B-5147
[1-7]

RESEARCH ARTICLE Indian Journal of Animal Research

Histochemical and Biochemical Developmental Studies on the Heart in Pre-natal Non-descript Sheep (*Ovis aries*)

S.K. Sahu[1], U.K. Mishra[1], S. Sathapathy[1], G.R. Sahoo[2], S.K. Joshi[3], S.S. Biswal[4], S.S. Behera[5], P.K.K. Mishra[2]

10.18805/IJAR.B-5147

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 histochemical and biochemical details of cardiac architecture especially in pre-natal sheep have 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 heart were processed by routine paraffin technique and cryo technique and after section cutting, the slides were stained by various histochemical staining methods. Further, fresh pieces of the heart samples were also processed for getting tissue extracts for the biochemical analysis.

Result: It was revealed that the PAS activity was observed in the cardiac wall, interatrial septum and interventricular septum in the sheep foeti at all stages of gestation. A variable degree of acid phosphatase activity was noted in the myocardium of heart wall in the sheep foeti at all stages of gestation. The lipid deposition also increased with advancing age in the myocardium of cardiac wall, interatrial septum and interventricular septum in the heart of sheep foeti. The activities of various enzymes like creatine kinase, alkaline phosphatase, serum glutamic-pyruvic transaminase and serum glutamic-oxaloacetic transaminase were high in left ventricle than the right ventricle in the heart of sheep foeti. Certain protein bands of specific molecular weight appeared in the ventricular tissue at specific age of gestation in addition to a group of common proteins which appeared in ventricular tissue of sheep foeti in all age groups under study.

Key words: Biochemical, Cardiac architecture, Histochemical, Prenatal sheep.

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.*, 2017). 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 histochemical and biochemical study of the cardiac architecture especially in pre-natal sheep has not yet been reported. Hence, the present histochemical and biochemical study was undertaken to elucidate the age wise development of cardiac architecture in prenatal non-descript sheep.

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. 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

[1]Department of Veterinary Anatomy and Histology, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar-751 003, Odisha, India.
[2]Department of Veterinary Biochemistry, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar-751 003, Odisha, India.
[3]Department of Livestock Production and Management, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar-751 003, Odisha, India.
[4]Department of ARGO, CVSc. and A.H., Odisha University of Agriculture and Technology, Bhubaneswar-751 003, Odisha, India.
[5]Department of Veterinary Surgery and Radiology, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar-751 003, Odisha, India.

Corresponding Author: S., Sathapathy. Department of Veterinary Anatomy and Histology, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar-751 003, Odisha, India.
Email: srinivas42.sathapathy@gmail.com

How to cite this article: Sahu, S.K., Mishra, U.K., Sathapathy, S., Sahoo, G.R., Joshi, S.K., Biswal, S.S., Behera, S.S. and Mishra, P.K.K. (2023). Histochemical and Biochemical Developmental Studies on the Heart in Pre-natal Non-descript Sheep (*Ovis aries*). Indian Journal of Animal Research. DOI: 10.18805/IJAR.B-5147

Submitted: 12-05-2023 **Accepted:** 01-11-2023 **Online:** 25-11-2023

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) with four animals in each age group. The heart samples were fixed in 10% buffered neutral formalin for 72 hours and processed by routine Paraffin technique (Bancroft and Stevens, 1964) to obtain 6µm thick paraffin sections. The sections were stained by PAS technique for neutral mucopolysaccharides, Alcian blue (pH: 1.0 and 2.5) for acidic mucopolysaccharides, Thionin method for mast cells and the staining for demonstration of argentaffin cells by Singh's modified Masson-Hamperl reaction for endocrine cells. Further, the fresh cut tissue samples were prefixed in formol calcium at 4°C for 24 hours to obtain the frozen tissue sections of 10 µm thickness in cryostat for demonstration of acid phosphatase activity by azo-dye coupling technique and for fat by Sudan Black B method (Bancroft and Stevens, 1964).

