A REPORT ON
PRESERVATION OF SOME INTERNAL ORGANS OF CHICKEN BY MODIFIED PLASTINATION TECHNIQUE

By:

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Roll: 16/31, Registration No: 01645

Intern ID: 28

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List of Abbreviation

Abbreviation	Elaboration
CVASU	Chattogram Veterinary and Animal Sciences University
CVH	Central Veterinary Hospital
Dr.	Doctor
DVM	Doctor of Veterinary Medicine
GPA	Grade point average
SAQTVH	Shahidul Alam Quadery Teaching Veterinary Hospital
TTPHRC	Teaching and Training Pet Hospital and Research Center
UVH	Upazila Veterinary Hospital

ABSTRACT

The study was planned to preserve some of the important internal organs of chicken through the modified plastination technique. Some freshly collected organs like the heart, syrinx from the adult chicken were used for this study. These collected specimens were fixed into 10% formalin which were immersed into acetone for dehydration, followed by washing into running tap water. Finally, forced impregnations of the specimens were done into melted paraffin which were hardened by the airdrying and sun drying process. In case of all specimens, a satisfactory outcome was found in this study. In the case of the heart all external and internal morphologies were visible clearly. Again, the excellent result was also found in the syrinx of the chicken with the little color change. This modified method of the plastination technique strengthens the appearance, clarifies the surface and internal anatomy, and makes durable and non-toxic specimens for undergraduate teaching. As this is very cost-effective, it might be a good and powerful tool for gross anatomy learning for veterinary students.

Keywords: *Modified plastination, internal organs, chicken*

INTRODUCTION

For anatomical preservation, many techniques have been used throughout the history. In ancient Egyptian civilization, mummification was used to prepare human remains for burial as the earliest tissue preservation method. This method is based on the dehydration technique, preceded by a treatment with chemical substance (Rorigues, 2005). But, the most recent technique used for tissue processing is plastination (Von Hagens *et al*., 1987).

The development of anatomical techniques for fixing and preserving cadavers was advanced by the appearance of the plastination technique, created by Prof. Gunther von Hagens, (1977) in Heidelberg, Germany.

This process can preserve tissue with a completely visible surface and prolonged durability. In this preservation technique, water and lipids are replaced by a curable polymer (Ottone *et al*., 2008), where a dry, odorless, and nontoxic specimen of any animal tissue can be prepared (Grondin *et al.*, 1994). That's why these can be handled with bare hand and no special laboratory arrangement or safety requirement is not required here (Weinhaus, 2007).

In the last years, a large evolution of plastination has taken place in gross anatomical and biological educations as teaching aids (Hagens, 1979). Over 250 institutes for Human Anatomy, Clinical Pathology, Biology, and Zoology worldwide now use plastination for preserving a biological specimen as teaching aids (Sora, 2005).

The devices which are used by the teachers to illuminate a subject to the students are called teaching aids (Collins, 2009). Different types of simple or complex teaching aids are used in teaching purpose like chalkboard or computer program. It is thought that an instructor can accomplished the actual teaching. But sometimes teaching not only depends on teachers' skill but also depends on the availability of teaching aids. Plastinated specimens are a relatively new and unique type of teaching aids obtained from natural specimens.

There are many protocols for preparing plastination samples. In each protocol, there are four main steps. 1st step is the fixation of the specimens by using formalin and 2nd step is dehydration of the specimen via acetone. In 3rd step, forced impregnation of the

specimens into paraffin is done to replace acetone. The final step is to hardening of the specimen by heat or curing grass. This protocol can be modified to get better results (Weiglein, 2005). Zheng *et al*., (2000) used a modified protocol to produce thousands of plastinated specimens at a lower cost than if they had used the standard materials and equipment.

Plastination is very important because various types of visceral organs like cardiovascular (heart) and Respiratory (Syrinx) of chicken can be preserved by impregnating into paraffin which will retain their original shape without losing any aspects of their physical appearance, such as color and size. This technique is also helpful to preserve samples through plastination which is useful in the improvement of the student as well as in morphological research along with application in clinical practices and surgery.

There are so many studies performed in the plastination technique to preserve the tissue sample throughout the world (Ameko *et al*., 2012). But, in Bangladesh, there are few studies documented in plastination technique. In addition, there is no documentation on the preservation of the internal organs of a chicken.

