MODIFIED PLASTINATION TECHNIQUE FOR TEACHING AND RESEARCH

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This is to certify that we have examined the above Master's thesis and have found that the thesis is complete and satisfactory in all respects and that all revisions required by the thesis examination committee have been made

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List of abbreviations

Abbreviations	Elaborations
AA	Arcuate Artery
AB	Apical Bronchus
AL	Apical Lobe
ALB	Apical Lobar Bronchus
AcL	Accessory Lobe
AcLB	Accessory Lobar Bronchus
AV	Arbor Vitae
CT	Computed Tomography
CVASU	Chattogram Veterinary and Animal Sciences University
DH	Dorsal Horn
DL	Diaphragmatic Lobe
DLB	Diaphragmatic Lobar Bronchus
DMS	Dorsal Median Sulcus
EAC	External Auditory Canal
GM	Grey Matter
IA	Interlobar Artery
ILA	Interlobular Artery
LH	Lateral Horn
LV	Lateral Ventricle
ML	Middle Lobe
MLB	Middle Lobar Bronchus
MRI	Magnetic Resonance Imaging
PB	Principal Bronchus
RA	Renal Artery
RC	Renal Cortex
RM	Renal Medulla
RP	Renal Pelvis
SAQTVH	Shahedul Alam Quadary Teaching Veterinary Hospital
SA	Segmental Artery
SB	Segmental Bronchus

SP	Septum Pellucidum
T	Trachea
VH	Ventral Horn
VMF	Ventral Median Fissure
WM	White Matter

Abstract

Plastination is recognized as one of the most proficient tissue preservation procedures that has a wide range of applications in education and research. This technique demands costly equipment and chemicals; which requires adapting this exquisite process in an affordable manner. This study intends to develop an affordable methodology for preparing luminal casts and sheet plastinates with commercial silicon sealant following the basic steps (fixation, dehydration, impregnation and curing) of traditional plastination procedures. The resulting luminal castings were environment-friendly, dry, odorless, flexible, non-irritant and provided three-dimensional representations of the bronchial tree, segmentation of renal artery and orientation of the external auditory canal of goat. On the other hand, sheet plastinates were semi-transparent, dry, portable and presented good anatomical details of brain, spinal cord and kidney sections. Students of the Gross Anatomy course were approached about the use of plastinated and formalin-preserved specimens through a structured questionnaire. Almost all of them (95.5%) felt unsafe during handling of formalin-fixed specimens, 91.1% of students regarded plastinated samples very safe during hand manipulation and none reported any odor or health threat from the plastinates. Furthermore, 51.1% of participants strongly suggested the plastinates in anatomy practical courses, while 48.9% recommended the plastinates in conjunction with fixed and fresh samples and 88.9% found the plastination technique very essential for studying anatomy. Finally, the modified procedure may be utilized for anatomy instruction and research at a lower cost than the traditional technique. However, this approach is time-consuming, requires expert staff and cannot substitute conventional hands-on dissection for comprehensive anatomical knowledge.

Keywords: Anatomy; modified plastination; research; silicon sealant; teaching.

Chapter 1

Introduction

Veterinary Anatomy is acknowledged as the foundation of veterinary science and the gateway toward clinical practice. Besides, dissection of animal carcasses is a must to learn veterinary anatomy in the Doctor of Veterinary Medicine (DVM) program. Moreover, dissection integrates clinical information with the study of anatomy in a practical setting. Comprehending the size, shape, form, consistency, correlations and three-dimensional orientation of various body components are critical for understanding regional and topographic anatomy (Khullar et al., 2012). It is also crucial to get a practical insight into the surface and regional anatomy of different body areas in order to establish the foundation for clinical and surgical treatments. Furthermore, it is difficult to be a good physician or veterinarian without having an adequate understanding of anatomy.

Since it is quite expensive to buy a fresh sample for dissection every time, the samples used to teach students in anatomy practical classes are usually fixed or preserved. Additionally, due to limits on animal slaughter set by the Institutional Animal Ethical Committee (IAEC) rules, dissection of animal carcasses is progressively being substituted by alternative resources, which leads to insufficient biological samples for various research projects (Dhanwate and Gaikwad, 2015).

However, the most frequent preservative to preserve anatomical samples is 10% buffered formalin (Ameko et al., 2012). Whereas, exposure to formaldehyde at levels of 0.25 to 3.0 ppm can lead to potential health risks such as asthma, irregular menstrual periods, chemical hypersensitivity eczema, eye, nose and throat pain (Wei et al., 2004; Kim et al., 2011). Moreover, formaldehyde has been shown to cause mutation in various primitive organisms (Slizynska, 1957; Nishioka, 1973) and in cultured mammalian cells (Ross and Shipley, 1980; Ragan and Boreiko, 1981). In addition, formalin preservation causes degradation and fading of biological specimens over time. Due to its offensive smell, it also restricts to close inspection and handling of specimens, eventually interfering with teaching and learning anatomy (Jong and Henry, 2007). Because of the detrimental effects of formaldehyde, a safe, student-friendly, eco-friendly and affordable alternative method to preserve carcasses, organs and tissue for teaching and research was a dire need for biologists (Dhanwate and Gaikwad, 2015).

Gunther von Hagens, a German anatomist, established a modern method of tissue preservation in 1979 to circumvent these limitations; known as 'plastination'. Plastination is the process of substituting water and lipids in biological tissues with polymers (such as silicone, polyester, and epoxy) that subsequently solidify to produce dry, resilient and hazard-free specimens called plastinates (Hagens, 1986; O'Sullivan and Mitchell, 1995; Pashaei, 2010). This method has proved to be a superior method for the preservation of gross specimens. Besides, plastinates are a great teaching tool as their use is not restricted to the dissection hall; slices or body parts allow studying anatomy in a portable manner. They also serve as excellent museum specimens because they are flexible, easy to handle and free of several toxic effects of chemicals like formalin. Additionally, a coordinated effort to employ macroscopic cross-sections of plastinated specimens and reference pictures of Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) can benefit teaching clinical anatomy and research (Zhang et al., 2002; Latorre and Rodríguez, 2007; Riederer, 2014). As a result, plastinated materials are becoming more popular because of their versatility and multidimensional use (Jones and Whitaker, 2009; Ravikumar, 2014). Furthermore, since plastinated models are aesthetic to look at, the development of such technique will make it easier to establish an enhanced anatomy museum using locally made specimens rather than importing them from abroad.

The academic or research institutions of lower or middle-income countries like Bangladesh find it more difficult to access the plastination process as it requires specialized types of equipment and costly chemicals. In Bangladesh, Akhter et al. (2021) documented room temperature plastination with Biodur products; while Sultana et al., (2019) prepared whole organ plastinates in a modified approach using paraffin wax. However, there was no documentation on the modified luminal cast and sheet plastination procedure in Bangladesh. Therefore, this study focused on developing affordable alternatives to traditional plastination techniques with the following objectives:

1.1 General objective

Establishment of luminal cast and sheet plastination technique in Chattogram Veterinary and Animal Sciences University (CVASU).

1.2 Specific objectives

1.2.1. To develop an affordable, economic and modified luminal cast and sheet plastination technique in CVASU

1.2.2. To demonstrate multiple dimensions of organs with plastinated models

1.2.3 Exhibition of developed models in anatomy museum

1.2.4 To reduce the cost of importing such models from abroad.

Chapter 2
Review of literature

This thesis intends to offer some literature related to current research that has been conducted worldwide and accordingly identify the research gaps and justify the present study. This section briefly describes standard and modified techniques of plastination.

2.1 Preservation and its aim

The decomposition of a dead body or biological tissue is a natural process occurred through a sequence of physical and chemical changes. Any biological tissue can be protected from putrefaction or decay by any method of preservation (Brooks, 2016).

2.1.1 Aim of preservation (Chhabra, 2020)

- Prevention of autolysis and putrefaction of dead body or tissue.
- Long-term storage of biological specimens in their original state.
- Maintenance of size, shape, color, appearance and volume of organ or tissue.

2.2 Preservation by plastination technique

A breakthrough technique called 'Plastination' for the preservation of cadavers was introduced in 1979 by German anatomist von Hagen (Hagens, 1979). It is a modern method of preservation that has the potential to prepare and preserve any biological specimens relatively close to their original condition (Riederer, 2014). This method is based on the concept that synthetic components such as polyester, silicone, epoxy etc. replace the lipid and water molecules in the carcass in order to produce durable, non-toxic and natural-looking dead bodies or part of it or specific organs (Weiglein, 2002; Pashaei, 2010). The anatomical detail and ease of handling of organs following the process make this approach popular over traditional formalin-based preservation (Mantri et al., 2017). Besides, plastinates remain life-like, non-toxic, odorless, and durable over time. It also allows close inspection and microscopic examination of tissue (Priya et al., 2007).

2.2.1 Background

In 1946 Romaniak first introduced unpolymerized resin in order to replace tissue fluid that was later modified by Gunther von Hagens who worked as a research assistant at the Anatomical Institute of Heidelberg University in Germany (Romaniak, 1946;

Hagens, 1979). He worked to improve the quality of renal specimens in the laboratory. Then he began experimenting with various sorts of plastics and after many attempts and errors on various tissues and organs, he obtained a grasp of the fundamentals of plastination using polymerized resin. The plastination procedures were then patented between 1977 and 1982 (Horst et al., 2019). Then he founded his own firm 'BIODUR', to promote his work by providing specific polymers, plastination equipment and technology to medical institutions all over the world (Hagens, 1986). Eventually, von Hagens demonstrate his plastinates in various parts of the world at 'Body Worlds Exhibits' (Tanassi, 2007; Jones and Whitaker, 2009; Khullar et al., 2012; Hayat et al., 2018; Farhat et al., 2021). Gradually plastinates become today's milestone in medical education and an ideal teaching tool not only in anatomy but also in pathology, obstetrics, radiology and surgery (Al-Zuhair et al., 1995).

2.2.2 Principle of plastination

Sargon and Tatar (2014) stated that plastination involves the gradual replacement of tissue fluids and lipids with dehydrating chemicals and polymers inside vacuum chambers. In these procedures, curable polymers such as silicone, epoxy resins and polyesters are used to replace water and lipids in biological tissues, resulting in plastinated specimens that are dry, odorless, hard and rigid in nature (Hagens, 1986). Following plastination, they are known as plastinates and the plastinates should be set in the optimal position to cure the polymer chains after replacing the water and lipids in the tissue (Asadi and Mahmodzadeh, 2004; Kocevski et al., 2010; Menaka, 2015; Kumar et al., 2019).

2.2.3 Properties of polymers used for plastination

The polymer used for plastination must have the following desirable properties (Saini, 2017):

- **a.** It must have the minimum viscosity while uncured.
- **b.** The polymer's refractive index should be different from that of the tissue
- **c.** The catalyst employed to cure polymers should have a reasonably lengthy action time or liquid phase to enable sufficient impregnation time.
- **d.** When the polymer is cured, its mechanical characteristics should be adequate.
- **e.** To resemble a natural or solid condition, it should have rubber-like properties.
- **f.** It should be affordable.

2.2.4 Types of plastination

Three types of plastination techniques are described by Prasad et al. (2015), Jadhav et al. (2016) and Khullar et al. (2012).

a. Whole body/Organ/ Silicon Plastination

This process involves plastination of the whole body or organ of a human, animal or any other specimen; hence, the whole structure and interrelation of the body or organ are preserved (Latorre et al., 2004). In the standard plastination technique silicon (S10), hardener (S3 or S6) or Polyester (P35 or P40 or P45) are commonly used to impregnate an entire body or organ and even the course of specific nerve in the body following fixation and dehydration in whole body/organ/silicon plastination method (Basset and Seleem; 2014; Sargon and Tatar, 2014; Hayat et al., 2018).

b. Luminal cast plastination

This is performed for hollow organs such as brain ventricles, air canal, lungs, vasculatures of heart, kidney, liver, spleen and lumen of the alimentary and reproductive tract (Jadhav et al., 2016). The concept of this procedure is to fill the lumen with cast material (e.g., silicon, resin, etc.) and dissolve the surrounding tissue (DeSouza et al., 2016).

c. Sheet/Epoxy plastination

It involves making thin, transparent or opaque sections of the body or any organ. These sheets are portable and display cross-sectional anatomy comparable to CT, MRI, ultrasound etc. (Skalkos et al., 1999; Sora and Cook, 2007). The standard technique of sheet plastination usually involves Epoxy (E10 or E12) and hardener (E1) to produce thin sheets of plastinates (Hagens et al., 1987). Silicon resin, polyester and araldite are also commonly used in sheet plastination (Barnett, 1997; Latorre et al., 2004; Ottone et al., 2016).

2.2.5 Basic steps of plastination technique

The fundamentals of plastination processes are the same across all forms, with a few differences and requirements for various specimens. Four basic steps of plastination were described by Khullar et al. (2012), Menaka et al. (2015), Farhat et al. (2021) and Yunus et al. (2021).

i. Fixation

To avoid putrefaction and autolysis, the biological samples should be fixed in a suitable fixative for 5-7 days before plastination (Ulmer, 1994). The typical fixatives employed for fixation include 5-20% formalin, 10% buffered formaldehyde, 4% paraformaldehyde and Kaiserling solution. The hollow organs must be dilated with cotton or stick during fixation process (Hagens, 1986; Lozanoff, 2002).

ii. Dehydration

This step serves as the replacement of water with a dehydrating fluid specifically acetone, which is used in standard plastination procedures because of its degreasing property and high vacuum pressure (Hagens, 1979; Hagens, 1986; Pereira- Sampaio et al., 2006). In the standard technique, the specimens are passed through three changes of acetone at -15 ˚C to -25 ˚C for a period of 3-4 day (Pereira- Sampaio et al., 2006).

iii. Forced impregnation

In this step, the intermediary solvent (acetone) in cellular and interstitial space is replaced with a curable polymer (silicon, polyester or epoxy resin) under vacuum pressure at -15 ◦C to -25 ◦C (Kessler, 1990; Menaka et al., 2015; Ganguly et al., 2018).

iv. Hardening or curing

After complete impregnation, the impregnated polymer must be cured either with a specific curing agent or at room temperature in order to harden it. Then the specimens are kept in a plastic bag for several weeks (Sargon and Tatar, 2014; Oyewopo et al., 2018).

2.2.6 Modified plastination techniques

Several adjustments to standard plastination techniques have been developed by researchers to make the procedure cost-effective (O'Sullivan and Mitchell, 1995; Zheng et al., 1998; Mehra et al., 2003; Sakamoto et al., 2006; Ottone et al., 2015).

