

Histomorphometric Study of Tunics of Ductus Arteriosus in Human Foetal Cadavers Using Image J Software

Meghna S. Manohar², Sundarapandian Subramanian¹, Balakrishnan Ramamoorthy¹, Ashma A. Latiff¹, Ezhilarasi B.¹

¹Department of Anatomy, SRM Medical College Hospital and Research Centre, SRM Institute of Science and Technology, SRM Nagar, Kattankulathur, Kanchipuram, Chennai, TN, India

²Panimalar Medical College Hospital and Research Institute, Varadharajapuram, Poonamallee, Chennai. Affiliated to The TN Dr. MGR Medical University, Chennai

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ABSTRACT

Introduction: Ductus arteriosus (DA) connects the pulmonary artery with the aorta during fetal life. Though it connects two elastic arteries, studies have shown that it is a muscular artery. There are very few studies on the histomorphometry of human fetal cadaveric DA. The changes in the tunics of the DA at various stages of fetal development have not been studied extensively. The aim of the present study is to observe the histomorphometric features of ductus arteriosus and its histological variations according to the gestational age of the fetus.

Methods: Ductus arteriosus dissected from 34 fetal cadavers of different gestational ages were stained with standard eosin and haematoxylin staining (H & E). The structure of ductus arteriosus was then studied under a light microscope. The thickness of each layer of DA was measured using Image J software.

Results: the thickness of the DA wall was directly proportional to the gestational age of the fetus. In each trimester distinct histological changes were observed in the tunics.

Conclusion: Formation of multiple mounds and increase in intimal thickness observed during the last trimester is mainly responsible for closure of the ductus after birth. Elastosis is associated with PDA. Disappearance of elastosis in the later gestational age ensures closure of ductus.

Keywords: Histomorphometry; Fetal cadavers; Ductus Arteriosus; H & E stain; Image J software.

Introduction

The ductus arteriosus develops from the distal part of the sixth aortic arch. It is a vascular structure connecting the proximal part of the descending aorta with the pulmonary trunk¹. The ductus arteriosus shunts most of the deoxygenated fetal blood returning from the head, upper extremities, and coronary sinus through the right ventricle into the descending aorta because of the high vascular resistance of the collapsed fetal lung^{2,3}. The ductus maintains its tubular shape with its lumen diameter and caliber increasing with an increase in the gestational week to reach the size equal to that part of the aorta with which it joins⁴. However, at birth rapid changes in size and shape leading to constriction and closure are observed. Although it directly connects two elastic arteries, the ductus is a muscular artery⁵. The DA has all the three generally recognized layers, tunica intima (TI), tunica media (TM) and tunica adventitia (TA). The functional closure of DA occurs immediately after birth in 90% of full-term babies within 48 hours of birth. The structural closure of DA occurs due to proliferation of intimal cushion (IC) and permanent closure occurs with complete obliteration in 1-3 months after birth.

The paucity of histomorphometric analysis along with histological correlation, on normal developmental

stages of ductus arteriosus lead to the current study. The aim of the current study was to observe and document,

- Histomorphometry of each tunic (tunica intima, tunica media, tunica adventitia) of ductus arteriosus.
- The microanatomy of ductus arteriosus at various levels: near the aorta, intermediate position, near the pulmonary trunk.
- The histomorphometric changes with gestational age.

Materials and Methods

Thirty-four aborted/dead born fetuses aged 9 - 40 weeks (2 months - 9 months) were collected from the Department of Obstetrics and Gynecology, SRM Medical College Hospital and Research Centre, Kattankulathur, India. These fetuses were embalmed in the Department of Anatomy, SRM Medical College Hospital and Research Centre, Kattankulathur, India.

Inclusion criteria: Spontaneously aborted fetuses from 9 weeks to 40 weeks, stillborn fetuses, and intra-uterine deaths.

Exclusion criteria: Fetuses with identified congenital anomaly.

