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Scanning Electron Microscopic Studies on the Auricular and Atrio-ventricular Valvular Architecture of Pre-natal Non-descript Sheep

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ABSTRACT

Background: Being the vital organ of circulatory system, the development of the heart before birth must be studied to safeguard the animal from the incidence of various developmental anomalies. The ultrastructural details of auricular and atrio-ventricular valvular 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 of auricle, bicuspid and tricuspid valves 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 auricles and valves were also taken at the ultrastructural level by the inbuilt software programming system. The recorded data were subjected to routine statistical analysis.

Result: It was revealed that the endocardium was the inner layer of the auricles of heart. The endothelial surface of the auricles was occupied by the simple squamous epithelium. The endothelial cells were elongated in shape in all the age groups. The subendothelial layer was consisted of connective tissue fibers and conducting fibers. The pectinate muscles covered the inner side of the right and left atria and were interconnected having a network like appearance except in mid prenatal stage, *i.e*. at 96 days of gestation, where they were arranged linearly. There was presence of pores among the pectinate muscles in both the atria in all the age groups under study. The endothelial surface of the bicuspid or mitral and tricuspid valves was lined by simple squamous endothelium that projected into the lumen of the valve. There was presence of pores among the endothelial cells of the valves. Further, the subendothelial layer was present just below the endothelium and comprised of connective tissue fibers and conducting fibers in both the valves. The width of the fibers in bicuspid valve was not uniform and was further categorized into thick and thin fibers especially in the mid prenatal stage, *i.e*. at 96 days of gestation. There was presence of clusters of pores among the endothelial cells of the tricuspid valve in the heart especially in the late prenatal stage, *i.e.* at 120 days of gestation period.

Key words: Auricle, Bicuspid valve, Pre-natal, Scanning electron microscope, Sheep, Tricuspid valve.

INTRODUCTION

The circulatory system plays a vital role in smooth functioning of the body of the cattle Janqueira and Carneiro, 2005) and Uttara fowl (Jaiswal *et al*., 2017a, Jaiswal *et al*., 2017b). 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* (Sahu *et al*., 2021a; Sahu *et al*., 2021b). 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 auricular and valvular architecture of heart especially in pre-natal sheep has not yet been reported. Hence, the present ultrastructural study was undertaken to elucidate the age wise development of auricles, bicuspid and tricuspid valves 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). The collected sheep foeti 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 of auricles 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 auricles, bicuspid and tricuspid valves of the hearts 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

The present SEM study showed that the myocardium and endocardium were the middle and innermost layers in the wall of the auricles of heart of non-descript sheep in prenatal stage. The present findings were in agreement with the reports of Leeson *et al*. (1985) in cattle, Eurell and Frappier (2006) in cattle, Dyce *et al*. (2009) in dog, Widmaier *et al.* (2011) in human, Konig *et al*. (2013) in domestic animals, Galfiova *et al*. (2017) in human and Varga *et al*. (2017) in human. The endothelial surface of the auricles was occupied by the simple squamous epithelium (Fig 1). The endothelial cells were elongated in shape in all the age groups. There were presence of pores among the endothelial cells in the auricles which were similar to the findings of Sizer *et al*. (2020) reported in the auricles of heart of Saanen goat.

The subendothelial layer was present in between the inner endocardium and middle myocardium. The present finding was in line with the reports of Myklebust *et al*. (1975) in sheep, Galfiova *et al*. (2017) in human and Sizer *et al*. (2020) in Saanen goat. It consisted of connective tissue fibers and conducting fibers (Fig 6). Further, the subendothelial layer continued with myocardium, the muscle layer of the heart (Fig 6). The present finding was in agreement with the reports of Saunders and Amoroso (2010) in human, Gauvin *et al*. (2013) in pig and Jaiswal *et al*. (2017b) in Uttara fowl. 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. The present finding was in agreement with the report of Jaiswal *et al*. (2017b) in Uttara fowl. The pectinate muscles covered the inner side of the right and left atria and were interconnected having a network like appearance (Fig 5). But they are arranged in linear fashion especially in mid prenatal stage, *i.e.* at 96 days of gestation (Fig 4).

