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Scanning Electron Microscopic Studies on the Ventricular Architecture of Pre-natal Non-descript Sheep

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ABSTRACT

Background: Heart is the principal organ of circulatory system that pumps blood into the blood vessels and performs many vital functions. Studies on its development before birth of utmost important to safeguard the animal from the occurrence and consequences of various developmental anomalies. The ultrastructural details of ventricular architecture of heart especially in pre-natal sheep has not yet been reported.

Methods: The collected foeti of sheep were divided into three age groups *viz.* early prenatal (up to 50 days), mid prenatal (51-100 days) and late prenatal (101 to 150 days). The samples from ventricles were processed for scanning electron microscopic study and subsequently, the samples were viewed and the photographs were taken in the facility available at Central Instrumentation Facility (CIF), OUAT, Bhubaneswar. The measurements of various parameters of ventricles were also taken at the ultrastructural level. The recorded data were subjected to routine statistical analysis.

Result: It was observed that the endocardium of the ventricles consisted of a simple squamous epithelium. The endothelial cells were elongated in shape and arranged linearly on the surface of ventricles in 33 days prenatal non-descript sheep. The boundaries among the endothelial cells were indistinct in this age group. There was presence of pores among the endothelial cells on the surface of the ventricles. In late prenatal period, the endothelial cells of the ventricles became elongated in shape. The subendocardial layer consisted of connective tissue fibers and conducting purkinje fibers, which were quite predominant in the late prenatal period, *i.e.* at the age of 120 days of gestation. The endocardial fibers were distinctly coiled around the endothelial cells of the ventricles in this age group. The myocardium comprised of cylindrical and highly branched cardiac muscle fibers with pores all over the surface of the myocardium of the ventricles. Each muscle bundle in the myocardium consisted of muscle fibers extending parallel to each other longitudinally and that these fibers made collateral connections with each other in some regions. The papillary muscles were ridge like structures projecting into the lumen of the ventricles of heart. The chordae tendinae connected the papillary muscles and the bicuspid and tricuspid valves. The endothelial surface of the chordae tendinae consisted of elongated endothelial cells with pores among them. The pores of various sizes were arranged linearly in patches on the endothelial surface of the chordae tendinae of the ventricles especially in the late prenatal period, *i.e.* at about 105 days of gestation.

Key words: Heart, Pre-natal, Scanning electron microscope, Sheep, Ventricle.

INTRODUCTION

The circulatory system plays a vital role in smooth working of the body of the animal (Jaiswal *et al*., 2017a, Jaiswal *et al*., 2017b and Janqueira and Carneiro, 2005). Heart is the central organ of circulatory system that pumps blood into the blood vessels and performs many vital functions (Sathapathy *et al*., 2013 and Sathapathy *et al*., 2014). The faulty development of heart may result in ectopia cordis, dextrocardia, hypoplasia, *etc*. Very often, these developmental anomalies of the heart cause foetal death and thereby severe economic loss to the farmers (Sahu *et al*., 2021). Due to close similarities in many of the systems between the animals and human being, the animals have always become a choice of interest for research purpose, which indirectly help the human being. The detailed ultrastructural study of the ventricular architecture of heart especially in pre-natal sheep has not yet been reported as evidenced from the available literature. Hence, the present ultrastructural study was undertaken to elucidate the age wise development of ventricles of heart in prenatal non-descript sheep.

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MATERIALS AND METHODS

The foeti of either sex of non-descript sheep were collected from the local slaughter houses situated at Laxmisagar and Jadupur of Bhubaneswar city during the period from July, 2020 to March, 2021 for the present study as a part of the doctoral research work. The adhering amniotic fluid from the body of the foeti was wiped by wet cotton. The crown

rump length (CRL) for each foetus was measured in centimetres (cm) with the help of non-stretchable nylon thread and graduated scale. Further, the CRL was placed on the standard CRL-Gestation Age Curve to estimate the approximate age of the foeti in days (Noden and Lahunta, 1985). Based on prenatal age, the collected sheep foeti were divided into three age groups *viz.* early prenatal (1-50 days), mid prenatal (51-100 days) and late prenatal (101 to 150 days). The samples of ventricles were processed for scanning electron microscopic study (Scanning Electron Microscope, Make: Hitachi and Model: S-3400N) and subsequently, the samples were viewed and the photographs were taken in the facility available at Central Instrumentation Facility (CIF), OUAT, Bhubaneswar. The measurements of various parameters of ventricles were also taken at the ultrastructural level by the inbuilt software programming system. The recorded data were subjected to routine statistical analysis (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

