“MORPHOMETRIC STUDY OF THE AORTA, PULMONARY TRUNK AND DUCTUS ARTERIOSUS WITH GROSS AND HISTOLOGICAL CORRELATIONS IN HUMAN FETUSES OF TAMILNADU POPULATION.”

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M.D. DEGREE

In

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CERTIFICATE

This is to certify that the dissertation “Morphometric Study of the Aorta, Pulmonary Trunk And Ductus Arteriosus With Gross And Histological Correlations In Human Fetuses Of Tamilnadu Population.” is an original work done by Dr. R. Jothi Ganesh, Post Graduate student, PSG Institute of Medical sciences and Research, Coimbatore, under my supervision and guidance.

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DECLARATION

I solemnly declare that this dissertation “Morphometric Study of the Aorta, Pulmonary Trunk and Ductus Arteriosus with Gross and Histological Correlations in Human Fetuses of Tamilnadu Population.” was done by me in the Department of Anatomy, PSG Institute of Medical sciences & Research, Coimbatore, under the guidance of Dr. M. Jamuna, M.S, Professor and Head of the Department, Anatomy, PSG Institute of Medical Sciences & Research, Coimbatore.

This dissertation is submitted to the Tamil Nadu Dr. M. G. R Medical University, Chennai in partial fulfillment of the university regulations for the award of degree of M.D Anatomy – Branch V examinations to be held in April 2016.

Place: Coimbatore

Dr. R. Jothi Ganesh

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Finally I wish to dedicate this work to My Parents who had gone with me through all the ups and downs of life.
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“Morphometric Study of Aorta, Pulmonary Trunk and Ductus Arteriosus with Gross and Histological Correlations in Human Fetuses of Tamilnadu Population.”

**INTRODUCTION**

Materno – Fetal healthcare is one of the most rapidly evolving fields in medicine focusing on the medical and surgical management of high-risk pregnancies. Technical advancements of the 21st century in the field of surgery allows for the correction of birth defects like congenital heart disease in-utero. Researches carried on the developmental aspects of fetus are much relevant in clinical and surgical terms of today and surely will lead to the better care of pregnant mother and her growing baby.

Knowledge of cardiovascular system remained as a great mystery during ancient times. Basic researches carried out earlier in the fetuses lead to an in-depth understanding of the structural and functional aspects of intrauterine life. Our present knowledge of fetal development and mal-development is much advanced owing to non-invasive monitoring of fetal health i.e. Ultrasonography and Fetal Echocardiography.
Fetal life in-utero begins with cellular fusion, differentiation and development that take place under the direct control of genes. Establishment of energy source for existence is achieved by the interstitial implantation of the blastocyst to the uterine endometrium. Depletion of nutrient material from uterine glands and tissues leads to formation of materno-fetal circulation through the placenta which provides a constant nutritional supply to the growing fetus. Further organogenesis and effective functioning of remaining systems are much dependent on the proper development of cardiovascular system.

Developmental milestones of the cardiovascular system in-utero starts around 3rd week of gestation with embryonic heart beating around the 4th week. Ebb and Flow model of blood flow precedes the primitive intra-embryonic circulation. Looped heart tube is observed from 6th week. Partitioning of heart into four chambers, morphogenesis of valvular apparatus, outflow tract septation and transformation of aortic arches takes place during the 6th – 9th week by the genetic expressions, migration of neural crest cells and local hemodynamic factors. Around 10th week heart is said to be structurally and functionally mature enough to handle the fetal circulation.
**Uniqueness of fetal circulation** lies in the oxygenation of blood that takes place in placenta instead of lungs as in adults and in the presence of transient fetal shunts i.e., ductus arteriosus, ductus venosus and foramen ovale which significantly reduces the functional overload to the developing lungs, liver and heart respectively. These features not only allow for better adaptation to the aquatic environment of the amniotic fluid but also undergo considerable changes post-natally to get acclimatized to the atmospheric environment.

**Structurally speaking,** the aorta and pulmonary trunk are classified as elastic type of arteries with predominance of elastic fibers arranged circumferentially whereas ductus arteriosus with predominance smooth muscle cells in the tunica media is classified as muscular type of artery. Elasticity and muscularity of the vessel depends not only on the proximity or remoteness of the vessel site in relation to heart but also on the association of the vessel with its neighboring structures as in the case of ductus arteriosus with left recurrent laryngeal nerve.

**Functionally speaking,** prenatally when blood pressure is more or less similar in the pulmonary artery and the aorta, the structure of the vessels is much similar. Post-natally, blood pressure increases leading to structural re-modeling of blood vessels i.e, the increase of tunica media thickness in aorta and pulmonary trunk.
From an **embryological perspective**, the cardiac primordium is derived from splanchnopleuric mesoderm. Specification, determination, patterning and differentiation of cardiomyocytes lead to the formation of the heart tube. Cardiomyocytes in particular from the secondary heart field have unique developmental fate compared to the atrial and ventricular myocytes especially after the migration of neural crest cells forming the cono-truncal ridge. Spatial changes like septations, rotation with absorptive changes between cono-truncal ridges and the primitive ventricles leads to the formation of right and left outflow tracts. Defects in these developmental aspects lead to relatively common congenital abnormalities like Truncus Arteriosus, Transposition of Great Arteries and Fallot’s Tetrology.

From a **topographical perspective**, the outflow tract is a conduit between contracting muscular heart chambers and the embryonic vascular network. Parts of the outflow tract are the proximal myocardial (conus), distal myocardial (truncus) and the intrapericardial (aortic sac) portion. Aortic sac portion is in continuity with the aortic arches which undergoes spatio-temporal regulations. The ductus arteriosus arises from the bifurcation point of the pulmonary trunk at the level of T4-T5. It is closely related to the thymus gland anteriorly, the left primary bronchus inferiorly and the left recurrent laryngeal branch of vagus nerve that enwraps it.
Changes in the topography of the cardiac outflow tract i.e., on holotopy, skeletotopy and sintopy during fetal periods can be used as reference parameters during the diagnostic ultrasonographic studies and in cardio-surgical interventions performed on fetuses. Developing embryo displays differential rates of growth in regard to time and space. Fetal growth can be quantified by using morphometric parameters.

The fact that there is a paucity of quantitative anatomical data regarding the normal developmental stages of outflow tract vessels in Tamil Nadu population justified the need for such a study. After considering the limitations observed during early studies pertaining to the study topic, a novel approach of morphometric analysis using ultrasound in-utero has been done to chart the developmental parameters which can be of use for radiological practice. Topographical relations with histological correlations of the Aorta, Pulmonary trunk and Ductus Arteriosus has also been undertaken for better correlation of structural changes with histological basis.
AIM AND OBJECTIVES

Aim:

To study the morphometric details of Aorta, Pulmonary Trunk and Ductus Arteriosus in the human fetuses between 14\textsuperscript{th} to 36\textsuperscript{th} week gestation: In-utero ultrasonographic study with histo-topographical correlations in aborted fetuses.

Objectives:

1) To quantify the developmental of the Aorta, Pulmonary Trunk, and Ductus Arteriosus and define the normal growth patterns using ultrasonographic measurement.

2) To study the histological changes in the great arterial vessels using Hematoxylin-Eosin & Verhoeff- Van Gieson staining techniques.

3) To identify the topographical relationship of great arterial vessels using fetal dissection method.

4) To include the expected incidental findings of the study.
REVIEW OF LITERATURE

Efforts have been made in the review of literature to make a theoretical framework and rationale for the study methodology.

Ebers Papyrus (1550 BC) mentions heart as the center of blood supply to which are attached all the vessels of the body. Early Egyptians considered heart as the meeting point of tears, blood, urine and semen.

The description of the heart in Atharva veda (1500-1000 BC) as inverted lotus bud with nine gates is much accurate. The nine gates are counted as 3 in right atrium, 4 in left atrium and 1 each in right and left ventricles. Nomenclature of structural entities in Sanskrit like Hridaya, Dhamanis and Shiras elucidates the conceptual knowledge of cardiovascular system during early days.

Empedocles (480B.C.) considered blood as innate heat and heart as the chief organ of both the pneuma and haima (breath and blood). He considered that breathing takes place through skin also thereby associating the skin and lungs to the blood flow.

Aristotle (384-322 BC) is the first one to describe the fetal pulsations in a chick embryo. The word 'Aorta' stems from greek word aeirō, meaning "I lift, raise". This term was applied by Aristotle while describing aorta.
Praxagoras (384-322 B.C.) studied anatomy and he was the first one to distinguish between arteries and veins. He thought of arteries as tubes carrying air like trachea which carried the pneuma. He believed that arteries emanated from the heart and veins from the liver.

