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Age related histomorphological changes in coronary arteries of pig (*Sus scrofa*)

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Abstract
The wall of coronary arteries was made up of three layers *viz*., tunica intima, tunica media and tunica adventitia. The tunica intima was made up of endothelium, subendothelial tissue and internal elastic membrane. The tunica media consisted of bundles of smooth muscle fibres intermingled with elastic and collagen fibres. The tunica adventitia was made up of loose connective tissue with collagen, elastic and nerve fibres. The thickness of tunica intima of coronary arteries increased with advancement of the age of the animal from group-I to group-II and reduced in group-III. Multiple splitting of the internal elastic membrane of coronary arteries was observed in Group-I and Group-II, duplicated, fragmented and discontinuous internal elastic membrane was observed in Group-III. The thickness of tunica media of the coronary arteries increased with advancement in age of the animal from group-I to group-III. The thickness of tunica media of right coronary artery was more than left coronary artery. The wall of right coronary artery was thicker than the wall of left coronary artery in all the three age groups.

Keywords: Coronary arteries**,** tunica intima, tunica media, tunica adventitia, internal elastic membrane

1. Introduction
The pigs are omnivorous animals and various aspects of their anatomical features are close to the human pattern with reference to the cardiovascular system. (Garg *et al.*, 2013) [8] Pigs possess a sparse, innate coronary collateral circulation consisting of a fine anastomotic network of endomural vessels similar to coronary collateral vessels in humans (Roth *et al*., 1990) [16]. Age related-changes in coronary artery anatomy and function represent an important cause of the age dependent impairment of the heart function (Amenta and Mione, 1988) [1].

2. Materials and Methods
The present study was conducted on right and left coronary arteries of SVVU-T-17 breed of pigs slaughter at ICAR-AICRP, Tirupati. The collected samples were divided into three groups based on their age i.e., Group I (upto six months), Group II (seven months to one year) and Group III (above one year) and in each group six animals were studied. The fresh tissue samples were fixed in 10% Neutral Buffered Formalin. Later these samples were processed for paraffin sections (Singh and Sulochana, 1997) [18]. About 5 µm thick paraffin sections were obtained and subjected to routine and special histological staining methods i.e Haemotoxylin and Eosin for routine histomorphology, Verhoeff's method for elastic fibres, Masson's trichome for collagen fibers, Wilder's method for reticular fibres and Bielschowsky method for nerve fibres (Singh and Sulochana, 1997) [18].

3. Results and Discussion
The wall of the coronary arteries consisted of three layers i.e., tunica intima, tunica media and tunica adventitia in all the three groups (Fig.1). Similar findings were reported by Gross *et al.* (1934) [10] in humans and Awal *et al.* (2001) [2] in black Bengal goat.

3.1 Tunica intima
The tunica intima was the inner most layer of the coronary arteries. The average thickness of tunica intima of right coronary artery in group-I was 15.7±1.98 µm, whereas in group-II it was 43.87±4.52µm and in group-III it was measured 21.53±2.13µm (Table.1). The average thickness of tunica intima of left coronary artery in group-I was 11.7±1.58 µm, in group-II it was 36.36±3.39 µm and in group-III it was 24.83±2.80 µm (Table.2). The thickness of tunica intima increased with advancement of age of animal from group-I to group-II and decreased in group-III. Similar findings were also observed by Tyagi and Dadgar (1978) [19] and Deopujari and Dixit (2010) [7] in humans.

The tunica intima was lined by a single layer of endothelial cells with prominent nuclei. A delicate layer of subendothelial connective tissue (Fig.1) was observed beneath the endothelial layer, which consisted of elastic and collagen fibres and smooth muscles. These observations are similar to the findings of Gross *et al.* (1934) [10] in humans. The thickness of subendothelial connective tissue layers increased with advancement of age of the animal.

The internal elastic membrane was placed between the tunica intima and tunica media. These observations were similar to the findings of Greep (1954) [9] in domestic animals and Copenhaver and Johnson (1958) [4] and Waller *et al.* (1992) [20] in humans. It was divided into multiple layers and formed the secondary internal elastic membrane in group-I and group-II (Fig.2). Similarly, Copenhaver and Johnson (1958) [4] observed splitting of internal elastic membrane during first decade in humans. But in group-III pigs, fraying, focal fragmentation, duplicated and discontinuous internal elastic membrane was observed. Similarly, Levene (1956) [14] reported fraying of internal elastic membranes with advancement of age in humans.

