

Gestational age-dependent changes in human ductus arteriosus: A cadaveric histomorphometric and developmental analysis

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Abstract. *Background and aim:* The ductus arteriosus (DA) is a critical vascular structure in fetal circulation, shunting blood from the pulmonary artery to the aorta. The study investigates the histomorphometric characteristics of the ductus arteriosus (DA) in human fetuses, exploring its developmental changes across gestational ages. *Methods:* This cross-sectional observational study examined 20 human fetal cadavers ranging from 17 to 37 weeks gestation, stratified into two groups: Group I (17th-24th week, n=10) and Group II (25th-37th week, n=10). Gross morphometric analysis included measurements of DA length, diameter at aortic and pulmonary ends, and angular orientation relative to the aorta. Histological examination involved H&E staining, with quantitative analysis of tunica media thickness. *Results:* Gross anatomical measurements showed significant growth of DA from Group I to Group II. Angular measurements showed more modest changes, with the upstream angle increasing from $52.9^\circ \pm 3.5^\circ$ to $61.0^\circ \pm 4.8^\circ$ and downstream angle from $92.9^\circ \pm 7.9^\circ$ to $103.8^\circ \pm 5.2^\circ$, exhibiting weak correlations with gestational age. Histologically, DA demonstrated a typical muscular artery structure. Group II fetuses exhibited more prominent internal and external elastic laminae. The tunica media showed notable differences, with Group I displaying a predominance of fibroblasts, while Group II fetuses had a thicker tunica media with more organized concentric lamellae. *Conclusions:* The relative stability of DA's angular orientation suggests evolutionary optimization of fetal hemodynamics. The progressive organization and thickening of the tunica media, along with the development of elastic laminae, indicate a structural maturation process likely underpinning the DA's functional capabilities. (www.actabiomedica.it)

Key words: ductus arteriosus, fetal development, histology, morphometry, gestational age

Introduction

The ductus arteriosus (DA) is a significant vascular structure in fetal circulation, serving as a vital shunt between the pulmonary artery and the aorta (1). This unique vessel plays a pivotal role in fetal hemodynamics by diverting approximately 85-90% of the right ventricular output away from the high-resistance pulmonary circulation and into the systemic circulation

(2,3). The proper development and function of the DA is essential for normal fetal growth and a smooth transition to postnatal life.

Embryological development of the ductus arteriosus

Embryologically, it originates from the sixth pair of pharyngeal arch arteries. During cardiovascular development, the left sixth arch artery persists as the

DA, while the right sixth arch artery regresses (4). This process is intricately regulated by genetic factors, growth factors, and hemodynamic forces, resulting in a structure that is histologically and functionally distinct from the adjacent great vessels (5,6).

Morphological characteristics

Throughout gestation, DA undergoes significant structural and functional changes. In early fetal life, it appears as a straight tube, but as pregnancy progresses, it develops a more curved configuration, often described as “hockey-stick” shaped (7). This morphological evolution is accompanied by changes in its histological composition, particularly in the muscular, tunica media layer, which is crucial for its eventual closure after birth (8,9).

Histological evolution

The closure of the DA is a complex physiological process that begins within hours of birth and typically completes within the first few days of life. This process involves initial functional closure through smooth muscle contraction, followed by anatomical closure through intimal thickening and fibrosis (10). Failure of this normal closure process results in patent ductus arteriosus (PDA), one of the most common congenital heart defects, affecting approximately 1 in 2000 full-term infants and up to 8 in 1000 pre-term infants (11). Understanding the normal development and histomorphometry of DA is important for several reasons. Firstly, it provides a baseline for identifying abnormal development, which may predispose to conditions like PDA or, conversely, premature closure of the channel in utero (12). Secondly, detailed knowledge of its structure and development can inform therapeutic strategies for managing PDA, particularly in preterm infants where the balance between maintaining ductal patency and encouraging closure is delicate (13,14). Lastly, insights into its development may have broader implications for understanding vascular biology and the mechanisms of vascular remodelling (15). Previous studies have explored various aspects of DA development using