2.2.6.1 Room temperature plastination

O'Sullivan and Mitchell (1995) were the first to conduct silicon (Biodur S10) plastination at room temperature. Later, different researchers (Glover et al., 1998; Zheng et al., 1998;) reported successful plastination at room temperature. Then gradually the porcine heart was plastinated by standard S10 technique at 25 ˚C by Darawiroj et al., (2010). Many researchers successfully performed plastination at room temperature with biodur products (Cires et al., 2012; Kumro et al., 2013; Ottone et al., 2014; Taghipour et al., 2016; Joshua et al.,2019; Akhter et al., 2021).

2.2.6.2 Use of alcohol during dehydration process

In a conventional plastination technique, an ascending grade of acetone is preferred (Ganguly et al., 2018). However, acetone is expensive hence increasing the cost of production. Studies by Sivrev and Usovich (2006), Srisuwatanasagul et al., (2010) and Cinaroglu and Keles (2020) suggested substituting acetone bath with ascending graded and absolute alcohol consequently to minimize the cost of plastination procedure. Though alcohol imposes greater shrinkage of tissue than acetone it may be an affordable substitute for conventional acetone-based dehydration (Srisuwatanasagul et al., 2010).

2.2.6.3 Impregnation with alternate chemicals

The standard plastination technique requires expensive curable polymers such as S10, E12, P40, P35 etc. (Horst et al., 2019). This exotic method of plastination using such special quality polymers is expensive and unavailable in most parts of Asia and the import procedure is also time-consuming (Sivagnanam et al., 2014). As a result, the modified plastination process using alternative indigenous chemicals has become increasingly popular (Sultana et al., 2019).

- **Modified silicon plastination techniques**

  Zheng et al., (1998) used locally available Chinese silicon known as Su-Yi Chinese Silicone solution instead of conventional S10 silicon polymer to impregnate biological specimens. Utilization of expanding foam instead of silicon was described by Arnautovic et al. (2005), Sawad et al. (2014) and Abumandour and El-Bakery (2019). Chandel et al. (2013) effectively modified silicon plastination with an acid-curing polymer 'melamyne' instead of S10. Furthermore, Mutturaj et al. (2014) and Ramkrishna and Leelavathy (2019) used recycled plastic tea cups and thermocol to plastinate biological samples. Dr. Fawzy Elnady recently developed

plastination with glycerin instead of polymers (Elnady et al., 2015; Elnady, 2016; Elnady, 2019). Mohsen et al. (2013) and Islam and Sultana (2021) adopted that modified method to preserve musculoskeletal specimens. Paraffin wax impregnation was described by Mihaly et al. (2014), Sultana et al. (2019). Narayanan (2015), described LAPOX^{TM} epoxy resin to prepare tracheobronchial cast. Pandit et al. (2015) utilized 'Orthocryl' to plastinate brain sections at room temperature. Satte et al. (2017) modified plastination method with gum Arabic solution (mixture of gum Arabic powder, glycerine and distilled water); Mishra and Sethi (2015) described a polymer polyvinylchloride (PVC).

- **Modified polyester plastination techniques**

Studies by Ezhilarasan et al. (2017) and Sivagnanam et al. (2014) presented modified polyester plastination method by impregnating cadavers and organs with locally available polyester resin. Bakici et al. (2022) preserved cross sections of the horse kidneys by modified polyester plastination with indigenous transparent moulding polyester resin instead of P35 or P40.

- **Modified epoxy resin plastination technique**

Ottone et al. (2016) manufactured plastinated sheets of human knee and leg using commercial epoxy resin under vacuum pressure rather than standard E12 polymer. Ari and Cinaroglu (2011) proposed alkyd resin as an affordable method for producing durable, odorless and flexible biological specimens. Cinaroglu and Ari, (2015) and Cinaroglu and Keles (2020) adopted the alkyd resin preservation technique to display urogenital organs of sheep and rat cadavers as an efficient teaching aid and museum specimen in anatomy. Sivagnanam et al. (2013) and Sivagnanam et al. (2014) reported a modified low-cost method of plastination with indigenous epoxy resin to plastinate lightweight poultry specimens and reproductive organs of animals. Georgieva et al. (2019) used an unsaturated polyester resin called 'norsodyne' to create plastinated slices of biological material as an alternative to the usual E12 procedure. Furthermore, Raj et al. (2018) and Leelavathy and Ramkrishna (2019) described Jelly wax sheet plastination technique; an alternative to the E12 method, as a useful tool for teaching sectional anatomy.

Chapter 3

Materials and methods

3.1 Study area and study duration

The cattle and goat samples were obtained from, government-approved slaughterhouses of Chawkbazar (22.35750˚ north and 91.84048˚ east) and Jhautola market (22.35836˚ north and 91.80749˚ east), Chattogram. While, with the owners' consent, the dead bodies of dogs and cats were collected from Shahedul Alam Quadary Teaching Veterinary Hospital (SAQTVH), Chattogram Veterinary and Animal Sciences University (22.36212˚ north and 91.80456˚ east) between January 2021 and November 2021 (Figure 3.1).

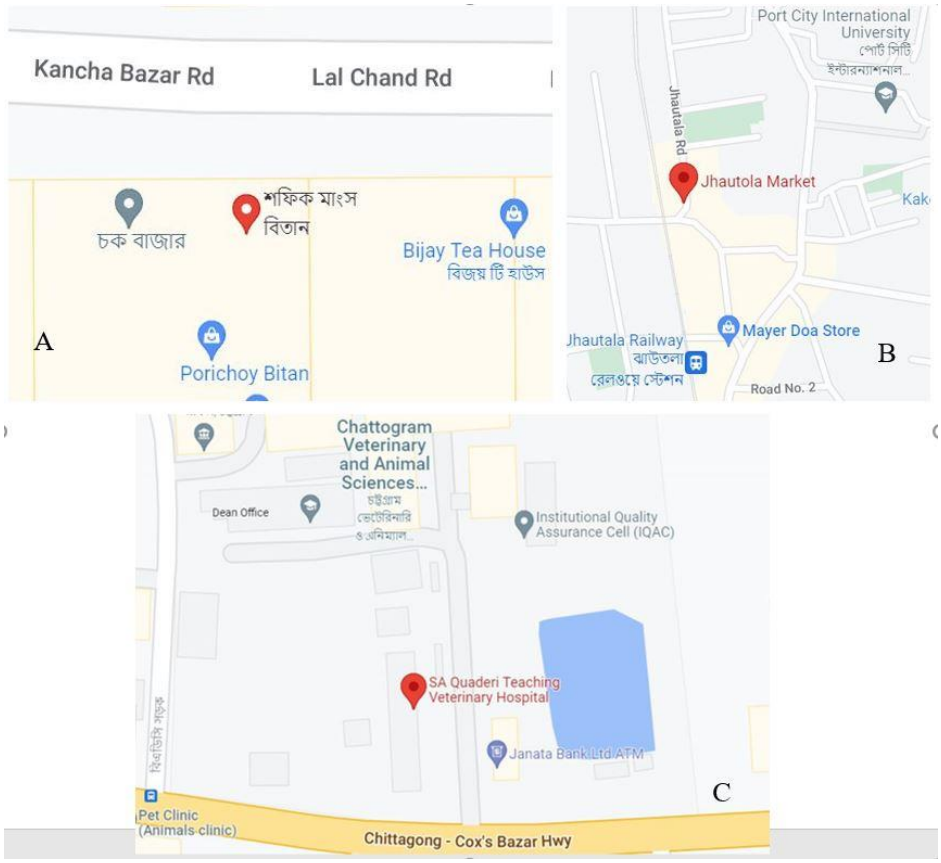


Figure 3.1: Study areas showing in map (red dot)

A. Chawkbazar; B. Jhautola market; C. SAQTVH, CVASU

3.2 Collection of specimens

The trachea with lungs, goat head with ears and renal artery with kidneys were selected for luminal cast plastination (Photograph 3.2). These specimens were collected directly from the abattoirs and promptly transported in a zip-lock bag to the Anatomy laboratory, Department of Anatomy and Histology, Chattogram Veterinary and Animal Sciences University (CVASU). The brain, spinal cord and kidney were selected for sheet plastination procedure and fixed in 10% buffered formalin immediately after collection. Additionally, we collected the dead bodies of dogs and cats that didn't exhibit unusual clinical symptoms following doctor's death certification and owner's permission and then brought to the Anatomy laboratory, Department of Anatomy and Histology, CVASU for further experimentation.

3.3 Lab preparations

3.3.1 Required instruments

i. Dissection tray
ii. Plain tissue forceps
iii. Curved-tip forceps
iv. Rat- tooth forceps
v. Cotton, gauze
vi. Applicator gun
vii. Plastic pipe
viii. Thread or string
ix. Nylon net
x. Plastic bowl
xi. Aluminium vessel
xii. Gas stove
xiii. Brush
xiv. Scrubber
xv. Aluminium foil
xvi. Beaker, measuring cylinder, volumetric flux
xvii. pH meter, electronic balance
xviii. Spatula, spreader
xix. Microtome blade
xx. Scalpel, scissors, clip etc.

3.3.2 Required chemicals

3.3.2.1 Chemicals for luminal cast plastination

Asian Paints Smartcare Xtremoseal^{GP} All Purpose Acetoxy Cured Silicon Sealant (White color) was used to obtain the luminal casts (Photograph 3.1 b). It is a cheaper variant of acetoxy cured general purpose silicon sealant that comes in paste form with different colors (e.g., White, transparent, black etc.) costing approximately 300 BDT. This acetic sealant is based on high-quality polysiloxanes (polymers made up of siloxane) which cure at atmospheric moisture to form a durable seal. The properties of the chemical are given below:

i. Formulated on an acetoxy basis
ii. Highly elastic
iii. 100% waterproof and UV resistant
iv. Not paintable with colors
v. Suitable temperature of application: +5 $^{\circ}$C to +40 $^{\circ}$C
vi. Temperature resistance -40 $^{\circ}$C to +120 $^{\circ}$C
vii. Curing time is about 15 minutes
viii. Emit acetic acid during curing thus slight odor found during curing process

3.3.2.2 Chemicals for modified sheet plastination procedure

i. Asian Paints Smartcare XtremosealGP All Purpose Acetoxy Cured Silicone Sealant (Transparent color) was the major chemical to obtain plastinated slices of the specimens. It also possesses the above mentioned properties.
ii. Distilled water
iii. Disodium hydrogen phosphate
iv. Sodium dihydrogen phosphate
v. 40% formaldehyde
vi. 10% buffered formalin
vii. Alcohol

3.3.3 Others

Surgical masks, gloves, goggles, tissue paper etc.

3.3.4 Preparation of 10% buffered formalin

About 900 ml of distilled water was measured in a measuring cylinder and then transferred into a volumetric flux. Up to 100 ml of commercial formaldehyde (40%) (Merck, Germany) was properly mixed with the distilled water. Further, 4.0 gm of sodium dihydrogen phosphate and 6.5 gm of disodium hydrogen phosphate were measured in Shinko electronic balance machine (Model DJ-600A) and then added to the mixture. Then the pH of the solution (6.5) was examined with a pH meter (HI2212 pH/ORP Meter, HANA instruments).

3.3.5 Setting up the applicator gun

A locally-made applicator gun was used in the study (Photograph 3.1 a). To obtain the casts, the gun was loaded with silicon sealant at first. With the aid of a sharp knife, the nozzle of the silicon cartridge was removed and firmly attached to the supplied cone-shaped cork. Further, the sealant tube was carefully set into the applicator frame with the cork facing away by pushing the gun's plunger (Photograph 3.1 b'). The trigger was then pushed two or three times to confirm the proper fit of the silicon sealant into the gun.

3.4 Preparation of specimens

3.4.1 Preparation of slaughterhouse specimens

Initially, the exterior surface of the collected organs (lungs, kidney, goat head) was washed under running tap water to eliminate dirt, hair, blood etc. The fascia, fatty tissues and other tissue remains were then removed using dissection instruments to ensure adequate impregnation and subsequent boiling of the organs (Photograph 3.4 a). The inner contents (e.g., blood clot, mucus) of the lungs and kidneys were then fully flushed under flowing tap water at medium flow through the respective lumens of the organs and then gently squeezed to remove the water, thereby cleaning the lumens (Photograph 3.4 b, c & e). This procedure was repeated several times until the mucus and blood clot drained out and clear water flowed from the organs (Photograph 3.4 f). The external auditory canals of the goat head were thoroughly washed under running tap water to remove the desquamated debris from the canal. This procedure was repeated several times. Then it was further flushed with a water-filled syringe 4-5 times (Photograph 3.4 d) and then excess water was mopped with gauze.

The brain, spinal cord and kidney samples; used for sheet plastination were fixed in 10% buffered formalin immediately after collection.

3.4.2 Dissection and preparation of hospital specimens

After collecting the dog and cat carcasses we initially looked for any obvious irregularities. After skinning, the costosternal joint was disarticulated to open the rib cage and the lungs were exposed. The tissue fluids were continuously wiped with cotton and gauze to visualize the organs properly. Finally, the trachea and lungs were precisely separated from the larynx in intact condition (Photograph 3.3). Further, the specimens were thoroughly examined for any pathological or anatomical defects and then the external surface was cleaned under running tap water to remove dirt, hair, blood etc. The fascia, fatty tissues and other tissue remnants were then removed using dissection instruments. The inner surface of the organs was fully flushed under flowing tap water at medium flow through the respective lumens of the organs and then gently squeezed to remove the water, thereby cleaning the lumens. This procedure was repeated several times until the blood clot and mucus drained out and clear water flowed from the organs.

3.5 Preparation of luminal cast

3.5.1 Fixation

Formalin is a regularly used fixative to avoid putrefaction by inhibiting decomposition and killing saprophytic microorganisms. Typically, organs are submerged in 10% buffered formalin for one to two weeks to ensure proper fixation. However, to facilitate tissue separation, we used fresh specimens rather than fixed samples. If the fresh organs cannot be used immediately, they can be kept in the freezer for a 2-3 hours without being fixed in formalin.

3.5.2 Drying

The specimens were wrapped in a nylon mesh and maintained inclined with the luminal end at lower level and then left to dry at room temperature (Photograph 3.5). This step ensures maximum drainage of water as the casting material could not reach the whole surface of the specimens without complete drainage of water from the lumen. This drying process took about 4 to 5 hours.