There were presence of pores among the pectinate muscles in both the atria in all the age groups under study

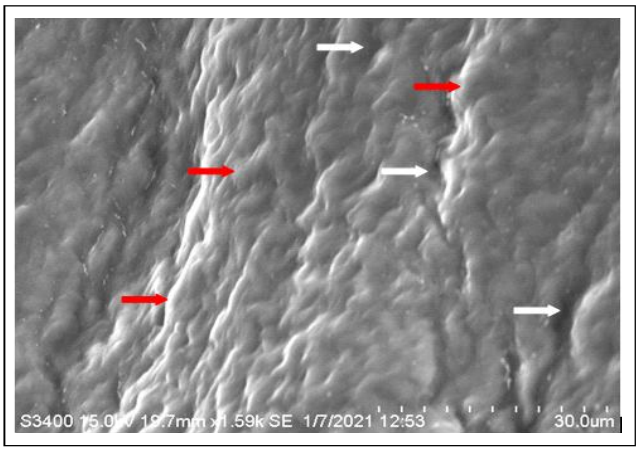


Fig 1: Photograph showing the endothelial cells (red arrows) along with pores (white arrows) in right atrium of 96 days prenatal non-descript sheep.

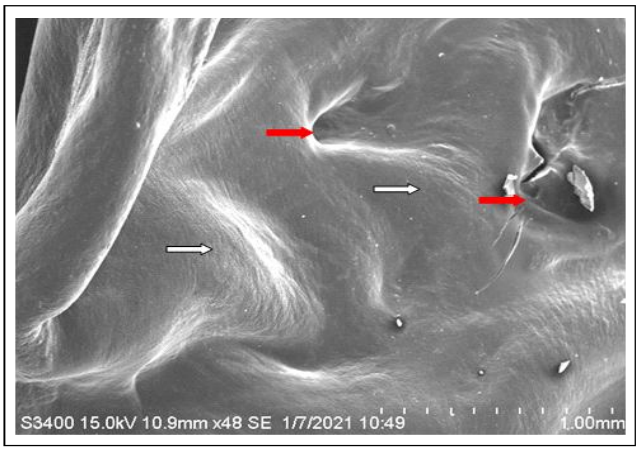


Fig 2: Photograph showing the pectinate muscles (white arrows) along with pores (red arrows) in right atrium of 33 days prenatal non-descript sheep.

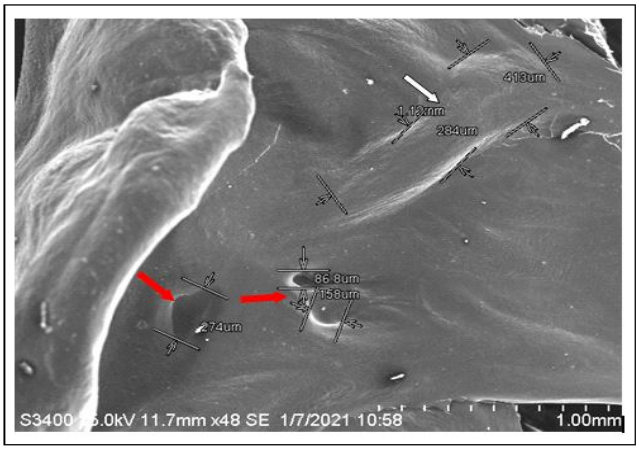


Fig 3: Photograph showing the length and width of pectinate muscles (white arrow) along with diameters of pores (red arrows) in right atrium of 33 days prenatal non-descript sheep.

