced their knowledge and understanding. Further, plastinated specimens helped the students to understand the complicated structures of anatomical specimens, the topographic relations of the organs and were utilized not only in the dissection hall but also in the lecture room, both individually and in groups.

The produced plastinated specimens were smooth in texture, clean and odorless with realistic shape but with

Table 1. Summary of the dehydration schedule.

Day 1	Wash specimens for 24 h in tap water
Day 2	Place specimens in an aliquot of -25°C acetone (>90%) 1:10 (specimen: acetone) ratio
Day 9	Measure and record purity of used acetone. Place the specimens into a fresh aliquot of new (99 – 100%) acetone (-25°C)
Day 16	Measure and record purity of used acetone. Place specimens into a fresh aliquot of acetone (-25°C)
Day 23	Measure and record purity of used acetone. Place specimens into a fresh aliquot of acetone (125°C)
Day 30	Check acetone purity and color as well as fat color: If purity is >98% and fat is negligible or opaque. Place specimens into cold impregnation mix

Table 2. Summary of the impregnation schedule.

Day 1	Load specimens and allow to equilibrate overnight
Day 2	Start pump, slowly decrease pressure to atmosphere to : 22 mm Hg
Day 3	Slowly decrease pressure, 1/3 current pressure to : 14 mm Hg
Day 4	Slowly decrease pressure, 1/3 current pressure to : 9 mm Hg. Air bubbles form but not continually rising
Day 5	If no bubbles rising, slowly decrease pressure 1/3 current pressure to 6 mm Hg
Day 6	If bubbles actively rising to the surface and bursting, do not decrease pressure by 1/3 current pressure to : 4 mm Hg
Day 7	Active bubbles, do not decrease pressure
Day x	When bubbles cease or slow dramatically, decrease pressure 1 mm Hg

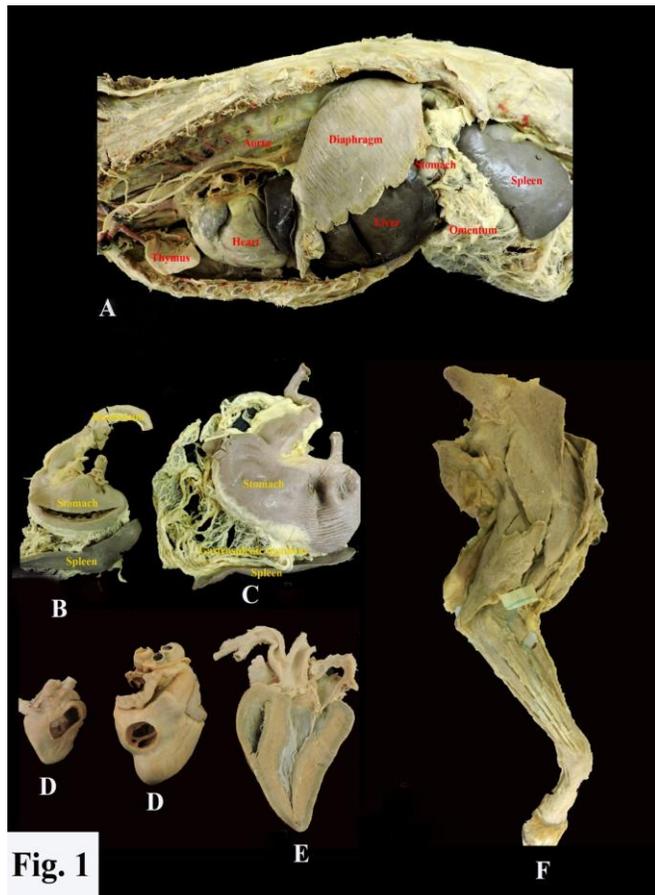


Fig. 1
 Figure 1. Plastinated specimens of horse, dog and pig. A. Left thoracic cavity of dog; B. stomach and spleen of dog; C. Stomach and spleen of pig; D. heart of dog; E. Longitudinal section of heart of horse; F. Thoracic limb of dog.