It was through Galen’s experiment (129-217 A.D.) that long held belief of arteries carrying air was overturned. Galen was able to demonstrate that arteries contain blood by his demonstration during vivisection of animals and careful observations on wounded gladiators. According to Galen, blood was created in the liver through food materials which flows to the right side of the heart where it gives off “sooty vapors” which are exhaled through the lungs. He also mentions about pores in the heart through which blood flows from the right to left side of the heart, where it receives “good vapors” which are inhaled through the lungs and further blood flows through the rete mirabilis which is located at the base of the brain before it is distributed to the whole body.

The earliest descriptions of the systemic and pulmonary circulatory was conceptualized and published in 1242 by Ibn al-Nafis. He wrote that blood flows through the pulmonary circulation instead of moving from right to the left ventricle as previously believed by Galen and his proponents.
**Andreas Vesalius** (1516-1564) with his evident based approach of dissections on human cadavers laid the groundwork for cardiovascular system that freed the academic world from Galen’s thought system of comparative understanding. He conducted *parallel dissections* in which an animal cadaver and a human cadaver are dissected simultaneously to demonstrate the anatomical differences and uncover the errors of early anatomists. He also discovered that there were no pores in the septum of heart which gave a basic framework for the systemic and pulmonary circulation.

Breakthrough in studies of cardiovascular system was done by **William Harvey** (1620-1684). Through his demonstration of *blood circulation* by tying a ligature on to the upper arm of a human being, he discerned the direction of blood flow by pushing up and down the blood in the vein. He identified the presence of valves (as little bumps) in the veins and their importance to unidirectional blood flow. By using mathematical proportions on the quantity of blood flow through the circulatory system he proved the overall impossible role of the liver as the origin of venous blood as believed early.

Animal experiments on rabbit embryos and fetuses of various developmental stages were undertaken by **Boyd** (1941). Using Cajal and Bielschowsky staining techniques specimens were studied which showed that ductus arteriosus possessed a sensory innervation similar to that of aorta and the carotid sinus. **Fine nerve fibres**, presumably motor were also found terminating in the muscular coat of the ductus.
Histogenesis of the arteries was studied by Hughes (1943) where wider inquiry into the development of the circulatory system in the chick embryo was done. An attempt to study structural and functional aspects of arteries was taken. Number of cell layers in the tunica media of the aortic arches at various points along the arches were measured and plotted against days of incubation. Study results helped in the classification of arteries into three main types based on histological features into Type I & Type II belonging to elastic arteries and Type III as muscular arteries.

Dussik and Keidel (1948) laid the foundation for the era of early diagnosis of congenital malformations in-utero by employing ultrasound in medical diagnosis of cardiac anomalies. With the advent of fetal echocardiography, mysteries concerning cardiovascular system have been demystified. Possibility of non-invasive monitoring of fetal growth and its wellbeing was enhanced by further discoveries.

In 1956, the Nobel Prize in Medicine for discoveries of cardiac catheterization was awarded to Courand, Forssmann and Richards.

Distribution, Connexions and Histology of baroreceptors in the pulmonary artery and sensory innervations in the ductus was investigated by Coleridge (1960). During the course of experiments on the dogs action potentials were recorded from afferent vagal fibers in a search for such receptors. Microscopic study showed that sensory endings or in contact with, the media resembled form of branches or coiled nerve fibers.
A comparative study of the lamellar unit of Aortic tunica media and its function on 10 mammalian species was undertaken by Harvey Wolinsky and Seymour Glagov (1967). They investigated the relationship between aortic diameter, medial thickness and vessel wall tension. He suggested that elastic lamella and the content of inter-lamellar zone represent the structural and functional unit of aorta.

Early hemodynamic studies were done on fetal circulation by Rudolph et al (1972). He concluded that the size of a blood vessel is directly proportional to the amount of blood flowing through it. Hence, cardiovascular malformations are expected to have an impact on the development of the great vessels during fetal life.

Histological changes in the tunica media of great arteries in relation to the internal diameter was quantified by Hildegonda van et al (1975) where packing density of elastic fibers and thickness of the ascending aorta’s tunica media doubled for a four-fold increase in internal diameter. He also noted that no significant changes were noted in the pulmonary trunk histology with increase in internal diameter and that the presence or absence of ductus arteriosus had no impact on the observed parameters.

Considering the differing opinions regarding closure of ductus arteriosus Yen Ho & Anderson (1978) undertook histological study in 35 specimens. They concluded that ductal obliteration is a gradual process which involves proliferation of tunica intima and media with formation of intimal mounds, mucoid filled spaces and fragmentation of internal elastic membrane.
Relationship between gestational age and histological maturation of ductus arteriosus was studied ultra structurally in cellular and sub-cellular levels during normal and abnormal ductus development by Tada T et al (1979). Ultra structural examination showed that intimal thickening was due to the migration of smooth muscle cells and not because of cellular proliferation. It is also to be noted that advanced stages of ductal degeneration showed intracellular and extracellular changes. Intimal cushion and interruption of elastic lamina were taken as the maturation indicator and fetuses above 29 weeks was considered to show more mature features.

Gittenberger-de Groot (1980) attempted to determine the histological difference between a normally closing human ductus arteriosus and a persistent ductus arteriosus. Histological features showed aberrant distribution of elastic material and a thick, wavy, unfragmented sub-endothelial elastic lamina supporting the view that an anatomical defect of the ductal wall is responsible for persistent patent ductus arteriosus.

To define the anatomical and histological feature of the aortic isthmus, a comparative study of descending aorta and aortic isthmus was done by Woezik and Krediet (1981). Parameters like thickness of tunica media and the packing density of elastic fibers in 69 specimens were studied. Results indicated that only 9% of the studied specimens showed marked narrowing with distinguishable histological features in the aortic isthmus.
Topographical relationship of Left Recurrent Laryngeal Nerve (RLN) and Ductus arteriosus is closely studied by Leonard (1983). He suggested that the relationship between the left recurrent laryngeal branch of the vagus nerve and ductus arteriosus is responsible for this histological difference on the grounds that nerve (which, in a stage 16 embryo, is very large in relation to the aortic arch system) lends a sling like supports as it enwraps the ductus, thereby permitting the ductus to develop as a muscular artery in comparison to Aorta and Pulmonary Trunk which are elastic.

Among normal infants and children histological study of pulmonary arterial tree was undertaken by Woezik et al (1987). He mentioned that in addition to the linear correlation found between body length and the internal diameters of the Aorta, Pulmonary Trunk & Ductus arteriosus there are parallel alterations like changes in thickness and packing density of the elastic fibers.

Normal diameter of the fetal aorta and pulmonary artery evaluation was done echocardiographically in utero by Cartier et al (1987). Correlation coefficient during systole and diastole was obtained (r=0.992 for aorta and r = 0.973 for pulmonary artery) in M-mode measurements. These normative data value can be used for comparison of standard growth with cardiac anomaly involving great vessels of heart or when abnormal vessel diameter was suspected.
Types of patent ductus arteriosus based on the insertion type into Conical, Window, Tubular, Complex and elongated duct was identified angiographically by Kirchenko (1989). He concluded that the size, configuration and relationship to adjacent structures are directly related to the degree of shunting and that it has greater implications during interventional closure of PDA.

Developmental studies on human fetuses by Ursell et al (1991) measured the vessel diameters at four developmental stages (10-26 weeks) in aborted fetuses. At each age, pulmonary valve diameter measured was greater than that of the aortic valve and diameter of the aortic isthmus was larger than that of the ductus arteriosus. She further added that measurements taken during fetal life are needed because fixation and post-agonal changes may have different effects on the caliber of elastic and muscular vessels.

According to Hornberger (1992) et al use of normal growth curves for the developing aortic arch helps in the early detection of aortic arch abnormalities, particularly aortic coarctation. In general there was progressive tapering of the aortic arch dimensions, with the smallest diameter noted at the aortic isthmus. The ratio of the transverse aorta, isthmus, descending aorta, and aortic root to the ascending aorta remained relatively constant with gestational age, with mean values of 0.94, 0.81, 0.96, and 1.13 respectively.
Brezinka (1994) studied on the flow velocity waveforms in ductus arteriosus during early pregnancy. She concluded that progressive changes in the vessel wall histology have a constricting effect on the lumen leading to ductal velocities being the highest in the fetal circulation. Data revealed increase in ductal waveforms from early pregnancy to mid-pregnancy. End-diastolic velocities (EDV) were not recorded until 13 weeks. EDV present in 50% at 15 weeks and are present in all the cases studied from 17 weeks. This knowledge of ductal anatomy and doppler waveform patterns is critical in assessing the effect of drug intake and is valuable in experimental studies.