3.5.3 Impregnation

3.5.3.1 Impregnation of lungs

At first, the air-dried specimens were placed in a tray. It was properly checked to assure the removal of water content as much as possible in order to impregnate the specimens properly. Then the trachea was inflated with air through a plastic tube to facilitate maximum filling of the lungs. Then the silicon sealant was applied slowly through the tracheal lumen by pressing the trigger of the applicator gun at 45° angle (Photograph 3.6 a). The specimens were filled with silicon sealant via the trachea until it reached all parts of the lungs. During this filling process, injecting the silicone sealant into the airways gradually becomes more difficult. Therefore, care was taken by applying gentle and continual pressure to the plunger of the gun to avoid overfilling or rupturing the airways. Shortly after application, filling of the trachea, bronchus and polygonal-shaped alveoli can be observed even with the naked eye. At last, the luminal end of the trachea was tied with a thread to avoid any leakage of the sealant (Photograph 3.7 a).

3.5.3.2 Impregnation of renal artery

Following air-drying, the renal arteries of the kidneys were identified accurately. Then the silicon sealant was applied through the renal artery of the bovine kidney by the applicator gun. But the size of the sealant's nozzle did not fit into the lumen of the caprine renal artery, thus the silicon sealant was transferred from the cartridge to a 10 ml syringe before it could be used to fill the goat kidney (Photograph 3.6 b). After then, the plunger of the syringe was gently pushed until the sealant had passed through the renal artery and reached the entire kidney. To prevent any leakage, the renal artery was finally secured with a thread and left in a tray for subsequent curing of the sealant.

3.5.3.3 Impregnation of external auditory canal of goat

The silicon sealant was injected directly into the external auditory canal through the auricle with the help of an applicator gun (Photograph 3.6 c).

3.5.4 Curing

The injected specimens (lungs, kidneys, ear) was left at room temperature for 4-5 hours in undisturbed conditions for curing (Photograph 3.7 a). The specimens except the goat head were then immersed in a bowl of water overnight to soften the tissue (Photograph

3.7 b). An aluminium vessel filled with water was then allowed to boil for 20 minutes at maximum temperature. After that, the lungs and kidney specimens were transferred to the boiling water and were allowed to boil at a mild heat for around 3-4 hours (Photograph 3.7 c). Then they were taken from the boiling water once the tissues of the trachea, lungs and kidneys had completely separated from the casts. Further, the casts were also allowed to cool before being thoroughly scrubbed in soap water with a scrubber. Then all the leftover tissues were carefully removed with a fine brush (Photograph 3.7 d). Finally, the casts were cleaned under running tap water and wiped with tissue paper once the residues had been completely removed. In case of the external ear canal, the luminal cast was removed with forceps after complete curing. Then it was properly washed with water and mopped with tissue paper (Photograph 3.8).

3.6 Preparation of plastinated sheets

3.6.1 Fixation

Sheet plastination was performed on the brain, spinal cord and kidney tissues. To avoid any health risks and putrefaction, intact samples were preserved in 10% buffered formalin solution for two weeks. We changed the solution three times at 48-hour intervals till clean fluid was obtained (Photograph 3.9 a).

3.6.2 Dehydration

The formalin-fixed samples were rinsed in running tap water for 24 hours and later soaked in blotting paper. The specimens were then dehydrated in five changes of alcohol (70% to 100%) at room temperature (Photograph 3.9 b). The specimens were then placed in another change of 100% alcohol at room temperature for 24 hours.

Table 3.1: Summary of the dehydration procedure

Step 1	Wash specimens under tap water for 24 hours
Step 2	Place specimens in 70% alcohol solution overnight
Step 3	Place specimens in 80% alcohol solution for 5-6 hours
Step 4	Place specimens in 90% alcohol solution for 5-6 hours
Step 5	Place specimens in 95% alcohol solution for 5-6 hours
Step 6	Place specimens in 100% alcohol solution for 5-6 hours
Step 7	Place specimens in 100% alcohol solution for 24 hours

3.6.3 Slicing and impregnation

After completion of the dehydration process, excess solvent was allowed to drain. The specimens were then sliced manually with a fine microtome blade- Feather A35 type (Photograph 3.1 d) to a maximum thickness of 5 to 6 mm (Photograph 3.10 a, b, c, d). An aluminium foil paper was then put on a flat surface. A thin coating of clear sealant was spread on the foil paper with a spreader. After 10 minutes, another coat of sealant was applied to the previous one to make the layer thicker. This layer was left undisturbed for an hour to reach a semi-hard state. The slices were then placed over this layer and lightly flattened with a spatula. A final layer of sealant was carefully applied over the slices to avoid any displacement or distortion (Photograph 3.11 A). Moreover, this layer was covered with another aluminium foil paper and slightly pressed with a spatula to remove the air bubbles. To remove the bubbles from another side, the foil sandwich model (foil- slice- foil) was turned over and pushed with the spatula. The sandwich model was then topped with a glass plate (Photograph 3.11 B).

3.6.4 Curing

Following impregnation, the sheets or slices were left undisturbed at room temperature for 4-5 days. Then the slices were carefully separated from the foil paper after complete curing and stored in a zip-lock bag (Photograph 3.12).

Precautions

- **a.** Throughout the entire procedure, we always wore an apron, hand gloves, face mask and goggles to get protection from direct chemical contact as well as from any infectious exposure from the specimens.
- **b.** Cut the nozzle of the sealant after loading it into the applicator gun and inject the silicon sealant immediately to avoid drying out the sealant.
- **c.** At the time of impregnation, it is important to avoid gaps between the layers to minimize formation of air bubble within the sheets.

3.7 Preparation of questionnaire

A standard 14-item questionnaire (Appendix A) was designed to assess students' experiences with anatomy learning using both plastinated and fixed samples. The prepared plastinated samples, as well as the fixed samples, were then demonstrated to

first-year DVM students of Gross Anatomy course, who completed the questionnaire (Photograph 3.13).

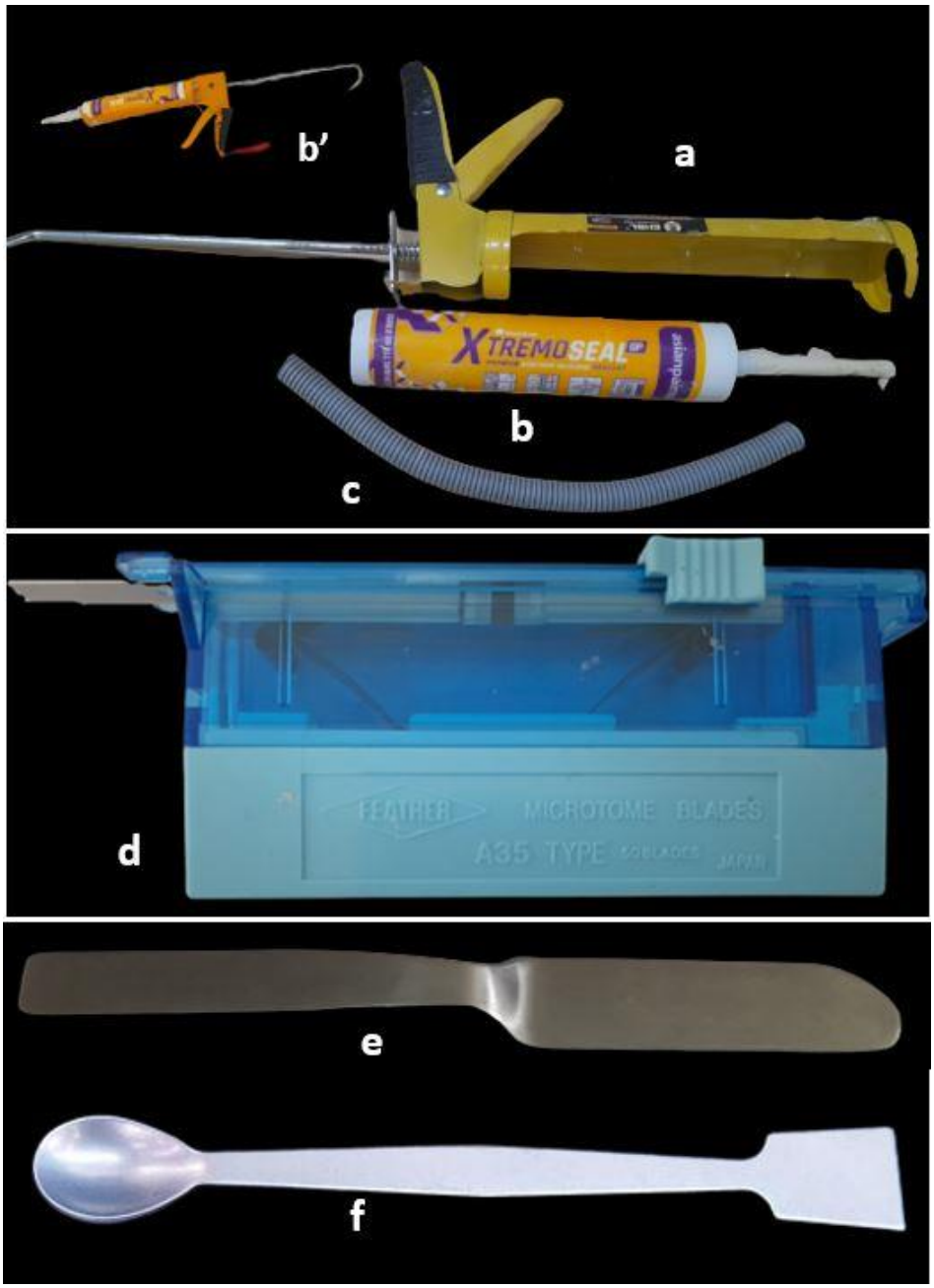
3.8 Statistical analysis

The responses of the students were stored in Microsoft Excel 2016 spreadsheet. The data was then coded, evaluated, tabulated and represented graphically.

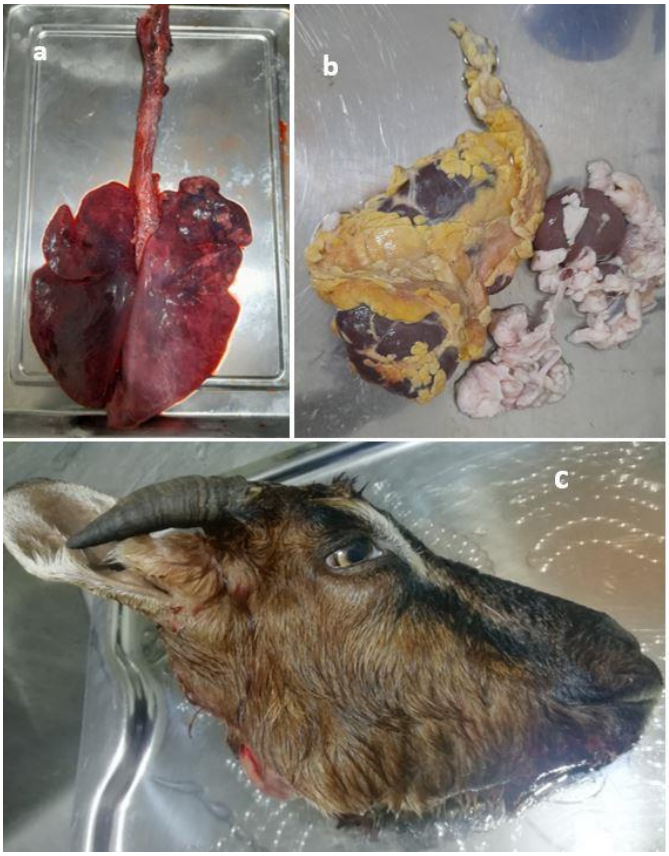
3.9 Photography

All the images related to this study were taken on a mobile device (Model Samsung A12). The images were slightly modified for better illustration of the study. And the sheet plastinated slices were photographed against a Yage light box (Model YG -T109 2102).