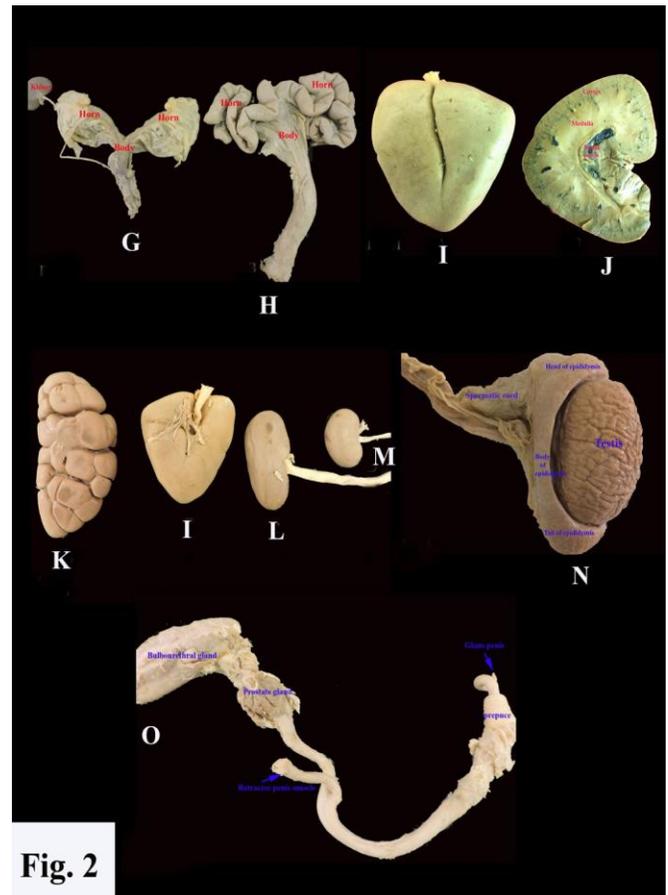


Fig. 2
 Figure 2. Plastinated specimens of horse, ox, pig and dog. G. Uterus of bitch; H. uterus of sow; I. Right kidney of horse; J. Longitudinal section in right kidney of horse; K Right kidney of ox; L. kidney of pig; M. kidney of dog; N. Testis of horse; O. Accessory genital glands and penis of boar.

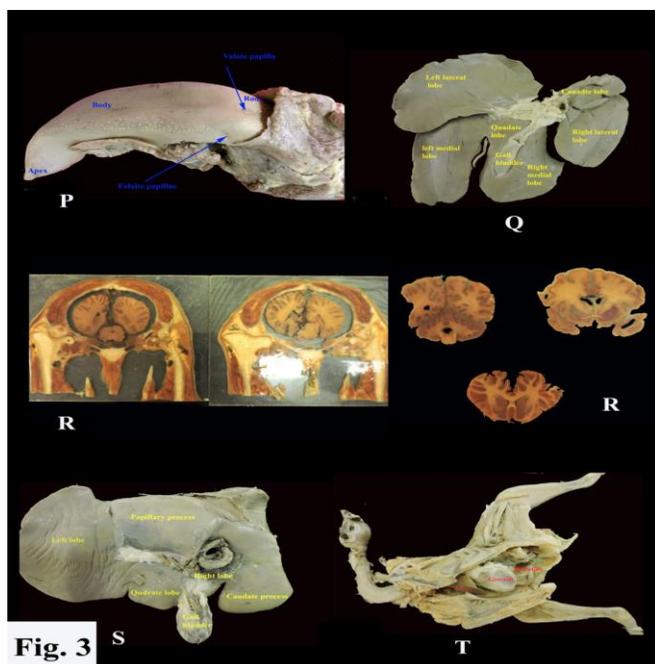


Fig. 3 A photograph showing some plastinated specimens of ox, pig and chicken. P. Tongue of pig; Q. Visceral surface of liver of pig; R. Cross sectional slices of ox brain; S. Visceral surface of liver of ox; T. Plastinated chicken.

slight difference in the color in some cases. On the other hand, testes showed slight shrinkage and a variation in texture and appearance (Figure 2/N).