For that reason, we planned to preserve some internal organs (Like heart, syrinx) from a chicken by a modified plastination technique. The study chose the modified technique for plastination due to a shortage of specialized equipment and expensive chemical.

So, the study is planned for the following objectives -

- Preservation of the internal organs of a chicken for further study
- Provide little knowledge on preservation of biological sample for long time with low cost

MATERIALS AND METHOD

There are different steps involved in the plastination procedure which are followed in this study. And these steps are described here.

2.1. Ethical approval

This study was done in the Department of Anatomy and Histology, Faculty of Veterinary medicine, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. All ethical clearance for all the experimental procedures of this study from the guidelines for 'the care and use of animals' by the Animal Welfare and ethics committee, CVASU were obtained.

2.2. Selection of Animals and Collection of Samples

The study selected a chicken without any detectable diseases and developmental disorders to remedy any drawback. This chicken was slaughtered, and immediately after slaughtering, some visceral organs of the chicken, i.e., heart and syrinx were collected.

There was performed the longitudinal section on the chicken heart to visualize all the important structures. Then the samples were immersed into 10% formalin as soon as possible to facilitate the transportation process into the laboratory.

2.2. Fixation of the Samples

Fixation of the collected sample is very important before plastination to prevent the enzymes that cause samples' autolysis. Fixation is also important for getting the final quality of the organs, like rigidity and steadiness of the plastinated samples.

The collected samples were also gone through the fixation technique. In this study, the collected samples were fixed into 10% formalin for several hours.

Finally, the fixed organs were washed with water to avoid excessive fixation.

2.3. Dehydration of the Samples

After completion of the fixation technique, the samples were removed and rinsed into running tap water. After that, the samples were immersed into 70% acetone solution

and kept them at 25^0 C for three days for dehydration of the collected samples. The acetone was changed three times in the dehydration process of the collected sample.

2.4. Defatting of the samples

In this step, the fat was removed by transferring the specimens from (-25ºC) to room temperature. The process was stopped when the fat started turning opaque.

2.5. Forced Impregnation of the Specimens

The samples were removed from the acetone solution after three days, and then these were washed in running tap water to remove excess acetone. After washing, the samples were blotted with tissue to dry up the samples.

Then these samples were immersed into an air-tight chamber containing melted paraffin. In this study, there were performed three dips in the melted paraffin that consisted of one minute each in an oven. Then the samples were kept at room temperature for hardening.

In addition, the samples were kept in the air-tight container that contains the silica gel after the complete impregnation.

2.6. Curing of the Samples

After completing the impregnation step, the specimens were removed from paraffin and detached of excess paraffin from the samples. Then the specimens were cured in heat at 50℃ temperature for three days in an oven.

The airdry and sun drying method were also performed along with the heat treatment.

RESULTS

In a developing country, plastination is a suitable technique to preserve different specimens because it is very cheap, highly durable, user-friendly, requires no wet preservation or special equipment, and the organs can be used to teach gross anatomy. It is very beneficial to attain dry, odorless, and robust actual biological specimens.

This study was performed to prepare a plastinated sample of heart and syrinx from chicken. All these collected samples were suitable for this study having strong anatomical quality and definition. No relevant artifacts affect the morphology of these organs.

In case of heart, the appearance of the external morphology was displayed having great anatomical details. The base and tip of the heart could be recognized very easily. In the base of heart, left and right atria with superior and inferior Vana-cava were found clearly. The left and right ventricles with coronary vessels and interventricular grooves were also visible in the plastinated heart.

The plastinated syrinx of the chicken was also visible with a good morphological structure. Three different parts of the syrinx were noticeable. The cranial part (tympanum), intermediate group, and caudal group (broncho syringeal) along the intrabronchial ligament (brachium) was found clearly.