It was revealed that the endocardium was the inner layer of the ventricles of heart. It covered the inner surface of the heart and consisted of a simple squamous epithelium. The endothelial cells were elongated in shape and arranged linearly on the surface of ventricles in 33 days prenatal non-descript sheep (Fig 1 and Fig 2). Further, the boundaries among the endothelial cells were indistinct in this age group. The endothelial cells of a row appeared cord like. As the age advanced, *i.e.* during mid prenatal period at about 96 days of gestation, it was noted that the shape and size of the endothelial cells varied significantly (Fig 3). They were elongated to oval in shape with significant variation in their sizes. There was presence of pores among the endothelial cells on the surface of the ventricles. In late prenatal period, the endothelial cells of the ventricles became elongated with pores among them.

The average longitudinal diameters of the endothelial cells were found to be 5.00±0.36µ and 17.51±1.48µ in the early and mid prenatal stages respectively in non-descript sheep at different magnifications. Further, the average transverse diameter of the endothelial cell was observed as 2.55±0.09µ in the early prenatal stage of non-descript sheep. There was presence of pores among the endothelial cells on the surface of the ventricles. The average diameters of the pores were found to be 1.03±0.11µ, 2.21±0.27µ and 2.87±0.28µ in the early, mid and late prenatal stages respectively in non-descript sheep at different magnifications.

The subendocardial layer was present in between the inner endocardium and middle myocardium. It consisted of connective tissue fibers and conducting purkinje fibers (Ghonimi *et al*., 2015 and Sizer *et al*., 2020). They were quite predominant in the late prenatal period, *i.e.* at the age of 120 days of gestation (Fig 4). The endocardial fibers were distinctly coiled around the endothelial cells of the ventricles in this age group (Fig 5).

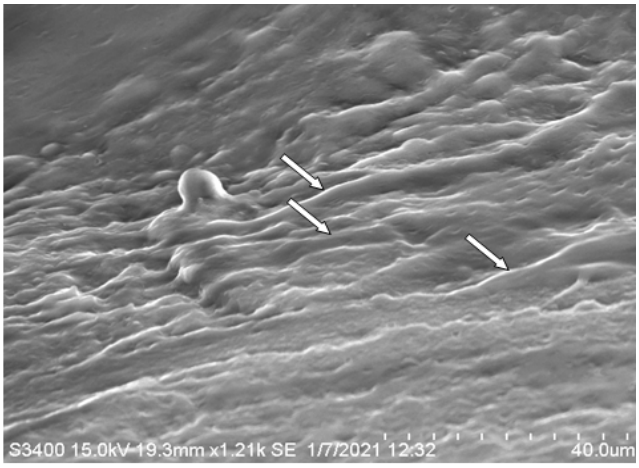

Fig 1: Photograph showing the linear arrangement of flat endothelial cells (white arrows) on the surface of left ventricle of 33 days prenatal non-descript sheep.

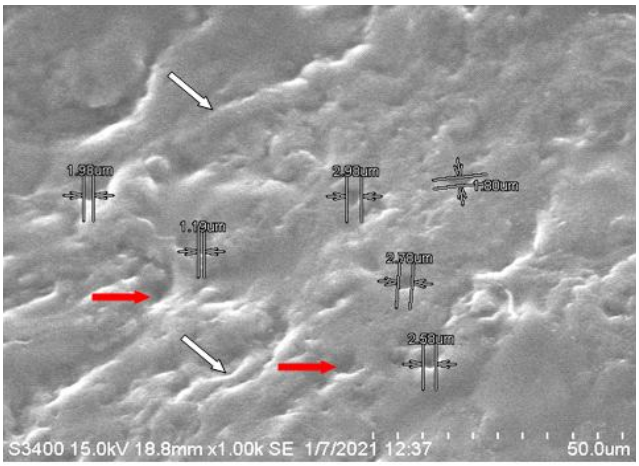

Fig 2: Photograph showing the indistinct boundaries among the endothelial cells (white arrows) along with pores (red arrows) on the surface of left ventricle of 33 days prenatal non-descript sheep.