Although there were many advantages in using plastinated specimens as a tool in teaching veterinary anatomy, the plastination technique is a sensitive and time consuming one. Findings show that the plastinated specimens were easy to work with in the classroom and laboratory but they could not completely replace the traditional dissection since students learn best by exploratory and hands-on dissection. Moreover, the wet specimens provided a more accurate visual representation of the body parts and can help develop skills that will aid the students when dealing with actual clinical cases in the future. Therefore, the plastinated and wet specimens can both be used depending on the topic being taught at the time. Therefore, at the U.W.I we aim to increase the number and quality of the plastinated specimens of different animal species and their organs.

DISCUSSION

The use of fresh specimens requires being fixed with 10% formalin. The many disadvantages in formalin embalming include deterioration of specimens over time, shrinkage of tissues and short term exposure limit (STEL) working with formaldehyde. In addition, formaldehyde is a

carcinogen, butagen, mutagen and a teratogen which limits its use in teaching and research. Storage of fresh specimens also poses a challenge as there was limited space in the lab to facilitate them. Also, over time, the formaldehyde turns to formic acid. With the above considerations the present study was taken to prepare the specimens which are durable, free of offensive odour and dry which can be used for teaching aids both in the classroom and in the clinical setting (Bickley et al.,1981; Latorre et al., 2001).

The plastinated specimens were prepared by using the S10/S15 technique as a superior standard than the silicone plastinated specimens as well as specimens preserved by other mechanisms. Unlike formalin fixed specimens, surface clarity was very good. The plastinated exhibited more flexibility. They were durable and free of offensive odor. These reports were concurrent with the findings of Henry (2004) which dealt with the specimens of domestic animals.

There was a natural progression from the gross dissected specimens to the final plastinated product. Specimen preparation was a key factor when starting the plastination process in the current study. The specimens to be used were well planned before starting the research with the consideration of how the final products should look, and we proceeded with dissections to carry out the plan. More flushing with water of the digestive tract was done which was necessary to remove all its contents. Plastination is a lengthy process and considering the time frame for the project, dilation of hollow structures in the digestive tract was done only for a short time. Rinsing with water for 24 h to remove the formalin was important because if that was not carried out, formalin may have leaked out of the specimen into the acetone. All the above processes were concurrent with the reports of Henry et al. (1997). Despite the short time used for the dilation of the digestive tract in the current study, it did not have any adverse effect on the final product.

The time for fixation with formaldehyde is depending on the organ and species of the animal in the plastination process. In this study, fixation was done for 3 weeks with 10% formaldehyde which is found to be more plastination friendly.

Dehydration was the major stage in the plastination process. The principles behind dehydration was that it replaced tissue fluid/water (both inter and intra-cellular) with an organic solvent. This solvent must be miscible with water and preferably volatile enough to serve as a volatile intermediary solvent which is the key to the

impregnation step. Common dehydration and intermediary solvents were acetone, alcohols and methylene chloride. Acetone was the universal dehydrating solvent for the plastination process because alcohol vapour pressure at -15°C is too low to be extracted gradually and continually and methylene chloride is not miscible with water. For tissues with a lot of fat methylene chloride can be used as a degreaser or defatting agent ([De Jong and Henry, 2007](#)).

The principles behind the impregnation stage or forced impregnation were the replacement of the volatile solvent in a biological specimen with a curable polymer. For this to occur, the volatile intermediary solvent must be miscible with the silicone polymer and must have a sufficiently high vapour pressure at -15°C to be gradually, continuously and completely extracted during the impregnation step of the plastination process. As the principle of dehydration was to replace tissue water/fluid with acetone, the impregnation principle was to replace the volatile intermediary solvent (acetone) with the reaction – mixture of S10/S3 ([De Jong and Henry, 2007](#)). The reaction mixture is too viscous to come to equilibrium with the solvent. Therefore, a forced vacuum is used to get the reaction-mixture inside the specimen in the current study.