different methodologies. Ultrasound studies have provided valuable in vivo data on its size and blood flow dynamics throughout gestation (16,17). Histological studies, primarily in animal models, have elucidated the cellular and molecular mechanisms underlying its maturation and closure (18). However, comprehensive histomorphometry analyses of human fetal DA across different gestational ages remain limited. Our study aims to bridge this gap by conducting a detailed histomorphometric analysis of DA using human fetal cadaveric specimens. This comprehensive approach will provide valuable insights into the structural basis of DA's function and maturation. By examining both macroscopic and microscopic features, we seek to characterize the structural changes that occur in the DA as gestation progresses. Our objectives include quantifying changes in the DA's length, diameter, and angular orientation relative to the aorta across different gestational ages and analysing the histological features of the DA, with a particular focus on the development and organization of the tunica media. Through this comprehensive approach, we aim to contribute to the growing body of knowledge on fetal cardiovascular development and provide valuable insights that may inform clinical practice in neonatal care. These findings may have significant implications for fetal medicine, neonatology, and pediatric cardiology, potentially informing new approaches to the diagnosis and management of DA related conditions.

Methods

Study design and ethical considerations

This cross-sectional observational study was conducted on human fetal cadavers following ethical guidelines and after obtaining parental consent. The study protocol was approved by the Institutional Ethics Committee (IEC:66/2023) and adhered to the Declaration of Helsinki principles for medical research involving human subjects, including fetal tissue. All specimens were obtained and handled in accordance with established bioethical standards for fetal research.

Sample acquisition and preparation

Human fetuses freshly acquired from the Department of Anatomy following spontaneous abortions and stillbirths. The specimens were collected within 2–4 hours of fetal demise. Dissection was carried out immediately after sample acquisition. A total of 20 fetuses (n=20), encompassing both sexes, with gestational ages ranging from 17 to 37 weeks were included in the study.

Inclusion criteria: Male and female fetuses from 17 to 37 weeks of gestation.

Exclusion criteria: Macerated fetuses, those with congenital anomalies -records were checked for the mention of any congenital defects specifically pertaining to cardiac and great vessel defects for fetal death. Such specimens were not included, or specimens with inadequate preservation of the cardiovascular system.

Preservation methodology

The specimens were immersed in 10% neutral buffered formalin solution within 30 minutes of acquisition to mitigate potential tissue degradation. 10% neutral buffered formalin was selected for its superior tissue preservation properties. The fixation process was initiated promptly to prevent autolytic changes and specimens were maintained at a consistent temperature of 4°C during initial preservation. To ensure specimen integrity, we implemented the following quality control steps of macroscopically examination for signs of tissue degradation, excluding macerated or significantly compromised specimens and carefully documenting the time between fetal demise and tissue preservation.

Age determination and cohort stratification

Gestational age was estimated using crown-rump length (CRL) measurements, following the method described by Streeter (1951). Measurements were taken using a digital caliper (precision: 0.01 mm) and corroborated with established growth charts. Based on estimated gestational age, specimens were stratified into two cohorts:

- Group I: 17th-24th week (n=10)
- Group II: 25th-37th week (n=10)

Gross morphometric analysis

Dissection and Exposure: The thoracic cavity was accessed via a midline incision, followed by careful removal of the pericardium and surrounding connective tissue to isolate the great vessels and DA.

Morphometric Measurements: All measurements were performed using high-precision digital calipers and validated using ImageJ software (NIH, USA; version 1.53k) on standardized digital photographs. Parameters measured included: (Figure 1)

- Length of DA: From the luminal junction with the aorta to the pulmonary trunk. (L)
- Diameter of DA:
 - a. At aortic end (d1)
 - b. At pulmonary end (d2)
- Angular measurements: Angular measurements were obtained through standardized macro-photography (Canon EOS R5, 100mm macro lens) and analyzed using ImageJ software with the angle tool, calibrated to known reference scales in each image. (Figure 2)
 - a. Upstream angle: Angle between DA and aortic isthmus (a1)
 - b. Downstream angle: Angle between DA and descending thoracic aorta (a2)

Histological analysis

Tissue Processing: The DA was carefully excised and processed for histological examination. The tissue was dehydrated through a graded series of ethanol, cleared in xylene, and embedded in paraffin wax. Sections of 5µm thickness were cut using a rotary microtome.