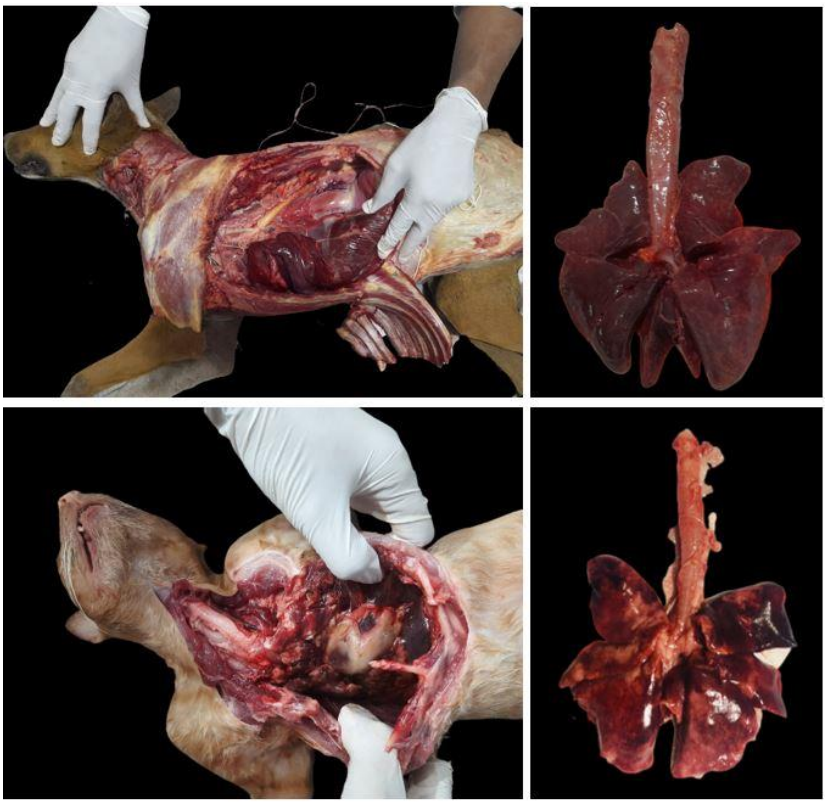
Picture Gallery



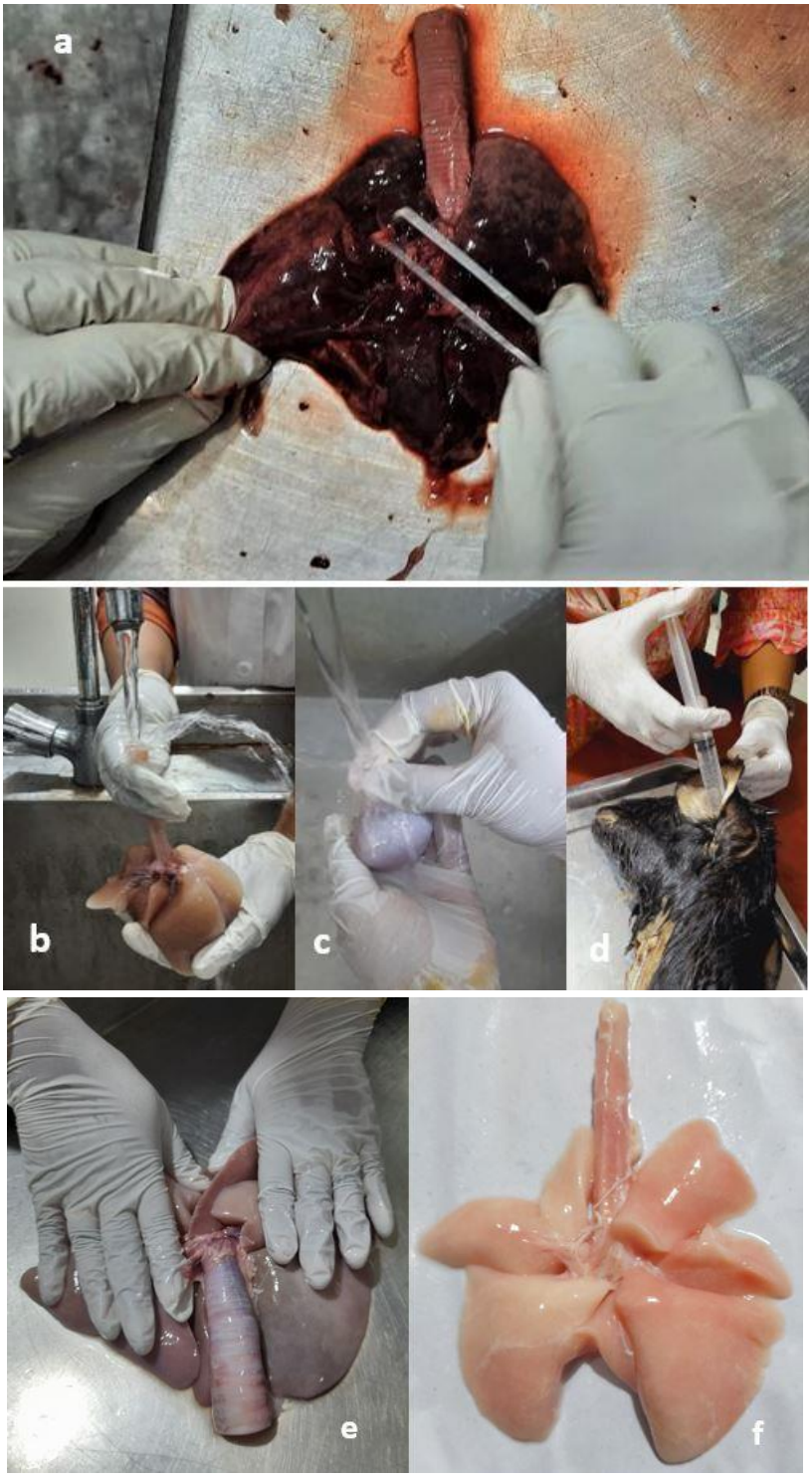
Photograph 3.1: Major chemical and tools used in the study
a. Applicator gun, **b.** Silicon sealant, **b'.** Sealant with gun, **c.** Plastic tube, **d.** Microtome blade, **e.** Spreader, **f.** Spatula



Photograph 3.2: Representative samples of slaughterhouse specimens for luminal cast plastination- a. Lungs, **b.** Kidney, **c.** Goat head



Photograph 3.3: Dissection and collection of lung from hospital specimens



Photograph 3.4: Preparation of samples for luminal cast plastination

a. Cleaning of the specimen, **b, c & d:** Washing the specimens through respective lumens of the organs, **e.** Squeeze out contents from the lumen,

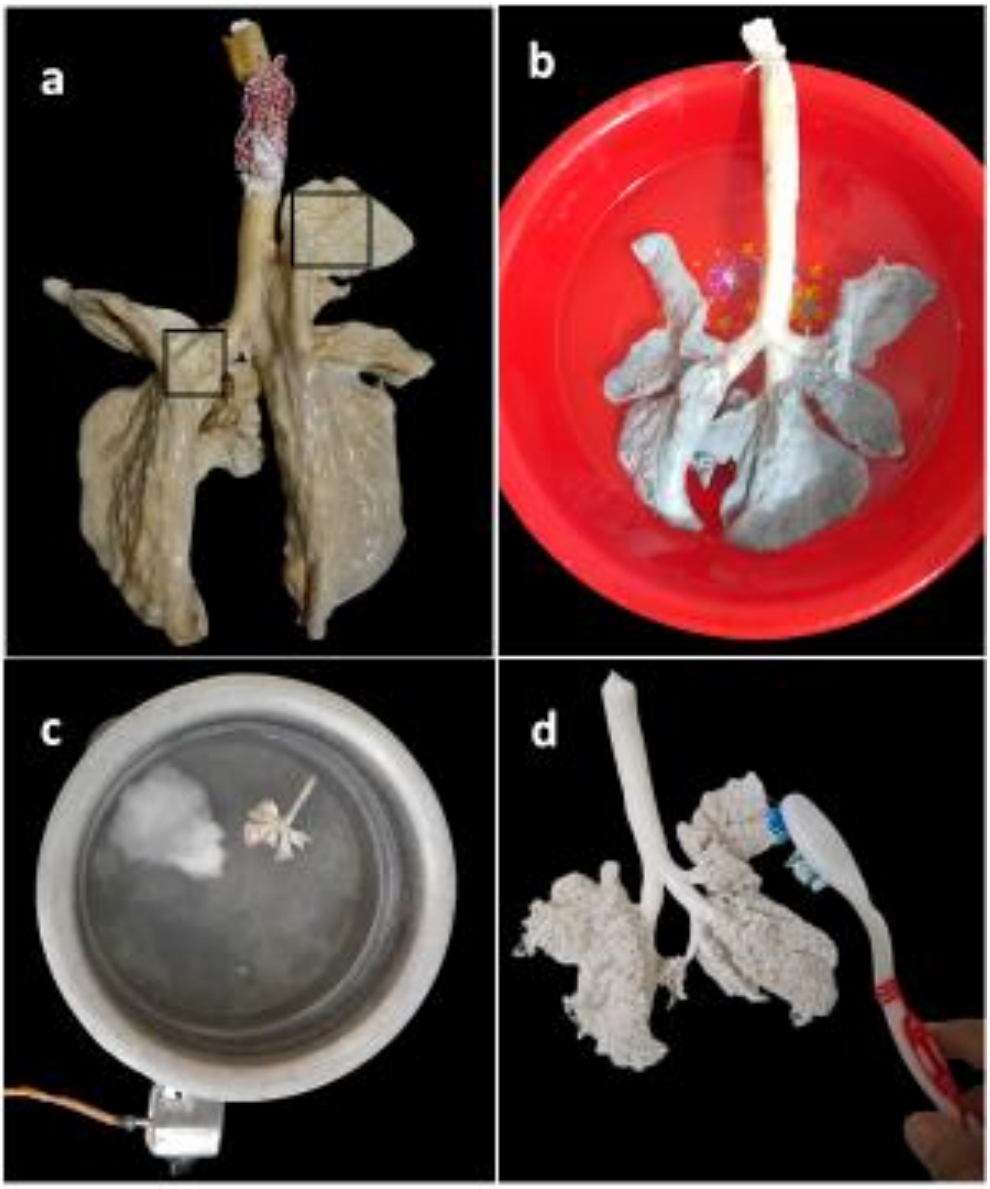
f. Representative sample after washing

Photograph 3.5: Inclined luminal end of representative organs for dehydration

Photograph 3.6: Impregnation of luminal organs-

a. Impregnation of lungs, **b**. Impregnation of Renal artery,

c. Impregnation of External auditory canal of goat

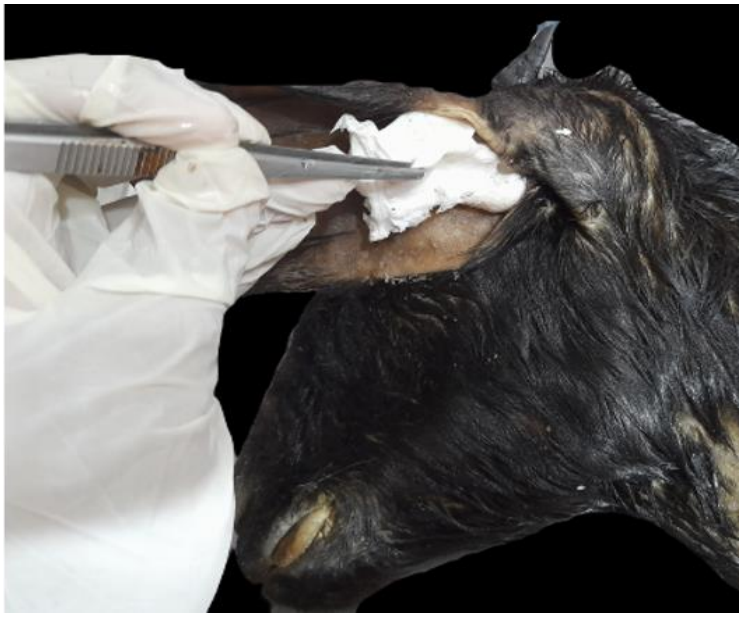


Photograph 3.7: Steps after impregnation

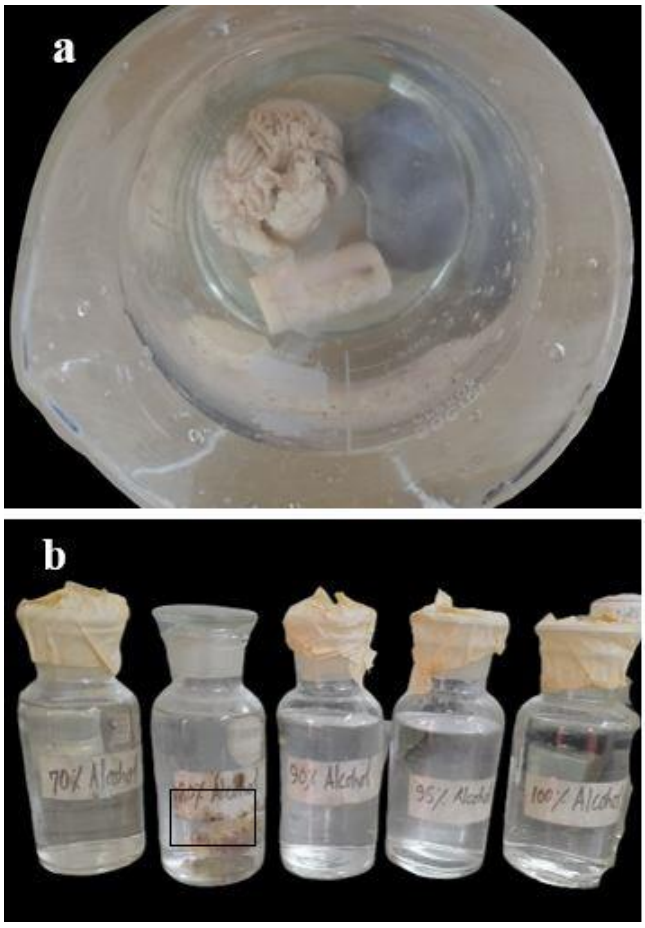
a. Impregnated lung sample (black rectangles showing polygonal-shaped alveoli),

b. Immersion of representative specimen in a bowl of water,

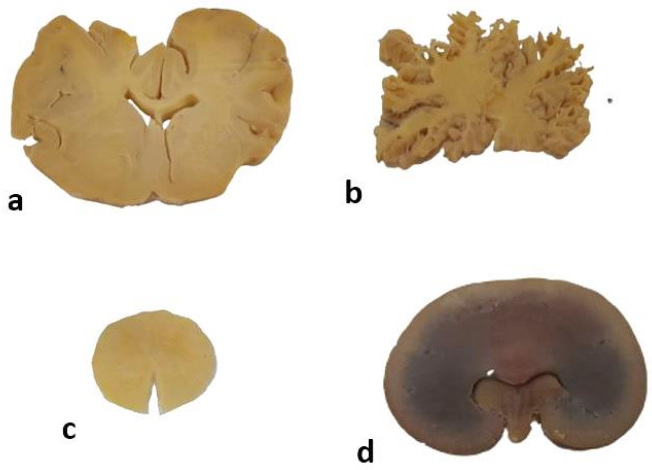
c. Boiling of representative sample, **d.** Cleaning of the cast with a fine brush



Photograph 3.8: Removal of EAC cast of goat with forceps

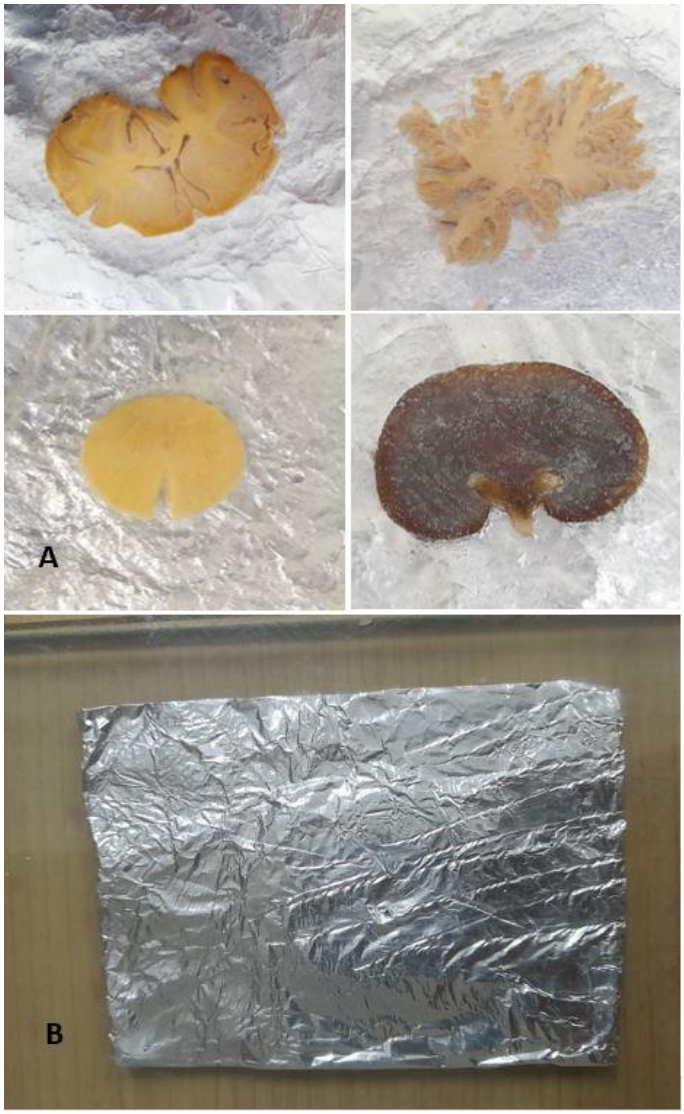


Photograph 3.9: Fixation and dehydration of samples for sheet plastination
a. Fixation of samples in 10% buffered formalin for sheet plastination,
b. Dehydration of samples in ascending graded alcohol