Categorization of the fetuses: Based on the gestational age, collected fetal samples were

categorized into 3 groups,

GROUP I - First trimester (9th to 13th week)

GROUP II- Second trimester (14th to 26th week)

GROUP III- Third trimester (27th to 40th week)

Dissection procedure: The thoracic cavity was opened with an inverted Y-shaped incision from the jugular notch to the xiphoid process. At the level of aortic and pulmonary roots, a transverse incision was made, and DA was dissected out along with the above-mentioned great arteries (Fig. 1).

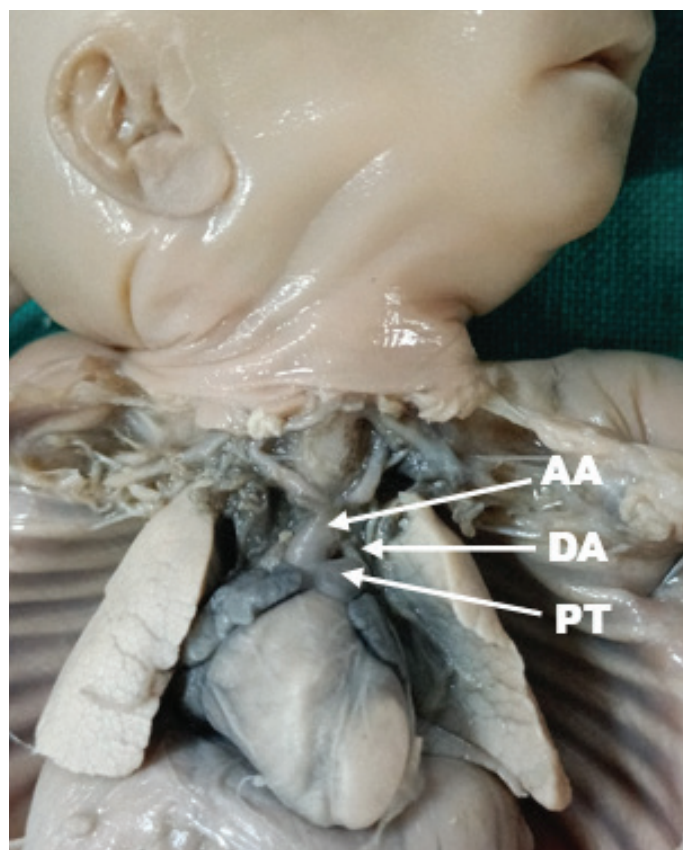


Figure 1. Dissection of thoracic cavity of fetus showing, AA- Arch of Aorta, DA- Ductus Arteriosus, PT- Pulmonary Trunk.

Histotechnique: Fixation of the tissue material in 10% formalin for 24 hours was done. After adequate fixation, 3 transverse sections were taken from each sample. The first section was DA near the pulmonary trunk, the second section was taken from the middle of the ductus and the third section was cut near to the aorta. The tissue was embedded in paraffin, serially sectioned at 5 microns, and stained with haematoxylin and eosin using standard procedure.

Table 1. Thickness of ductal wall with respect to gestational age of fetus.

Trimester	Wks. of gestation	Sample size	Thickness of DA layers (mm)									Total
			Tunica intima			Tunica media			Tunica adventitia			
			Max	Min	Avg.	Max	Min	Avg.	Max	Min	Avg.	
I	9-13	3	0.0371	0.0186	0.0279	0.2540	0.1814	0.2177	0.1253	0.0697	0.0975	0.3431
II	14-26	28	0.0991	0.0340	0.0666	0.7797	0.1910	0.4854	0.1458	0.0937	0.1198	0.6718
III	27-40	3	0.3767	0.1080	0.2424	0.9981	0.2833	0.6407	0.2361	0.1679	0.2020	1.0851

Histomorphometric analysis: “Image J” software was used for histomorphometric analysis. Photographs of slides were taken from the light microscope after focusing in low (40X), high (100X) and very high (400 X) magnifications. Photographs were taken using a mobile phone camera with Carl Zeiss lenses. The thickness of each layer of the ductal wall was analyzed using Image J software. The thickness of intima, media and adventitia were taken at three different points of the vessel wall and three values were obtained for each layer in each sample. The mean of the three values was taken as the thickness of that tunic. The measurements were then correlated with the gestational age of the fetus and the histological findings in the respective age.