Staining Protocol: Hematoxylin and Eosin (H&E): For general morphology and quantitative analysis.

Microscopic Examination and Quantification: Stained sections were examined using an Olympus BX53 microscope equipped with a DP74 digital camera. The thickness of the tunica media was measured using cellSens software (Olympus) under 10X magnification (Figure 4). For each specimen, measurements

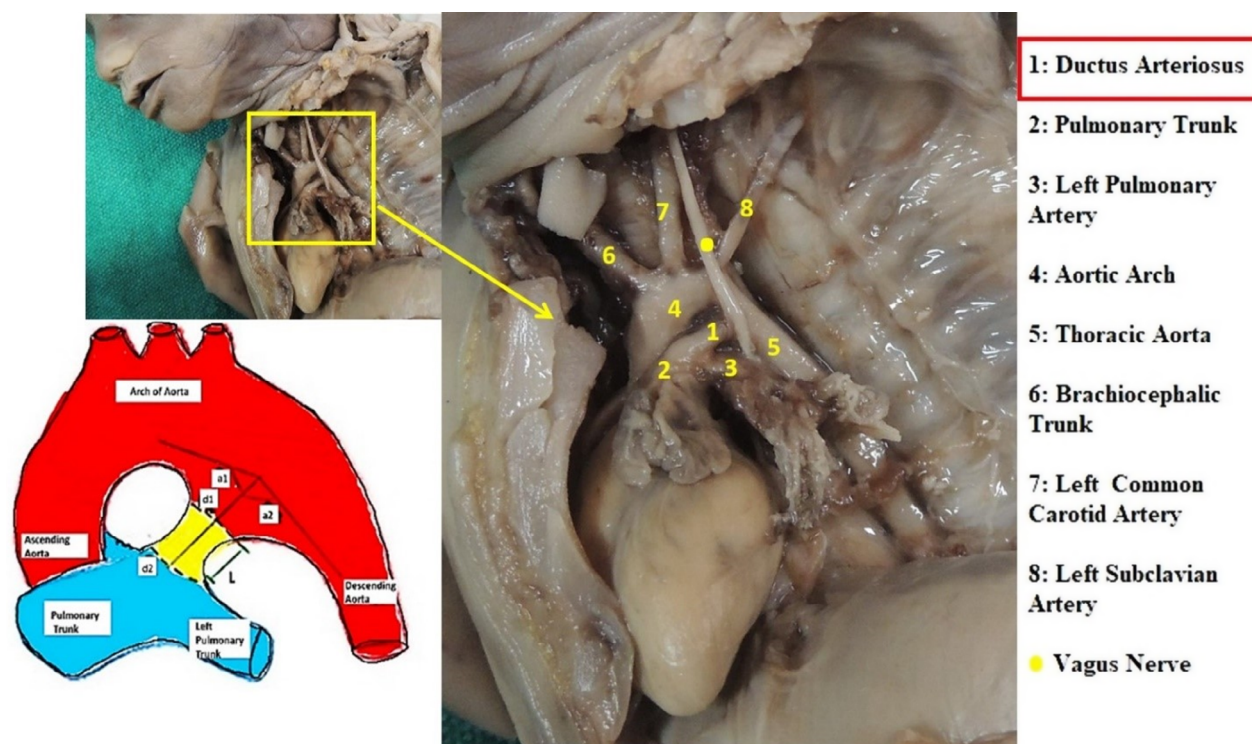


Figure 1. Cadaveric image and digital illustration of the ductus arteriosus of a fetus aged 25 weeks showing the parameters measured.