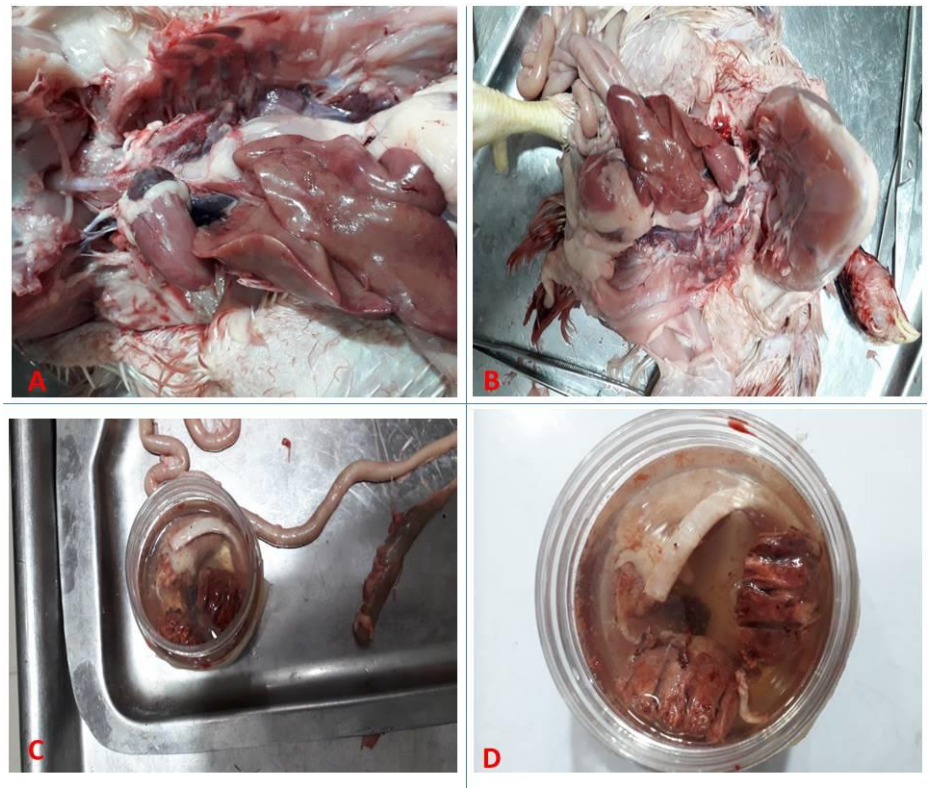

Figure 1: Dissection and collection of samples from a chicken,
(A, B) Heart with intact external morphology like color, size and shape. (C, D) Organs preserved in 10% formalin to facilitate transportation.

Figure 2: Fixation and Dehydration of the collected samples. (A, B) Fixation of samples (syrinx and heart) in 10% formalin. (C, D) Dehydration of samples by using 70% acetone which were repeated for three times and each for three days.

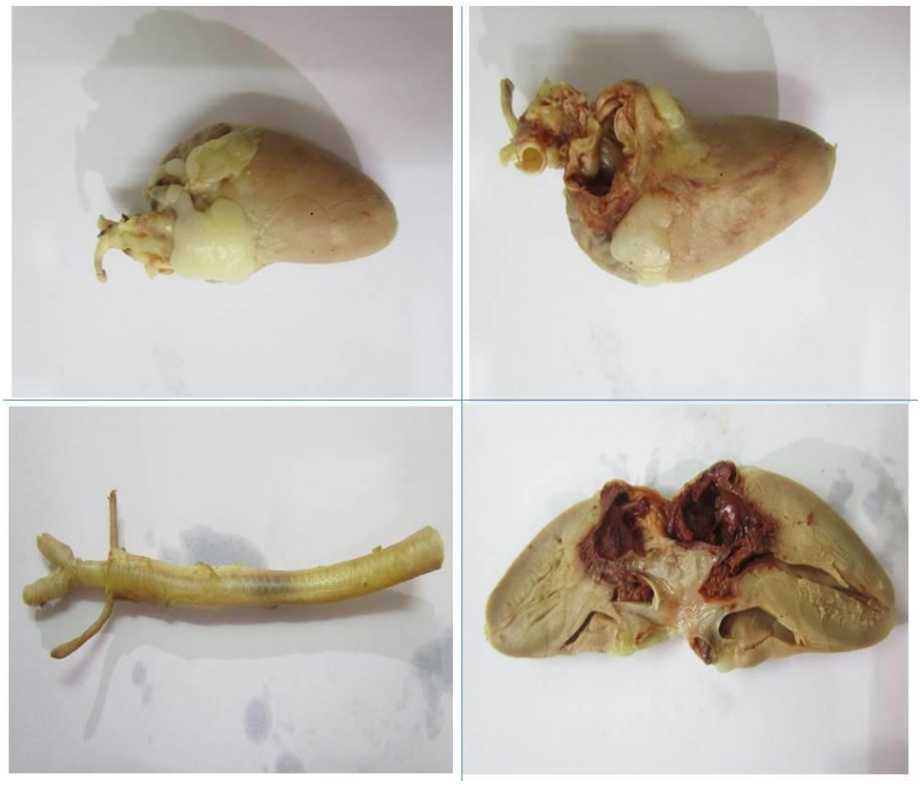

Figure 3: Modified plastinated samples (heart and syrinx) of a chicken, plastinated whole heart in 1st and 2nd images, plastinated syrinx in 3rd image and plastinated heart in longitudinally dissected section in 4th image.