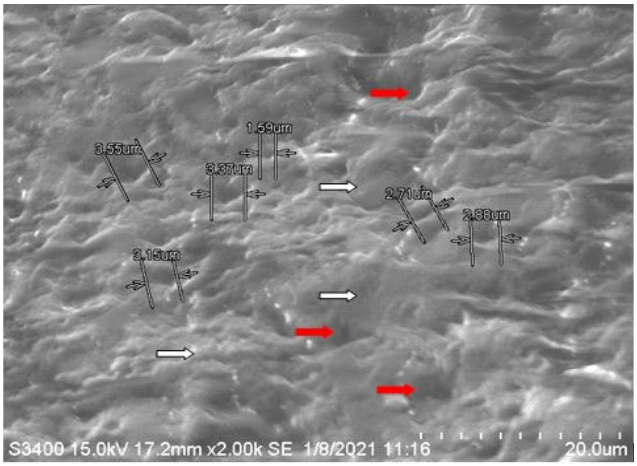

Fig 3: Photograph showing the irregular shaped endothelial cells (white arrows) along with pores (red arrows) on the surface of right ventricle of 96 days prenatal non-descript sheep.

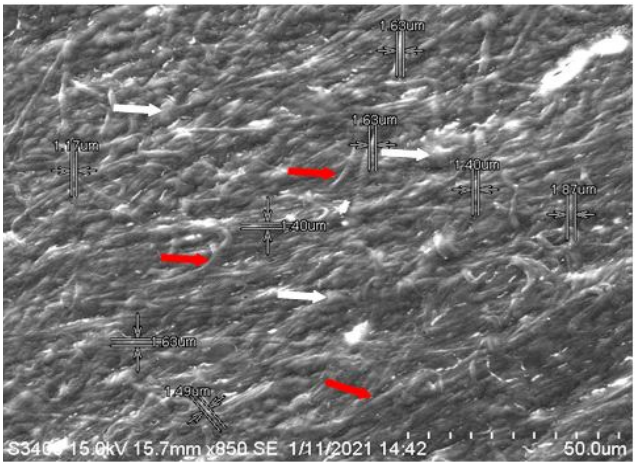

Fig 4: Photograph showing the endothelial cells (white arrows) and fibers (red arrows) in left ventricle of 120 days prenatal non-descript sheep.

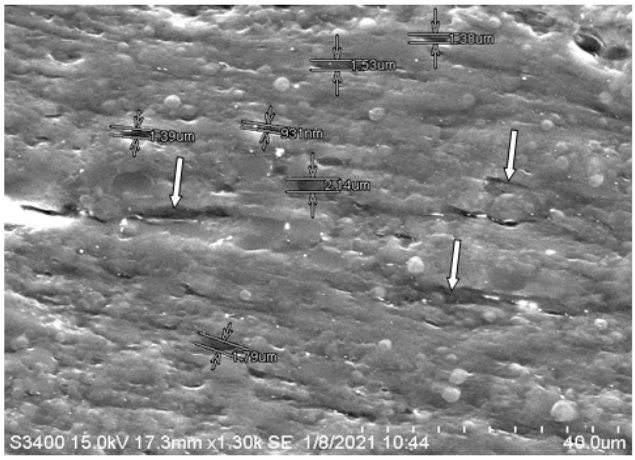

Fig 6: Photograph showing the longitudinal view of cardiac muscles along with pores (white arrows) in right ventricle of 105 days prenatal non-descript sheep.

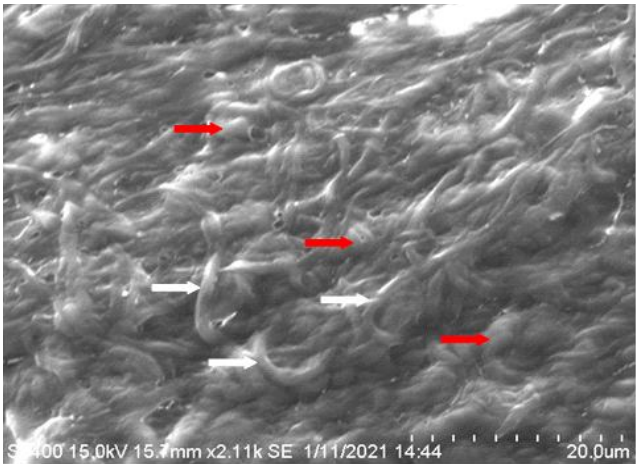

Fig 5: Photograph showing the coiling of fibers (white arrows) around the endothelial cells (red arrows) in left ventricle of 120 days prenatal non-descript sheep.