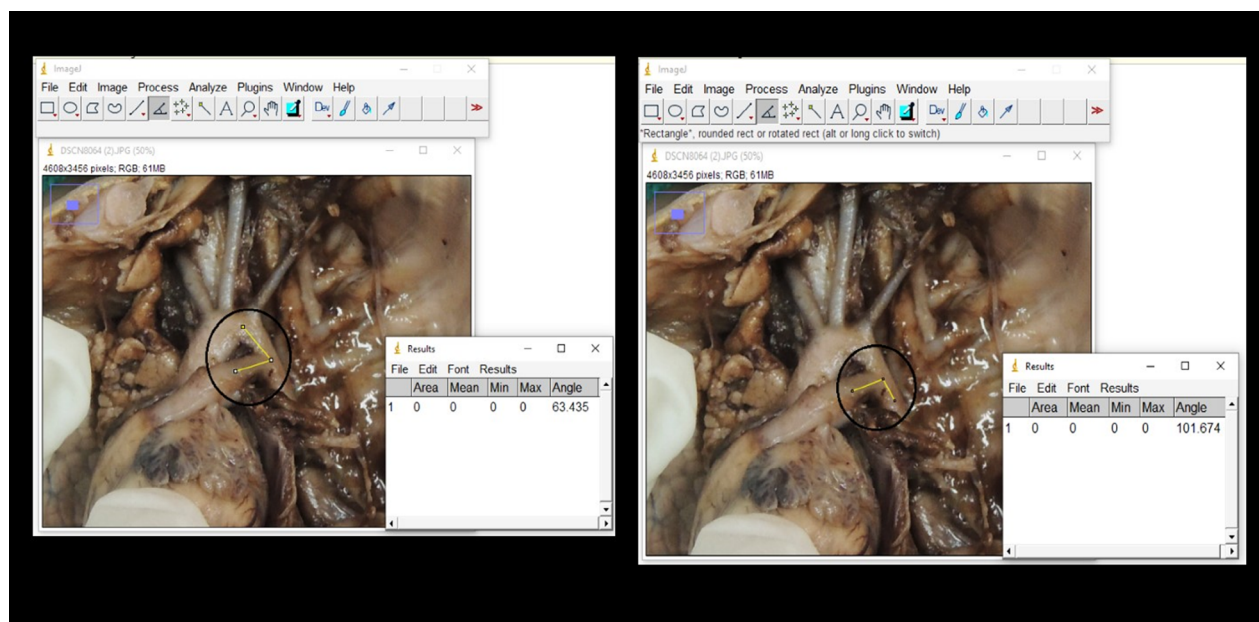


Figure 2. Photograph showing measurement of upstream and downstream angle of ductus arteriosus using Image J software.

were taken at 12 equidistant points around the circumference of the DA to account for potential asymmetry.

Statistical analysis

Data were analyzed using SPSS version 27.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics including mean, standard deviation, were calculated for all measured parameters. Normality of data distribution was assessed using the Shapiro-Wilk test. Inferential statistics including Pearson's correlation coefficient (r) was calculated to assess relationships between measured parameters and gestational age.

Quality control

To ensure reliability, all measurements were performed by two independent observers. Inter-observer variability was assessed using intraclass correlation coefficients. In cases of significant discrepancy, measurements were repeated and consensus was reached through discussion.

Results

Gross anatomical measurements

Significant growth of the DA was observed from Group I to Group II (Table 1). The mean values of the length of DA increased from 4.9 ± 0.6 mm to

7.1 ± 2.2 mm, strongly correlating with gestational age. The mean diameter was equally pronounced with advancing gestational age from 2.9 ± 0.3 mm to 4.0 ± 0.9 mm in the pulmonary end and 2.9 ± 0.3 mm to 4.3 ± 0.9 mm in the aortic end. In contrast, angular measurements showed more modest changes with the upstream angle increasing from $52.9^\circ \pm 3.5^\circ$ to $61.0^\circ \pm 4.8^\circ$ and the downstream angle from $92.9^\circ \pm 7.9^\circ$ to $103.8^\circ \pm 5.2^\circ$, exhibiting weak correlations with gestational age.

