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Efficacy of different plastination techniques in lungs of goat: A comparative morphometric study

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Plastination is a procedure through which living specimen can be processed and preserved in their near-natural state. In this, the lipids and the water present in the lung specimens is replaced with synthetic components like polyester or silicone etc., and then after the hardening of these materials they are ready to be used for teaching and research purposes. In this study the impregnation and curing materials used were the ones which were cheap and readily available in the market. The most striking feature of this technique over conventional formalin-based preservation is the anatomical detail and ease of handling of specimen without being carcinogenic or cause of contact dermatitis and conjunctivitis (Sittel *et al.* 1997). Plastinated specimens are preferred to models and organs preserved in formaldehyde for teaching and research purposes (Sethi *et al.* 2008). These plastinates can be used to explain the structural details in better way in museum display, demonstration and discussion than in glass jars (Mohan *et al.* 2020). The present study was undertaken to observe the compare the gross morphological and biometric parameters on the formalin fixed and plastinated specimens of adult goats, and to determine the relative efficiency of both the plastination methods by morphometric analysis by calculating the percentage of shrinkage and morphological difference related to color, texture and elasticity of the specimens.

Collection and fixation of samples: Lung specimens from 12 apparently healthy adult goats were collected immediately after slaughtering and fixed in 10% neutral buffered formalin (NBF) for 1 month. First of all the lungs were cleaned and observed for gross morphological details such as colour, consistency, shape and number of different lobes in right and left lungs. The length (in cm) of both the lungs was measured from apex to base by an inelastic thread and a calibrated scale and its weight (g) by an electric balance.

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Methods of plastination: After gross biometrical study of formalin fixed lungs, the animals were divided into two groups, i.e. group I and II with 6 animals in each group. In present study, the lungs of group I were processed by glycerine method of plastination (Girish *et al.* 2022) and those of group II by indigenous plastination /low cost innovative technique (Ramakrishana and Nanjappa 2018, Bansal *et al.* 2022). Steps involved in the standard protocol of plastination were followed in both the techniques i.e. fixation, dehydration, impregnation and curing.

Glycerine technique of plastination: Fixation: Right and left lungs specimens of group I were fixed in 10% neutral buffered saline for about 1 month.

Dehydration: It was done by using 100% acetone for 2-3 changes in a glass container with a tight lid. Each change lasting for about 2 weeks till there was no change in color of acetone.

Impregnation: The specimens were immersed in glycerine for two changes of 15 days each. The specimens were taken out and hanged to allow drainage of residual glycerine.

Curing: It was done by entirely covering the specimens with corn flour and wrapped in layers of muslin cloth to absorb excess glycerine which took 15 days to completely cured. The specimens were now permanent preserved organs, which to be used for years, when not in use can be simply covered in a plastic bag and sealed.

Indigenous plastination technique: Fixation and dehydration: Right and left lungs specimens of group II were processed by similar method as mentioned in method I.

Impregnation: The specimens were dipped in modified indigenous plastination solution (150 g thermocol and 500 g petroleum Jelly in 10 L of chloroform) for two changes of 15 days each. The excess of impregnation material was drained from plastinates by hanging them for few days till complete drainage. The plastinates obtained by this technique took approximately 5 to 8 days for complete drainage of impregnation material.

After plastination, the length and weight of the lungs was measured and compared with the formalin fixed

and plastinated ones prepared by Method I and II. The morphological details and biometrical data obtained by two methods was statistically analysed to observe the shrinkage of the specimens. The data was subjected to one way analysis of variance (ANOVA) to find out significant differences between the formalin fixed and plastinated specimens and was calculated by applying 't' test to observe the significant difference when p-value was less than 0.05 ($p<0.05$).