Results

Histomorphometry: The thickness of the three tunics of the ductus arteriosus in the three trimesters observed is given in Table 1. The total thickness of the wall of DA increased with gestational age. The thickness of each tunic also increased with gestational age. The thickness of intima is increased by nearly 4 folds from 2nd trimester to 3rd trimester. This confirms the appearance of intimal mounds in third trimester.

Group I: 2 - 4 months (9 - 13 weeks) of gestational age (Fig.2):

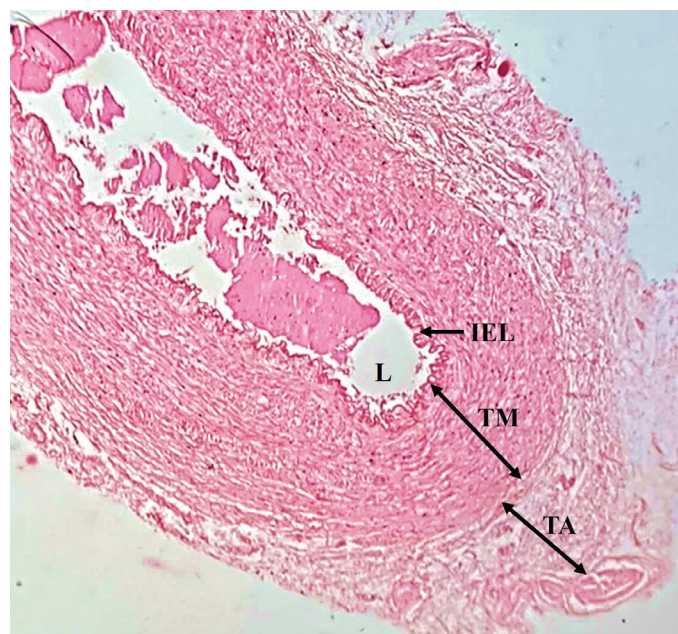


Figure 2. High magnification (400x) of DA from 2 - 4 months (9 - 13 weeks) of gestational age. L- Lumen, IEL- Internal Elastic Lamina, TM- Tunica media, TA- Tunica Adventitia.

In this group there were 3 fetuses. In tunica intima, internal elastic lamina was very prominent. The absence of intimal cushion was the distinctive feature of this trimester. Tunica media was made up predominantly of smooth muscle cells with very rare and frail elastic fibers and was well demarcated from the other tunics. Tunica adventitia was made of connective tissue and elastic fibers.

Group II: 4 - 6 months (14 - 26 weeks) of gestational age (Fig.3):

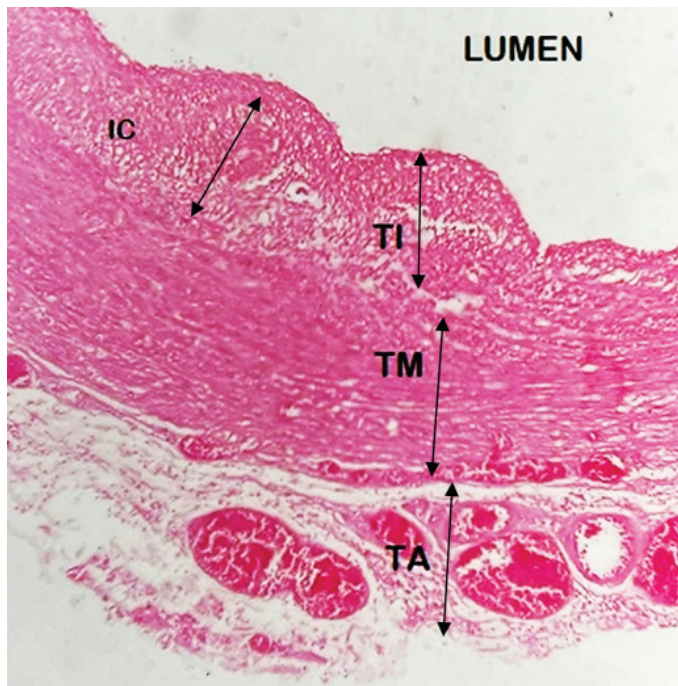


Figure 3. High magnification (400x) of the tunics of ductus arteriosus, 4 - 6 months (14 - 26 weeks) of gestational age. TI- Tunica intima, TM- Tunica media, TA- Tunica Adventitia, IC- Intimal Cushion.