The solvent filled (acetone) specimens were submerged in the liquid silicone reaction-mixture in the plastination chamber which was kept in a -15°C deep freezer. Applying vacuum to the reaction-mixture and the specimens caused the solvent to vaporize/boil (at a known pressure) and left the specimen, passed through the reaction-mixture and finally the vaporized solvent was pumped out through the vacuum pump exhaust. This is manifested by bubbles rising through the reaction mixture. Vaporization of the acetone from the specimen leaves a tissue void or negative pressure inside the specimen and the reaction-mixture is drawn into the specimen. For this exchange to take place there must be enough time for the viscous polymer-mixture to enter the cells of the specimen. Therefore, if the pump speed is too great and generates excess vacuum too quickly, too much acetone will leave and the tissue will collapse. The collapsed tissue will not allow the viscous reaction-mixture to enter. This will result in shrinkage and desiccation of the specimen ([Henry, 2004](#)). Two fine adjustment needle-valves were used for delicate control of pressure in the present study.

Preparation of the reaction-mixture was important step in the process of plastination. The S10 or S15 polymer was

mixed with S3 (catalyst with chain extender) at 100:1 to prepare the reaction-mixture. Thorough stirring was necessary; a normal stick can be used. During mixing, the reaction-mixture first turned opaque then clear. This polymer-catalyst mixture was deaerated by placing the mix in a vacuum chamber and reducing pressure to 3 mm Hg. The air was boiled out and the reaction-mixture was ready to be used at -15°C for impregnation or to be stored in the deep freezer preferably less than -25°C . In the present study the readymade mixtures were used to ensure proper curing.

Monitoring of bubbles in the impregnation stage of the plastination process was very important. The too quick evacuation of acetone/solvent result in incomplete impregnation of the specimen with the polymer-mix and shrinkage ([De Jong and Henry, 2007](#)). To avoid the shrinkage pressure was reduced slowly (1/3 of the current reading) over a four to six day period to the point where the intermediary solvent began to vaporize/boil and leave the tissue at a slow steady rate. This allowed the reaction-mixture to enter the tissue void created by the vaporizing and exiting acetone. The pressure at which the solvent reaches its boiling point depends on the temperature and the solvent used. When at boiling point, 1cm bubbles rose continually to the surface and burst. Before this point a few sporadic bubbles rose but were likely to sit on the polymer surface. For acetone, this steady extraction of solvent at -15°C occurs around 3 mm Hg pressure or 1.5 mm Hg pressure at -25°C . If bubbles were actively rising to the top of the polymer and bursting, do not decrease pressure. It was found that decreasing pressure slowly to be effective than at a faster rate. Acetone removal and have polymer impregnation at cold temperature will take 3 to 5 weeks depending on volume of specimens and pump speed. When bubbles ceased to rise, slowly and incrementally the needle valve was closed to decrease pressure until active bubbles started to rise again. Usually it is necessary to lower pressure only 1–2 mm Hg to continue active bubble production. It took a few minutes after incremental valve closure before bubble production was observed. All these steps were performed as report of [De Jong and Henry \(2007\)](#). The impregnation reaction-mix within the specimen is cross-linked and the specimen made dry during curing. This is a two-step process consisting of chain extension and cross linkage of polymer. Chain extension of the silicone molecules is an end to end alignment, thus forming longer chains using the chain extender portion of the S3 impregnation-mixture which is now in the specimens. This first reaction, known as pre-curing, results from the reaction of the S3 with the S10. This is known as slow

cure. It is advantageous to pre-cure for at least two or three days. Longer pre curing is even better and may yield more flexible specimens ([De Jong and Henry, 2007](#)). In the current study the curing was done as fast cure which also yielded good results.