Morphology and biometry of formalin fixed specimens: Morphological examination of formalin fixed lungs from all goats showed that the lungs of goat were cone shaped with narrow apex and broad base. The fresh lungs were spongy to touch and reddish pink in colour, (Fig. 1A) which became dull and reddish grey in colour after fixation (Fig. 1B). The right lung was divided into four lobes i.e. apical, cardiac, intermediate and diaphragmatic lobe, whereas the left lung was having two lobes i.e. apical and diaphragmatic lobe (Fig. 2A and 2B). As there was no clear cut demarcation by interlobar fissure between apical and cardiac lobes and is described as apico-cardiac lobe (Vij *et al.* 2018). The apical lobe of the right lung was mostly divided into cranial and caudal parts. The division of apical lobe of right lung was observed in 9 samples out of 12. But in few samples (3), there was no division of apical lobe in right lung. The variation in the division of apical lobe may be due to variation in breeds. Yousif and Dawood (2019) noticed undivided apical lobe in right lung, but divided one in left lung in goats.

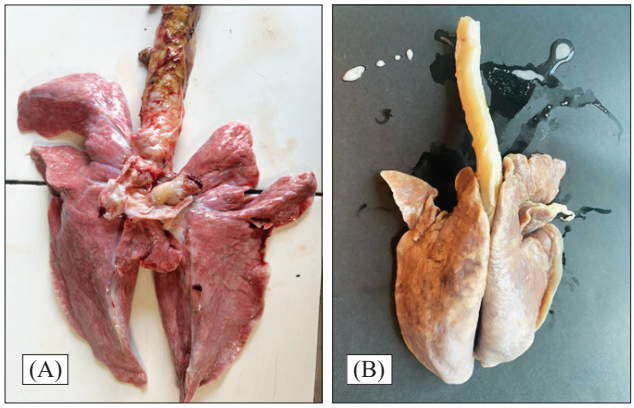

Fig. 1. (A) Fresh goat lung specimen; (B) Formalin fixed goat lung specimen.

The average weight of the lungs was 368.83 g, which was almost half than the that of Gaddi goat as 711.00±48.17 g as recorded by Vij *et al.* (2018), and in goats as 816.00±194.00 g and 650.00-700.00 g reported by

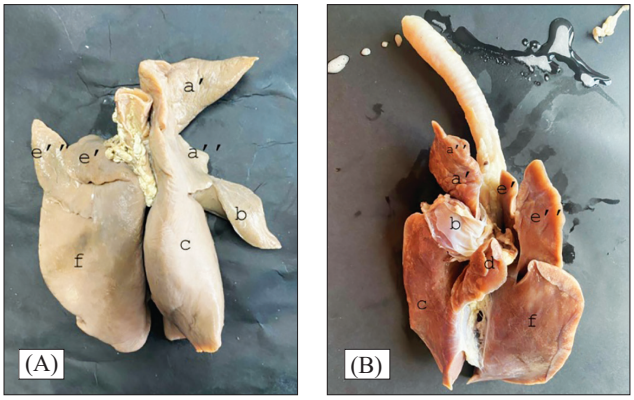

Fig. 2. (A & B) Showing parietal and visceral view of different lobes of goat lung: a' (right apico cranial lobe), a''(right apico caudal lobe), b (right cardiac lobe), c (right diaphragmatic lobe), d (right intermediate lobe), e'(left apico cranial lobe), e'' (left apico caudal lobe), f (left diaphragmatic lobe).

Alam *et al.* (2011) and Al-Sadi (2005), respectively. The average length of right and left lung from base to apex was found to be 21.33±0.44 cm and 18.4±0.73 cm, respectively. The difference in the base to apex length of lungs was higher in the right than the left one. This may be due to larger size and lobes of the right lung than the left one as reported by Vij *et al.* (2018) in goat.

Comparison of formalin fixed and glycerine plastinates: The glycerine plastinates were soft to touch with minimal color change and were nearly normal in texture (Fig. 3). All the demarcation between different lobes were observed without any distortions on the lung borders. The study conducted by Silvia *et al.* (2011) also showed that the glycerine is a good preservative to maintain the appearance, consistency and visibility of the structures in the central nervous system of animals as compared to formalin.

Fig. 3. Glycerine plastinated goat lung specimen.