28 fetuses were studied under this group. The ductus arteriosus of this group resembled a muscular artery. In the 3 - 5 months group, initial stages of the proliferation of tunica intima were seen. In the 5 - 6 months sections, the histological features leading to ductal closure were observed. The internal elastic lamina was fragmented. The appearance of intimal clear spaces was observed, and the beginning of the formation of intimal cushions or thickenings were seen. The intimal cushions were seen protruding towards the lumen in this stage. The tunica media showed predominantly smooth muscle cells irrespective of the gestational age in this group. The adventitia was made up of connective tissue with plenty of vasa vasorum.

Group III: 6 - 9 months (27 - 40 weeks) of gestational age (Fig.4, Fig.5):

In this group, the three layers of ductal walls were clearly visible. The tunica intima increased in thickness with the presence of well-formed intimal cushions made of elastic and smooth muscle fibers protruding into the lumen. The 6 - 7 months fetuses showed obliquely oriented smooth muscles migrating from tunica media to intima. In the full-term fetuses,

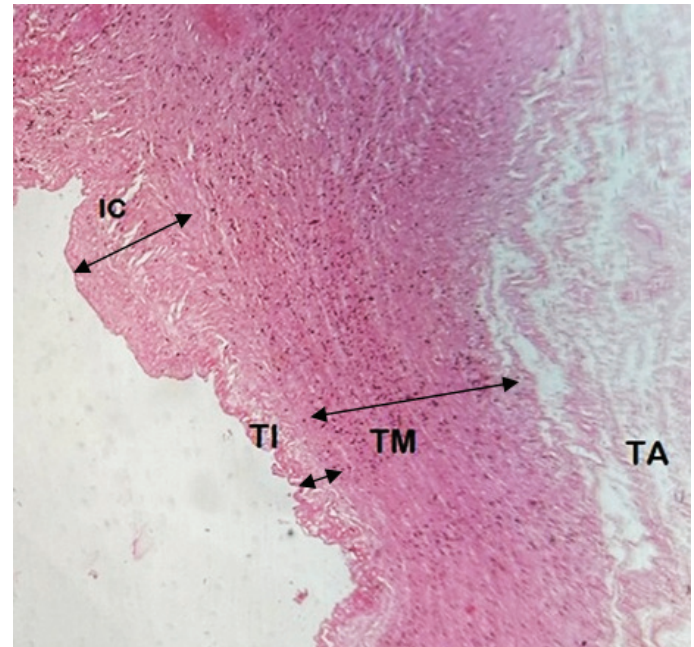


Figure 4. High magnification (400x) of the tunics of DA, 6 - 9 months (27 - 40 weeks) of gestational age. TI- Tunica intima, TM- Tunica media, TA- Tunica Adventitia, IC- Intimal Cushion.