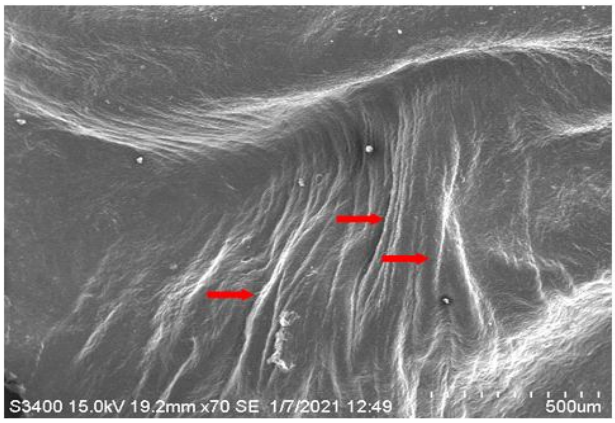


Fig 4: Photograph showing the parallel arrangement of pectinate muscles (white arrows) in left atrium of 96 days prenatal non-descript sheep.

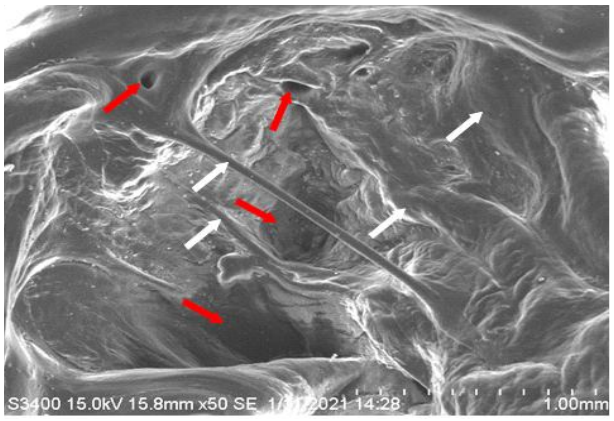


Fig 5: Photograph showing the pectinate muscles (white arrows) along with pores (red arrows) in left atrium of 105 days prenatal non-descript sheep.

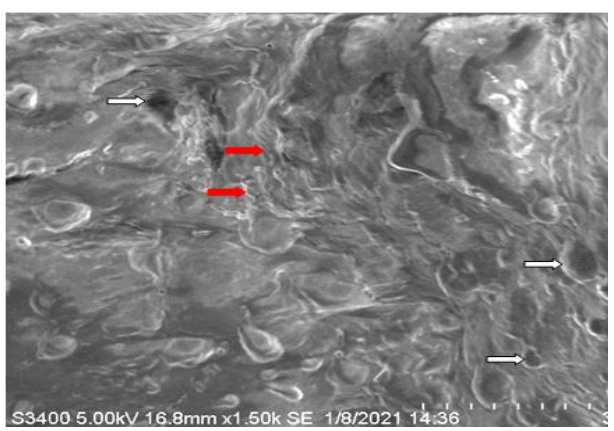


Fig 6: Photograph showing the cross sectional view of cardiac muscles along with a. pores (white arrows) and b. Subendothelial fibers (red arrows) in right atrium of 120 days prenatal non-descript sheep.

(Fig 2 and Fig 3). The present finding was in agreement with the report of Sizer *et al*. (2020) in Saanen goat. The average diameters of the pores were found to be 172.75±26.12 µ, 282.25±52.12 µ and 305±13.72 µ in the early, mid and late prenatal stages respectively in non-descript sheep at different magnifications.

The endothelial surface of the mitral valve was lined by simple squamous epithelium that projected into the lumen of the valve (Fig 7 and Fig 8). The present findings were in line with the reports of Hurle *et al.* (1985) in human, Icardo *et al.* (1993) in mouse, Dohmen *et al.* (2003) in juvenile sheep, Brazile *et al*. (2015) in pig, Markby *et al*. (2017) in dog and Sizer *et al*. (2020) in Saanen goat. Further, the average diameters of the endothelial cells were found to be 3.39±0.24 µ and 8.60±0.74 µ in the early and mid prenatal stages respectively in non-descript sheep at different magnifications.