Histological findings

GENERAL STRUCTURE

The DA demonstrated a typical muscular artery structure with three distinct layers from the lumen to the periphery as tunica intima, tunica media, and tunica adventitia (Figure 3). Clear separation between all of the tunics was not feasible in the current histological research.

TUNICA INTIMA

The findings revealed distinct structural changes across fetal development. In Group II fetuses (25-37 weeks), the internal elastic lamina (IEL) and external elastic lamina became more prominent, particularly at 36 weeks gestation. This suggests a progressive maturation of the arterial wall structure (Figure 4).

Table 1. Morphometric parameters and correlations with gestational age of the ductus arteriosus in Group I and II fetuses. Values are presented as mean $\pm$ standard deviation (range). Correlation (r) represents Pearson correlation coefficient with gestational age.

Parameter	Group I (17 th -24 th weeks)	Correlation (r)	Group II (25 th -37 th weeks)	Correlation (r)
Length L (mm)	4.9 ± 0.6 (2.7-5.2)	0.74	7.1 ± 2.2 (5.6-9.6)	0.67
Diameter at aortic end (d1) (mm)	2.9 ± 0.4 (2.5-3.2)	0.67	4.0 ± 0.9 (2.4-5.4)	0.63
Diameter at pulmonary end (d2) (mm)	2.9 ± 0.3 (2.6-3.2)	0.64	4.3 ± 0.9 (2.8-5.8)	0.68
Upstream angle (a1) (degrees)	52.9 ± 3.5 (48.3-58.2)	0.11	61.0 ± 4.8 (53.4-68.4)	0.27
Downstream angle (a2) (degrees)	92.9 ± 7.9 (71.4-98.1)	0.15	103.8 ± 5.2 (97.2-115.8)	0.19

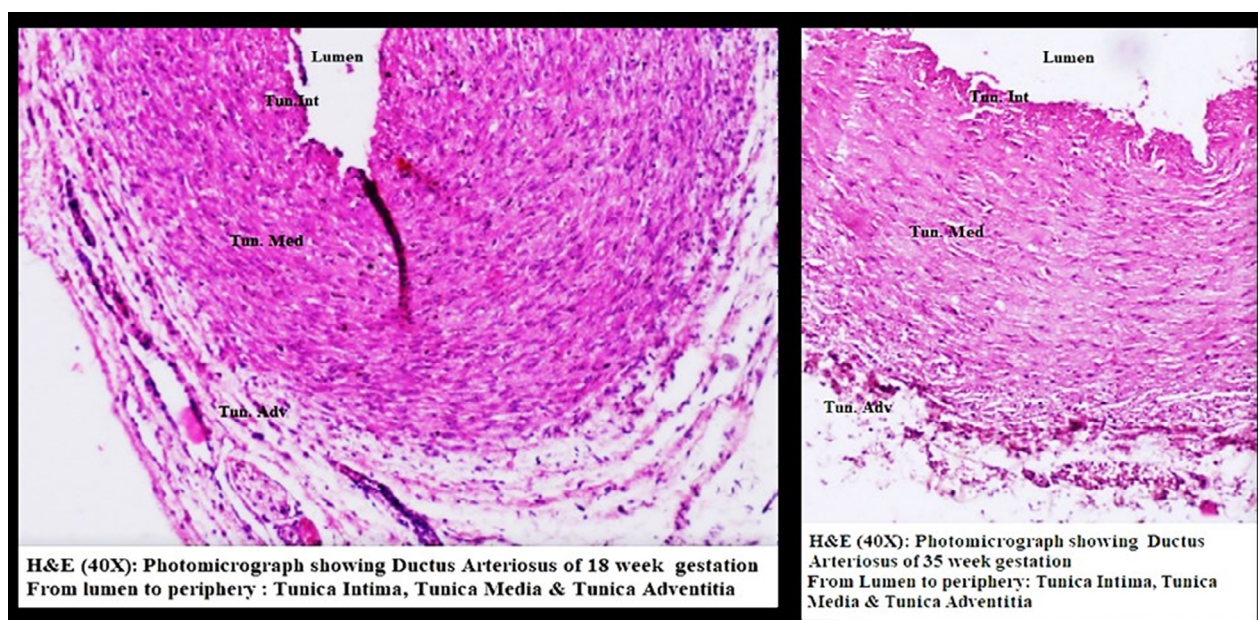


Figure 3. Photomicrograph showing the 3 layers of the DA from the lumen to the periphery - tunica intima, tunica media, and tunica adventitia at 18 weeks and 35 weeks gestation period.

