*Original Research*

# Organ Embalming by Perfusion Technique: A Humane Alternative for Animal Sacrifice in Veterinary Education

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**Abstract**

*A perfusion technique was used to embalm the heart, lungs, liver and kidneys collected from a humanely slaughtered Large White Yorkshire pig. These organs serve as valuable specimens for teaching veterinary anatomy and as an alternative* for procuring *the organs from a purposely sacrificed and embalmed healthy animal. They were perfused with 10% formalin individually and later immersed in 10% formalin for a week till complete fixation was ensured. All the specimens grossly appeared normal in colour and consistency. Subsequent storage of the specimens was done in a bottle cooler at 4°C, thus avoiding wet formalin storage. Tissue pieces from these organs were collected after a month to ensure the effect of fixation. Routine histological processing and sectioning were done. The stained sections showed normal histoarchitecture which confirmed that organ embalming by perfusion technique fixed the tissues, similar to the fixed organs collected from a purposely sacrificed and embalmed animal. The organs prepared in this manner can be used for teaching both gross and microscopic anatomy. These organs can further be used for preparation of dried specimens by plastination techniques.*

**Key words:** Alternative, Embalming, Histology, Pig, Perfusion

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## Introduction

Veterinary education traditionally involved sacrifice and embalming of healthy animals, both for dissection and collection of visceral organs for demonstration in practical classes. Concern with the conservation of bodies existed for more than 500 years; several researchers tried to discover techniques for storage of bodies (eSilva *et al*., 2008). Ethical sourcing of animal cadavers for education and training was also defined in the International Network for Humane Education (inter NICHE) policy (Jukes and Martinson, 2008). University Grants Commission in India repeatedly urged the universities to stop sacrifice of animals for

teaching and suggested use of alternative mechanisms available to provide hands-on-experience to the students ensuring a close observance of high ethical standards, to prohibit the use of sacrificed animals for dissection. Alternate modes for dissection and demonstration were proposed with a lot of challenges ahead for anatomists (Pederson, 2002). Skill laboratories were proposed to be developed by the institutions to train students on interactive alternative models (Jukes and Chiuia, 2003).

In this regard, Willed-Body programme was established in this department to minimize sacrifice of animals for dissection and to prepare humane alternatives in veterinary education. In the earlier practices, unfixed organs were collected from cadavers and stored in formalin. Spot injection of formalin was also carried out for fixation of solid organs. But these methods did not ensure adequate fixation and the organs could not be stored for long term use in the gross anatomy laboratory. The inadequately fixed tissues were also not suitable for preparation of microscopic slides.

As a replacement for organs of animals for demonstration in practical classes, in addition to the resin teaching models being used, teakwood models of visceral organs were also prepared in this department (Kishore, 2018), however, these artificial models did not provide a realistic experience to the students. Therefore, organ embalming by perfusion was done to preserve the heart, lungs, liver and kidneys collected from a humanely slaughtered Large White Yorkshire pig, in life-like condition as much possible, for teaching veterinary anatomy and to provide hands-on-experience to the students.

**Materials and Methods**

As part of implementing the Willed-Body programme and other humane alternatives in veterinary education, the heart, lungs, liver and kidneys were collected immediately from a humanely slaughtered Large White Yorkshire pig in the Department of Livestock Products Technology at NTR College of Veterinary Science, Gannavaram. These organs were embalmed and preserved using the perfusion technique. The organs were washed with water to remove the blood stains and debris. Perfusion of 10% formalin was initially done into the heart, liver and kidneys using a large syringe. Perfusion of heart was done through the aorta and pulmonary artery after ligation of the stubs of the anterior vena cava, posterior vena cava and pulmonary veins till the chambers and vessels were distended. Perfusion of liver was done through the portal vein by directing the flow through all the lobar branches ensuring that all lobes got filled. Perfusion of kidney was done through the renal artery.

Perfusion of lungs was done through trachea using a cadaver injector till the lungs distended to full size. The perfused specimens were immersed in 10% formalin for a week till complete fixation was ensured. The perfused formalin was later drained out, enabling the specimens ready for use, in a life-like condition. The specimens were then preserved in a bottle cooler at 4°C to avoid long term storage in formalin. Tissue pieces from these perfused organs were collected after a month to determine the effect of fixation. Routine





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histological processing and sectioning of these tissues were done. These sections were stained to observe their normal histoarchitecture.

**Results and Discussion**

In the traditional method, which involves sacrifice of healthy animals, bleeding and embalming of the whole body is being done through the common carotid artery. In the recent past, this method attracted ethical concerns from various animal welfare organizations. Hence, several attempts were made by researchers to overcome this concern. In this context, the perfusion technique was employed for embalming and preservation of organs as an alternative to procuring the organs from a live and healthy animal purposely sacrificed and embalmed for laboratory teaching. In addition, the perfused organs were stored in a bottle cooler at 4°C as an alternative to storage in hazardous formalin tanks as Banoo *et al*. (2016) reported that the use of formalin causes respiratory problems. The developed specimens were realistic, soft and flexible and had no offensive odour. These organs retained a life-like condition to provide hands-on-experience to the students (Fig. 1). They served as valuable specimens for teaching veterinary anatomy. This technique can be used for preparation of anatomy specimens in future from the organs discarded after slaughter or from naturally dead donated/disposed cadavers. In this perfusion technique, except for 10% formalin no other chemicals were used. This is advantageous compared to the expensive technique of preparation of glycerinated and plastinated bodies (eSilva *et al*., 2008) and the Elnady technique which also preserves tissues but as dry specimens with the use of many chemicals (Elnady, 2016).