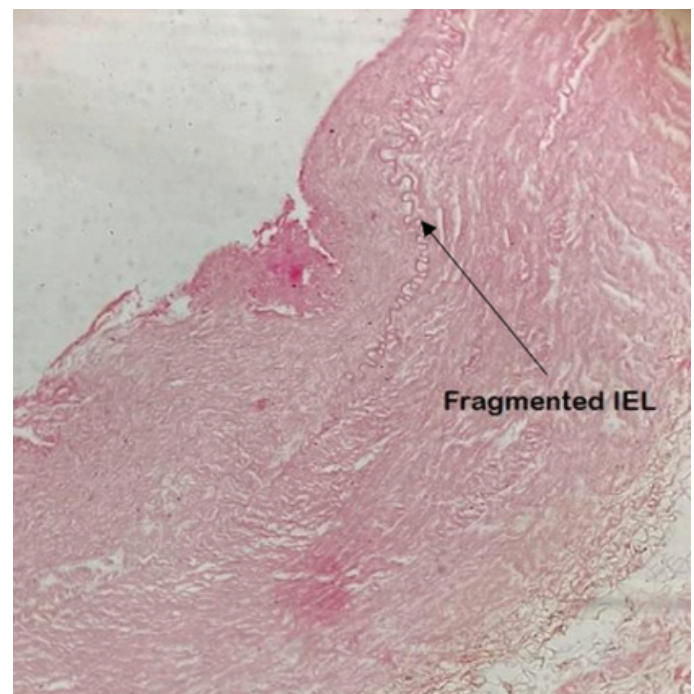


Figure 5. High magnification (400x) of the tunics of DA, 6 - 9 months (27 - 40 weeks) of gestational age. IEL- Fragmented Internal Elastic Lamina.

intimal cushions were well-formed and proliferation of subendothelial connective tissue was seen. The internal elastic lamina was disrupted. The tunica media was made of smooth muscle fibers. The thickness of tunica intima was found to be increased significantly. There were not many changes noted in the thickness of tunica media in this group. The adventitia was made of connective tissue and vasa vasorum.

Histology at the transitional zones (Near the junction of DA with pulmonary trunk and near the junction of DA with aorta):

At the junction between ductus and pulmonary trunk, the section largely resembled an elastic artery with tunica media comprising of elastic fibers with very few smooth muscle cells. In the transitional zone between ductus and the aorta, the tunica media of ductus blended with the tunica media of the aorta and concentric lamellae of elastic fibers were seen in the media. The histology of transition zone was similar in all the three groups with no significant differences except for decrease in elastic fibers in media nearing term.

Ductus Elastosis:

12 out of 34 specimens contained elastic fibers in the tunica media and showed laminar elastosis or aortification which is a primary characteristic associated with patent ductus arteriosus.

Discussion

Microanatomy of DA: In the present study, ductus arteriosus showed characteristics of a muscular artery that was different from that of the aorta and pulmonary trunk which are elastic arteries. This matches with the findings of Hughes AF, whose study aided in the classification of arteries developing from the aortic arches into three basic categories based on histological features: Type I & Type II belonging to elastic arteries and Type III as muscular arteries⁶. The observation of ductus arteriosus being a muscular artery in the present study relates to the results of the study conducted by Yokoyama Utako *et al.*⁷. The reason for this was attributed in their study to the suppression of elastogenesis by EP4 signaling in the DA but not in the aorta when LOX protein was degraded, resulting in PGE2. Leonard ME *et al.*⁸. observed that the left recurrent laryngeal branch of the Vagus nerve enwrapping the ductus arteriosus as a sling like support is responsible for the histological difference, allowing it to develop as a muscular artery in contrast to the arteries that develop from the adjacent unsupported aortic arches, which are elastic.

Tunica intima: Lining endothelium was visible without a prominent subendothelial connective tissue in the present study which is like the observations of Jothi GR in which the subendothelial connective tissue was barely visible⁹. The appearance of intimal cushions from the second trimester were also observed by Desligneres S, Larroche JC⁵. It was noted that the intima is thickened with 'cushions' made of cells and metachromatic substances and these cushions are a thickening of media rather than intima. The intimal thickening with disrupted internal elastic lamina and smooth cells from media migrating towards the intima were also noted by Meera Kuganathan *et al.*¹⁰. The discontinuity was attributed to the intimal smooth muscles showing connections with cells of tunica media.

Tunica media: The tunica media of the ductus arteriosus was observed to be made of predominantly smooth muscle cells by Gournay Véronique as in our present observation¹¹. In the present study, the tunica media in almost all samples was composed of circularly arranged smooth muscle fibers. But in some cases, outer longitudinal bundles were seen in sections surrounded by connective tissue near the adventitia⁹. The tunica media is mostly comprised of smooth muscle cells and very few elastic fibers⁵. They also found clear spaces in between the muscle fibers filled with metachromatic substances which were not found in the present study. Costa M *et al.* observed well defined external elastic lamina. But in our observations, the external elastic lamina was ill defined or absent⁴.

Tunica adventitia: In the present study, the tunica adventitia comprises more of connective tissue elements, vasa vasorum and vasa nervosum. These observations are in accordance with the observations of Meera Kuganathan *et al.*¹⁰.