There was presence of pores among the endothelial cells of the bicuspid valve (Fig 9) in all the age groups under

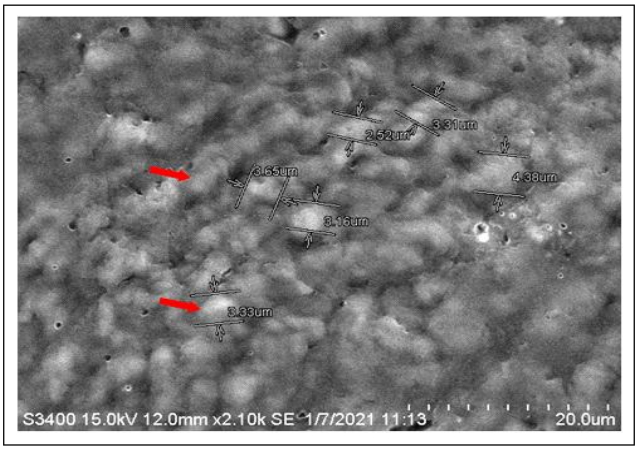


Fig 7: Photograph showing the endothelial cells (red arrows) of bicuspid valve in the heart of 33 days prenatal non-descript sheep.

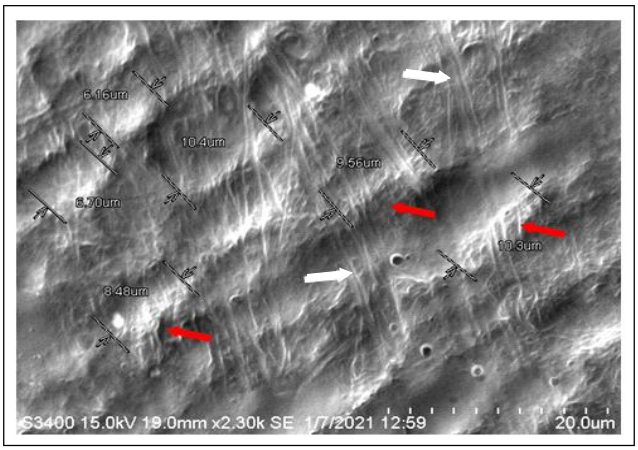


Fig 8: Photograph showing the endothelial cells (red arrows) and subendothelial fibers (white arrows) of bicuspid valve in the heart of 96 days prenatal non-descript sheep.

study. The average diameter of the pores was found to be 1.60±0.16µ in the late prenatal stage of the non-descript sheep.

The subendothelial layer was present just below the endothelium and comprised of connective tissue fibers and conducting fibers (Fig 6). The present observations were in accordance with the findings of Ghonimi *et al*. (2015) in camel and Sizer *et al*. (2020) in Saanen goat. The fibers were interwoven with each other forming a network (Fig 10). The width of the fibers was not uniform and was further categorized into thick and thin fibers especially in the mid prenatal stage, *i.e*. at 96 days of gestation (Fig 10). The average thickness of thick and thin fibers was measured as 397.5±59.5 nm and 119±20.81 nm respectively at 96 days of gestation.

The tricuspid valve guards the right atrio-ventricular orifice, *i.e*. the opening between the right atrium and right ventricle. The endothelial surface of the valves was lined by simple squamous endothelium that projected into the lumen of the valve (Fig 11 and Fig 13). The present findings were in line with the reports of Hurle *et al.* (1985) in human, Icardo *et al.* (1993) in mouse, Dohmen *et al.* (2003) in juvenile sheep, Brazile *et al*. (2015) in pig, Markby *et al*. (2017) in dog and Sizer *et al*. (2020) in Saanen goat. Further, the average diameters of the endothelial cells were found to be 6.23±0.40 µ and 4.86±0.24 µ in the early and mid prenatal stages respectively in non-descript sheep at different magnifications. The average longitudinal and transverse diameters of the endothelial cells were recorded as 5.82±0.32 µ and 3.41±0.10 µ respectively in the late prenatal stage of the non-descript sheep at different magnifications.