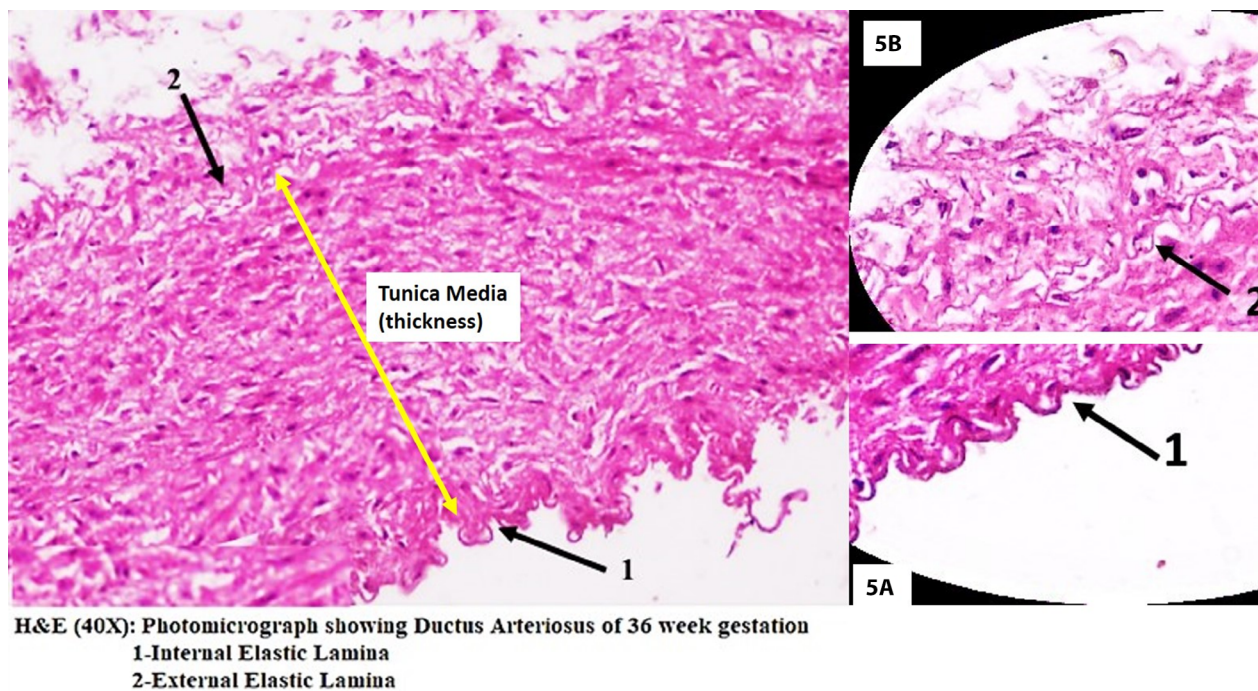


Figure 4. Photomicrograph showing the internal (5a) and external elastic lamina (5b) of the DA at 36 weeks' gestation period. Line representing the thickness of the tunica media measured.

TUNICA MEDIA

The tunica media showed notable differences with advancing gestational age. Group I fetuses exhibited a predominance of fibroblasts (Figure 3), while Group II fetuses displayed a thicker tunica media with more organized concentric lamellae. Collagen fibers, vasa nervosa, and vasa vasorum were visible in tunica adventitia.

TUNICA ADVENTITIA

The tunica adventitia contained collagen fibers, vasa vasorum, and vasa nervosa. No significant differences were observed between the two groups in this layer.