Table 1. Biometrical data on shrinkage of formalin fixed and glycerine plastinated specimens

Lung parameter	Formalin fixed	Glycerine plastinates	Shrinkage percentage
Weight (g)	368.83±9.67[a]	185.23±17.26[b]	49.77%
Length RBA (cm)	20.8±0.34[a]	17.9±0.92[a]	13.94%
Length LBA (cm)	18.4±0.73[a]	15.75±0.81[a]	14.40%

Data having at least one similar superscript do not differ significantly ($p>0.05$).

Table 2. Shrinkage in individual lobes of formalin fixed and glycerin plastinates

Lung lobe	Ln(ff)	Ln(gt)	% shrinkage	Bd(ff)	Bd(gt)	% shrinkage	Th(ff)	Th(gt)	% shrinkage
Right apico-cranial lobe	6.37±0.63	5.36±0.78	15.86	9.23±0.59	8.9±0.98	3.58	1.66±0.12	1.3±0.57	21.69
Right apico-caudal lobe	5.4±0.26	4.73±0.40	12.41	6.7±0.5	6.53±0.5	2.54	1.04±0.16	1±0.1	3.85
Right cardiac lobe	5.13±0.63	4.7±0.58	8.38	8.73±0.17	7.6±0.86	12.94	1.68±0.1	1.5±0.05	10.71
Right intermediate lobe	6.53±0.6	6.4±0.81	1.99	3.6±0.26	2.66±0.38	26.11	1.27±0.21	1.12±0.04	11.81
Right diaphragmatic lobe	12.86±0.5	10.03±0.31	22.01	9.8±1	7.9±0.56	19.39	3.63±0.13	2.83±0.17	22.04
Left apico-cranial lobe	6.6±0.87	5.53±0.03	16.21	5.46±0.29	5.03±0.18	7.88	0.96±0.11	0.8±0.15	16.67
Left apico-caudal lobe	4.53±0.38	3.6±0.75	20.53	9.2±0.32	7.9±0.02	14.13	1.36±0.13	1.08±0.10	20.59
Left diaphragmatic lobe	11.66±0.17	10.13±0.56	13.12	10±0.34	8.5±0.75	15.00	3.3±0.30	2.86±0.38	13.33

The biometrical data of plastinated and formalin fixed specimens indicated that the mean weight of lungs in group I was 368.83±9.67 g which reduced to be 185.23±17.26 g in glycerine mounts showing 49.77% shrinkage in the weight. The average base to apex length of right (RBA) decreased from 20.8±0.34 cm to 17.9±0.92 cm, and that of left (LBA) from 18.4±0.73 cm to 15.75±0.81 cm. The percentage of shrinkage was slightly more in left lung than the right one. Table 1 showed the shrinkage observed in weight of lung specimens plastinated by glycerine technique of plastination showed significant difference ($p<0.05$) from that of formalin fixed specimens. However, the apex to base length of right and left lung did not show any significant difference in formalin fixed and glycerine plastinates.

Lobe-wise shrinkage of formalin fixed and glycerine plastinates: The length, breadth and thickness of all the lobes of right and left lungs was measured before and after plastination. The data was compared and it was found that the maximum shrinkage occurred in the length of right diaphragmatic lobe and minimum in right intermediate lobe. The breadth and thickness wise shrinkage was minimum in right apico-caudal lobe. Maximum shrinkage was noticed in the breadth in right intermediate lobe and thickness of left apicocaudal lobe. The biometrical data representing the average length (Ln), breadth (Bd), thickness (Th) in centimeters of different lobes of goat lungs in formalin fixed (ff) and glycerin plastinates (gt) in Table 2.

Comparison of Formalin fixed and Indigenous plastinates: The morphological characteristics of indigenous plastinates were very hard to touch, totally lost their elasticity, distorted lung borders and the color changed to whitish grey (Fig. 4). Similar findings have been recorded by Shamasundar (1996). The average weight of formalin fixed lungs in this group was recorded as 276.83±12.83 g which decreased to 43.72±5.01 g in indigenous plastinates. The average length from base to apex in right and left lung was 21.33±0.44 cm and 17.93±0.41 cm in formalin fixed specimens, which decreased as 13.81±0.94 cm and 11.73±0.43 cm in indigenous plastinates, respectively.