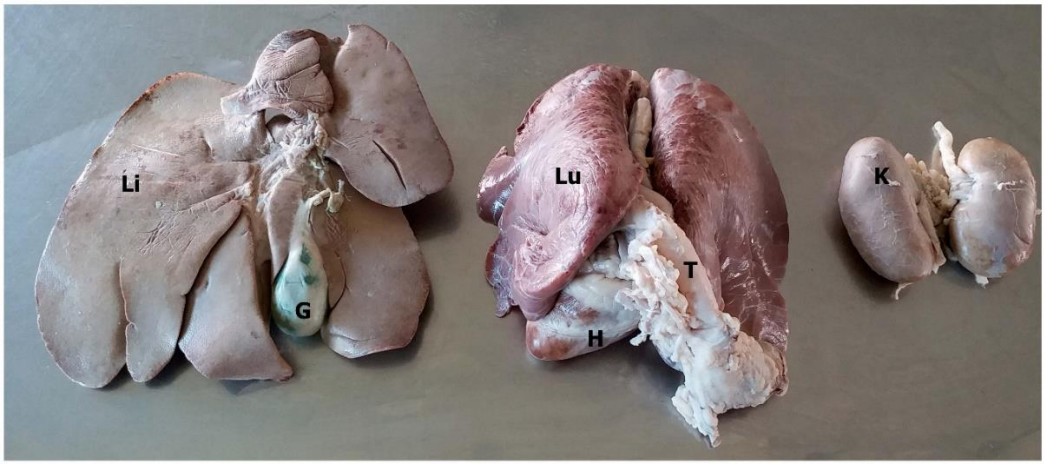


**Fig. 1:** Gross photograph of the embalmed visceral organs of Pig
(H- Heart, Lu- Lung, T- Trachea, Li- Liver, G- Gall bladder, K- Kidney)

In the traditional embalming method, the circulation in the intact animal body is closed and complete which allows the flow of formalin in both halves of the heart. But in the present perfusion technique to embalm the heart, two different approaches were employed as the heart was removed from the intact body leading




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to incomplete circuit of circulation. The formalin perfused through the pulmonary artery fixed the right chambers and that perfused through the aorta, fixed the left chambers along with the wall of the heart through the coronary circulation. Routinely the pig is slaughtered by opting the puncture of heart so that the animal could be brought to death after profuse bleeding but it was replaced and preferably a cut was taken on the throat to have similar bleeding but without damage of heart. Hence, in the present method bleeding was done by a slit in the throat to collect the heart. The intact heart collected in this manner embalmed by the perfusion technique was stored in a bottle cooler at 4°C. This organ was only used for gross studies.

However, tissue pieces were collected from the perfused lungs, liver and kidneys for routine histological procedure. The stained sections of all these organs showed normal histoarchitecture. In routine practice, the fresh tissue or the tissue from a cadaver is collected for processing. The perfusion technique revealed a better histoarchitecture of inhaled lung due to the better fixation after infiltration of 10% formalin up to alveolar level. On the contrary, the partially distended and deflated lung reveals a compact arrangement of parenchyma (Fig. 2). Inhalation in the lung results in expansion of the airways and alveoli in the lung parenchyma (Frandson *et al*., 2009).

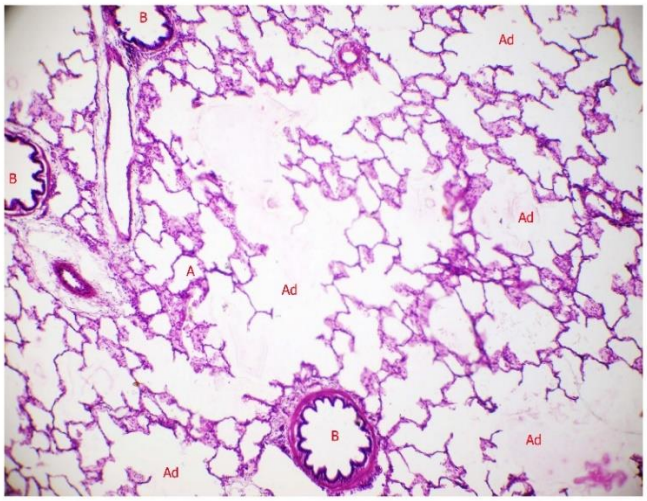


**Fig. 2:** Photomicrograph of the embalmed lungs of Pig
(B- Bronchiole, A- Alveolus, Ad- Alveolar duct)

The liver receives blood both through the hepatic artery and portal vein. In the traditional method of embalming, formalin reaches the liver through the hepatic artery. However, in the present study, perfusion of liver was done through the portal vein as it had a larger diameter offering less resistance and similar distribution as the hepatic artery. This resulted in the better infiltration up to the sinusoidal level giving a good histological detail (Fig. 3).