For estimation of various enzymes like Alkaline Phosphatase, Creatine Kinase, Serum Glutamic-Pyruvic Transaminase, (SGPT), Serum Glutamic-Oxaloacetic Transaminase (SGOT), fresh heart tissue was employed for preparation of tissue extract. For preparation of tissue extract, fresh tissue sample was collected from wall of left and right ventricles of heart. The tissue was then, mixed with 10 ml of PBS and triturated by a tissue homogenizer. The homogenized tissue was centrifuged at 5000 rpm for 10 min and the tissue extract (supernatant) was used for estimation of different cardiac enzymes in a manner similar to techniques used in serum. The enzyme estimation was done using Kits (manufactured by Coral Clinical Systems, Tulip Diagnostics (P) Ltd., Goa, India) as per the standard protocol given by the manufacturer of the Kit itself and the protein estimation was carried out by SDS-PAGE method. The biochemical data obtained from the present investigation were subjected to routine statistical analysis (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The present histochemical and biochemical studies revealed significant results with regards to the micro architecture and enzymatic activity in different structures of the heart in prenatal non-descript sheep.

(i) Histochemical study

(a) Right atrium

The endocardium of auricle showed mild PAS activity in all ages of gestation. The PAS activity was fair in the myocardium of auricle in foeti aged 33 days to 49 days of gestation and it became strong during 59 days to 149 days of gestation in sheep. The endocardium of pectinate muscle illustrated mild PAS activity at all ages of gestation. The PAS activity was fair in the myocardium of pectinate muscle in sheep foeti aged 33 days to 49 days of gestation that became strong from 59 days of gestation and onwards till term end. The endocardium of crista terminalis showed mild PAS activity in all ages of gestation. The PAS activity was fair in the myocardium of crista terminalis between the age of 33 days and 49 days of gestation that became strong during 59 days to 149 days of gestation. The endocardium of intervenous crest showed mild PAS activity in all ages of gestation. The PAS activity was fair in the myocardium of intervenous crest in sheep foeti aged 33 days to 49 days of gestation that became strong from 59 days to 149 days of gestation. The neutral mucins are reported to yield a positive reaction and chiefly reflect a carbohydrate component or a glycoprotein in the tissue. The initial low content of the PAS positive material in the early gestation age of the sheep foetus followed by a moderate rise of the material within the myocardium may suggest for maintaining the membrane integrity as well as myocardial activity with increasing age of the sheep foetus. An absence of an alcianophilia suggests that probably the acid mucopolysaccharides and sulphated mucopolysaccharides do not play a role during cardiac development in animals.

Few mast cells were encountered in the interstitial connective tissue of the myocardium of the pectinate muscles in all stages of gestation. Though the exact role of such a sparse mast cell population in unclear, but they are reported to bear a wide variety of chemicals such as histamine, heparin, cytokines and growth factors. They are likely to play a role in the immune status of the cardiac tissue. Appearance of this cell in the heart during prenatal period might reflect acquiring immuno-competency during embryogenesis. Few argentaffin cells with endocrine granules were observed in the myocardium of the pectinate muscles in foeti of 72 days of age (Fig 1). These cells are probably playing an endocrine role to regulate the extracellular fluid volume during embryogenesis of the cardiac tissue.

The nerve fibres were scanty in the endocardium of auricle of heart of sheep foeti from age of 33 days to 49

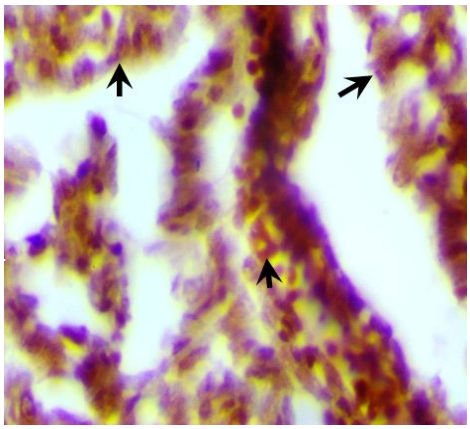

Fig 1: Photomicrograph of right atrium of heart of sheep foetus aged 120 days showing distribution of argentaffin cells in the myocardium (arrow).

days of gestation and the fibres became fair in number from 59 days to 149 days of gestation. The nerve fibres were few in the myocardium of auricle in foeti aged 33 days to 49 days of gestation and they were fair in frequency from age of 59 days to 96 days of gestation. They became moderate in amount from 105 days to 149 days of gestation. The nerve fibres did not appear in the endocardium of pectinate muscles between the age of 33 days and 49 days of gestation. They were few in the said location by the age of 59 days till the age of 149 days of gestation. The nerve fibres were few in the myocardium of pectinate muscles during 33 days to 49 days of gestation which became fair in number from the age of 59 days to 96 days and moderate in amount from the age of 105 days till term end. The nerve fibres could not be traced in the endocardium of crista terminalis at 33 days and 38 days of gestation. By 43 days and 49 days of gestation, the fibres were sparse. They were few in the endocardium of the foeti aged 59 days and onwards till 149 days of gestation. The nerve fibres were scanty in the myocardium of crista terminalis between 33 days and 49 days of gestation. They were fair in amount during 59 days to 96 days of gestation and moderately branching fibres were seen in foeti aged 105 days and onwards. The nerve fibres were occasional in the endocardium of intervenous crest in foeti of from age of 33 days to 49 days of gestation. They were scanty in foeti aged 59 days to 96 days of gestation and were few in amount from the age of 105 days to 149 days of gestation. The nerve fibres were scanty in the myocardium of intervenous crest from 33 days of age to 49 days of gestation. They were few in foeti by the age of 59 days to 96 days of gestation and fair in amount from age of 105 days to 149 days of gestation. The increase in neuronal supply is suggestive of an increased neural control over the differentiating and maturing cardiac tissues.