David et al (1997) states that mismatch of high pulmonary artery/aortic ratio and the small size of the aortic isthmus were the main elements suggestive of abnormalities of the aortic arch, especially in the early prenatal period based on his retrospective study of 43 cases of abnormal dimensions of the left cardiac chambers and vessels compared with 102 normal fetuses. Study data revealed that the ratio of pulmonary artery to aortic dimension was comparatively much higher in the fetuses with coarctation of the aorta than with functional disequilibrium or normal fetuses.
Quantification of structural and functional **alterations of elastin** in the arterial media was evaluated by Avolo et al (1998). Images of pressure-fixed aortic sections stained for elastin were obtained from postmortem specimens of 35 animals belonging to different species. A directional fractal curve was generated for each image. Study analysis showed that loss of medial elastic function was associated with combined contribution of age and heart rate variables leading to increased luminal wall stress with further endothelial damage and predisposition to atherosclerosis.

Achiron (1998) undertook **fetal aortic arch measurement** study in 14-38 weeks of gestation using ultrasound examination. Reference ranges for Transverse Aortic arch and distal aortic isthmus diameter were established for earlier detection of aortic arch abnormalities. Considering the examination time for its visualization he suggested that it can be integrated into the routine screening fetal cardiac examination. Another study conducted by him the same year on measurements of fetal aorta and pulmonary trunk diameters during first trimester revealed the aortic diameter (AD) and pulmonary artery diameter as a function of gestational age (GA). The correlation was found to be highly significant (p < 0.0001) for both great vessels.
Fate and function of the **mammalian neural crest cells** in the development of the aortico-pulmonary septum formation was studied by **Jiang et al (2000)** using genetically labeling technique in the mouse neural crest cell population. Study results showed evidence of labeled cells populating the aortic-pulmonary and cono-truncal region prior to and during the division of outflow tract.

**Genetic analysis on septal defects** like ASD, VSD and PDA are investigated by **Vaughan (2000)**. Molecular analyses showed the basic genetic abnormalities on several Mendelian forms. For example **TBX 5** transcription factor gene mutation is associated with ASD, VSD and Holt- Oram syndrome all being inherited in autosomal dominant pattern. A mutation of **NKX 2.5** transcription factor gene is also associated with complex congenital heart disease. Recent analyses of autosomal dominant Char syndrome, which is characterized by PDA, hand and craniofacial malformations. Analysis revealed that CHAR syndrome is caused by mutations of transcription factor gene - TFAP2B.

**Smooth muscle cell behavior** during closure of the ductus arteriosus after birth was studied in rabbits using histo-chemical analysis by TUNEL technique by **Imamura (2000)**. Study results suggested smooth muscle cell involvement substantiated by the SM2 MHC mRNA expression in the media of DA.
A study on microstructure of adult human arteries was conducted by Kumar et al (2000). Density of elastic fibers and smooth muscle fibers was observed in the ascending aorta, pulmonary trunk, pulmonary arteries and coronary arteries. Data analysis revealed that arterial elasticity was directly proportional to vessels proximity to the heart and its muscularity directly proportional to its distance from the heart.

Massoudy et al (2002) studied the relationships of the aorta and pulmonary trunk and found significant association with certain patterns of coronary arterial branching and commissural mismatch. Five types of variations noted were:

Type I- Aorta was anterior and to the right relative to the pulmonary trunk.
Type II- Aorta was directly anterior to pulmonary trunk.
Type III- Aorta was side to side in relation to pulmonary trunk.
Type IV- Aorta was anterior and to the left of pulmonary trunk.
Type V- Aorta was posterior and to the right of pulmonary trunk.

Histological study of the human ductus arteriosus during the last embryonic week of gestation was done by Szyszka-Mróz & Woźniaki (2003). A comparative analysis was done with Aorta & Pulmonary trunk histology. Study concluded that the ductus arteriosus in early development has a structure similar to that of the Aorta & Pulmonary Trunk with slight structural differences in the tunics of the ductus was observed.
Molecular analysis of **TbX2 for morphogenesis** and septations of outflow tract was studied extensively in mouse embryos by **Harrelson et al (2004)**. Targeted mutagenesis method was used to investigate Tbx2 function. Group of heterozygous embryos for a Tbx2 null mutation appear normal but another group of homozygous embryos reveal a crucial role of Tbx2 during cardiac development. Molecular analysis reveals that Tbx2 is required to repress chamber differentiation in the atrio-ventricular canal at 9.5 days post coitus (dpc).

Variations in the **shape and angulations of ductus arteriosus (DA)** during last trimester of pregnancy has been reviewed in a study by **Brezinka (2003)**. Ductus arteriosus and aortic arch are two arched tubes in an elliptical fashion with different radiuses. Place of fusion takes place at different levels and the angle formed between them are inconstant. In extreme cases of differing angulations it has been observed that it leads to tricuspid regurgitation and right ventricular hypertrophy.

The etiology of patent ductus arteriosus in different dog breeds was extensively studied by **Buchanan et al (2003)**. He used 3-Dimensional histological approach to study the changes in the ductal architecture. Multi-centric studies in different veterinary clinics also showed statistically significant data’s in other breed types with a strong association to familial mode of inheritance. He suggested further to screen for PDA the relatives of dogs affected with the condition and mentioned that dogs with PDA should not be used for the purpose of breeding.
Castillo et al (2005) measured the dimensions of aorta and pulmonary trunk in normal human fetal hearts between 13–20 weeks post-fertilization in 103 fetuses. External diameters were studied under stereoscopic magnification and measured. Data analysis showed linear growth against gestational age. Median values of pulmonary artery were: 2.2–4.2 mm and for aorta was 2.1–4.2 mm. Diameter of the ductus arteriosus was 1.2–2.45 mm. The diameter of the ascending aorta, descending aorta, the right pulmonary artery and left pulmonary artery was also studied. These reference parameters concerning the dimensions and growth of great arteries of heart can be of use for pediatric echo cardiographers.

Galindo (2006) et al analyzed the fetal echocardiographic findings of absent pulmonary valve syndrome (APVS), its association with anomalies and its outcome. Cardiac evaluation included assessment of cardiothoracic ratio, diameter of pulmonary arteries and doppler flow values in the pulmonary trunk. In the conclusion of the study he states that Absent Pulmonary Valve Syndrome can be accurately diagnosed by fetal echocardiography compared to ultrasound study.

Szpinda (2006) worked on the morphometric measurements of ductus arteriosus in humans where measurements like length, external diameter, volume of the DA in 131 human fetuses were studied by means of anatomical, digital and statistical methods. Length of the DA ranged from 3.95mm to 12.20mm, mean values of the diameter of the DA ranged from 1.34 to 3.49mm and the mean values of the DA volume ranged from 5.08 mm$^3$ to 117.30mm$^3$ for the 15$^{th}$ to 34$^{th}$ week gestational groups, respectively.
Wong et al (2007) suggested a simple screening tool for evaluation of fetal outflow tract abnormalities during second trimester by Pulmonary artery/Aorta ratio in three vessel view plane by USG established the reference range for pulmonary artery diameter as 2.1-4.93mm and for aorta as 2.1-5.2 mm.

Hemodynamic investigations on the embryonic aortic arches during late gestation was done in-vitro using a physiological model and compared with computational fluid dynamic (CFD) models by Pekkan (2008). CFD models allows for quantitative evaluation of hemodynamic values across different time periods. In the study normal arch pattern flow was compared to flow patterns of Pulmonary Atresia, Tetralogy of Fallot, Coarctation of Aorta and Hypo plastic Left Heart Syndrome. Flow patterns in-vitro and in Computational Fluid Dynamics models correlated in time and space with different conditions studied. Data analysis showed that irregular blood flow patterns usually happen during congenital heart disease like the above mentioned conditions directly influences the structure and function of the blood vessels.

A review article by Khuffash et al (2008) highlighted the role of Brain Natriuretic Peptide (BNP) and N terminal pro BNP (NTpBNP) as diagnostic markers for Patent Ductus Arteriosus (PDA). BNP and NTpBNP have diagnostic roles in the adult population. In children, these markers serve as indicators of cardiovascular disease and may be used to monitor therapeutic efficacy. They can be used as adjunct to echocardiography in the diagnosis of PDA and persistent pulmonary hypertension of the newborn.
Study on case selection technique and complications during ductal stenting were done by Alwi et al. (2008). He explained that the ductus in cyanotic heart disease has many morphological variations. For example, the ductus may arise occasionally from the subclavian artery or more proximally under the aortic arch giving rise to a long and vertical ductus with much of tortuosity rendering stent implantation technically impossible. Hence angiographic evaluations to delineate these morphological features are considered as the basis for case selection.