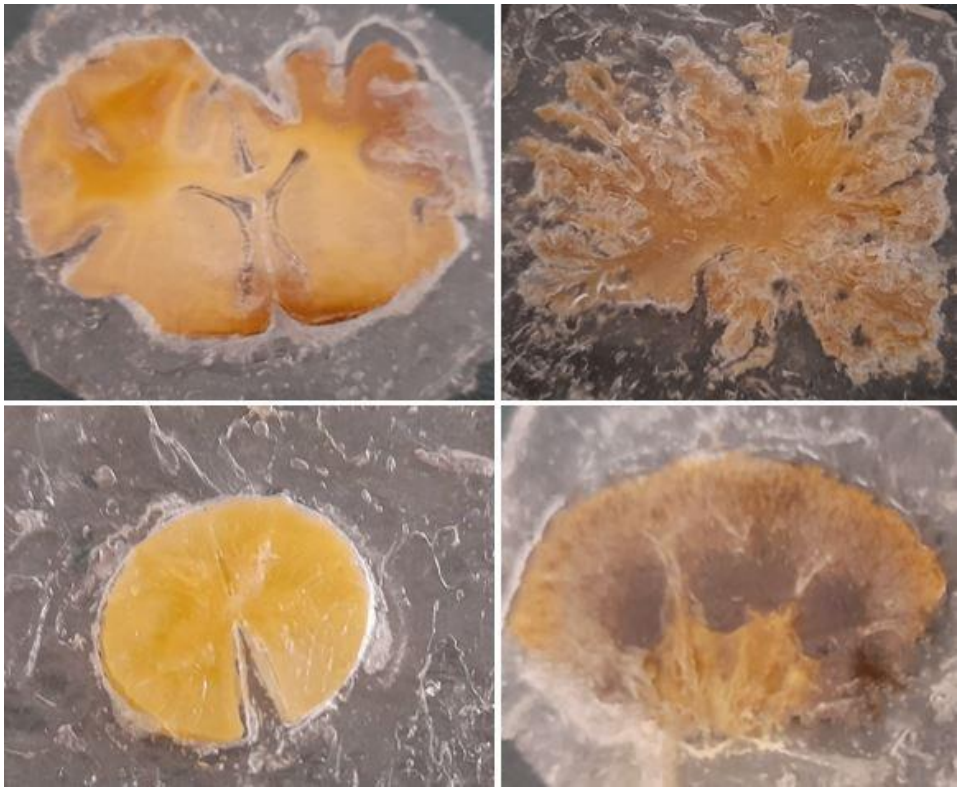


Photograph 3.10: Representative slices for sheet plastination

a. Slice of cerebrum, **b.** Slice of cerebellum, **c.** Slice of spinal cord, **d.** Slice of kidney



Photograph 3.11: Impregnation of sheets- A. Impregnation of representative slices with transparent silicon sealant, **B.** Foil-slice-foil sandwich topped with glass plate



Photograph 3.12: Representative sheet plastinated specimens after curing process

Photograph 3.13: Demonstration of prepared plastinated samples in anatomy practical class

Chapter 4

Results

4.1 Luminal cast of lungs

The gross anatomical examination of the lungs of goat, dog and cat revealed four lobes on the right side and two lobes on the left. In the casts, we observed differences in segmental structure between the right and left lungs, as well as discrepancies in the tracheobronchial tree among the species studied.

4.1.1. Luminal cast plastination of goat lungs

Photograph (4.1 A and 4.1 B) clearly exhibit trachea with its cartilaginous markings and tracheal division from principal bronchi to alveoli was readily distinguishable from the casts. Two principal bronchi (right and left) were seen in the goat lungs (Photograph 4.1 A). Besides, an additional bronchus on the right side called tracheal bronchus/apical bronchus/apical lobar bronchus arises directly from the trachea above tracheal bifurcation (Photograph 4.1 B). Which branches into segmental bronchi to ventilate the apical lobe of the right lung. Then the right (rt.) principal bronchus was divided into a middle lobar bronchus, a diaphragmatic lobar bronchus and an accessory lobar bronchus. Whereas, the left (lt.) principal bronchus gave off an apical lobar bronchus and a diaphragmatic lobar bronchus. These lobar bronchi further split into several segmental bronchi and ventilate subsequent portions of the lung (Photograph 4.1 B). Differences in the branching pattern of right and left lungs is showed in the table 4.1.

Table 4.1: Differences in segmental structure between the rt. and lt. lungs of goat

Criteria	Right lung	Left lung
Tracheal/apical lobar bronchus	Present	Absent
Middle lobar bronchus	Present	Absent
Accessory lobar bronchus	Present	Absent

4.1.2 Luminal cast plastination of dog lungs

A distinct bronchial tree pattern with cartilaginous tracheal marks was visible in the tracheobronchial cast of canine lungs and the trachea was bifurcated into right and left principal bronchi (Photograph 4.2 A). The right principal bronchi were splited into apical, middle, diaphragmatic and accessory lobar bronchus while the left principal bronchus was divided into an apical lobar bronchus and a diaphragmatic lobar bronchus

(Table 4.2). Additionally, a number of segmental bronchi arise from each lobar bronchus to ventilate the respective lung lobes (Photograph 4.2 B).

Table 4.2: Differences in segmental structure between the rt. and lt. lungs of dog

Criteria	Right lung	Left lung
Middle lobar bronchus	Present	Absent
Accessory lobar bronchus	Present	Absent

4.1.3 Luminal cast plastination of cat lungs

The results obtained from the tracheobronchial cast showed that the segmental structure between right and left lungs varies to some extent (Table 4.3). The right and left principal bronchi emerge as a result of the tracheal bifurcation (Photograph 4.3 A). Four lobar bronchi- apical, middle, diaphragmatic and accessory lobar bronchi were derived from the right principal bronchi. The segmental bronchi split off from the lobar bronchi which divided into bronchiole and finally ended in tiny bristle-like alveoli (Photograph 4.3 B). The left principal bronchus was divided into an apical lobar bronchus and a diaphragmatic lobar bronchus which terminate into segmental bronchi and ventilate respective lung lobes (Photograph 4.3 B).

Table 4.3: Differences in segmental structure between the rt. and lt. lungs of cat

Criteria	Right lung	Left lung
Middle lobar bronchus	Present	Absent
Accessory lobar bronchus	Present	Absent

It is evident from the casts that the apical/accessory bronchus that ventilates the apical lobe of the right lung was a distinguishable feature of goat lungs. Because it was absent in case of dog and cat (Table 4.4). Such unique traits that are easily demonstrated by the luminal cast technique also facilitate comparative anatomical research.

Table 4.4: Key differences in the tracheobronchial tree of the examined species

Criteria	Goat	Dog	Cat
Tracheal/apical bronchus in right lung	Present	Absent	Absent
Alveoli	Fine	Fine	Tiny bristle shaped

4.2 Luminal casts of renal artery

The major renal artery and its intra-renal segments were distinctly observed in the three-dimensional casts of bovine and caprine kidneys (Photograph 4.4 A & B).

4.2.1 Intra-renal segmentation of cattle kidney

The renal artery of the cattle kidney was initially divided into segmental arteries. The segmental arteries detached two to three interlobar arteries (Photograph 4.4 A). Further examination of the cast showed that interlobar arteries gave off multiple arcuate arteries (Photograph 4.4 A).

4.2.2 Intra-renal segmentation of goat kidney

Two segmental branches of the renal artery were clearly discernible in the prepared silicon casts. Interlobar arteries were detached by each segmental artery (Photograph 4.4 B). It then splits into a number of arcuate arteries and ultimately terminated in numerous interlobular arteries. The branches of the renal artery did not exhibit any anastomosis (Photograph 4.4 B).

4.3 Luminal cast of external auditory canal of goat

The cast exhibited an unprecedented three-dimensional configuration of the cavity, with the auricle laterally and the tympanic membrane of the external ear medially. The ear canal cast was a tortuous long tube that curved cranially, medially and ventrally (Photograph 4.5).

4.4 Modified sheet plastination technique

The overall quality of the plastinated sections obtained with transparent-colored Smartcare Xtremoseal^{GP} silicon sealant was very good. The finished silicon sections were semi-transparent, durable, dry, odorless and stored at room temperature (Photograph 4.6 A, B, C & D). There was minimal change in the color, slight shrinkage and dislocation of structures in the plastinated sheets. Additionally, in this technique we could not completely eliminate small bubbles in the slices. Whereas, photograph 4.7 displays moist surface of the preserved sample, altered color and distortion of structures due to handling.

4.4.1 Plastinated sections of goat brain and spinal cord

The obtained sheets were of good quality, thin and semi-transparent. The color and consistency of these sections remained almost unaltered though in the case of formalin-fixed sample we observed alteration of color (Photograph 4.7). The fine details of many anatomical structures could be observed and easily identified. The plastinated transverse sections of brain and spinal cord presented good contrast between grey and white matter (Photograph 4.6 A & B). Besides, the lateral ventricle and septum pellucidum of the cerebrum and arbor vitae of cerebellum, horns (dorsal, lateral and ventral), dorsal median sulcus and ventral median fissure of the spinal cord was also easily identified in the respective sheets (Photograph 4.6 A, B & C).

4.4.2 Plastinated sections of goat kidney

The kidney sections prepared by transparent colored silicon sealant were found to be durable and semi-transparent (4.6 D). The slices were flexible, dry, odorless and had smooth surfaces. In addition, plastinated slices exhibited distinct morphological features with relative convenience and minimum expenditure. No obvious shrinkage or distortions were observed in the slices. With background lighting, light-colored renal cortex, dark-colored renal medulla with pyramids and renal pelvis were easily distinguished in the prepared sections of goat kidney (4.6 D).

4.5 Students' experience with plastinated samples

We displayed the prepared plastinates to 45 first-year (Gross Anatomy course) DVM students who attended anatomy practical classes on a regular basis and gave them the prepared questionnaire. In response to the questionnaire, 100% of respondents stated that, formalin-preserved biological samples are frequently demonstrated in the classes and 95.6% of them feel uncomfortable during handling of those samples (Figure 2).

In contrast, table 4.5 showed 91.1% of participants safely and satisfactorily handled the plastinated specimens without gloves and all the participants found the plastinates odorless and hazard-free.

Table 4.5: Percentage of satisfaction with plastinated and preserved specimens

Questions	Options	Percentage (%)
Is the supplied plastinated sample safer to handle without gloves than the formalin preserved or fresh sample?	a. No b. At some extent c. Very safe and satisfactory	a. 0 b. 8.9 c. 91.11
Do you detect any odor or health risks while handling the Plastinated samples?	a. Yes b. No	a. 0 b. 100

We found 51.1% of the participants highly recommended plastinated casts and sheets in anatomy practical classes; while 48.9% recommended the plastinates along with fixed and fresh samples. Besides, 93.3% considered plastinated aesthetic enough to display in the museum (Figure 4.1).

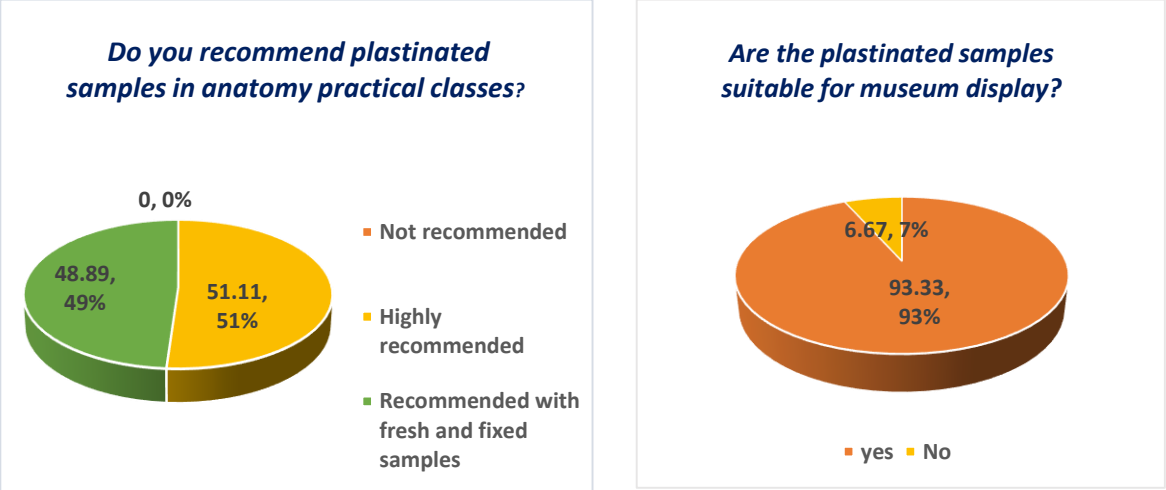


Figure 4.1: Recommendations from the participants

However, our study found 86.7% of the students were very little and 13.3% students were little satisfied with formalin preserved specimens. Whereas, 88.9% found plastination technique very important to study anatomy (Figure 4.2).