The acid phosphatase activity was feeble in the endocardium of pectinate muscles between 59 days and 149 days of gestation. The myocardium of crista terminalis and intervenous crest showed mild acid phosphatase activity during 33 days to 49 days of gestation and a fair activity was found from 59 days to 149 days of gestation. This enzyme is known to impart a lytic function and therefore, presence of this enzyme in higher concentration at a later age of the foetus within the myocardium may ask for a role in the process of differentiation and cell growth.

A mild grade lipid deposition was seen in the endocardium of auricle during 105 days to 149 days of gestation. The lipid deposition was mild in the myocardium of auricle from the age of 33 days up to 49 days of gestation which became fair from 59 days to 96 days of gestation and a moderate lipid deposition was evident during 105 days to 149 days of gestation. A feeble lipid deposition was seen in the endocardium of pectinate muscles during 59 days to 96 days of gestation and it became mild during 105 days to 149 days of gestation. The lipid deposition was feeble in the myocardium of pectinate muscles from age of 33 days to 49 days of gestation that became mild in foeti aged 59 days to 96 days of gestation. A mild lipid deposition was noted in foeti aged 105 days up to age of 149 days of gestation. The exact nature of this kind of age dependent distribution is not clear though the present findings corroborate with the reports of Sathyamoorthy (2003) in pigs.

(b) Right ventricle

The PAS activity was moderate in the myocardium of crista supraventricularis from 33 days of age to 49 days of age of sheep foetus as well as from the age of 105 days to 149 days of gestation. The PAS activity was moderate in the myocardium of chordae tendineae from 33 days to 49 days and from 105 days to 149 days of gestation. Further, the PAS activity was moderate in the myocardium of the ventricular wall and in the moderator band from 33 days to 49 days and from 105 days to 149 days of gestation. The PAS activity was moderate in the myocardium of papillary muscle in sheep foeti aged from 33 days to 49 days as well as in foeti aged from 105 days to 149 days of gestation. Intense PAS activity was observed in the epicardium of right ventricle in the sheep foeti aged 33 days of age (Fig 2). The neutral mucins are reported to yield a positive reaction and chiefly reflect a carbohydrate component or a glycoprotein in the tissue.

Few mast cells were encountered in the myocardium of the papillary muscle in all stages of gestation. Few argentaffin cells with endocrine granules were noted in the myocardium of the papillary muscle in all stages of gestation. The nerve fibres were few in the myocardium of crista supraventricularis from 33 days to 49 days of gestation. They were fair in foeti aged 59 days to 96 days of gestation and became moderate in amount from 105 days to 149 days of gestation. The nerve fibres were sparse in the myocardium of chordae tendineae from the age of 33 days to 49 days of gestation. The nerve fibres were fair in the endocardium of chordae tendineae of foeti aged 59 days to 96 days of

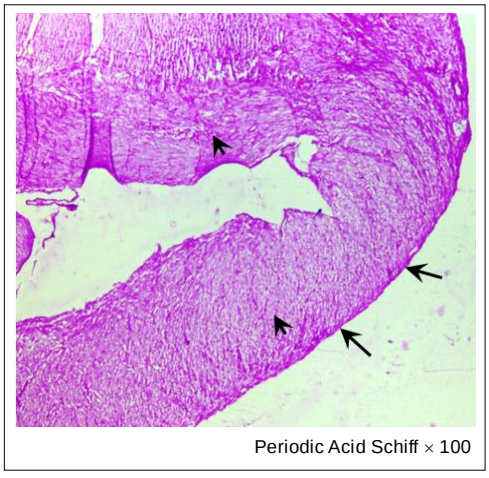

Fig 2: Photomicrograph of right ventricle of heart of sheep foetus aged 33 days showing intense PAS activity in the epicardium (arrow) and a moderate activity in the myocardium (arrow head).

gestation and moderate in amount in foeti of age 105 days to 149 days of gestation. Similarly, fair numbers of nerve fibres were observed in the myocardium of moderator band from the age of 33 days to 49 days of gestation. The nerve fibers were fair in number in the endocardium of papillary muscles in foeti from age of 59 days to 96 days of gestation and moderate in amount in foeti of 105 days, 120 days, 136 days and 149 days of gestation.