The smooth muscle cells in the tunica intima were arranged in longitudinal direction and their number also increased with advancement of age in pigs i.e., from group-I to group-III. The number of elastic fibres were decreased with advancement of age in the tunica intima i.e., from group-I to III animals**.** The number of collagen fibres in the tunica intima increased with age and their number was more in group-III followed by group-II and group-I.

Further, all the layers of the tunica intima appeared wavy and undulated due to the contractions of the artery after the death. Similarly, Copenhaver and Johnson (1958) [4] in humans and Dellmann and Carithers (1997) [6] in domestic animals also observed undulating or wavy pattern of internal elastic membrane.

3.2 Tunica media

The tunica media was the thickest layer in the coronary arteries and it formed the bulk of the arterial wall. Copenhaver and Johnson (1958) [4] in humans also observed that the tunica media was the thickest coat and consisted of 25-40 layers of circularly arranged muscle fibres, whereas, Khan *et al.* (2006) [12] also reported concentric layers of smooth muscles in tunica media of buffalo, goat, rabbit and rat.

The average thickness of tunica media of right coronary artery in group-I was 91.8±72 µm, in group-II it was 253.80±9.14 µm and in group-III it was 259.5±11.42 µm (Table.1). The average thickness of tunica media of left coronary artery in group-I was 53.2±7.32 µm, in group-II it 210.63±5.92 µm and in group-III it was 234.4±16.82 µm (Table.2).

The tunica media predominantly consisted of smooth muscle cells which were oriented in circular and oblique fashions with a centrally located nuclei. Cormack (1987) [5] also reported circularly arranged smooth muscles in humans.

The number of smooth muscle cells varied with age of animal in both the right and left coronary arteries. In the present study the tunica media had more number of smooth muscle layers in the right coronary artery than that of left coronary artery. Further, elastic and collagen fibres were also observed between the smooth muscle cells. Awal *et al.* (2001) [2] in black Bengal goats reported that the tunica media primarily composed of smooth muscle cells with some fine elastic fibres.

The elastic fibres were irregular in their distribution and they were present in greater concentration and appeared more prominent towards the inner half of tunica media when compared to the outer half of the tunica media. The elastic fibres in the tunica media were abundant in group-I and group-II (Fig.2), but comparatively fewer elastic fibres were observed in group-III animals (Fig.3). Barry *et al*. (2003) in humans reported the loss of elastic laminae of the tunica media with advancement of age as noted in the present study.

3.3 Tunica adventitia

The tunica adventitia was the outermost layer of the coronary arteries and thinner than tunica media. Contrary to this Dellmann and Carithers (1997) [6] in domestic animals observed that the tunica externa and tunica media have same thickness.

The average thickness of tunica adventitia of right coronary artery in group-I was 36±3.88 µm, whereas in group-II it measured 85.85±5.84 µm and in group-III it was 87.43±3.55 µm. (Table.1) The average thickness of tunica adventitia of left coronary artery in group-I was 28.3±2.79 µm, whereas in group-II it was 90±6.82 µm and in group-III it was 85.46±6.97 µm. (Table.2)

The tunica adventitia predominantly consisted of collagen fibres and some elastic fibres and smooth muscle cells. A band of elastic fibres were observed at the junction of tunica media and adventitia (Fig.3). These findings are similar to the observations of Gross *et al.* (1934) [10], Copenhaver and Johnson (1958) [4] in humans and Awal *et al.* (2001) [2] in black Bengal goats.

In the tunica adventitia of coronary arteries of pigs numerous blood vessels (Fig.4), few reticular fibres, (Fig.5) and nerve fibres were observed (Fig.6). Ham and Leeson (1961) [11] and Waller *et al.* (1992) [20] in humans, Cormack (1987) [5] in domestic animals and Samuelson (2007) [17] in domestic animals also noted similar findings.

The amount of collagen fibres in the tunica adventitia increased with advancement of age of the animal. The number of collagen fibres were high in tunica adventitia when compared to tunica intima and tunica media (Fig.7). Kumar (2010) [13] opined that the coronary arteries of humans, buffaloes, pigs, goats, dogs and rabbits have more density of collagen fibres in tunica adventitia, followed by in tunica media and tunica intima.