Comparative demonstration of micro calcifications within the atherosclerotic plaque of rabbit aorta with Optical Coherence Tomography (OCT) and Intravascular Ultra Sonography - Virtual Histology (IVUS- VH) was done by Mancuso et al (2008). Optical Coherence Tomography as an intravascular imaging technology provides high-resolution, cross-sectional images of normal histological and pathological arterial structure. Comparison with intravascular ultrasound (IVUS), showed that OCT can better identify structures of the arterial wall, such as the internal and external elastic laminae and provide precise measurements of the thickness of tunica intima, media and adventitia.
The ability to selectively manipulate the mouse genome to model human diseases provides valuable insight into the genetic processes underlying developmental disorders. The use of transgenic mouse models to study the development of the Pharyngeal Arch Arteries and other malformations affecting the great arteries has provided key information to understanding the pathology of Pharyngeal Arch Arteries (PAA) -related abnormalities (Kameda et al, 2009). This has been the case for Di-George Syndrome (22q11 deletion syndrome).

Piyarat et al (2009) prepared useful tools for the assessment of fetal aortic arch abnormalities. In her study of Fetal Aortic Arch Measurements from 14th to 40th weeks of gestation using spatiotemporal Image Correlation Volume Data Sets, reference range for transverse aortic arch diameter (TAD) and distal aortic isthmus diameter (DAID) was established. The best regression models for TAD were –1.01 + 1.69 mm and for DAID was 0.85 + 1.54 mm. Further a table of nomogram for 5th, 50th, and 95th percentile ranges were constructed.

Nowak (2011) studied the development of the aortic isthmus during the 4th to 8th months of fetal life in 233 human fetuses. Study data showed that the dimensions of the aortic isthmus increased linearly in relation to time, ratio of the aortic isthmus to ascending and thoracic aorta decreases as time proceeds, whereas the aortic isthmus diameter to that of the DA increases and that there was no significant difference between the two sexes.
An observational study on microscopic structure of aorta in relation to different age groups was carried out to identify the histological changes of abdominal aorta in different age group ranging from new born to 70 years by Gupta et al (2011). During infancy mean thickness of tunica media was noted as 335 µs (range of 240-385 µs). Study findings showed degenerative changes like hypertrophy of intima and fibrosis of tunica media with increasing age leading to hardening of the arteries and other associated changes.

Topography of the heart and major mediastinal vessels during human ontogenesis was quantitatively evaluated by Zhelezev LM (2013). 176 human fetuses aged 16-23 weeks was dissected by N.I. Pirogov’s method and histotopographical sections were taken in 3 mutually perpendicular planes. Recorded data’s are suggested as reference parameters for ultrasonographic and tomographic studies of fetuses.

Current understanding of the cardiac outflow tract development and pathogenesis was reviewed by Neeb et al (2013). He mentioned that the roles of neural crest cell population and second heart field derivatives during outflow tract development have been clarified and that experimental studies in animals with genetic investigations are yet to explain the molecular regulations and causes of anomalies during morphogenesis of outflow tract.
**Kowalski et al (2013)** studied the window period during which critical changes take place in the **patterning of aortic arches** using in-vivo data’s and Computational Flow Dynamic model predicted data’s like Flow and Wall Shear Stress (WSS) patterns. Studies on the chick embryo in vivo using fluorescent dye injections at stage 21 of Hamburger-Hamilton staging showed that the pattern of aortic arch diameter coincides with the changes in Wall Shear Stress parameter. Relationship between hemodynamic changes and its relation to aortic arches can lead us to a better understanding of the anomalies associated with it.

**Mapping of human Congenital Heart Disease genes** (CHD) was discussed by **Srivastava and Olson (2013)**. Understanding the genetical basis of cardio-genesis allows the possibility of cardiac repair through genetic reprogramming of cells by changing their fate. Method of Genomic sequence profiling will allow genetic screening for identification of CHD gene mutations and polymorphisms. Comparative analysis of the concerned genes can enable disease genes to be identified in humans from studies in other species and vice versa.

Studies on **molecular regulations of elastogenesis** - formation of elastic fibers in aorta, pulmonary trunk and ductus arteriosus was done by **Yokoyama in 2014**. During fetal life increase in the packing density of elastic fibers prevents the vascular wall collapse in aorta and pulmonary trunk. Data analysis suggested that degrading of LOX protein leading to PGE$_2$ via EP4 signaling inhibits elastogenesis in the DA, but not in the aorta. The lower levels of elastic fibers are directly associated with the obliteration process of the DA after birth.
Ductus arteriosus (DA) from six fetuses of different gestational period (20-30 weeks) were studied histologically to observe the process of closure by Hydrina D Silva (2014). Gradual increases in length, outer and inner diameter of ductus was observed. Length of DA was noted to be 2.5 -10.33 mm. Outer & inner diameter was measured using ocular scale and was noted to be 1.423-3.385 mm & 0.648-1.912 mm. Tunics of the ductus showed sequential structural changes in the arrangement of muscle fibers, collagen and elastic fibrils.

Anatomical closure of the Ductus Arteriosus: A study in 30 specimens of 13-40 weeks was undertaken by Meera Kuganathan et al (2014) were measurements of DA (Length, Diameter and Thickness) was studied which showed that gestational age is directly proportional to these parameters. DA was found to originate either from left pulmonary artery (83.33%) or from bifurcation of pulmonary trunk (16.66%). Histological observations showed the obliteration process of duct to start by 20 weeks of gestation.

Ultra-structural studies on the aortic media by Julius et al (2014) concluded that conventional concepts of Medial Lamellar Unit (MLU) should be revised to Musculo-Elastic Fascicle (MEF). To accommodate MEF, the mosaic structure comprising of interaction between collagen, smooth muscle cell and elastin structure.
Molecular studies on regenerative capability of epicardium and its underlying mechanism was studied by Wong et al (2015). Regenerative ability of the mesothelium has been studied extensively considering its clinical implications. Experimental study using genetic approach in zebra fish, showed that epicardium is critical for proliferation and regeneration of cardiomyocytes after injury and that the mechanism is related to Hedgehog signaling pathway which originated from the outflow tract.

Marantz P and his colleagues (2015) had discussed about the possibilities of In-utero cardio-fetal interventional procedures in their study. Mentioning that interventional aspects studied in animal models with its limitations needs far more time span before its practical feasibility for human application. Multi-centric researches on fetal heart intervention with new perspectives are promising. It is possible that fetal aortic valvuloplasty may find its place in the near future among the treatment strategies of congenital heart disease.
MATERIALS AND METHODS

Study proposal titled as “Morphometric study of the aorta, pulmonary trunk and ductus arteriosus with gross and histological correlation in human fetuses of Tamil Nadu population” was submitted to Institutional Human Ethics Committee. Approval to conduct the study was granted with a proposal no. : 14/171 for a period of 1 year. 100 samples were collected from PSG Hospitals, out of which 50 aborted fetuses and 50 ultra-sonographic images of fetuses aged between 14-36 weeks of gestation was collected. Parental consent and permission to retrieve images from Picture Archive Communication System (PACS) was collected accordingly.

Methodology for the study was arrived after detailed discussions with guide based on the feasibility, available infrastructure and allotted time frame. Standardized protocols for CRL measurements, Dissection steps, Staining procedures and USG measurements were established. Inclusion and Exclusion criteria’s were formed. Levels at which histological sections take were shown

1. Level of Aortic Section. 2. Level of Pulmonary Section 3. Level of ductus arteriosus section
Based on the gestational age of the fetuses, collected samples were categorized into 3 groups

GROUP I – 14\textsuperscript{th} -20\textsuperscript{th} week.

GROUP II- 21\textsuperscript{st} – 30\textsuperscript{th} week.

GROUP III- 31\textsuperscript{st} to 36\textsuperscript{th} week.

Aborted Fetuses were fixed in 10\% formalin and dissections were carried out in relatively fresh specimens. Tissue processing was done in Automatic Tissue Processor, followed by wax embedding in labeled plastic cassettes and sections of 6 micron thickness was taken from aorta, pulmonary trunk and ductus arteriosus and were stained with \textbf{Hematoxylin and Eosin (H&E), Verhoeff - Van-Gieson (VVG)} for delineation of elastic laminae and thereby aid in the measurement of tunica media thickness.

Ultrasound examination was done by qualified and experienced sonographers. Ultrasonographic images were retrieved from the PACS system. Measurements were taken from the widest portion of aorta, common pulmonary trunk and ductus arteriosus using electronic onscreen calipers.
STUDY METHODOLOGY

Preparatory Phase
- Identification of research topic.
- Submission of Study Protocol to Ethics Committee.
- Receipt of approval letter for initiation of the study.
- Intimation to MGR University regarding thesis topic.