The myocardium of chordae tendineae showed moderate acid phosphatase activity in the foeti between 105 days and 149 days of age. Similarly, the acid phosphatase activity was moderate in the myocardium of moderator band, ventricular wall and papillary muscle from 105 days of age to 149 days of gestation. The lipid deposition was fair in the myocardium of papillary muscle in foetus from the age of 105 days to 149 days of gestation (Fig 3). Moderate lipid deposition was revealed in the epicardium during 96 days to 149 days of gestation. The exact nature of this kind of age dependent distribution is not clear though the present findings corroborate with the reports of Sathyamoorthy (2003) in pigs.

(c) Left atrium

The PAS activity was moderate in the endocardium of auricle and myocardium of pectinate muscle during 59 days to 96 days of gestation. Few mast cells were encountered in the myocardium of the pectinate muscle and interatrial septum in all stages of gestation (Fig 4). Few argentaffin cells with endocrine granules were observed in the myocardium of the pectinate muscle in all stages of gestation. The nerve fibres were few in foeti from age of 59 days to age of 96 days and became fair in frequency during 105 days of age to 149 days of gestation in the endocardium of auricle. Similarly, the nerve fibers were moderate in number in the myocardium of auricle, pectinate muscle and interatrial septum in foeti aged 105 days to 149 days of gestation, whereas fair amount of nerve fibers were noted in the endocardium of pectinate muscle at the age of 105 days to 149 days of gestation.

The acid phosphatase activity was moderate in the myocardium of auricle, atrial wall, pectinate muscles and interatrial septum during 59 days to 96 days of gestation, whereas strong activity was noted in 105 days to 149 days of gestation (Fig 5). The epicardium revealed moderate acid phosphatase activity reaction by 59 days to 81 days of age followed by an intense reaction by 96 days of age till term end. The lipid deposition was fair in the myocardium of auricle during 105 days to 149 days of gestation. The myocardium of pectinate muscle, interatrial septum revealed fair amount of lipid deposition in foeti aged 59 days to 96 days of gestation, whereas moderate lipid deposition was observed in foeti aged 105 days to 149 days of gestation. The epicardium revealed moderate deposition of lipid during 96 to 149 days of gestation. The exact nature of this kind of age dependent distribution is not clear though the present findings corroborate with the reports of Sathyamoorthy (2003) in pigs.

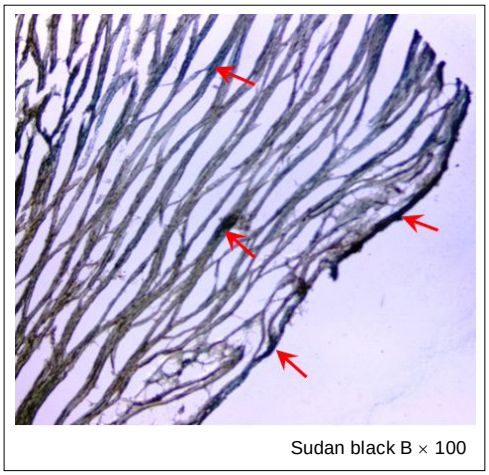

Fig 3: Photomicrograph of right ventricle of heart of sheep foetus aged 149 days showing distribution of lipids in the epicardium (arrow) and myocardium (arrow head).

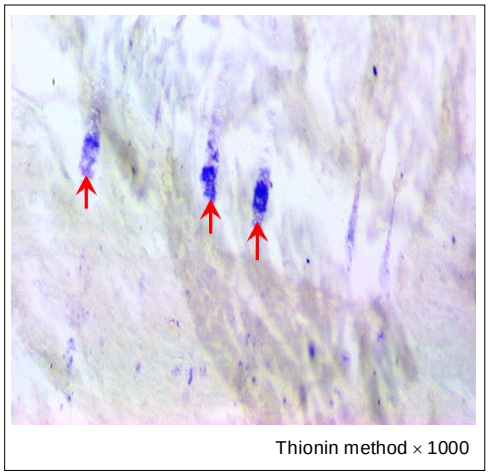

Fig 4: Photomicrograph of left atrium of heart of sheep foetus aged 149 days showing the distribution of mast cells (arrow) within the interstitium of the myocardium.