In the present study the external elastic membrane was ill-defined and not appreciated in the coronary vessels. Parmar (2002) [15] in the muscular arteries of goats also reported similar observations. Contrary to the present findings, Waller *et al.* (1992) [20] in humans and Dellmann and Carithers (1997) [6] in domestic animals observed external elastic membrane in large muscular arteries.

In the present study the maximum thickness of wall of right coronary artery was observed in group-III i.e., 368.77±16.58 µm, followed by group-II 336.33±15.21 µm and group-I 145.3±11.23 µm. These observations suggested that the thickness of wall of coronary arteries increased with advancement of the age of the animal.

The maximum thickness of wall of left coronary artery was observed in group-III 328.93±17.65 µm, followed by group-II i.e., 277.36±9.33 µm and group-I i.e., 86.5±7.38 µm. The above findings suggested that the wall thickness was comparatively less in left coronary artery than that of right

coronary artery. This might be due to the longer course of the left coronary artery than the right coronary artery and supplies larger part of the heart.

Table 1: Showing average thickness of wall of right coronary artery and its different layers in group-I, group-II and group-III.

S. No.	Group-I	Group-II	Group-III
Tunica intima	15.7±1.98 µm	43.87±4.52 µm	21.53±2.13 µm
Tunica media	91.8±72 µm	253.80± 9.14µm	259.5±11.42 µm
Tunica adventitia	36±3.88 µm	90±6.82 µm	87.43±3.55 µm
Total wall thickness	145.3±11.2 µm	368.77±16.5 µm	336.33± 15.2µm

Table.2: Showing average thickness of wall of left coronary artery and its different layers in group-I, group-II and group-III.

S. No.	Group-I	Group-II	Group-III
Tunica intima	11.7±1.58 µm	36.36±3.39 µm	24.83±2.80 µm
Tunica media	53.2±7.32 µm	210.63±5.92 µm	234.4±16.82 µm
Tunica adventitia	28.3±2.79 µm	84.13±6.34 µm	85.46±6.97 µm
Total wall thickness	86.5±7.3 µm	277.36±9.33 µm	328.33±17.6 µm

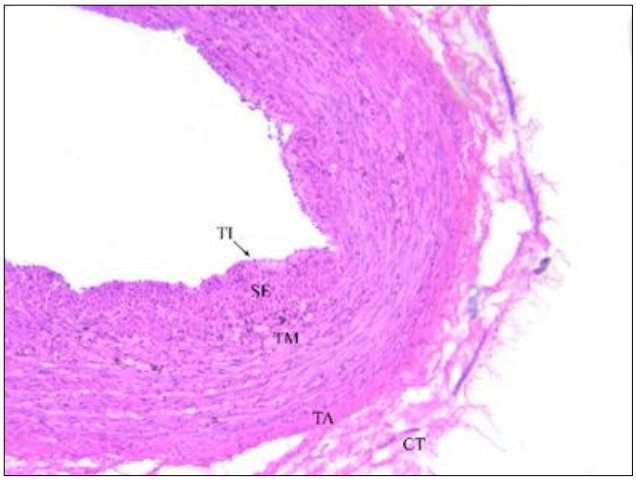

Fig 1: Photomicrograph of right coronary artery of pig of group-III showing tunica intima (TI), subendothelium (SE), tunica media (TM), tunica adventitia (TA) and CT- connective tissue. (Haematoxylin and Eosin X 100)

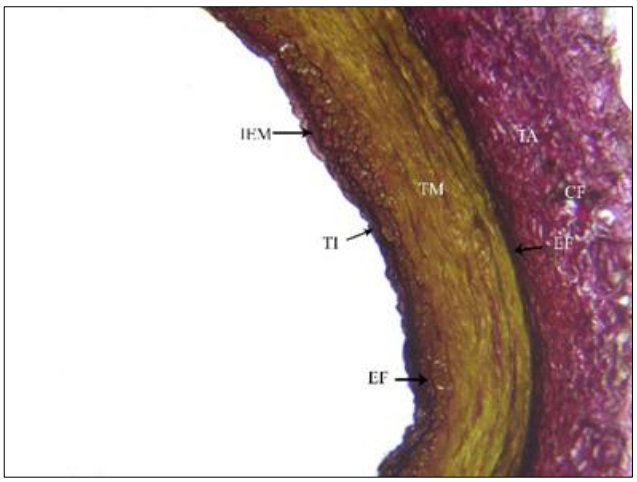

Fig 2: Photomicrograph of right coronary artery of pig of group-II showing splitting of internal elastic membrane (IEM) of tunica intima (TI), elastic fibres (EF) in tunica media (TM), TA- tunica adventitia and CF- collagen fibres. (Verhoeff's method X 100)