Study Phase
- Collection of parental consent from the selected group (for USG scans & Aborted Fetuses).
- Retrieval of USG saved images from PACS followed by measurements of internal diameter of the vessels.
- Dissection of the aborted fetus and removal of outflow tract vessels.
- Histological processing of the tissue followed by microscopic observation.

Analysis Phase
- Statistical Analysis of the collected data and its interpretation.
- Discussions with Guide and Thesis submission.
MORPHOMETRICS IN-UTERO (USG)

Ultrasound examination was performed with a transabdominal 2.5-5 MHz curvilinear transducer (HDI3500 or HDI5000; Siemens, USA) by experienced sonographers. Freeze frame images and electronic onscreen calipers were used for measurements.

1) Three vessel view plane was obtained during routine ultrasound examination, this was obtained by sliding cranially from the 4-Chamber view.

2) In the transverse plane, common pulmonary trunk and aorta was identified.

3) Spatial arrangement was confirmed by identifying the pulmonary trunk and aorta which were arranged in a straight line, decreasing order in size from the left anterior to the right posterior aspect of the mediastinum.

4) Calipers were then placed on the lines that defined the vessel wall from the widest portion of common pulmonary trunk and the widest portion of the aortic diameter.

5) Ductus arteriosus was identified as the first branch from the left pulmonary artery or at the point of bifurcation of pulmonary trunk and measurement was taken.

Graphs are planned to be established by evaluating pulmonary trunk and aorta ratio of fetuses, with an idea to use it as a potential screening tool for outflow tract abnormalities.
ULTRASONOGRAPHIC VIEWS.

Three Vessel View

S – SVC; A – Aorta;
P – Pulmonary Trunk.

RV – Right Ventricle
RA – Right Atrium
LV – Left Ventricle
LA – Left Atrium

Left Ventricular Outflow Tract
Right Ventricular Outflow Tract
MORPHOMETRIC MEASUREMENTS IN-UTERO

29 wk of Gestation

28 wk of Gestation
**STEPS USED DURING DISSECTION OF ABORTED FETUSES**

1. An inverted Y shaped incision extending from the jugular notch till xiphoid process with lateral extensions below the rib cage to expose the thoracic cavity was done.

2. Careful removal of thymus was made to expose the heart with its great vessels.

3. Chambers of the heart, aorta, pulmonary trunk, ductus arteriosus with pericardium in situ was identified.

4. Attachment of heart to the diaphragm was detached by cutting through the septum transversum.

5. Transverse incision above the diaphragm was made to free the esophagus and the descending thoracic aorta and another incision to free trachea and esophagus was made. at the level of the root of neck

6. Heart in-toto with lungs, great vessels, trachea, esophagus, descending thoracic aorta and left vagus was dissected out.

7. Stripping of pericardial reflection over the aorta, pulmonary trunk and the dense connective tissue over the ductus arteriosus was done carefully.

8. Careful dissection of the pulmonary hilum was done to detach the lobes of the lungs.

9. Heart was placed in left lateral position the left recurrent laryngeal nerve enwrapping the ductus arteriosus was carefully freed.

10. A transverse incision was made at the level of aortic and pulmonary root and ductus arteriosus was dissected out separately.
ILLUSTRATION OF DISSECTION STEPS

FIG: 1 SKIN INCISION MARKINGS

26 WEEK OF GESTATION

Fig 1a: EXPOSURE OF THORACIC CAVITY

28 WEEK OF GESTATION

Inverted Y shaped marking

Lung

Heart

Pericardium

Thymus
ILLUSTRATION OF DISSECTION STEPS

Fig 1b: EXPOSURE OF GREAT VESSELS

PERICARDIAL STRIPPING DONE

IDENTIFICATION OF GREAT VESSELS

30 WEEK OF GESTATION
Following fixation of the tissue material in 10% formalin for 2-3 weeks, chemical processing was done in Automatic Tissue Processor and prepared further for the paraffin embedding.

**DEHYDRATION:** Dissected tissue blocks are placed in cassettes and are numbered with lead pencil. Considering the size & type of blood vessel, 100% acetone processing is done for 60 minutes i.e., 3 changes for 20 mts. in each jar.

**CLEARING:** Considering the tissue section thickness of 6 microns each, xylene processing is done for 40 minutes i.e., 2 changes for 20 mts. in each jar.

**INФILTRATION:** Tissue is then transferred into next jar for a bath in molten paraffin wax. Temperature of 54-56 degree Celsius was maintained for 40 minutes i.e., 2 changes for 20 mts. in each jar.
HEMATOXYLIN & EOSIN STAINING PROTOCOL

**OBJECTIVE:** Basic demonstration of tissue details i.e.; nucleus, cytoplasm, connective tissue and muscle.

**PRINCIPLE:** Hematein demonstrates the cell nuclei by process of differentiation and blueing up and eosin binding to protein within the cytoplasm and the connective tissue elements.

**REAGENTS:** Harris Hematoxylin Solution (Hematoxylin powder, alcohol, aluminium, mercuric oxide & distilled water) & Eosin.

**PROCEDURE:**

1) Deparaffinize section through xylene changes (I, II & III)
2) Dehydrate through graded alcohols to water.
3) Stain with harris hematoxylin solution for 4-5 minutes.
4) Dip in running tap water- 2-3 times.
5) Differentiate in 1% acid alcohol - 5-10 seconds.
6) Wash in running tap water.
7) Counter stain using eosin solution for 1 minute.
8) Dehydrate quickly through alcohols.
9) Blot to remove excessive stain.
10) Clear in xylene; mount the tissue section using coverslip and DPX.

**EXPECTED RESULTS**

Nuclei- Blue; Cytoplasm – Pink; Erythrocytes – Orange.

38
VERHOFF – VANGIESON’S STAINING PROTOCOL

OBJECTIVE: To differentiate elastic fibers in a tissue section.

PRINCIPLE: Verhoff’s Hematoxylin stains the elastic fibers.

REAGENTS: Wiegert’s Iron Hematoxylin Solution, Ferric Chloride, and Lugol’s iodine (staining solution) and Van-Gieson solution (aqueous picric acid & acid fuschin solution)

PROCEDURE:
1) Deparaffinize, clear and hydrate to water.
2) Cover with staining solution for 10 minutes.
3) Rinse in distilled/tap water.
4) To differentiate in 2% aqueous ferric chloride until elastic fibers appear black on a grey background.
5) Rinse in water.
6) Rinse in 95% alcohol to remove excessive staining
7) Counter stain using Van-Gieson solution.
8) Blot to remove excessive stain.
9) Dehydrate quickly through alcohols.
10) Clear in xylene and mount the tissue section.

EXPECTED RESULTS
Elastic fibers- Black, Collagen – Red.
HISTOLOGICAL STUDY OF AORTA, PULMONARY TRUNK AND DUCTUS ARTERIOSUS.

IEL: Internal Elastic Lamina  EEL: External Elastic Lamina
OBSERVATIONS

Systematic observations were carried out with multiple recordings to minimize observational bias. Grouping was done based on the criteria of Gestational Age (GA) to plot the data’s in accordance with development. Further comparison of the parameters was done to plot the growth pattern curve of vessels and correlate with histological findings.

Morphometric analysis was studied by grouping the human fetuses under 3 gestational age groups by using Hadlock’s criteria of Gestational Age assessment in Ultrasound.

- Group A: Gestational Age of 14-20 weeks. (n=10).
- Group B: Gestational Age of 21-30 weeks. (n=30).
- Group C: Gestational Age of 31-36 weeks. (n=10).

Histo-topographical correlations were done by grouping the aborted fetuses under 3 gestational age groups by using Crown-Rump Length measurement. Considering the difficulties encountered during the histological staining measurements were taken in 30 specimens alone out of the 50 studied specimens.

- Group 1: Gestational Age of 14-20 weeks (n=10).
- Group 2: Gestational Age of 21-30 weeks (n=10).
- Group 3: Gestational Age of 31-36 weeks (n=10).
Parameter of **internal diameter** for Aorta, Pulmonary Trunk and Ductus Arteriosus was recorded at different levels in Ultrasound as diagrammatically shown. Parameter of **thickness of tunica media** for Aorta, Pulmonary Trunk and Ductus Arteriosus was observed in Carl Zeiss light microscope with Axio Cam provision and measurements under 10X magnification in millimeter scaling was done using computerized software programme.

Recorded parameters were tabulated in Microsoft Excel 2007 and data’s were further entered into the software package - Statistical Package for the Social Sciences (SPSS) version 17 for statistical analysis. **ANOVA test** for comparison of internal diameter of vessels with Gestational Age was done and **Pearson Correlation co-efficient test** was done to quantify the strength of association between the thickness of tunica media and gestational age groups. Efforts have been made in the observation section to present the complex morphometric data’s in simplified graph and table formats.
Group A: 14-20 weeks.