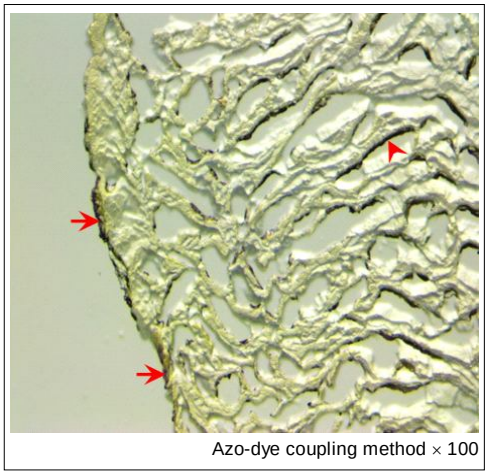

Fig 5: Photomicrograph of left atrium of heart of sheep foetus aged 149 days showing distribution of acid phosphatase activity in the epicardium (arrow) and in the myocardium (arrow head).

(d) Left ventricle

The PAS activity was intense in the myocardium of the ventricle and papillary muscle at the age 149 days of the sheep foeti. The endocardium of interventricular septum showed moderate PAS activity during 59 days to 96 days of gestation, whereas the activity was strong in the myocardium of interventricular septum from the age of 33 days to 49 days of gestation. Few mast cells were encountered in the myocardium of the papillary muscle and interventricular septum in all stages of gestation. Few argentaffin cells with endocrine granules were observed in the myocardium of the papillary muscle in all stages of gestation.

The nerve fibres were fair in quantity in the endocardium of chordae tendineae in foeti between 105 days and 149 days of gestation. The myocardium of chordae tendineae consisted of fair amount of nerve fibres from the age of 59 days to 96 days of gestation and they were moderate in frequency in foeti aged 105 days to 149 days of gestation (Fig 6). The nerve fibres were moderate in frequency in the myocardium of papillary muscle and interventricular septum from 59 days to 96 days of gestation and became abundant from 105 days to 149 days of gestation.

The acid phosphatase activity was moderate in the myocardium of chordae tendineae, papillary muscle in foeti aged 105 days and onwards till 149 days of gestation. The lipid deposition was fair in the endocardium of papillary muscle in foeti aged 105 days to 149 days of gestation. The lipid deposition was moderate in the myocardium of ventricle, papillary muscle and interventricular septum from the age of 105 days and onwards till term end. The epicardium revealed moderate deposition of lipids in sheep foeti during 105 days to 149 days of gestation. The exact nature of this kind of age dependent distribution is not clear though the present findings corroborate with the reports of Sathyamoorthy (2003) in pigs.

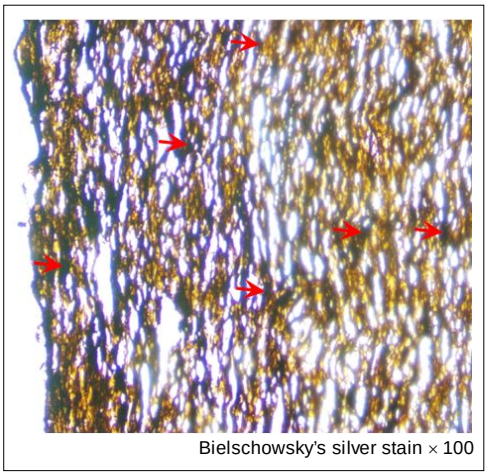

Fig 6: Photomicrograph of left ventricle of heart of sheep foetus aged 149 days showing distribution of nerve fibres.

(ii) Biochemical study

The creatine kinase activity in right ventricle of heart of the sheep foetus was measured as 752.13±72.31 U/l, 2285.24±210.45 U/l and 6723.85±662.27 U/l in early prenatal, mid prenatal and late prenatal stages respectively. The activity of creatinine kinase in left ventricle of heart of the sheep foetus was found to be 895.21±83.24 U/L, 3267.43±310.41 U/l and 9730.80±968.21 U/l in early prenatal, mid prenatal and late prenatal stages respectively (Fig 7). The increase in creatine kinase activity in both the ventricles with age resulted in higher energy reserves in the myocardium for better pumping efficiency of heart in the late prenatal life of the sheep foeti (Hildegard *et al.*, 1990). The average alkaline phosphatase activity in right ventricle of heart of the sheep foetus was estimated as 10.36±0.13 U/l, 19.59±1.73 U/l and 51.53±4.73 U/l in early prenatal, mid prenatal and late prenatal life respectively. The average alkaline phosphatase activity in left ventricle of heart of the sheep foetus was 10.96±0.93 U/l, 20.64±1.63 U/l and 53.12±4.71 U/l in early prenatal, mid prenatal and late prenatal stages respectively (Fig 8).