QUANTITATIVE ANALYSIS OF TUNICA MEDIA THICKNESS

Quantitative analysis showed significant thickening of tunica media from Group I to Group II. The mean thickness of tunica media increased from 0.29 ± 0.04 mm to 0.57 ± 0.049 mm, with a strong positive correlation to gestational age (Group I: $r = 0.63$; Group II: $r = 0.71$).

Discussion

The findings reveal a significant increase in key morphometric parameters throughout gestation, aligning with and extending previous findings in the field. Analysis showed a considerable linear increase in parameters like length and diameter which aligned with the histological findings of our study. With increasing gestational age, there was a significant increase in the DA's length. The data are similar to the results of previous morphological studies (Table 2). The diameter was determined at both the proximal and distal attachment sites. Our results, which show comparatively constant sizes at the proximal and distal ends of the DA are consistent with the findings among fourth to eighth-month fetuses (21). However, an ultrasound-based study by Mielke and Buda (2000) (16) reported that the proximal DA was approximately 2.5% smaller than

the distal section, a subtle difference that may not have been detectable in our study. The slight discrepancy in our macroscopic study and the previously performed ultrasound examination may be due to potential methodological differences.

Until now, there have been very few studies concerning prenatal assessment of angle related to DA. The findings on the angular orientation of the DA align with previous anatomical studies. Brezinka et al. (1994) (22) reported upstream angles between 30° and 80° and downstream angles between 80° and 120° in their postmortem study of fetuses from 8 to 19 weeks gestation. Similarly, present results showed acute upstream angles and obtuse downstream angles throughout the second and third trimesters. However, unlike Brezinka et al. (1994) (22) who found no significant correlation with gestational age, the present study observed a modest increase in both angles as gestation progressed. This is partially consistent with findings by Mielke and Buda (2000) (16), who reported upstream angles of $45.58 \pm 9.38^\circ$ and downstream angles of $98.48 \pm 12.58^\circ$ in 2D-echocardiographic evaluations of fetuses from 13-41 weeks, although they observed a significant correlation with gestational age. This discrepancy may be due to differences in sample size or measurement techniques. Nevertheless, accurately determining this angle using either sonography or morphological methods presents challenges due to the aortic arch's curved structure (16). Although less extensively studied, the stability of its angulation has important clinical implications. Abnormal morphology and angulation, potentially resulting from ductal lumen constriction or kinking, have been associated with tricuspid regurgitation and other cardiac anomalies (7,16). These findings suggest that significant deviations from these angles, particularly in late gestation, may warrant further investigation for potential pathology. The histological findings of our study reinforce DA as a muscular artery providing crucial insights into its structural maturation. This aligns with earlier work by Szyszka-Mróz and Woźniak (2003) (9), who studied DA histologically during early intrauterine development in humans, and observed an endothelium and several circular cell layers forming the primordium of the tunica media. The observation of prominent internal and external elastic laminae in third trimester

Table 2. Comparative table summarizing previous studies on the ductus arteriosus (DA) development with present study findings.

Study	Location	Number of Specimens	Detection Method	Key Findings
Present study	South India	20 fetuses (17-37 weeks)	Gross morphometric analysis, histological examination (H&E and Masson's trichrome staining)	- Significant increase in DA length and diameter with gestational age - Modest changes in angular measurements - Thickening of tunica media from 0.29 ± 0.04 mm (Group I) to 0.57 ± 0.049 mm (Group II) - Progressive organization of connective tissue elements
Mielke and Buda 2000 (16)	Germany	329 fetuses (13-41 weeks)	2D-echocardiography	- Upstream angle: $45.58 \pm 9.38^\circ$ - Downstream angle: $98.48 \pm 12.58^\circ$ - Proximal DA approximately 2.5% smaller than distal section
Brezinka et al. 1994 (22)	Netherlands	Fetuses (8-19 weeks)	Postmortem study, sonography	- Upstream angles: 30° to 80° - Downstream angles: 80° to 120° - No significant correlation with gestational age
Szpinda et al. 2007 (19)	Poland	128 fetuses (15-34 weeks)	Digital-image analysis system	- Linear increase in DA length with advancing gestational age - Positive correlation between DA length and gestational age
Kugananthan et al. 2014 (20)	India	30 stillborn fetuses (20-40 weeks)	Histological examination	- Prominent internal and external elastic laminae in third trimester specimens - Progressive closure of DA lumen with advancing gestational age
Nowak et al. 2011 (21)	Poland	41 fetuses (8-19 weeks)	Morphometric measurements	Constant DA diameters at proximal and distal ends from fourth to eighth month of gestation
Szyska-Mróz and Woźniak 2003 (9)	Poland	Embryos and fetuses (5-8 weeks)	Histological examination	Endothelium and circular cell layers forming primordium of tunica media in early development