Relationship between the Internal diameter and Gestational Age

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Age of the Fetus</th>
<th>Mean Internal Diameter of Great Vessels (mm)</th>
<th>Ratio</th>
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<tr>
<td></td>
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<td>Aorta</td>
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<td>N=10</td>
<td>16-20 wks</td>
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### DATA SHEET -1

**Group A: 14-20 weeks**

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### Group B – 21-30 weeks

Relationship between the Internal diameter and Gestational Age

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<th>Age of the Fetus</th>
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**Group B - 21-30 wks**

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Group C – 31-36 week

Relationship between the Internal diameter and Gestational Age

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<th>Ratio</th>
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Group C - 31-36 wks

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## Analysis of Variance Test (ANOVA)

### DESCRIPTIVE STATISTICS

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<th>Std. Error</th>
<th>95% Confidence Interval for Mean</th>
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<td>.50195</td>
<td>.09164</td>
<td>3.2459</td>
<td>3.6208</td>
<td>2.60</td>
</tr>
<tr>
<td>31-36 wks</td>
<td>10</td>
<td>5.7700</td>
<td>.46679</td>
<td>.14761</td>
<td>5.4361</td>
<td>6.1039</td>
<td>4.70</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>3.6820</td>
<td>1.22286</td>
<td>.17294</td>
<td>3.3345</td>
<td>4.0295</td>
<td>1.80</td>
</tr>
</tbody>
</table>

### Multiple Comparisons

<table>
<thead>
<tr>
<th>(I) GA (wks)</th>
<th>(J) GA</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-20 wks</td>
<td>3.00</td>
<td>-1.09333*</td>
<td>.16684</td>
<td>.000</td>
<td>-1.4290</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>-3.43000*</td>
<td>.20433</td>
<td>.000</td>
<td>-3.8411</td>
</tr>
<tr>
<td>21-30 wks</td>
<td>1.00</td>
<td>1.09333*</td>
<td>.16684</td>
<td>.000</td>
<td>.7577</td>
</tr>
<tr>
<td></td>
<td>3.00</td>
<td>-2.33667*</td>
<td>.16684</td>
<td>.000</td>
<td>-2.6723</td>
</tr>
<tr>
<td>31-36 wks</td>
<td>1.00</td>
<td>3.43000*</td>
<td>.20433</td>
<td>.000</td>
<td>3.0189</td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>2.33667*</td>
<td>.16684</td>
<td>.000</td>
<td>2.0010</td>
</tr>
</tbody>
</table>

* The mean difference is significant at the 0.05 level.
Relationship between the Internal diameter of Aorta and Gestational Age

Aorta: Regression equation: \(-1.553 + 0.209 \times \text{GA}\)

Mean internal diameter of aorta as recorded during 14-20 wks is 2.3 mm.
Mean internal diameter of aorta as recorded during 21-30 wks is 3.4 mm.
Mean internal diameter of aorta as recorded during 31-36 wks is 5.7 mm.

Analysis of the internal luminal diameter reveals the growth of aorta to be linear in accordance to the gestational age. With the expressed regression equation it can be elucidated that mean value regresses just -1.1553 and +0.209 from minimum and maximum values respectively.
Relationship between the Internal diameter of P.T. and Gestational Age

Regression equation for PT: \(-2.409 + 0.256X_{GA}\)

Mean internal diameter of P.T. as recorded during 14-20 wks is 2.5 mm
Mean internal diameter of P.T. as recorded during 21-30 wks is 3.7 mm
Mean internal diameter of P.T. as recorded during 31-36 wks is 6.5 mm

Analysis of the internal luminal diameter reveals that the growth of the pulmonary trunk to be linear in accordance to the gestational age. With the expressed regression equation it can be elucidated that the mean value regresses -2.409 and +.256 from min. and max. values respectively.
Relationship between the Internal diameter of P.T. and Gestational Age

Regression equation for DA : -1.156 + 0.152XGA

Mean internal diameter of D.A. as recorded during 14-20 wks is 1.9 mm
Mean internal diameter of D.A. as recorded during 21-30 wks is 2.3 mm
Mean internal diameter of D.A. as recorded during 31-36 wks is 4.2 mm

Analysis of the internal luminal diameter reveals that the growth of the ductus arteriosus to be linear in accordance to the gestational age. With the expressed regression equation it can be elucidated that the mean value regresses -1.156 and +.152 from min. and max. values respectively.
Relationship between the Tunica Media thickness and Gestational Age.

GROUP - 1

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Number</th>
<th>Thickness of tunica media (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-36 wks</td>
<td>30</td>
<td>Aorta 0.35  P.T. 0.38  D.A. 0.25</td>
</tr>
</tbody>
</table>
Relationship between the Tunica Media thickness and Gestational Age

GROUP – 2

<table>
<thead>
<tr>
<th>No</th>
<th>Age of the Fetus</th>
<th>Mean Thickness of tunica media (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Aorta</td>
</tr>
<tr>
<td>N=30</td>
<td>14-36 wks</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Gestational age in weeks (14-36 wks)
Relationship between the Tunica Media thickness and Gestational Age

GROUP – 3

Ductus Arteriosus

<table>
<thead>
<tr>
<th>No.</th>
<th>Age of the Fetus</th>
<th>Mean Thickness of tunica media (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Aorta</td>
</tr>
<tr>
<td>N=30</td>
<td>14-36 wks</td>
<td>0.77</td>
</tr>
</tbody>
</table>
DESCRIPTIVE STATISTICS

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Tunica thickness</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aorta</td>
<td>.5550mm</td>
<td>.18562</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>PT</td>
<td>.6080 mm</td>
<td>.20577</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>DA</td>
<td>.3460</td>
<td>.09328</td>
<td>30</td>
</tr>
</tbody>
</table>

PEARSON CORRELATION CORRELATION COEFFICIENT ANALYSIS

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Tunica thickness</th>
<th>Statistical Test</th>
<th>r value</th>
<th>r² value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aorta</td>
<td>Pearson Correlation</td>
<td>0.92**</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sig. (2–tailed)</td>
<td>.000</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>PT</td>
<td>Pearson Correlation</td>
<td>0.91**</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.000</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>DA</td>
<td>Pearson Correlation</td>
<td>0.82**</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.000</td>
<td></td>
</tr>
</tbody>
</table>