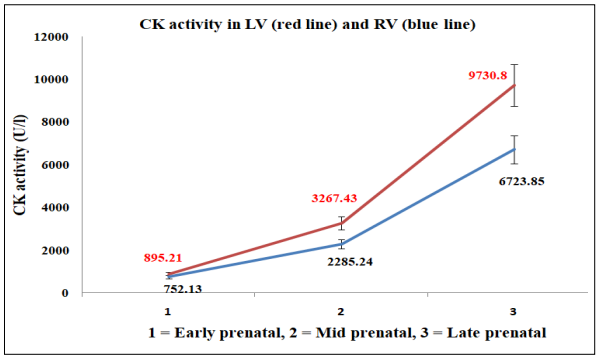

Fig 7: Photograph showing Creatine kinase (CK) activity in the wall of left ventricle (LV) and right ventricle (RV) of heart of sheep foeti during early, mid and late prenatal period.

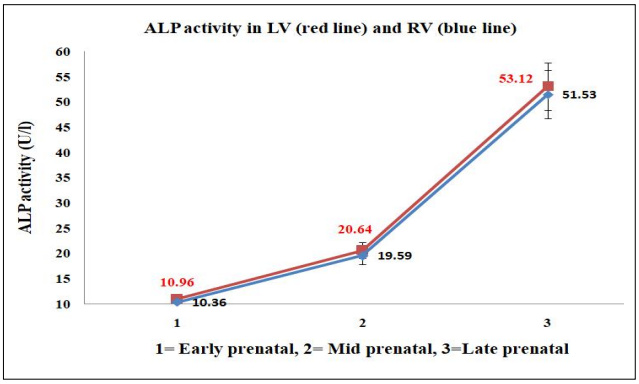

Fig 8: Photograph showing Alkaline phosphatase (ALP) activity in the wall of left ventricle (LV) and right ventricle (RV) of heart of sheep foeti during early, mid and late prenatal period.

The average SGPT activity in right ventricle of heart of the sheep foetus was determined to be 5.90±0.53 U/l, 70.34±6.12 U/l and 112.88±10.31 U/l in early prenatal, mid prenatal and late prenatal stages respectively. The average SGPT activity in left ventricle of heart of the sheep foetus was registered to be 10.44±0.91 U/l, 84.11±7.63 U/l and 151.51±14.23 U/l in early prenatal, mid prenatal and late prenatal stages respectively (Fig 9). The average SGOT activity in right ventricle of heart of the sheep foetus was calculated as 4.00±0.31 U/l, 16.56±1.34 U/l and 211.67±20.34 U/l in early prenatal, mid prenatal and late prenatal stages respectively and the average SGOT activity in left ventricle of heart of the sheep foetus was seen to be 5.82±0.54 U/l, 86.08±7.11 U/l and 314.18±28.32 U/l in early prenatal, mid prenatal and late prenatal stages respectively (Fig 10).

The cardiac tissue extracts from either ventricles of the heart of the sheep foeti of early, mid and late prenatal stages was subjected to 10 % SDS-PAGE for analysis of different proteins during prenatal cardiogenesis. The protein ladder

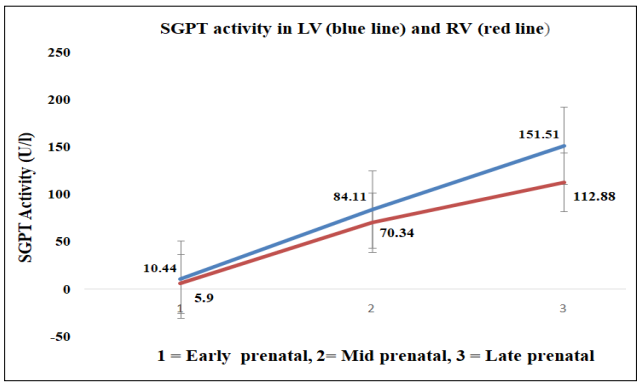

Fig 9: Photograph showing Serum glutamic pyruvic transaminase (SGPT) activity in the wall of left ventricle (LV) and right ventricle (RV) of heart of sheep foeti during early, mid and late prenatal period.