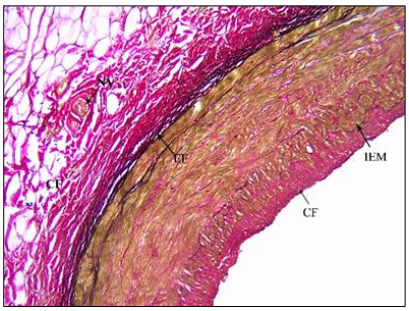

Fig 3: Photomicrograph of left coronary artery of pig of group-III showing fragmented and discontinuous internal elastic membrane (IEM) and collagen fibres in tunica intima (TI) and elastic fibres, collagen fibres, nervi vasorum in tunica adventitia (TA), EF-Elastic fibres, CF- Collagen fibres and NV-Nervi vasorum. (Verhoeff's stain X 100)

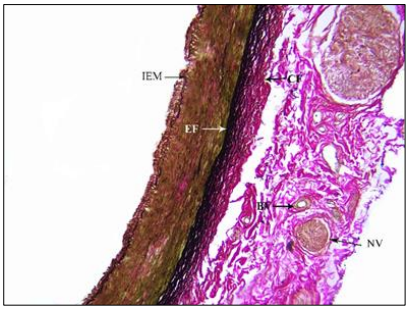

Fig 4: Photomicrograph of left coronary artery of pig of group-III showing fragmented and discontinuous internal elastic membrane (IEM) of tunica intima (TI), elastic and collagen fibres, vasa vasorum (VV), nervi vasorum (NV) in tunica adventitia (TA), EF-elastic fibres and CF- collagen fibres. (Verhoeff's stain X 100)

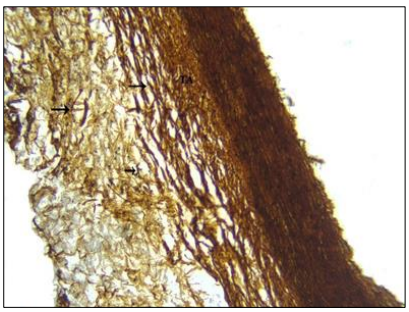

Fig 5: Photomicrograph of left coronary artery of pig of group-II showing reticular fibres (arrow) in tunica adventitia (TA). (Wilder's method X 100)

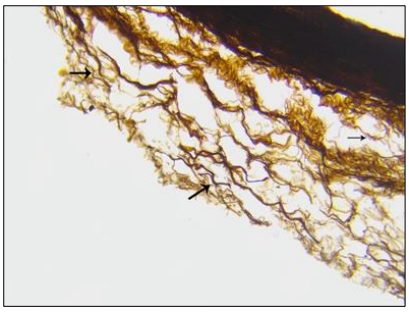

Fig 6: Photomicrograph of left coronary artery of pig of group-III showing nerve fibres (arrow) in tunica adventitia (TA). (Bielschowsky method X 100)

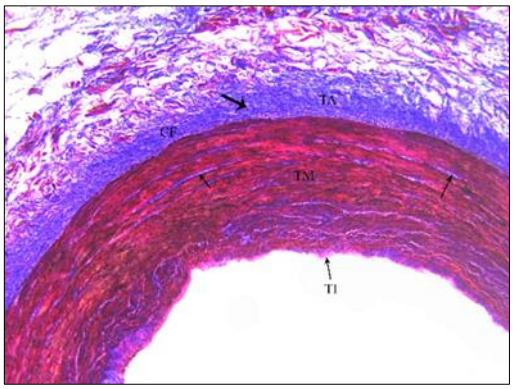

Fig 7: Photomicrograph of right coronary artery of pig of group-III showing tunica intima (TI), collagen fibres (CF) in tunica media (TM) and tunica adventitia (TA). (Masson's Trichrome X 100)

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