Transitional zones: Ågren P *et al.*, on chicken ductus arteriosus had similar findings as current study in the transitional zone¹². The presence of elastic fibers in the outer segment of the media and muscle fibers in the inner segment of the media was noted in some samples. At the junction between the pulmonary artery and ductus arteriosus, the elastic fibers from the pulmonary artery continued to the external part of the media of ductus near the adventitia while the inner part of media shows the typical features of the ductus Desligneres S, Larroche JC⁵. Danesino *et al.* observed that the tunica media was composed of circularly arranged smooth muscle fibers and interspersed mucoid material¹³.

Histomorphometry: The thickness of the three tunics was measured in two other studies and the findings were like the current study^{10,14}. These studies noted an increase in thickness of the wall of DA with the increase in gestational age of the fetus.

Closure of ductus: Fetuses above six months of gestational age showed histological differences that lead to the obliteration of DA. The observations of Yokoyama *et al.* suggested that the obliteration of the DA after birth is significantly related to the decreased amounts of elastic fibers⁷. Fetuses in early trimesters showed elastosis in the current study while late trimester showed reduction in elastic fibers. This supports the theory of patent ductus arteriosus being associated with elastosis. This was noted in the studies conducted by Toda T *et al.*, Chaqui B and S Desligneres and J Cl. Larroche^{15,16,5}.

Veronique Gournay noted that in the media of the ductus arteriosus there are predominantly smooth muscle cells, rather than the circumferentially oriented layers of elastic fibers that make up the media of the aorta and pulmonary artery¹¹. The lumen of the ductus arteriosus narrows and the ductus arteriosus shortens due to the smooth

muscle cell contraction present in the media which leads to obliteration. The beginning of intimal thickening is seen as early as the fourth month of the second trimester with disruption of the internal elastic lamina in the present study, while in a similar study by Desligneres S, Larroche JC, the appearance of intimal cushions and the disruption of the internal elastic lamina is observed only after 32 weeks of gestation which contributes to the closure of DA⁵. The appearance of intimal mounds as a focal thickening on one side of the wall in the early months of the second trimester and the formation of multiple mounds in the later stages are like the observations made by Meera Kuganathan *et al.*¹⁰. The initiation of the obliteration process of DA is marked by the migration of smooth muscle fibers from tunica media to tunica intima wherein we can observe the oblique direction of muscle fibers entering the intima disrupting the internal elastic lamina. Hence, we can see the fragmented internal elastic lamina¹⁷.

In this study on histomorphometry of DA in fetal cadavers, thickness of each layer of DA was directly proportional to the gestational age of the fetus. In each trimester distinct histological changes were observed. The appearance of intimal mounds was first observed in the second trimester as a focal thickening at any one side of the wall. The formation of intercellular spaces, migration of smooth muscles from media to intima and disruption of internal elastic lamina occurred gradually with an increase in gestational age of fetuses. Formation of multiple mounds and increase in intimal thickness was observed during the last trimester which marks the onset of obliteration process of DA.

Ethics Statement

This study was approved by the Institutional Ethics Committee of SRM Medical College Hospital and Research Centre. Approval number 2885/IEC/2021. As the fetal cadavers were voluntarily donated by the parents, consent is waived for the study.

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Mini Curriculum and Author's Contribution

1. Meghna S Manohar – MSc Medical Anatomy. Contribution: Effective intellectual participation for the study; technical procedures; data acquisition.
2. Sundarapandian Subramanian– M.D. Anatomy, PhD. Contribution: Conceptualization of the study; effective intellectual participation for the study; critical review and final approval. ORCID: 0000-0001-8796-6681

3. Balakrishnan Ramamoorthy- M.D. Anatomy. Contribution: Data interpretation; critical review. ORCID: 0000-0002-4301-8978
4. Ashma A Latiff- M.D. Anatomy. Contribution: Data interpretation; preparation and draft of the manuscript. ORCID: 0000-0002-0931-3404
5. Ezhilarasi B- MSc Lab Technology. Contribution: Technical procedures.

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Corresponding author
Ashma A Latiff
E-mail: ashmaarul@gmail.com