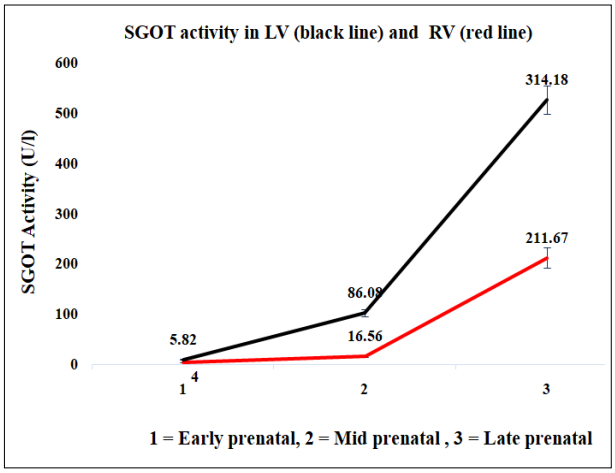

Fig 10: Photograph showing Serum glutamic oxaloacetic transaminase (SGOT) activity in the wall of left ventricle (LV) and right ventricle (RV) of heart of sheep foeti during early, mid and late prenatal period.

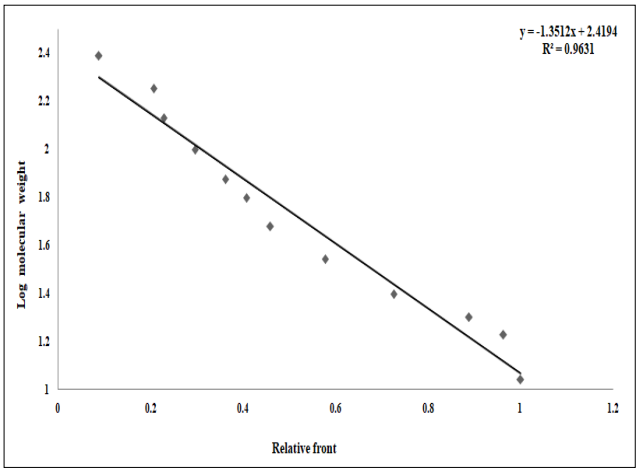

Fig 11: Photograph showing calibration curve for determination of molecular weight of protein bands obtained from tissue extract from the of wall left ventricle (LV) and right ventricle (RV) of heart of sheep foeti during early, mid and late prenatal period.

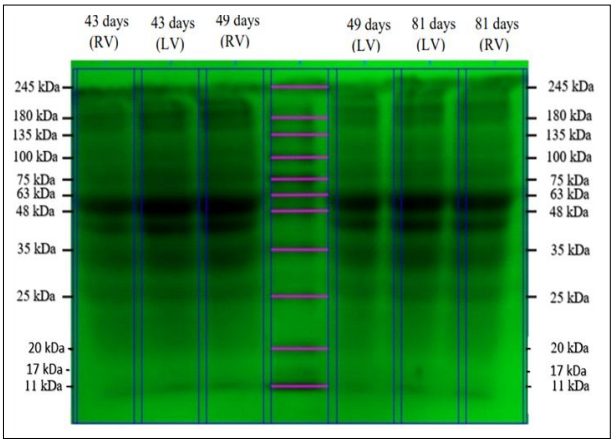

Fig 12: Gel electrophoresis of the ventricular tissue extract of heart of sheep foetus showing the protein bands of different molecular weights at the age of 43 days, 49 days and 81 days of gestation.

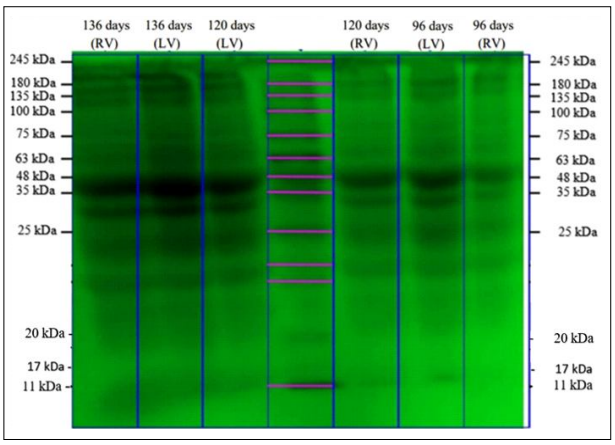

Fig 13: Gel electrophoresis of the ventricular tissue extract of heart of sheep foetus showing the protein bands of different molecular weights at the age of 96 days, 120 days and 136 days of gestation.