r value : 0.76-1 : High Correlation;
r value : 0.51-0.75 : Moderate Correlation;
r value : 0.1-0.5 – Low Correlation
<table>
<thead>
<tr>
<th>S.No.</th>
<th>Age of the Fetus</th>
<th>Thickness of tunica media (mm)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Aorta Max-Min</td>
<td>P.T. Max-Min</td>
</tr>
<tr>
<td>1</td>
<td>16 wk</td>
<td>0.40 - 0.31</td>
<td>0.36- 0.29</td>
</tr>
<tr>
<td>2</td>
<td>16wk</td>
<td>0.38 – 0.30</td>
<td>0.39- 0.28</td>
</tr>
<tr>
<td>3</td>
<td>16wk</td>
<td>0.36 – 0.27</td>
<td>0.42 – 0.31</td>
</tr>
<tr>
<td>4</td>
<td>17wk</td>
<td>0.39 - 0.31</td>
<td>0.41 – 0.36</td>
</tr>
<tr>
<td>5</td>
<td>17wk</td>
<td>0.37 - 0.29</td>
<td>0.39 - 0.33</td>
</tr>
<tr>
<td>6</td>
<td>18wk</td>
<td>0.41 – 0.28</td>
<td>0.40 – 0.31</td>
</tr>
<tr>
<td>7</td>
<td>18wk</td>
<td>0.44 - 0.30</td>
<td>0.47 – 0.37</td>
</tr>
<tr>
<td>8</td>
<td>18wk</td>
<td>0.48 – 0.31</td>
<td>0.49 - 0.35</td>
</tr>
<tr>
<td>9</td>
<td>19wk</td>
<td>0.46 - 0.29</td>
<td>0.50 – 0.38</td>
</tr>
<tr>
<td>10</td>
<td>20wk</td>
<td>0.48 - 0.31</td>
<td>0.51 – 0.37</td>
</tr>
<tr>
<td>11</td>
<td>21wk</td>
<td>0.50 – 0.34</td>
<td>0.58 - 0.39</td>
</tr>
<tr>
<td>12</td>
<td>21wk</td>
<td>0.58 – 0.39</td>
<td>0.61- 0.43</td>
</tr>
<tr>
<td>13</td>
<td>22wk</td>
<td>0.66 – 0.38</td>
<td>0.69 – 0.40</td>
</tr>
<tr>
<td>14</td>
<td>23wk</td>
<td>0.69 - 0.41</td>
<td>0.72 – 0.44</td>
</tr>
<tr>
<td>15</td>
<td>24wk</td>
<td>0.66 - 0.39</td>
<td>0.70 - 0.48</td>
</tr>
<tr>
<td>16</td>
<td>26wk</td>
<td>0.72 – 0.44</td>
<td>0.74 – 0.55</td>
</tr>
<tr>
<td>17</td>
<td>26wk</td>
<td>0.70 - 0.42</td>
<td>0.72 – 0.53</td>
</tr>
<tr>
<td>18</td>
<td>28wk</td>
<td>0.69 – 0.40</td>
<td>0.71 - 0.55</td>
</tr>
<tr>
<td>19</td>
<td>28wk</td>
<td>0.77 - 0.44</td>
<td>0.79 – 0.58</td>
</tr>
<tr>
<td>20</td>
<td>30wk</td>
<td>0.79 - 0.48</td>
<td>0.83 – 0.62</td>
</tr>
<tr>
<td>S.No.</td>
<td>Age of the Fetus</td>
<td>Thickness of tunica media (mm)</td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>----------------</td>
<td>--------------------------------</td>
<td>------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aorta Max-Min</td>
<td>P.T. Max-Min</td>
</tr>
<tr>
<td>21</td>
<td>31wk</td>
<td>0.74 – 0.56</td>
<td>0.82- 0.61</td>
</tr>
<tr>
<td>22</td>
<td>31wk</td>
<td>0.76 – 0.68</td>
<td>0.81- 0.70</td>
</tr>
<tr>
<td>23</td>
<td>31wk</td>
<td>0.69 – 0.51</td>
<td>0.77 – 0.62</td>
</tr>
<tr>
<td>24</td>
<td>33wk</td>
<td>0.79 - 0.66</td>
<td>0.86 – 0.74</td>
</tr>
<tr>
<td>25</td>
<td>33wk</td>
<td>0.75 - 0.70</td>
<td>0.79 - 0.69</td>
</tr>
<tr>
<td>26</td>
<td>34wk</td>
<td>0.79 – 0.68</td>
<td>0.84 – 0.72</td>
</tr>
<tr>
<td>27</td>
<td>34wk</td>
<td>0.85 - 0.77</td>
<td>0.89 – 0.83</td>
</tr>
<tr>
<td>28</td>
<td>34wk</td>
<td>0.92 – 0.81</td>
<td>0.96 - 0.91</td>
</tr>
<tr>
<td>29</td>
<td>35wk</td>
<td>0.97 - 0.85</td>
<td>1.04 – 0.99</td>
</tr>
<tr>
<td>30</td>
<td>35wk</td>
<td>0.99 - 0.83</td>
<td>1.08 – 0.97</td>
</tr>
</tbody>
</table>
TOPOGRAPHICAL CORRELATIONS

Aorta and Pulmonary Trunk are considered as the great vessels of the heart with the Superior Vena Cava. Pulmonary Trunk arises from the right ventricle in a relation of anterior aspect to the aorta and in a direction towards the left shoulder. Division of pulmonary trunk into right and left pulmonary artery is the main morphological feature that differentiated it from ascending aorta.

Ascending aorta – outflow tract vessel of the left ventricle, is present in the posterior aspect and to the right side of pulmonary trunk. As it emerges it is directed to the right shoulder. Division of arch of aorta into brachio-cephalic trunk, left common carotid and left subclavian artery was noted in all dissections.

Correlation of ductus arteriosus to the great vessels was elucidated clearly in a left lateral position of heart. Post exposure of pericardial reflections over it. It emerged from the proximal part of the left pulmonary artery or at the pulmonary trunk bifurcation point. In most of the cases it joined with the aorta antero-laterally just beneath the origin of the subclavian artery. Other relations noted were the thymus gland lying anteriorly, left primary bronchus inferiorly and the left recurrent laryngeal nerve that loops it.
ILLUSTRATION OF DISSECTION STEPS

Fig. 1c: DUCTUS ARTERIOSUS

LOOPING OF L. RLN SEEN

- L. RLN: Left Recurrent Laryngeal Nerve
DISCUSSION

Non-invasive monitoring of fetal health using ultrasonography has evolved as the mainstream practice in materno-fetal healthcare. Considering the lack of quantitative anatomical data regarding the outflow tract vessels in Tamilnadu population, the study design was primarily aimed to **quantify and correlate the developmental parameters** like internal diameter and tunica media thickness of Aorta, Pulmonary Trunk and Ductus Arteriosus with gestational age. Spatio-temporal variations in the observed parameters, general trend of growth and exceptions noted are discussed with histological basis.

Efforts have been taken in this section to critically examine the study findings and compare it with the existing knowledge on the topic. Interpretation of statistically significant data with its underlying mechanism, limitations of the study, clinical relevance and directions for future research has been highlighted. Similarities and differences of the findings from earlier studies are tabulated.
Morphometric measurements in ultrasonography

An approach to study normal fetal anatomy using ultrasonography was undertaken for most commonly in medically induced abortion post mortal intrauterine retention leads to autolysis and maceration of visceral organs which directly affects the measurements. Practical difficulties encountered during short and long duration formalin fixation led many researchers to focus on noninvasive in-utero measurements of heart and its vessels as strongly suggested by Ursell and Achiron.

Comparison between the mean diameters of Pulmonary Trunk and Aorta from 14 weeks gestation is crucial for early detection of severe cardiac and outflow tract abnormalities. In the present study mean aortic and pulmonary internal diameters were 2.3, 2.5 mm respectively for 14-20 weeks of gestation. From 21-30 weeks it was 3.4, 3.7 and for 31-36 weeks it is 5.7 and 6.5 mm. These measurements are within the reference range and can be compared with studies conducted by Cartier, Ichida and Hornberger.
Individual and ethnic variations in cardiac growth usually presents a wide scatter of normal values with values lying usually away from the regression curve. Standardisation for such a limitation is done by calculating the ratio of the pulmonary trunk to aorta for each subject which is interestingly much constant. During 16-24 weeks of pregnancy the PT/A ratio and its S.D. is 1.16 (0.18) as plotted by Wong and throughout infancy and childhood it is 1.06 (0.06) except the first 24 hours of life, when the value is larger 1.29 (0.12) but within one week it decreases to the normal ratio as found in the older age groups as plotted by Ichida.

In our present study the ratio of pulmonary trunk to aorta in different age group is:

Group A: 14-20 weeks – PT/A ratio (S.D.) was 1.08.

Group B: 21-30 weeks – PT/A ratio (S.D.) was 1.08.

Group C: 31-36 weeks – PT/A ratio (S.D.) was 1.13.

Nowadays PT/A ratio is commonly used as a simple screening tool to detect outflow tract abnormalities in foetuses which is easily measured during 3- vessel view and which consumes much lesser visualisation time in expert hands.
High correlation was found between measurements of aorta and pulmonary trunk against gestational age as derived from Karl-Pearson Co-efficient analysis in the present study which can be compared with the studies of Cartier and Hornberger.

Cartier and his colleagues using In Utero Echocardiographic evaluation using 2D real time during systole and diastole gave a value of $r = 0.994$ for aorta and $r = 0.996$ for pulmonary artery and M-mode measurements value of $r = 0.992$ for aorta, $r = 0.973$ for pulmonary artery.

Hornberger in his echocardiographic study of the morphology and growth of the aortic arches in human fetuses presented correlation coefficients for the diameter of each aortic arch segment when related to gestational age varied from $r = 0.87$ to $r = 0.94$ for each.

In the present study the correlation coefficient for Aorta and Pulmonary Trunk was $r = 0.92$ and $r = 0.91$ respectively.
Achiron et al. in his ultrasonographic study during first half of gestation measured the Aortic diameter (AD) and Pulmonary Artery diameter (PD) as a function of gestational age (GA) in terms of 14-26 weeks was expressed by the regression equation and $r^2$ value of:

- $AD = -1.603 + 2.256 \times GA; \ r^2 - 0.94$ (High Correlation)
- $PD = -1.476 + 2.402 \times GA; \ r^2 - 0.94$ (High Correlation)

In the present study the regression equation for aortic diameter (AD) as a function of gestational age (GA) in terms of 14-36 weeks was expressed by the regression equation and $r^2$ value of:

- $AD$: Regression equation: $-1.553 + 0.209 \times GA; \ r^2 - 0.86$ (High Correlation)
- $PD$: Regression equation: $-2.409 + 0.256 \times GA; \ r^2 - 0.86$ (High Correlation)

Limitations of the present study is a low sample size of 50 and in-equal clustering of groups into (10, 20 & 30 fetuses in Group A, B and C respectively) which can be considering the limited time frame of 1 year for the study. Strength of the study lies in the practical exposure and the new learning experience of the researcher to on-screen caliper instrumentation tool encountered during measurement in USG images.
HISTOLOGICAL CORRELATIONS

Comparison between the tunica media thickness of Aorta, Pulmonary Trunk and Ductus Arteriosus against gestational age was done. Quantification of the parameter was done using Verhoeff-Vangieson stain that delineated internal and external elastic laminae. Measurement was made in millimetre scaling. Accompanied histological changes in the ductus arteriosus especially the obliteration process was also discussed in this section.