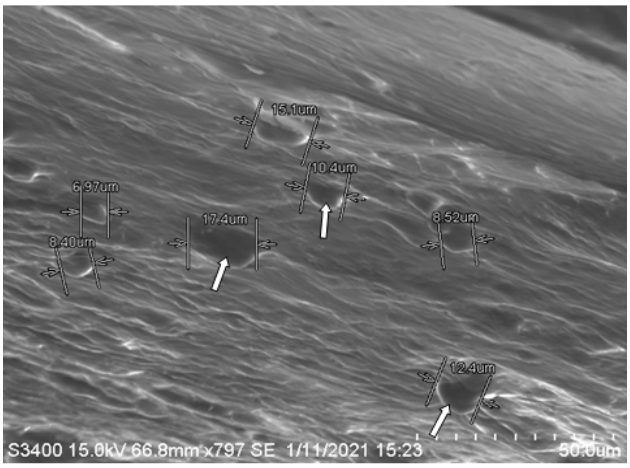

Fig 7: Photograph showing the myocardium along with pores (white arrows) in left ventricle of 120 days prenatal non-descript sheep.

The myocardium was the middle layer of the ventricles of heart present between the inner endocardium and outer pericardium (Myklebust *et al*., 1975 and Galfiva *et al*., 2017). It consisted of cylindrical and highly branched cardiac muscle fibers (Fig 6). It was observed that each muscle bundle in the myocardium consisted of muscle fibers extending parallel to each other longitudinally and that these fibers made collateral connections with each other in some regions (Jaiswal *et al*., 2017b). There was presence of pores all over the surface of the myocardium of the ventricles (Fig 7). The average diameters of the pores were found to be 5.13±0.48µ and 11.31±1.45µ in the mid and late prenatal stages respectively in non-descript sheep at different magnifications.

The papillary muscles were ridge like structures projecting into the lumen of the ventricles of heart (Fig 8). Further, there were presence of bands of chordae tendinae that connected the papillary muscles and the bicuspid and

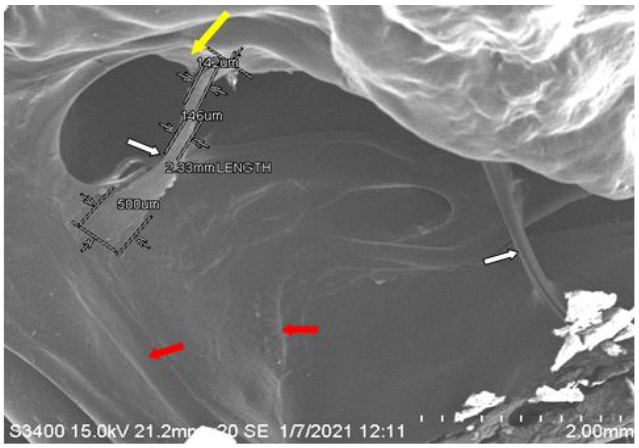

Fig 8: Photograph showing the chordae tendinae (white arrows) connecting the papillary muscles (red arrows) and the tricuspid valve (yellow arrow) in right ventricle of 33 days prenatal non-descript sheep.

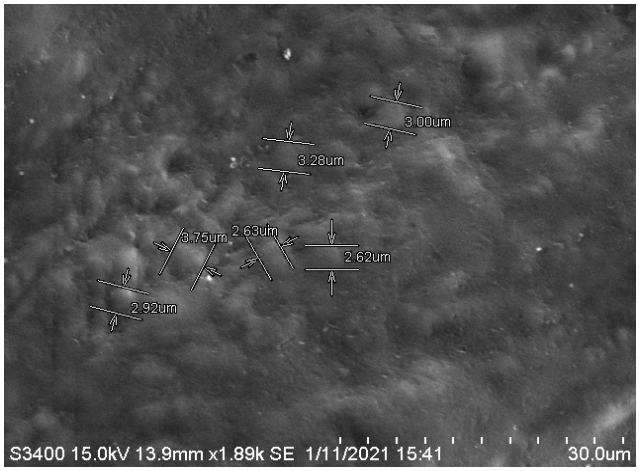

Fig 9: Photograph showing the oval shaped endothelial cells (white arrows) on the surface of chordae tendinae in right ventricle of 33 days prenatal non-descript sheep.

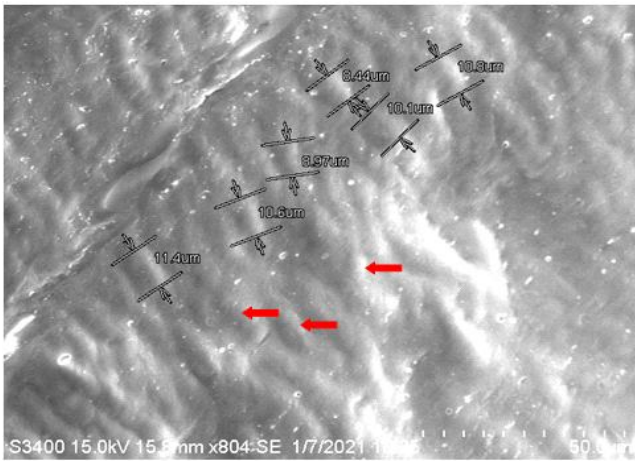

Fig 11: Photograph showing the elongated endothelial cells on the surface of chordae tendinae (white arrows) in right ventricle of 120 days prenatal non-descript sheep.