(Himedia, India) was run along with the extracted proteins for calculation of molecular weight of different proteins of cardiac wall of the non-descript sheep foeti of Odisha state in question. The molecular weight of the electrophoretic separated proteins was calculated by using the standard calibration curve (Fig 11). At 43 days of gestation, tissue extract from the wall of right and left ventricle revealed fourteen number of protein bands with molecular weight range from 10.90 kDa to 223.50 kDa. At 49 days of gestation, tissue extract from the wall of right and left ventricle showed fourteen number of protein bands with molecular weight range from 10.90 kDa to 223.50 kDa. At 81 days of gestation, tissue extract from the wall of right and left ventricle discerned fifteen number of protein bands with molecular weight ranging from 10.90 kDa to 223.50 kDa (Fig 12). At 96 days of gestation, tissue extract from the wall of right and left ventricle documented seventeen number of protein bands with molecular weight ranging from 8.70 kDa to 223.50 kDa. At 120 days of gestation, tissue extract from the wall of right and left ventricle illustrated nineteen number of protein bands having molecular weights range from 8.70 to 223.50 kDa. At 136 days of gestation, tissue extract from the wall of right and left ventricle confirmed nineteen number of protein bands with molecular weight ranging from 8.70 to 223.50 kDa (Fig 13). The increased in the number of protein bands with age in the sheep foeti might be contributing to the increased interaction of actin-myosin and might be involved in providing structural support or elasticity to the cardiac muscles (Hartzell and Sale, 1985).

CONCLUSION

The cardiac histochemistry and biochemistry showed significant variations among different ages in the pre-natal sheep. Further, the present study provided a detailed baseline data on the age wise histochemical and biochemical development of different cardiac structures in pre-natal sheep that could help in studying various congenital developmental anomalies in different animals.

ACKNOWLEDGEMENT

The authors are grateful to the Officer In-charge, Central Instrumentation Facility (CIF), OUAT, Bhubaneswar for providing necessary facilities and support for the successful completion of this research work within time.

Conflict of interest: None.

REFERENCES

Bancroft, J.D., Stevens, A. (1964). Theory and Practice of Histological Techniques, Churchill Livingstone, Edinburgh, New York, USA.

Hartzell, H.C. and Sale, W.S. (1985). Structure of C protein purified from cardiac muscle. The Journal of Cell Biology. 100: 208-2015.

Hildegard, D.J., Hasselbaink, W.T.L., Moorman, A.F.M. and Lamers, W.H. (1990). Creatine kinase isozyme expression in prenatal rat heart. Anatomy and Embryology. 182:195-203.

Jaiswal, S., Singh, I., Mahanta, D., Sathapathy, S., Mrigesh, M., Pandit, K., Tamil selvan, S. (2017). Gross and morphometrical studies on the heart of Uttara fowl. Journal of Entomology and Zoology Studies. 5(6): 2313-2318.

Janqueira, L.C., Carneiro, J. (2005). Basic Histology Text and Atlas. (11th Edn.), The McGraw-Hill Companies. pp. 245.

Noden, D.M., Lahunta, A.D. (1985). Embryology of Domestic Animals: Developmental Mechanisms and Malformations. Williams and Wilkins, Berlin, Germany. pp. 2.

Sahu, S. K., Mishra, U.K., Sathapathy, S. (2021). Morphometrical Studies on the Exterior of the Heart of Pre-natal Non-descript Sheep. Indian Journal of Animal Research, DOI: 10.18805/IJAR.B-4400.

Sathapathy, S., Khandate, S.P., Dalvi, R.S., Charjan, R.Y., Salankar, A.M. (2013). Biometry of the heart and its vessels in young and adult of local non-descript goats (*Capra hircus*). Indian Journal of Veterinary Anatomy. 25 (2): 111-112.

Sathapathy, S., Dalvi, R.S., Joshi, S.K., Singh, M.K. (2014). Biometry of the heart and its vessels in kids of local non-descript goats (*Capra hircus*). Pantnagar Journal of Research. 12 (3): 416-418.

Sathyamoorthy OR. (2003). Anatomy of the heart in pigs (*Sus domesticus*). Ph.D. Dissertation. Chennai, India.

Snedecor, G.W., Cochran, W.G. (1994). In “Statistical Methods” (8th Edn.), Oxford and IBH Publishing House, Calcutta, India.