In the present histological study, clear distinction between all the tunics was not possible. Few endothelial cells nuclei were seen as it was lost mostly during tissue preparation phase. Sub-endothelial connective tissue was inconspicuous. The tunica media of aorta and pulmonary trunk showed elastic lamellae which were circumferentially arranged. Smooth muscle cells and collagen fibers were also seen. Tunica media of ductus arteriosus showed inner circular and outer longitudinal layer of smooth muscle cells. Similar histological descriptions were made by Robert et al (1964) when they compared aorta, pulmonary trunk and ductus arteriosus at different ages.
**Mean thickness of the tunica media** for Aorta, Pulmonary Trunk and Ductus Arteriosus is tabulated as follows.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Number</th>
<th>Thickness of tunica media (mm)</th>
<th>Aorta</th>
<th>P.T.</th>
<th>D.A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-20 wks</td>
<td>10</td>
<td>0.35</td>
<td>0.38</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>21-30 wks</td>
<td>10</td>
<td>0.54</td>
<td>0.60</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>31-36 wks</td>
<td>10</td>
<td>0.77</td>
<td>0.83</td>
<td>0.43</td>
<td></td>
</tr>
</tbody>
</table>

Our present study findings are in line with the histomorphometric studies conducted by **Meera Kuganathan and Hydrina D Silva.**

The association of gestational age with thickness of aorta showed high correlation with a $r$ value of 0.926 and $r^2$ value of 83%.

The association of gestational age with thickness of pulmonary trunk showed high correlation with a $r$ value of 0.914 and $r^2$ value of 83%.

The association of gestational age with thickness of ductus arteriosus showed moderate correlation with a $r$ value of 0.828 and $r^2$ value of 68%.

$r$– Correlation Coefficient; $r^2$– Coefficient of Determination

**Meurs Van Woezik** investigated the bodily growth alterations and change in internal diameters, with parameters of tunica media thickness and in the packing density of elastic fibers. Our study findings showed a highly positive correlation between gestational age groups and tunica media thickness with a positive trend line which are similar to the Woezik’s conclusions.
Incidental findings of Intimal Cushions and IEL fragmentation

Early hypothesis concerning ductal closure like traction exerted on the ductus by Chevers, valve development at the aortic end of ductus arteriosus by Strassman was nullified through histological studies. Structural changes in the tunics of ductus arteriosus formed the histological basis for its anatomical closure, i.e., by the migration of smooth muscle cells into the intimal layer, accumulation of ground substance and hyalinosis process leading to the ductal closure and its conversion into ligamentum arteriosum. Yen Ho et al (1979) suggested that proliferation of tunica media by the SMC and the contraction of tunica adventitia was the reason behind the fragmentation of the internal elastic lamina (IEL) and the formation of intimal mounds.

Histologically it was Swensson (1939) who first noted these cushions in 230 mm CRL fetuses approximating to a GA of 26 weeks. Daniseno et al (1955) considered that the intimal cushions usually appear around 24 weeks of gestation. Desliqneres (1970) mentioned about the absence of intimal cushions below 28 weeks of gestation. Gittenberger et al reported a period of 17-21 weeks for the formation of intimal mounds.

In the present study well developed intimal cushions and interrupted internal elastic lamina (IEL) were observed in fetus of a 20 week gestational age which is in line with the studies of Gittenberger.
INCIDENTAL FINDINGS OF THE HISTOLOGICAL STUDY.

Fig. 4: Showing Smooth Muscle Cell Migration

H&E stain (10X): Ductus Arteriosus of 20 wk gestation

IEL - Internal Elastic Lamina; SMC - Smooth Muscle Cell.
CONCLUSION

Measurements of Aorta, Pulmonary Trunk and Ductus arteriosus taken in ultrasound addressed the paucity of quantitative anatomical data concerning the growth of these vessels in our Tamilnadu population and as intended fulfilled the aim of study. Based on the study findings, it is advised to visualize the outflow tracts using the extended basic cardiac examination which is much critical for evaluation of congenital heart diseases and outflow tract disorders whenever feasible.

An attempt to correlate the growth parameters with histological basis and topographical relations as undertaken by the measurement of tunica media thickness showed high correlation with regard to gestational age for Aorta and Pulmonary Trunk. Incidental findings of ductal closure by formation of the intimal mounds and interruption of internal elastic lamina can further be extended to an ultra structural level. Influence of the left recurrent laryngeal nerve on the ductal musculature could have been addressed using on immuno-histochemical techniques.

Materno-Fetal health care as addressed through this study will be explored more by the researcher in the future....
**SUMMARY**

**TITLE:** Morphometric Study Of The Aorta, Pulmonary Trunk And Ductus Arteriosus In Human Fetuses Of Tamilnadu Population with Histo-Topographical correlations.

**INTRODUCTION:** Basic researches carried out in the fetus leads to an in-depth understanding of its structural and functional aspects eventually for the better care for pregnant mother and her growing fetus. Our present knowledge of fetal development and mal-development is much advanced owing to non-invasive monitoring of fetal health.

Fetal growth can be quantified by using morphometric parameters. An approach of morphometric analysis using ultrasound in-utero has been done to chart the developmental parameters which can be of use for radiological practice. Topographical relations with histological correlations of the Aorta, Pulmonary trunk and Ductus Arteriosus has also been undertaken for better correlation of structural changes with histological basis.
AIM: To study morphometric details of Aorta, Pulmonary Trunk & Ductus Arteriosus in the human fetuses between 14th to 38th week gestation in Tamilnadu Population: in-utero Ultrasonographic and Foetal study with Gross and Histological correlation.

OBJECTIVE:

- To identify the topographical relationship of Great Arterial Vessels with its associated structures. (Fetal dissection).
- To identify the Histological Changes in the Great Arterial Vessels in fetal specimen. (Hematoxylin & Eosin Stain & Verhoeff-Van Gieson stain).
- To estimate the Morphometric parameters of Great Arterial Vessels in live fetuses. (Ultrasonography).

JUSTIFICATION FOR THE STUDY: To address the paucity of quantitative anatomical data concerning the normal developmental stages of great arterial vessels in Tamilnadu Population with gross & histological correlation.

STUDY DESIGN: Observational study./Cross-Sectional study design.

DATA COLLECTION METHODS:

1. Spontaneously aborted fetuses from labour room, Department of Obstetrics & Gynaecology, PSG Hospitals.
2. USG Scan details of Pregnant Population from PACS, Department of Radiology, and PSG Hospitals.
**STUDY POPULATION:**

1. Spontaneously aborted fetuses from 9th to 36th week from labour room of PSG Hospitals.

2. USG Scan details of pregnant women population from 14th to 36th week from Dept. of Radiology, PSG Hospitals.

**STUDY LOCALE (GEOGRAPHIC AREA):** Coimbatore, Tamilnadu.

**SAMPLE SIZE:**

1. 50 spontaneously aborted fetuses that are received from June 2014 to August 2015, Department of Obstetrics & Gynaecology, PSG-IMS&R.

2. 50 USG Scan details of Pregnant women population from June 2014 to August 2015, Department of Radiology, PSG-IMS&R.

**Total:** 100. (50 Fetal / 50 USG Scan)

**SAMPLING METHOD:**

1. Collection of spontaneously aborted fetuses from labour room of PSG hospital.

2. Collection of USG Scan details from PACS - Department of Radiology PSG Hospital, Coimbatore.
**INCLUSION CRITERIA:**

1. Spontaneously aborted fetuses from 9 weeks to 40 weeks.

2. Still born fetus.

3. Dead born fetus.


**EXCLUSION CRITERIA:**

1. Macerated fetus.

2. Fetus with congenital heart anomaly as identified by USG from previous scan.

**STATISTICAL ANALYSIS:**

Internal diameter of Aorta, Pulmonary Trunk, Ductus arteriosus and their thickness of tunica media was observed and analyzed. ANOVA test for comparison of internal diameter of vessels with gestational age showed high statistical significance and Pearson Correlation co-efficient test was done to quantify the strength of association between the thickness of tunica media and gestational age groups. It showed high correlation for Aorta and Pulmonary Trunk and moderate correlation for ductus arteriosus.

**PERSON TAKING CONSENT:** Principal Investigator, English & Tamil version for Fetus & Scan details
BIBILOGRAPHY