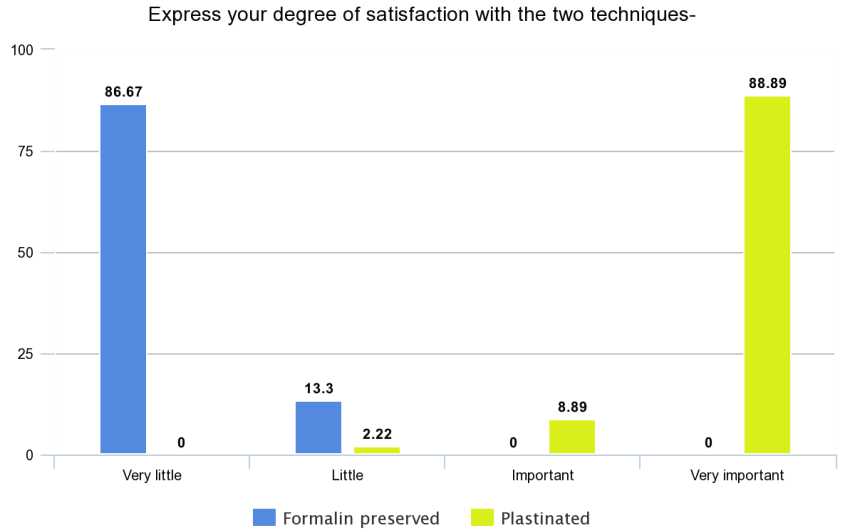


Figure 4.2: Degree of satisfaction with preserved and plastinated sample

According to 97.8% of the students the lung casts were more effective in describing three-dimensional orientation and segmentation of the tracheobronchial tree than the animated video or image or preserved samples (Figure 4.3). Further, 82.2% found the casts highly suitable for comparative study (Figure 4.3).

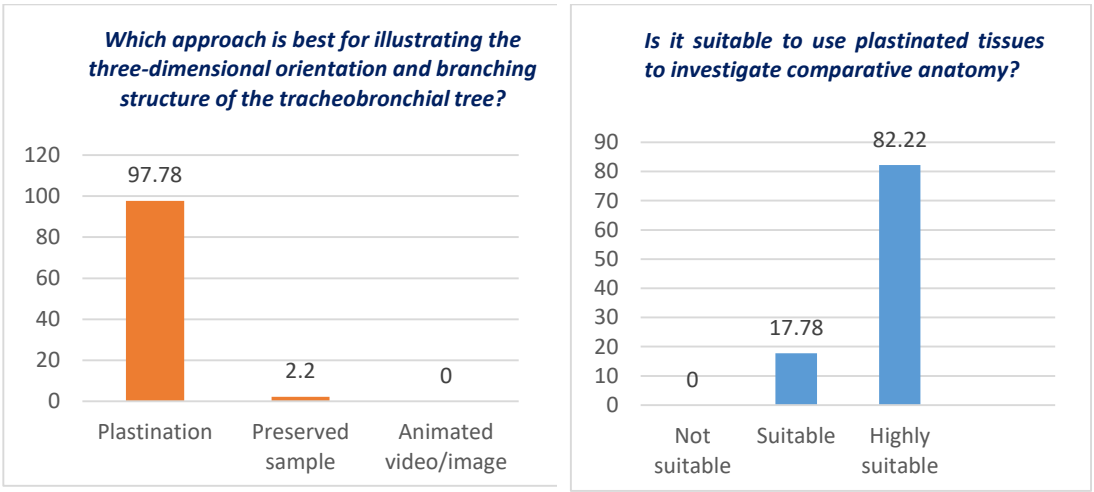


Figure 4.3: Students' perception with luminal casts of lungs

Figure 4.4 depicted that 88.9% of students completely understand the intra-renal segmentation of the renal artery (RA) by the prepared renal casts.

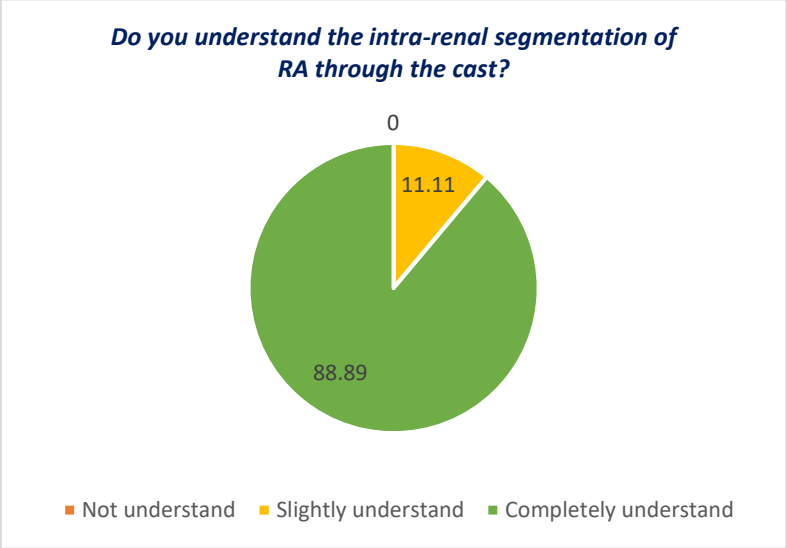


Figure 4.4: Degree of clarity of intra-renal segmentation through renal cast

Figure 4.5 showed all the participants found the cast very useful tool to understand the complicated orientation of EAC.

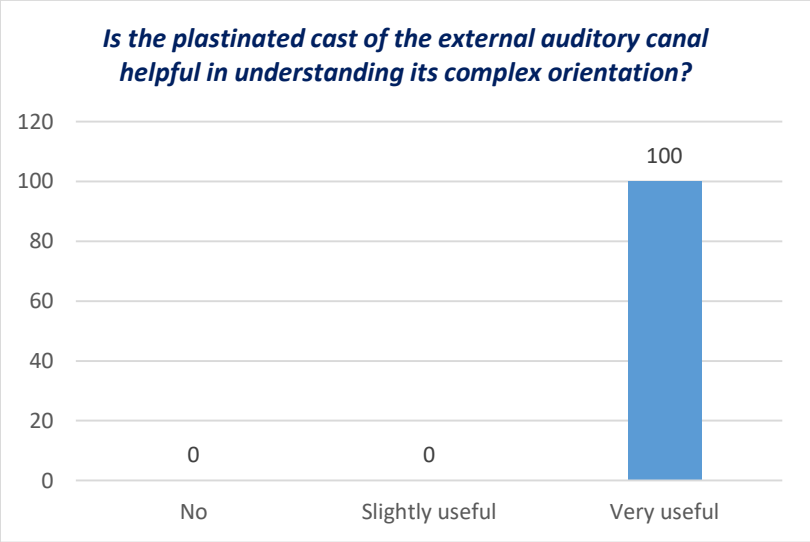


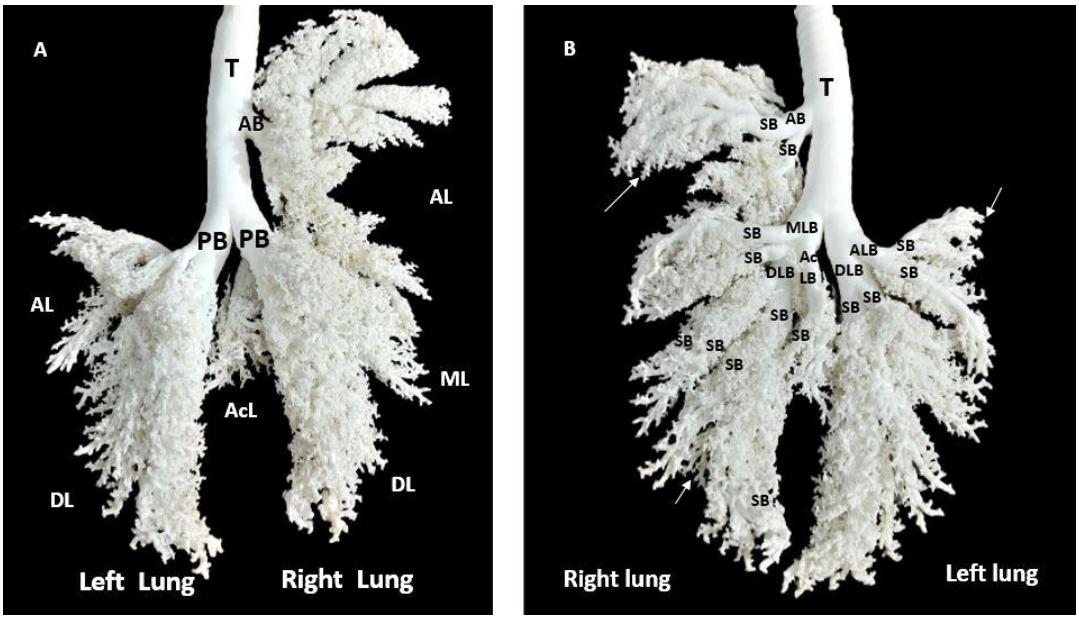
Figure 4.5: External auditory canal (EAC) cast as teaching tool

Student's experience with the plastinated slices depicted in the table 4.6. Table 4.6 displayed 97.7% of the students stated, the sheet plastinated samples better exhibits the internal structure, color and orientation of the supplied slices than the fixed samples. The participants also found the slices flexible, highly portable and suitable for close inspection compared to the preserved slices (Table 4.6).

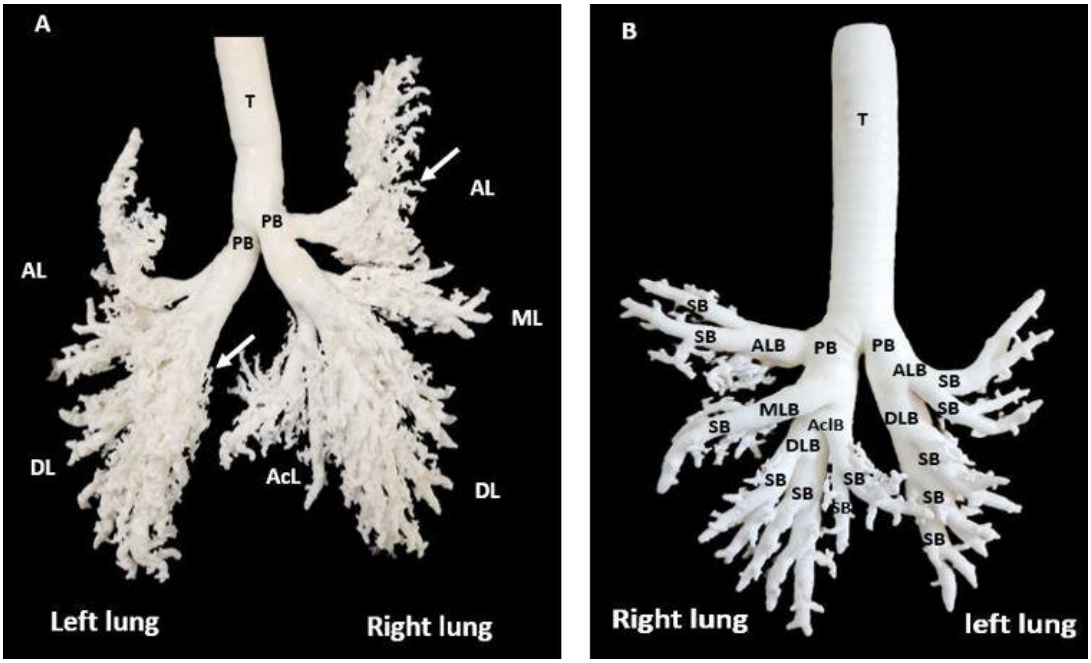
Table 4.6: Students' experience with modified sheet plastinated slices

Questions	Options	Percentage (%)
Which sample best represents the internal structure, color and orientation of the provided brain, spinal cord and kidney slices?	a. Fixed samples b. Sheet plastinated samples	a. 0 b. 97.7%
Which sample is suitable for handling and flexible enough for close examination?	a. Preserved b. Sheet plastinated	a. 100 b. 0
Are the sheets portable enough to be studied outside of the practical class?	a. No b. Slightly portable c. Highly portable	a. 0 b. 0 c. 100

Picture Gallery

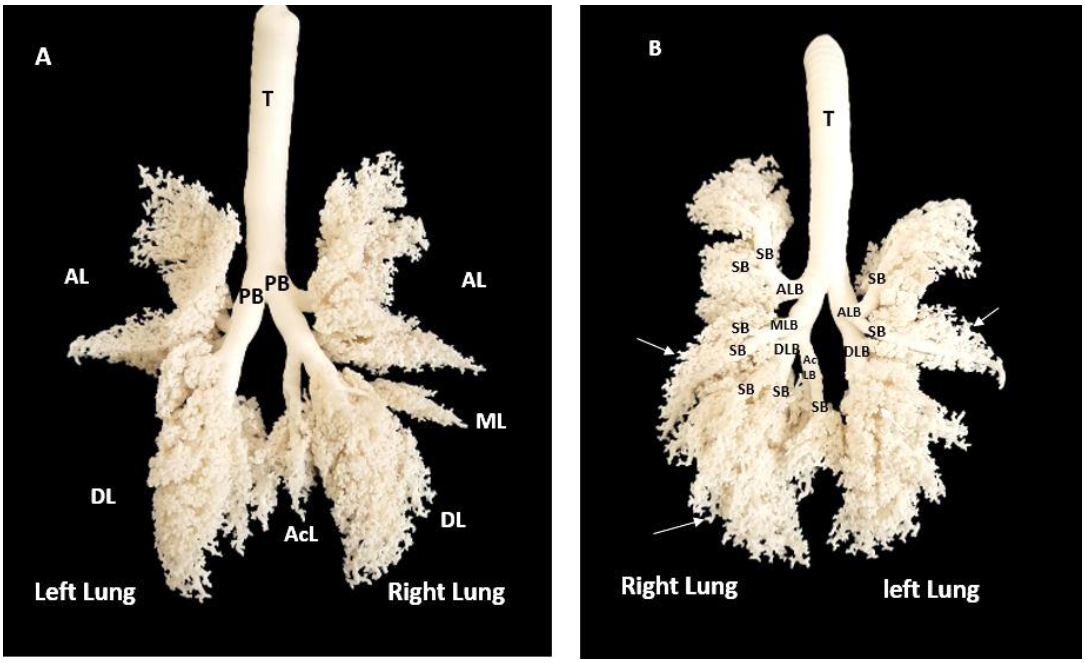


Photograph 4.1: Luminal cast of goat lungs- A. Dorsal view of the cast showing- T= Trachea, AB= Apical bronchus, PB= Principal bronchus, AL= Apical lobe, ML= Middle lobe, DL=Diaphragmatic lobe and AcL= Accessory lobe**;**
B. Ventral view of the cast showing- AB=Apical lobar bronchus, MLB= Middle lobar bronchus, DLB= Diaphragmatic lobar bronchus, AcLB= Accessory lobar bronchus, SB= Segmental bronchus and alveoli (white arrow)