There was presence of pores among the endothelial cells of the tricuspid valve (Fig 11 and Fig 13) in all the age groups under study. The present finding was in agreement with the report of Sizer *et al*. (2020) in Saanen goat. The average diameters of the pores were found to be 650±56.29 nm and 2.27±0.37 µ in the early and mid prenatal stages respectively in non-descript sheep at different magni

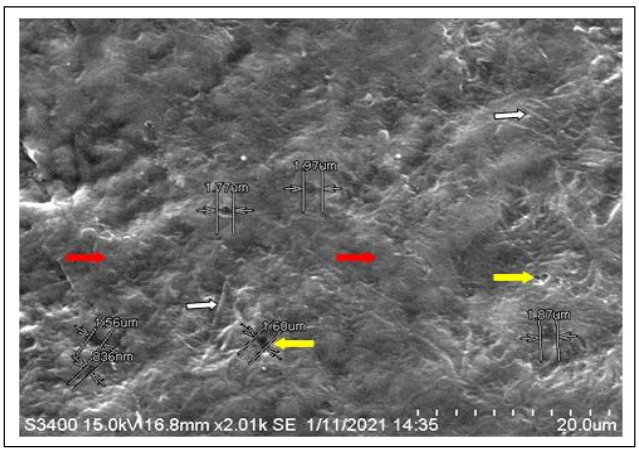


Fig 9: Photograph showing the endothelial cells (red arrows) along with pores (yellow arrows) and subendothelial fibers (white arrows) of bicuspid valve in the heart of 120 days prenatal non-descript sheep

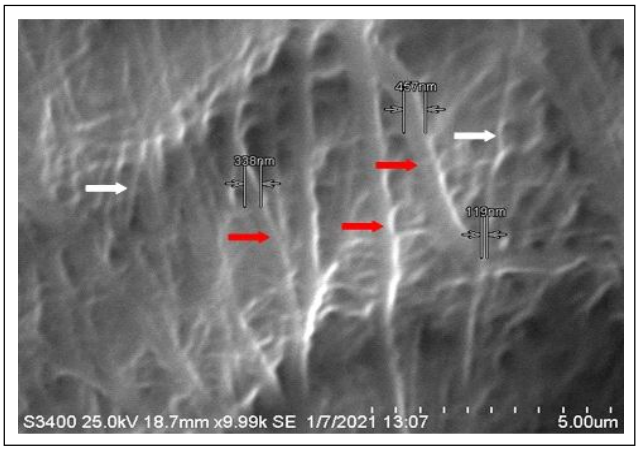


Fig 10: Photograph showing the thick (red arrows) and thin (white arrows) fibers in the subendothelial layer of bicuspid valve in the heart of 96 days prenatal non-descript sheep.

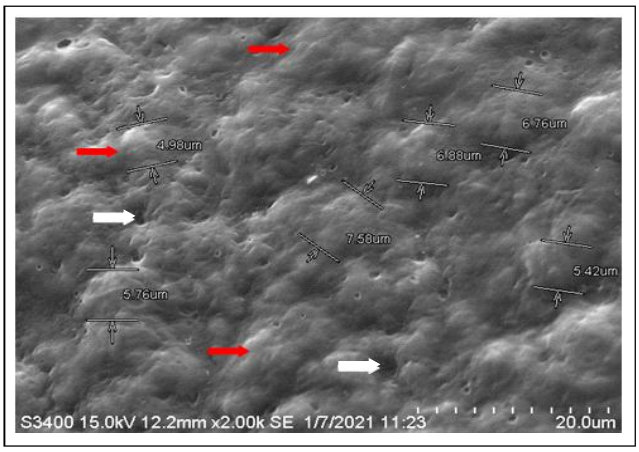


Fig 11: Photograph showing the endothelial cells (red arrows) along with pores (white arrows) of tricuspid valve in the heart of 33 days prenatal non-descript sheep.