Fig. 4. Indigenously plastinated goat lung specimen.

Table 3. Biometrical data on shrinkage of formalin fixed and indigenous plastinated specimens

Lung parameter	Formalin fixed	Indigenous plastinates	Shrinkage percentage
Weight (g)	276.83±12.83[a]	43.72±5.01[b]	84.20%
Length RBA (cm)	21.33±0.44[a]	13.81±0.94[a]	35.25%
Length LBA (cm)	17.93±0.41[a]	11.73± 0.43[a]	34.57%

Data having at least one similar superscript do not differ significantly (p>0.05).

The percentage of shrinkage was 84.20% in weight, while remained almost same (35% approximately) in length of right and left lung as depicted in Table 2. The present data showed that there was a significant difference (p<0.05) in the weight of formalin fixed and indigenously plastinated specimens. But the length from apex to base did not vary significantly in formalin fixed and plastinated specimens of right and left lungs.

Comparison of glycerine plastinates and Indigenous plastinates: Morphological analysis showed that the glycerine fixed lungs elucidated the minimal changes in color, texture and flexibility as compared to the modified plastinated method. According to Shetty *et al* (2023), formalin-fixed soft tissue were dull and light in colour whereas plastinated specimen appeared bright and glossy. The consistency of soft tissue fixed in formalin was firm, and slightly flexible in plastinates. Tables 1 and 3 showed that the shrinkage percentage in weight was 49.77% in glycerine mounts which increased by indigenous method as much as 84.20%. After plastination, soft tissues showed average shrinkage of 3.49% with the range of 0.80–7.90% in comparison to the original size. The shrinkage of length of right and left lung was 2.5 times more in method II than I. Similarly, Hassan and Sawad (2021) observed that the pelvic girdle of dogs remained in natural color and shape without any shrinkage in glycerine mounts.

When compared the biometrical data of plastinated specimens with that of their formalin fixed specimens, shrinkage in terms of weight and length was more evident in samples plastinated by modified plastination technique than glycerine fixed specimens. The study conducted by Pandit *et al. (*2015) also showed that Orthocryl and Epoxy resins retained maximum colour with minimal shrinkage while maximum discoloration was with polypropylene plastinates. Mohan *et al.* (2020) compared the four indigenous methods of plastination and concluded that polyester dye-cast resin gave best results than other three methods of plastination.

The present study showed that the morphological details were nearly normal with comparatively less shrinkage in glycerine plastinates in goat lungs. So, it is recommended that the glycerine technique of plastination should be preferred to indigenous method.

SUMMARY

The study was conducted on apparently healthy caprine lungs to evaluate the efficacy of different plastination techniques by gross biometrical and morphological analysis of the formalin fixed and plastinated specimens. The gross biometrical analysis was made on all the formalin fixed lungs and after that the samples were divided as Group I and Group II with 6 animals in each group. In method I, after proper fixation, the specimens were dehydrated in three changes of acetone (two weeks each), then impregnated in two changes of glycerol for 15 days each and cured in muslin cloth containing corn starch. In method II, modified plastination solution was used as impregnation solution. The plastinates prepared by method I were soft to touch, odorless, the elasticity and colour of lung tissue was near normal, whereas the lungs plastinated by method II were hard to touch and their color turned to whitish grey. The biometric data showed that the shrinkage in weight of lungs was 47.99% in method I and 85.46% in method II. The shrinkage in length of apex to base of right lung was 14.36% and that of left lung was 16.18% by method I. In method II, the length from apex to base was reduced to be 35.84% and 34.83 in right and left lungs, respectively. It was concluded from present study that the plastinated glycerine specimens (Method I) showed lesser deviations from their near natural state as compared to the plastinates prepared by Indigenous method (Method II). The shrinkage was more evident in plastinates prepared Indigenous method (Method II) than by glycerine method (Method I). So, it is recommended that glycerine method of plastination should be preferred to any other method of plastination.

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