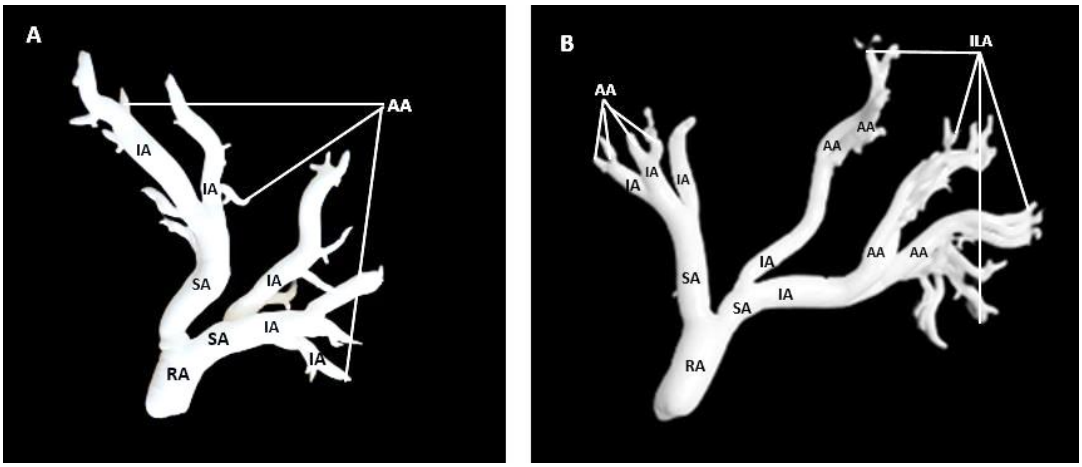


Photograph 4.2: Luminal cast of dog lungs- A. Dorsal view of the cast showing- T= Trachea, PB= Principal bronchus, AL= Apical lobe, ML= Middle lobe, DL=Diaphragmatic lobe, AcL= Accessory lobe and alveoli (white arrow);

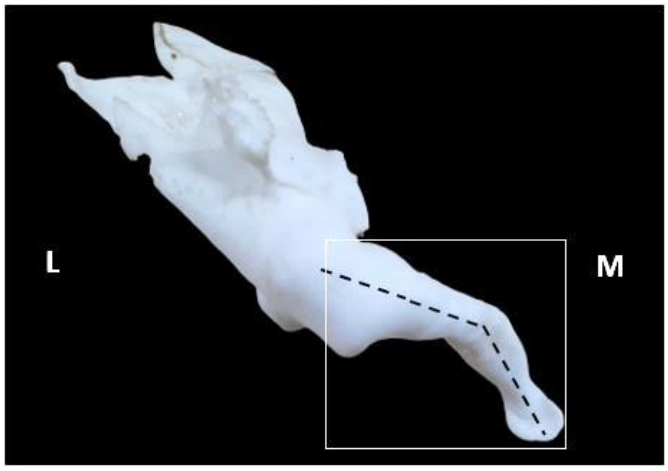
B. Ventral view of the cast showing- ALB=Apical lobar bronchus, MLB= Middle lobar bronchus, DLB= Diaphragmatic lobar bronchus, AcLB= Accessory lobar bronchus, SB= Segmental bronchus



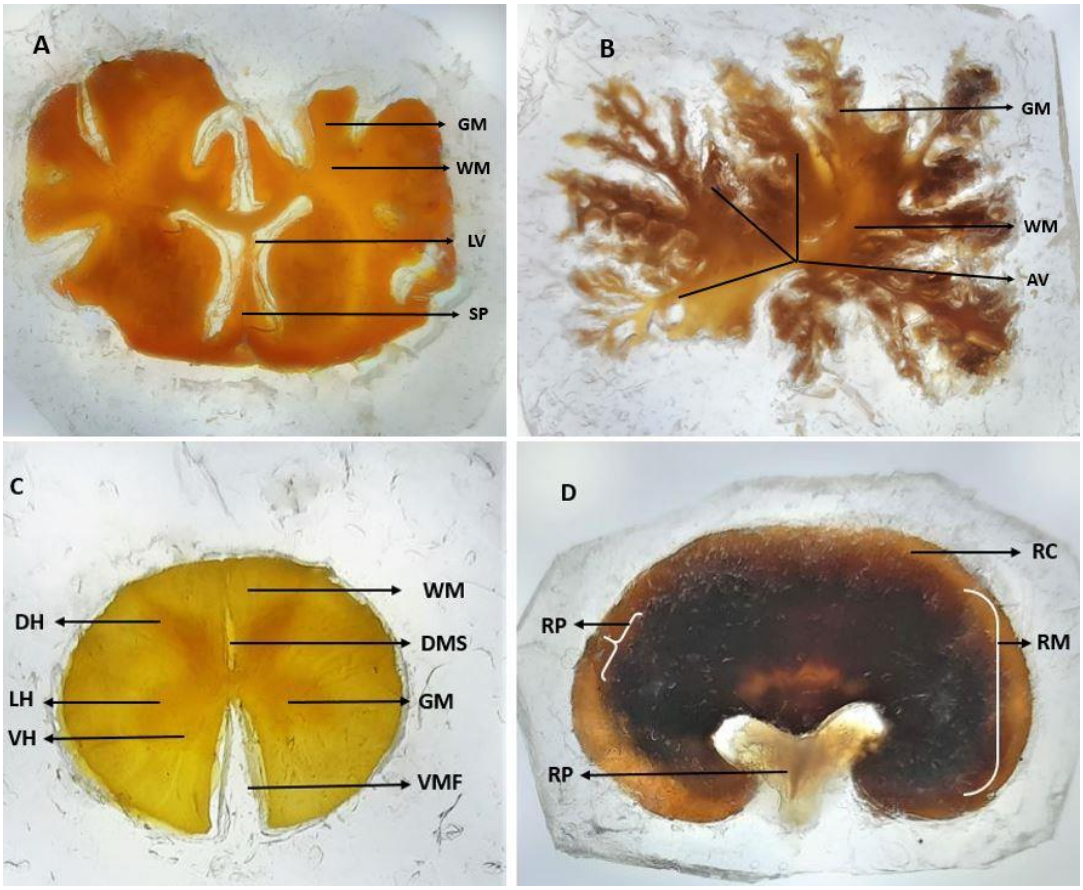
Photograph 4.3: Luminal cast of cat lungs- A. Dorsal view of the cast showing- T= Trachea, PB= Principal bronchus, AL= Apical lobe, ML= Middle lobe, DL=Diaphragmatic lobe and AcL= Accessory lobe;
B. Ventral view of the cast showing- ALB=Apical lobar bronchus, MLB= Middle lobar bronchus, DLB= Diaphragmatic lobar bronchus, AcLB= Accessory lobar bronchus, SB= Segmental bronchus and alveoli (white arrow)



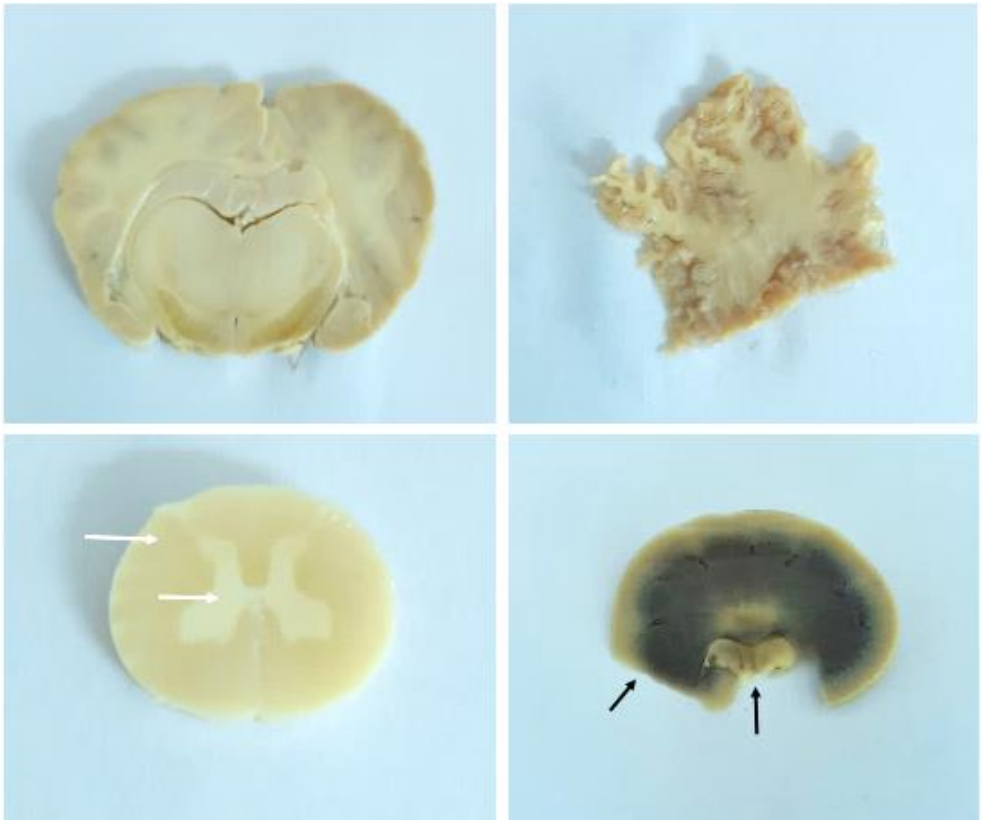
Photograph 4.4: Luminal casts of RA- A. Intra-renal segmentation of renal artery of cattle showing- RA= Renal artery, SA= Segmental artery, IA= Interlobar artery and AA=Arcuate artery; **B.** Intra-renal segmentation of renal artery of goat showing- RA= Renal artery, SA= Segmental artery, IA= Interlobar artery, AA=Arcuate artery and ILA= Interlobular artery



Photograph 4.5: Luminal cast of external auditory canal of goat (white rectangular area) showing its curvature (cranially, medially and ventrally) in black dots;
L= Lateral, M= Medial



Photograph 4.6: Sheet plastinates- A. Plastinated sheet of cerebrum showing- GM= Grey matter, WM= White matter, LV= Lateral ventricle and SP= Septum pellucidum; **B.** Plastinated sheet of cerebellum showing- GM= grey matter, WM= White matter and AV= Arbor vitae; **C.** Plastinated slice of spinal cord showing- WM= White matter, GM= Grey matter, DMS= Dorsal median sulcus, VMF= Ventral median fissure, DH= Dorsal horn, LH= Lateral horn and VH= Ventral horn; **D.** Sheet Plastinated goat kidney showing- RC= Renal cortex, RM= Renal medulla and RP= Renal pelvis



Photograph 4.7: Formalin fixed slices of studied organs showing-
wet surface, altered color (white arrow) and distortion of structures on hand manipulation (black arrow)

Chapter 5
Discussion

5.1 Luminal cast plastination with silicon sealant (white color)

The technique of luminal cast plastination is beneficial for studying the dimensions and morphology of tubular components. The basic concept is to fill the lumen with polymer and dissolve the surrounding tissue to construct the cast. Fixation is not necessary for this approach, hence we used fresh samples to obtain luminal casts. Studies by Menaka et al. (2015), Sah et al. (2017), Jabbar et al. (2018) and Ramkrishna and Leelavathy (2019) also stated fixation was not mandatory for this type of plastination. Conversely, Bhandari et al. (2016) preserved the specimens in formalin for the luminal cast technique. We selected 'Asian Paints Smartcare Xtremoseal^{GP} All Purpose Acetoxy Cured Silicone Sealant' as the most cost-effective commercial silicon sealant (Photograph 3.1 b). Dahiphale et al. (2013), Menaka et al. (2015), DeSouza et al. (2016) and Dawood (2022) also used a similar silicon sealant to produce flexible and elastic silicon casts. However, Narayanan (2015) stated that epoxy resin was more durable than silicon since resin casts were rigid and non-elastic, unlike silicone casts. In this study, the impregnated tissue was finally destroyed in boiling water following proper curing (Photograph 3.6 c). Though Wang and Karman (1988) immersed the tissue in sodium hydroxide for alkali corrosion and Narayanan (2015) utilized diluted nitric acid for decomposition.

We found the prepared silicon casts were dry, flexible, odorless, safe, long- lasting and suitable for teaching, research and exhibition. Besides, the casts excellently exhibited the three-dimensional orientation of the tracheobronchial tree, intra-renal segmentation of renal artery and external air canal (Photograph 4.1-4.3). Such findings were consistent with the study of Akbari et al. (2011), Nassar (2013), Mantri et al. (2017) and Laishram and Sathvika (2017).

5.1.1 Tracheobronchial cast of lungs

The tracheobronchial casts of goat, dog and cat indicated that the trachea was bifurcated into two principal bronchi, one for each lung. The principal bronchi were separated into three lobar bronchi in goat (middle, diaphragmatic and accessory); four lobar bronchi in dog and cat (apical, middle, diaphragmatic and accessory) in the right side of lung

and two lobar bronchi (apical and diaphragmatic) in the left side of lung (Photograph 4.1-4.3). The lobar bronchi were then ramified into segmental bronchi, which ended in alveoli. However, above the tracheal bifurcation, an extra bronchus (apical lobar bronchus) was detected only in the right lung of goat, which ventilates the apical lobe of the corresponding lung (Photograph 4.1 A). These findings were comparable to these findings (Sisson and Grossman, 1975a; Sisson and Grossman, 1975b; Henry, 1992, Nassar, 2013; Ananya et al., 2019; Lokanathan et al., 2020).

5.1.2 Luminal cast of renal artery

The renal artery (RA) is a branch of the abdominal aorta that provides arterial blood to the kidney. Each kidney is supplied by the corresponding renal artery (RA). Bailey (1978), Richards (1984), Jabbar et al. (2018), Szymanski et al. (2018) described the RA enters the kidney at hilus and split into some large branches. These branches divide into a number of interlobar arteries. These give off several short arteries known as arcuate arteries. Which eventually sends off a large number of finer branches, the interlobular arteries. In the current investigation, renal casts of cattle and goat also showed similar division of RA; which divided into segmental arteries; that branched into interlobar arteries. Which gave rise to many arcuate arteries and terminate into interlobular arteries (Photograph 4.4 A & B).