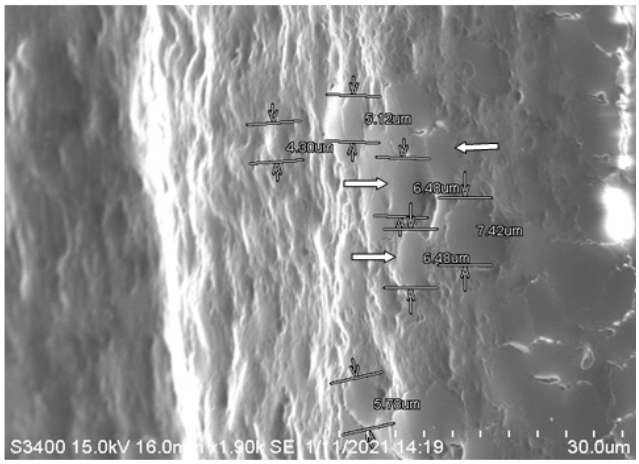

Fig 10: Photograph showing the elongated endothelial cells on the surface of chordae tendinae (red arrows) in left ventricle of 96 days prenatal non-descript sheep.

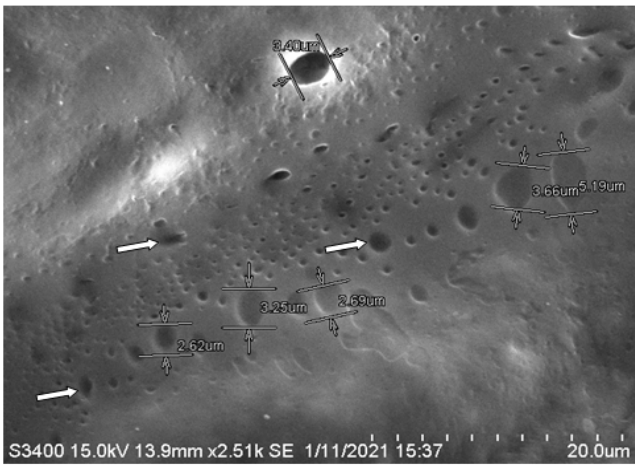

Fig 12: Photograph showing the linear arrangement of pores (white arrows) on the surface of chordae tendinae (white arrows) in left ventricle of 105 days prenatal non-descript sheep.

tricuspid valves (Fig 8). The sizes of the chordae tendinae varied within the ventricles of the animal. The average length of chordae tendinae was measured as 2.33±0.15mm in the early prenatal stage. Similarly, the average width of chordae tendinae was recorded as 500±20.21µ, 146±6.09µ and 142±7.25µ at the base, middle and apex respectively in the early prenatal stage at different magnifications.

It was noted that the endothelial surface of the chordae tendinae consisted of endothelial cells that varied significantly in size among the age groups (Morse *et al*., 1984). The endothelial cells were oval in shape in early prenatal stage (Fig 9), which gradually became elongated in the mid and late prenatal stages (Fig 10 and 11). The average diameters of the endothelial cells were found to be 3.03±0.17 and 10.05±0.46µ in the early and late prenatal stages respectively in non-descript sheep at different magnifications. The average longitudinal and transverse diameters of the endothelial cells were noted as 5.93±0.45µ and 3.06±0.35µ respectively in the mid prenatal period of the animal at different magnifications.

It was observed that the pores of various sizes were arranged linearly in patches on the endothelial surface of the chordae tendinae of the ventricles especially in the late prenatal period, *i.e.* at about 105 days of gestation (Fig 12). The pores were categorized as larger and smaller types. The average diameters of the larger and smaller pores were found to be 3.48±0.46µ and 533.5±62.53nm respectively in the late prenatal stages of the animals under study at different magnifications.

CONCLUSION

The ultrastructural ventricular architecture showed significant variations among different ages in the pre-natal sheep. Further, the present study provided a detailed baseline data

on the age wise ultrastructural development of ventricles of heart in pre-natal sheep that could help in studying various congenital developmental anomalies in different animals.

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