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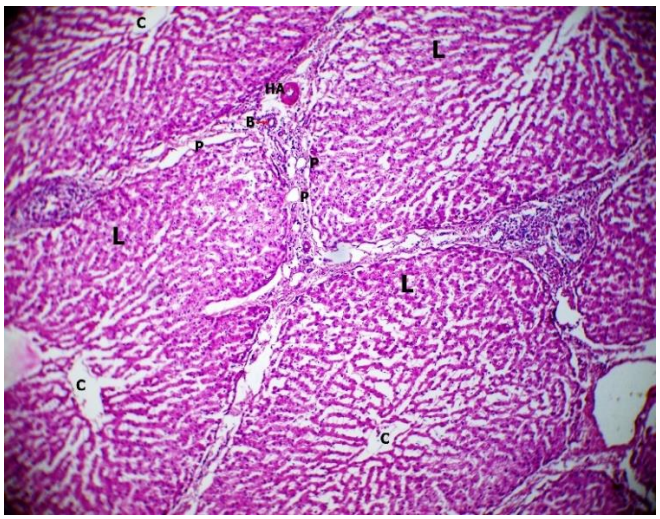


**Fig. 3:** Photomicrograph of the embalmed liver of Pig
(L - Lobule, C - Central vein, P- Portal vein, B- Bile duct, HA– Hepatic artery)

In the traditional method of embalming the animal body, formalin reaches the kidney parenchyma through the renal artery. In this study, the same approach was adopted in the removed kidney and it resulted in complete fixation exhibiting the normal histoarchitecture (Fig. 4).

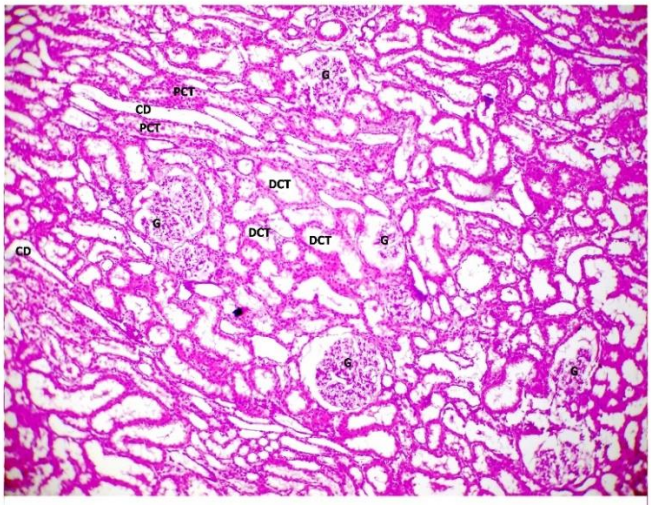


**Fig. 4:** Photomicrograph of the embalmed kidneys of Pig
(G - Glomerulus, PCT - Proximal convoluted tubule, DCT - Distal convoluted tubule, CD - Collecting duct)

These observations confirmed that organ embalming by perfusion fixed the tissues, similar to the fixed organs collected from a purposely sacrificed and embalmed animal. The organs prepared in this manner can therefore be used both for gross anatomy and histology teaching.

Clinical procedures and hands-on-training for surgery; cardiac interventions, lobectomy of lung and renal procedures can also be practiced on these specimens to provide comparable results with a great potential




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for enhancing teaching technological tools against digital and resin alternatives. This technique is cost-effective; it satisfies ethical concerns by replacing the use of purposely sacrificed healthy animals for collection of organs, as in the present study the organs were collected from slaughtered animals and preserved successfully. This method also minimizes water usage and the repeated washing of specimens taken out from the formalin tank for use in the laboratory. They can further be used for preparation of dried specimens by plastination techniques.

**Conclusion**

Organ embalming by perfusion is an alternative for procuring the organs by sacrificing and embalming a healthy animal in veterinary education. The storage of these organs in a bottle cooler at 4°C for periodical use is also an alternative for the long-term storage of such organs in hazardous formalin tanks. Histological sections prepared from these organs after a month's storage in a bottle cooler at 4°C showed normal histoarchitecture confirming that organ embalming by perfusion fixed the tissues, similar to the fixed organs collected from a sacrificed and embalmed animal. The feedback received from the students was positive as these specimens were in a life-like condition with no foul odour/fumes of formalin routinely experienced through conventional methods of embalming. This method also minimizes water usage and the repeated washing of specimens taken out from the formalin tank for use in the laboratory. The organs prepared in this manner can therefore be used for teaching both gross and microscopic anatomy. These organs can further be used for preparation of dried specimens by plastination techniques.

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