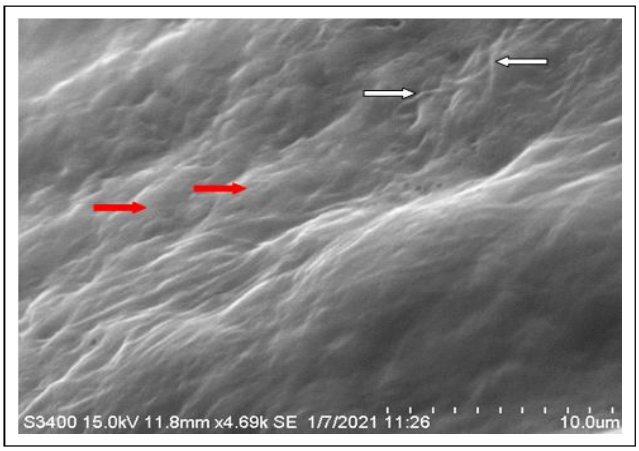


Fig 12: Photograph showing the endothelial cells (red arrows) along with interwoven subendothelial fibers (white arrows) of tricuspid valve in the heart of 96 days prenatal non-descript sheep.

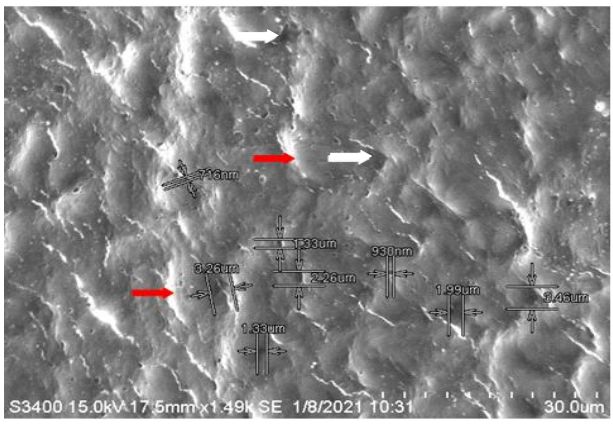


Fig 13: Photograph showing the endothelial cells (red arrows) along with pores (white arrows) of tricuspid valve in the heart of 96 days prenatal non-descript sheep.

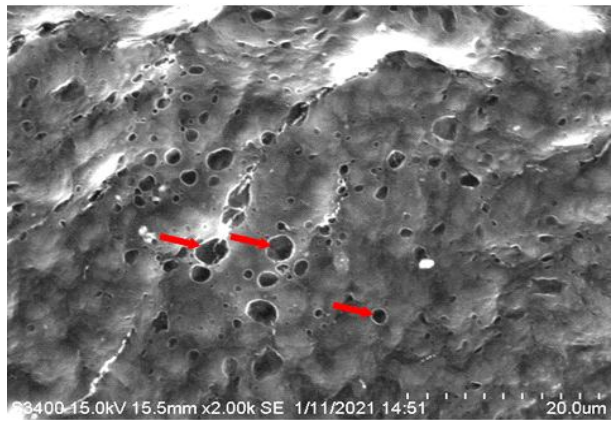


Fig 14: Photograph showing the clusters of pores among the endothelial cells (red arrows) of tricuspid valve in the heart of 120 days prenatal non-descript sheep.

-fications. There was presence of clusters of pores among the endothelial cells of the tricuspid valve in the heart especially in the late prenatal stage, *i.e*. at 120 days of gestation period (Fig 14). Further, in late prenatal stage, a lot of variations were recorded in the size of the pores present on the endothelial surface of the tricuspid valve. They were categorized into smaller, medium and larger pores. The average diameters of the smaller, medium and larger pores located among the endothelial cells of the tricuspid valve were noted as 513.83±31.16 nm, 1.61±0.07 µ and 3.06±0.15 µ respectively in the 120 days gestational age of the foetus at different magnifications.

The subendothelial layer was present just below the endothelium of the tricuspid valve and comprised of connective tissue fibers and conducting fibers (Fig 12). The present finding was in agreement with the report of Sizer *et al*. (2020) in Saanen goat. The subendothelial fibers were interwoven with each other forming a network (Fig 12). The average thickness of the fibers was found to be 2.90±0.24 µ in the 105 days gestational age of the foetus.

CONCLUSION

The ultrastructural auricular and valvular 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 auricles, bicuspid and tricuspid valves of heart in pre-natal sheep that could help in studying various congenital developmental anomalies in different animals.

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Conflict of interest

All authors declare that they have no conflict of interest.

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