DISCUSSION

There was found a little alteration of exact color for modified plastinated samples of the chicken. These color changes were occurred by formalin fixation. The heart became light brown color where the normal color of this organ should be reddish-brown. In this study, no artificial color was added to these organs. These findings were similar to the finding of (Baker, 1958).

But, according to Mikosova and Miklos (2004), the color of the organs changed due to unsuitable temperature, old bad fixed formaldehyde specimens, and too long gas–curing procedures.

The fatty tissue was removed from the collected sample for better visualization. The same procedure was performed previously by many authors (Shahar *et al*., 2007; Sora and Cook, 2007).

The total time required to perform this study was about two weeks. But this time can vary according to the specimens' size, especially the time required for forced impregnation. The larger the specimens, the longer the required time for impregnation, which can be extended to 2/3 days.

But the time requirement was more for preparing adult human plastinated heart by the other author like Baptista and Conran (1989).

They showed that adult human heart plastinates were prepared using Klotz Solution as the fixative, dehydrated in cold acetone for five weeks, impregnated with standard silicone resin, and cured with the gas cure agent for 4 - 6 days. Klotz solution is the preparation that preserves the natural color of the specimen, and it consists of multiple concentrations of Formalin fixative (Ulmer, 1994).

Baptista and Conran's plastinated human heart (1989) was dissected to clearly show the Right ventricle, pulmonary trunk, left ventricle, Aorta, Left auricle, Right auricle. In this study, we have dissected the chicken heart that also showed almost all-important structures clearly.

Many authors like Baker (1958) and Sakamoto *et al.*, (2006) used silicone polymer to the force impregnation process. But, in this study, the melted paraffin was used due to the lack of the expensive polymer.

This study performed the hardening process by the combined heating, sun drying, and air drying. The results of the hardening process of these samples were found similar to the finding of other authors (Ulmer, 1994, Shahar *et al*., 2007; Sora and Cook, 2007).

LIMITATIONS

Though the study was done carefully, there were some limitations. Specimen selection was not satisfactory here in this study. The size of specimens was so small that it created some difficulties in different steps of the plastination process. If the specimens were larger, it would be more visible of different structures contained in each organ. A shorter study period, lack of funds and unavailability of expensive chemicals also created limitations on this study.

CONCLUSION

The plastinated heart, stomach, and syrinx were preserved in a dry, rigid, and odorless condition by maintaining general shapes and structure. The levels of shrinkage of specimens were significantly better than the procedure used in many previous studies. But the alteration of exact color has occurred in the formalin fixation process. These plastinated specimens can be used as teaching aids to facilitate teaching and learning. These specimens can also be used in future morphological research. This simple and cost-effective sample preservation method may be applied in gross veterinary anatomy teaching.

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Author,

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BIOGRAPHY

Homaira Pervin Heema, Daughter of Shamsul Alam and Mostofa Begum, was born on 1st July, 1997. She passed her Secondary School Certificate Examination from Kutubdia Model High School, Chattogram, in 2012 (GPA 5.00). Then she passed her Higher Secondary School certificate examination from Chattogram college, Chattogram, in 2014 (GPA 5.00). Now she is completing her one-year-long internship program for fulfilling the requirement of a Doctor of Veterinary Medicine (DVM) degree in Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. During her internship period, she received her clinical training on Veterinary Medicine from UVH Sadar Cox's Bazar, SAQTVH, CVASU, Teaching & training Pet Hospital and Research Center (TTPHRC), CVASU, CVH, FV & FC, Dhaka, Chattogram, and Dhaka Zoo and managemental training from Chattogram based farm and Chattogram based Pharmacy, etc.

Her primary research interest is in wild animal parasites, especially parasites of captive animals. But she feels much interest in working on coccidiosis of different animals. She also feels immense interest in exploring new techniques to contribute to the development of the veterinary field in Bangladesh.