5.1.3 Luminal cast of external auditory canal of goat

The EAC is a cavity that has the auricle laterally and leads medially towards the tympanic membrane of the middle ear (Sisson and Grossman, 1975a). The prepared goat EAC cast displayed that its curvature showed a cranial, medial and ventral direction (Photograph 4.5), which was found similar with the description of Ghosh (1995).

5.2 Modified sheet plastination technique with silicon sealant (transparent color)

We adopted indigenous tools and chemicals to accomplish the four fundamental stages of sheet plastination outlined by Ottone et al. (2018). This simplified technique was also carried out at room temperature rather than freezing condition which was in line with a similar study by Raj et al. (2016).

5.2.1 Fixation: Proper fixation of a biological sample is critical for producing a high-quality plastinated specimen. The most common fixative used in the plastination

process is 5-20% formalin (Sora, 2016). Although Sora and Cook (2007) stated that fixation is not mandatory for the production of plastinates and also identified some drawbacks of fixation, including discoloration of organs. However, in our investigation, the samples were fixed in 10% buffered formalin for maximum color retention of the studied organs (Photograph 3.9). Sivagnanam et al., (2013) reported that Keiserling solution is the best fixative for plastination procedure because of its excellent color preservation quality which can be tested in future research.

5.2.2 Dehydration: Dehydration of the fixed or fresh specimens is required to replace the tissue water with an intermediate solvent. Both alcohol and acetone can be employed as dehydrating agents in the plastination process (Pereira-Sampaio et al., 2006). Studies by Pendovski et al. (2008), Chaudhary (2013), Latorre et al. (2019) and Bakici et al. (2022) regarded cold temperature (-15 ˚C to -25 ˚C) acetone as the optimal intermediate solvent because of its excellent dehydration capability and minimal organ shrinkage However, since acetone is more expensive than alcohol, we dehydrated the samples with ascending graded (70% to 100%) alcohol (Photograph 3.9 b). Brown et al. (2002), Sivrev and Usovich (2006) and Srisuwatanasagul et al., (2010) also mentioned acetone replacement with ascending graded alcohol in the plastination procedure. So, despite shrinkage, alcohol might be a feasible alternative for an affordable plastination procedure.

5.2.3 Slicing and impregnation: Slicing is thought to be the most significant step of sheet plastination. Quality of the plastinated sheets greatly relies on the thickness of the slices. Multiple researchers agreed that specialized saws such as ‘diamond saw’, ‘band saw’, and ‘shark band blade’ were suitable for cutting specimens for conventional sheet plastination (Eckel et al., 1993; Alston et al., 1997; Latorre and henry, 2007; Sora and Cook, 2007; Ottone et al., 2018). These saws were not locally available and relatively expensive. Therefore, we sliced the dried samples using a sharp microtome blade (Feather A35) with a maximum thickness of about 6 mm (Photograph 3.1 d). Such modification with meat slicer was reported by Pendovski et al. (2008). Studies by (Raj et al. (2016) and Leelavathy and Ramkrishna (2019) demonstrated regular knife as a cheaper alternative to cut pieces for plastination.

The key stage in sheet plastination is impregnation, which involves filling the cellular and interstitial tissue spaces with an impregnation mixture (Latorre and Henry, 2007).

In our study, brain, spinal cord and kidney slices were impregnated with an available cheap acetic acid polymer (Asian Paints Smartcare XtremosealGP All Purpose Acetoxy Cured Silicone Sealant). This modified strategy was contrasted with other adjustments conducted by Raj et al. (2016), Georgieva et al. (2019), Leelavathy and Ramkrishna (2019) and Bakici et al. (2022). The main benefit of our process was that it can be performed at ambient temperature and pressure rather than vacuum pressure and controlled temperature. Besides, no additional activator was required to initiate the linking process of the polymer and no mold was necessary to shape the sections.

5.2.4: Curing: In our experiment, the curing process takes approximately 4-5 days at room temperature and no additional curing agents were applied to accelerate the process. In contrast, the traditional method involved specific curing agent and curing chamber made of glass sheets to complete the step (Bickley and Townsend, 1984; Lattore et al., 2004; Weber et al., 2007; Elnady and Sora, 2009; Sargon and Tatar, 2014). Whereas current study demonstrated a simple method for easy and quick hardening of the plastinated sheets.

Finally, the cured slices were odorless, soft, dry, environmentally safe and stayed intact at room temperature for the last three months. Inspection of the produced sheets using a background light revealed semi-transparent brain, spinal cord and kidney slices with fine macroscopic detail of the organs (Photograph 4.6). These findings were also in line with the study of Raj et al. (2016), Leelavathy and Ramkrishna (2019), Rabiei et al. (2014). Sheets obtained by this method were also useful research tool because of its capacity to provide precise detail. Additionally, such transparency of the slices made them important, low-cost visual assistance for diagnostic imaging procedures (CT scan, MRI etc.). Minimal color change and shrinkage were noticed, which might be attributed to the formalin fixation and alcohol dehydration processes (Brown et al., 2002). In addition, retention of bubbles might be a drawback of the developed technique. Rabiei et al. (2014) also reported numerous bubble formations in the modified procedure with synthetic resin.

5.3 Plastination technique in anatomy education

Anatomy is a fundamental field that every student or health professional must learn in medical science courses. It is essential to use a variety of teaching strategies to assist

students in comprehending a vast subject like anatomy. Fresh and well-preserved specimens, textbooks and three-dimensional images/videos are common aid for anatomical study (Ameko et al., 2012). Purchasing and euthanizing of new animal each time to demonstrate fresh samples is both costly and morally unsound. Moreover, texts or dissection cannot demonstrate the three dimensions of the organs and it is also difficult to understand intricate structures using animated pictures or videos (Sora et al., 2019). According to our study, majority of the biological samples exhibited in the anatomy practical class were preserved in formalin and nearly all (95.6%) respondents found those preserved samples unsafe during handling. These findings were consistent with the studies by Mantri et al. (2017) and Sultana et al. (2019). Due to such feedback from the learners, 'Plastination' technique developed by Dr. Gunther von Hagens is now widely applied in teaching and learning anatomy. But the expenditure of standard plastination procedures is highly expensive and non-affordable to most institutions. As a result, affordable modification of the standard technique was developed in many countries. The current study developed luminal cast and sheet plastination method using indigenous chemicals for the first time in Bangladesh. The prepared casts and sheets were dry, flexible, clear, non-irritant and free of offensive odor. Further, almost all (91.1%) of the students were satisfied and preferred these modified plastinates over formalin-fixed samples and none of them noticed any undesirable odor during handling (Table 4.5). These findings also agreed with a survey from the anatomy students of the School of Medicine, University of Granada, Spain (Roda-Murillo et al., 2006). Besides, demonstrated plastinated specimens were highly recommended by 51.11% of learners while 48.9% recommended the samples with fresh and preserved organs (Figure 4.1). Our investigation also observed 86.7% and 13.3% students were very little and little satisfied with formalin preserved samples. In contrast, 88.9%, 8.9% and 2.2% of the participants found plastinated specimen very important, important and little important to learn anatomy (Figure 4.2). A similar response was described by Latorre et al. (2016) conducted at University of Cambridge, UK. Even a vast majority of them (93.3%) considered the plastinates were suitable for museum exhibitions (Figure 4.1). Furthermore, almost all (97.8%) of the participants mentioned that the trachea-bronchial casts were more effective than animations or preserved samples in illustrating the three dimensions of the bronchial tree (Figure 4.3). A larger proportion of them (82.2%) agreed that the casts also facilitate comparative anatomy studies. These findings agreed with those of Bhandari et al. (2012), Trivedi et al. (2016) and Mantri et

al. (2017). The segmentation of RA was acknowledged to be entirely understood through these casts by 88.9% of the students (Figure 4.4). Further, all the participants of this investigation found the EAC cast very useful to comprehend the complex structure of the EAC (Figure 4.5). Sivagnanam et al. (2014), Bhandari et al. (2016), Trivedi et al. (2016), Mohamed and John (2018) and Sora et al. (2019) also found plastinates as useful teaching material in a wide range of subjects including human anatomy, veterinary anatomy, gynecology, pathology, surgery etc. The overall responses of the learners suggested that the modified plastination approach could be an effective teaching and learning resource in the practical lessons. Further, feedback from the students also showed that plastinated slices beat preserved samples in terms of inner structure, color and orientation preservation. Moreover, all of them agreed that, the plastinates are markedly flexible, portable and safe for close inspection (Table 4.6). These results correspond with that of Roda-Murillo et al. (2006). Finally, adopting this technique can greatly reduce the need for animal sacrifices for practical demonstration.

5.4 Plastinates as a research tool

Cook (2007) stated, sheets or plastinated sections are mostly applied research tools in anatomy. Transparent and semi-transparent plastinated slices provide a precise comprehension of complicated anatomical structures as well as extensive organ examination. These slices were reported to be effective for morphological and topographic studies by Rafiemanzelat et al. (2021). The preservation of tissue sections without collapse or dislocation is another key use of plastination in the research sector (Dev et al., 2002). Additionally, these transparent sheets also provide relevant information for diagnostic imaging modalities. However, the typical epoxy or polyester process of producing sheets is quite expensive. Leelavathy and Ramkrishna (2019) introduced jelly wax sheet plastination to investigate sectional anatomy in an affordable way and Ottone et al. (2016) reported sheet plastination technique with commercial epoxy resin. Whereas, for the first time we adopted the locally available silicon sealant to yield plastinated sections which was frequently used for luminal cast plastination. Moreover, jelly wax is a paraffin wax with melting point of 30 $^{\circ}$C to 45 $^{\circ}$C (Chaichan et al., 2021); whereas the chemical used in this study has a melting point of 120 $^{\circ}$C. Thus the adopted procedure of the study was more reliable and thermolabile. Photograph 4.6 A, B, C & D offered precise view of different anatomical structures that were suited in-depth macroscopic research. Haenssgen et al. (2014) stated, a clean

corrosion cast was useful during X-ray photography and micro computed tomography (μCT) related research. The low-cost luminal casts obtained in the study could also facilitate such works. Zaidi et al. (2019) and Coutinho et al. (2021) reported the EAC cast of goat act as a suitable model for otological surgery in human. So, the semi-transparent sheets developed with transparent silicon sealant might be a valuable and affordable visual aid for diagnostic imaging techniques (CT scan, MRI etc.) and surgical procedures. While the flexible silicon casts could be a feasible research aid in anatomy.

Finally, adoption of such low-cost approach would significantly reduce the need of euthanization of animals for teaching and research as well as importation of the plastinates from foreign countries. Furthermore, large-scale development of the presented technique will allow distribution of the plastinates to various institutions to aid teaching and research.

Limitations

The procedure has the following constraints-

1. The technique is more sensitive, laborious and time-consuming than the conventional chemical fixation procedure.
2. It may take a number of trials and errors to get the desired outcome, which might result in the wastage of samples; thus, the processes required well-trained personnel.

Conclusion

The prepared plastinated specimens were odorless, dry, non-irritant and non-toxic; thereby ideal for close inspection. These advantages made the plastinates desirable among learners and biologists. Plastinated specimens, however, did not totally replace traditional guided animal dissection. The use of plastinated specimens in conjunction with standard wet specimens may be a modern way of teaching and learning veterinary anatomy. The current study provided an affordable, safe and long-lasting method of luminal cast and sheet plastination with commercial silicon sealant. The obtained plastinates could be an efficient tool for gross anatomy instruction, diagnostic imaging and morphological research.

Chapter 6

Recommendations and future perspectives

7.1 Recommendations

The present study suggests the following recommendations-

1. During boiling of the lung specimen, it should be covered with a layer of cotton to ensure complete immersion of the lung in water.
2. An electric or automated slicer is recommended to cut homogeneous sections for sheet plastination.

7.2 Future perspectives

In our future study we intend to-

1. Develop a whole body or whole organ plastination technique with indigenous chemicals
2. Compare the efficacy of different alternate chemicals utilized for modified plastination.

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Appendix A

A questionnaire to evaluate student's perception on plastinated samples

(Part of MS thesis on 'Plastination technique for teaching and research of anatomy')

Year and semester: **Practical Course name:**

1. Which type of samples are commonly demonstrated in the anatomy practical class?

a. Preserved b. Fresh c. Plastinated

2. Do you feel safe/comfortable during handling of those samples?

a. Yes b. No

3. Is the supplied Plastinated sample safer to handle without gloves than the formalin preserved or fresh sample?

a. No b. At some extent c. Very easy and safe

4. Do you detect any odor or health risks while handling the Plastinated samples?

a. Yes b. No

5. Which approach is best for illustrating the three-dimensional orientation and branching structure of the tracheobronchial tree?

a. Plastinated cast b. Preserved sample c. Animated video/image

6. Do you understand the intra-renal segmentation of RA through the cast?

a. Not at all b. Slightly understand c. Completely understand

7. Is the plastinated cast of the external auditory canal helpful in understanding its complex orientation?

a. No b. Slightly useful c. Very useful

8. Which sample best represents the internal structure, color and orientation of the provided brain, spinal cord and kidney slices?

a. Fixed samples b. Sheet plastinated samples

9. Which sample is suitable for handling and flexible enough for close examination?

a. Preserved b. Sheet plastinated

10. Are the sheets portable enough to be studied outside of the practical class?

a. No b. Slightly portable c. Highly portable

11. Are the plastinated samples suitable for museum display?

a. Yes b. No

12. Do you recommend plastinated samples in anatomy practical classes?

a. not recommend b. Highly recommended c. Recommend along with fixed and fresh sample

13. Is it suitable to use plastinated tissues to study comparative anatomy?

a. Not suitable b. Suitable c. Highly suitable

14. Express your degree of satisfaction with the two techniques:

	Very little	Little	Important	Very Important
Formalin	1	2	3	4
Plastination	1	2	3	4

Biography

Farnaz Kader Nova received Doctor of Veterinary Medicine (DVM) degree in 2019 from the Faculty of Veterinary Medicine, Chattogram Veterinary and Animal Sciences University, Khulshi, Chattogram-4225, Bangladesh. She completed her Higher Secondary Certificate Examination from Bangladesh Mohila Samiti Girls' High School and College in 2013 with GPA 5.00 and Secondary School Certificate Examination from Chittagong Govt. Girls' High School with GPA 5.00. Mrs. Nova is now a MS fellow of Anatomy in Department of Anatomy and Histology at Chattogram Veterinary and Animal Sciences University. She has been awarded the National Science and Technology Fellowship from the University Grants Commission, Bangladesh during her MS course. She is the 2nd daughter of Mohammad Abdul Kader and Hamida Khanam.