The specimens were drained for a few days before curing. Fast cure at this timing will likely take at least one week to complete. The specimens were allowed to drain at room temperature for a few days to allow maximum chain extension. During this period, the specimens were kept in the anatomically correct position and dilated to ensure correct position as chain extension progresses. Theoretically, chain extension starts when the S3 (catalyst and chain extender) and S10 polymer were mixed. This reaction slowed down dramatically by cold temperature (less than -15°C). At room temperature, the elongation occurred at an increased rate. The above findings are in concurrence with the reports ([Henry, 2004](#)).

Veterinary anatomy is a vast subject which encompasses very extensive contents and needs sufficient time for its understanding and memorizing, therefore teaching of veterinary gross anatomy using different methods such as books, videos and dissections as well as different types of cadaveric and plastinated specimens are important for the students to understand anatomy. Using the traditional teaching methods such as didactic lectures and dissection of the cadavers for certain topics such as gastrointestinal and reproductive organs as well as brain and heart are not enough for optimum student learning; as such organs were plastinated and demonstrated to students for better understanding of these topics ([Holladay and Hudson, 1989](#); [Henry et al., 1997](#)).

Handling specimens preserved by traditional methods are discouragingly difficult ([Ravi and Bhat, 2011](#)). The plastination process was used for a variety of species for producing plastinated specimens which are used as teaching aids in many veterinary schools. Plastination allowed specimens to be durable, dry, odorless, non-irritant, and easy to hold in the hand, nontoxic, prevent the exposure to formalin hazard and may even have a beautiful color ([Bhandari et al., 2016](#); [Rabiei et al., 2016](#)). However, one of the limitations was the size of the impregnation chamber ([Dawson et al., 1990](#); [Nicaise et al., 1990](#); [Hawley et al., 1991](#); [Pond et al., 1991](#); [Eckel et al., 1993](#); [Shamim, 2012](#)).

Although minimal technical skills were needed to get plastinated specimens, another problem was the costs involved in purchasing the chemicals such as polymers, curing agents and building the chambers ([Daviau et al.,](#)

[1997](#)). The plastination process, as a new teaching tool, led to the better understanding of the complex structure of the animal body but could not replace the traditional method of dissection ([Roda-Murillo et al., 2006](#); [Riederer, 2014](#)).

The anatomical relationship between the different structures of the thoracic and abdominal cavities and limbs of the dog were demonstrated using plastinated specimens, similar to [Menaka et al. \(2010\)](#) who demonstrated the relationship between the structures of the neck, abdominal region and lateral thoracic wall of the whole goat kid cadaver.

Most of the plastinated specimens kept their original shape, however, some specimens showed shrinkage in size such as testes which may be due to incomplete dehydration of the organ or the type of silicon used and this will require trying other polymers ([Weiglein, 1997](#); [Ali and Al-Thnain, 2007](#)).

Plastination specimens did not completely replace the traditional guided animal dissection; similar result was mentioned by [Klaus et al. \(2018\)](#). Moreover, the combination of the plastinated specimens with the traditional wet specimens in the teaching and learning of veterinary gross anatomy can be beneficial depending on the topic being taught at the time; similar result was mentioned by [Latorre et al. \(2016\)](#).

CONCLUSION

Plastination of well dissected specimens provided good quality plastinated specimens which were a perfect tool for prolonged preservation. Plastinated specimens were odorless, dry, non-irritant, nontoxic and easy to hold in the hand. The plastinated specimens were appreciated by the staff members and students. Plastinated specimens gave a new teaching method and tool for the veterinary gross anatomy teaching and learning as well as better understanding of anatomy of the animals. Plastination specimens did not completely replace the traditional guided animal dissection. Some drawbacks were that the process was time consuming, high cost of the chemicals used and limited size of the impregnation chamber.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTION

The first author dissected the specimens and wrote the manuscript. The second author prepared the specimens. The two authors revised manuscript.

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