specimens (Figure 4) is consistent with the findings of Kuganathan et al. (2014) (20). This elastin deposition may contribute to the DA's capacity for rapid constriction after birth (6). The tunica media showed notable differences between the two groups. Group I fetuses exhibited a predominance of fibroblasts, while Group II fetuses displayed a thicker tunica media with more organized concentric lamellae (Figure 3). The presence of fibroblasts within the smooth muscle layers of the tunica media in Group I fetuses may indicate an active process of cellular proliferation and migration. This could contribute to the observed increase in wall thickness and potentially play a role in the preparation for postnatal DA closure. The significant thickening of the tunica media from 0.29 ± 0.04 mm in the second trimester to 0.57 ± 0.049 mm in the third trimester, with strong positive correlations to gestational age ($r = 0.63$ and 0.71 , respectively), reflects the complex maturational changes occurring in the DA wall. This thickening is crucial for the DA's eventual closure after birth and involves the development of intimal cushions and changes in smooth muscle orientation (Gittenberger-de Groot et al. 1980; Gittenberger-de Groot et al. 1985; Slomp et al. 1992) (5,23,24). The thickening of the tunica media not only contributes to the vessel's ability to constrict postnatally but also plays a role in its responsiveness to prostaglandins and oxygen tension, key factors in DA patency and closure (2).

Limitations and future direction

While this study provides valuable insights, it has some limitations. The sample size could be expanded in future research to allow for more detailed subgroup analyses. Additionally future studies using advanced imaging techniques on live fetuses to obtain more precise measurements would enhance the morphometric findings.

Conclusion

This comprehensive histomorphometric study of the DA in human fetuses provides valuable insights into the structural development of this critical fetal

vessel. These findings demonstrate significant changes in both gross anatomical features and histological structure of the DA as gestation progresses, contributing to a more nuanced understanding of fetal cardiovascular development. The DA undergoes substantial growth in length and diameter during fetal development, with more pronounced changes observed in the third trimester. This growth pattern likely reflects the increasing demands on fetal circulation as gestation advances. The relative stability of the DA's angular orientation concerning the aorta throughout the studied gestational period suggests an evolutionary optimization of fetal hemodynamic. The progressive organization and thickening of the tunica media, along with the development of the internal elastic lamina, indicate a structural maturation process that likely underpins the DA's functional capabilities. The correlation between gross anatomical measurements and histological findings, particularly the relationship between DA diameter and tunica media thickness, highlights the intricate interplay between form and function in fetal vascular development. Detailed characterization of DA development provides a foundation for improved understanding of DA related pathologies, potentially informing clinical management strategies for preterm infants and those with congenital heart defects. Such studies could potentially lead to novel therapeutic approaches for managing DA related conditions in neonates.

Ethical Approval: This study was conducted in full compliance with ethical standards and approved by our Institutional Ethics Committee (IEC:66/2023). All procedures involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

Authors Contributions: PA: conception and design of the work, acquisition, analysis, and interpretation of data, and writing of the initial and final draft; RP: study design, provision of research

materials, data organization, and revision of the initial draft; SGK: drafting, critical revision for important intellectual content, and final approval of the version. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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