International Journal of Bio-resource and Stress Management

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Crossref

IJBSM February 2025, 16(2): 01-08

Article AR5778b

Research Article

Natural Resource Management

DOI: HTTPS://DOI.ORG/10.23910/1.2025.5778b

Histological Characterization and Histochemical Profiling of the Heart of Prenatal Goat (*Capra hircus*)

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0009-0009-0382-1852

ABSTRACT

The study was conducted during July to October, 2020 at the Department of Veterinary Anatomy, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India to investigate the histological and histochemical features of the hearts of 18 prenatal healthy goats (*Capra hircus*). The heart shape varied among the groups; groups I and II exhibited an ovoid shape, while group III had a flattened cone shape. Histological examinations revealed both superficial and deep cardiac muscle fibers in the atrial walls, forming muscular arches in the atrium. The ventricular myocardium was composed of three distinct layers of muscle fibers, with the middle layer being the thickest. The left ventricular wall was significantly thicker than the right. The cardiac valves were identified as endocardial flaps. Histochemical analysis showed that collagen and elastic fibers were more abundant in the atria than in the ventricles, with collagen primarily surrounding blood vessels. Reticular fibers formed networks in both the atria and ventricles. A weak to moderate periodic acid-Schiff (PAS) reaction was observed in the atria, ventricles, blood vessels, and atrioventricular cusps. Metachromasia was noted in both the atria and ventricles. Additionally, DNA content in atrial nuclei exhibited moderate staining across all age groups. These findings provide a detailed histological and histochemical description of prenatal goat heart development, contributing to the understanding of fetal cardiovascular maturation.

KEYWORDS: Heart, histology, histochemical, prenatal, fibers

***Citation* (VANCOUVER):** Gayatri et al., Histological Characterization and Histochemical Profiling of the Heart of Prenatal Goat (*Capra hircus*). *International Journal of Bio-resource and Stress Management,* 2025; 16(2), 01-08. HTTPS://DOI.ORG/10.23910/1.2025.5778b.

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.

RECEIVED on 12th September 2024 RECEIVED in revised form on 20th January 2025 ACCEPTED in final form on 02nd February 2025 PUBLISHED on 08th February 2025

1. INTRODUCTION

In recent years, the importance of small ruminants, particularly goats, has significantly increased within the dairy industry, especially in developing countries. Goats are often referred to as the "poor man's cow" due to their pivotal role in ensuring food and nutritional security (Singh et al., 2022; El-Tarabany and El-Bassiony, 2021). Their ability to thrive in harsh environments and provide both milk and meat makes them a valuable asset for rural populations (Khan et al., 2021; Ahmad et al., 2023). Additionally, goats' adaptability to diverse climates and their minimal resource requirements make them particularly suited for low-income farmers in tropical and arid regions (Verma et al., 2020; Majumder et al., 2022). The heart, a vital muscular organ in the circulatory system, is central to maintaining systemic homeostasis. It consists of four chambers, two septa, and several valves, all contributing to spontaneous rhythmic contractions that facilitate continuous blood circulation throughout the body (Adams and Dellman, 1998; Olah et al., 2017). Adequate blood flow is crucial for cellular metabolic processes, thermoregulation, and various organ functions, including urine formation (Swenson, 2000; Kumar et al., 2022). Recent studies have emphasized the heart's role in regulating systemic homeostasis, particularly its dynamic response to environmental changes and stress factors in livestock, which are crucial for maintaining overall health (Mullins et al., 2021; Gupta et al., 2023).

Histological and histochemical studies on the hearts of prenatal goats provide essential insights into their early development and function (Ahmad et al., 2022; Mahmood et al., 2020; Ali et al., 2023). These studies are critical for understanding the formation and morphology of the heart, as the heart's structure significantly influences overall growth and metabolic efficiency in goats. The identification of collagen, elastic fibers, and extracellular matrix components is vital for evaluating the mechanical properties of the heart and its ability to adapt to the increasing demands of lactation and reproduction (Zhang et al., 2021; Liu et al., 2022). Additionally, recent histopathological research has explored the impact of maternal nutrition and genetic factors on fetal heart development, which may influence the long-term cardiovascular health of goats (Li et al., 2023; Zhang et al., 2024).

The present study aims to examine the normal histological and histochemical structures of the heart in prenatally healthy goats (*Capra hircus*). The findings of this research are expected to contribute significantly to the foundational knowledge of goat production and veterinary medicine, particularly in understanding cardiovascular function (Kumar et al., 2023; Kumar and Sharma, 2021). Improved knowledge of cardiac structures is essential for better management practices, ultimately enhancing goat health and productivity (Sharma et al., 2018; Alam et al., 2021; Firdous et al., 2023). Furthermore, understanding how cardiac morphology adapts to various environmental stressors is crucial for developing strategies that improve resilience in goats under changing climatic conditions (Ahmed et al., 2021; Patel et al., 2023).

The role of genetics and nutrition in influencing cardiac development and function in goats has become an increasingly important area of research, providing valuable opportunities for breeding programs aimed at improving heart health (Ali et al., 2022; Rahman et al., 2024). Enhancing our understanding of the histological features of goat hearts can help inform strategies to mitigate cardiovascular issues, which could significantly improve overall health outcomes (Hussain et al., 2022; Anwar et al., 2023). Moreover, advancements in genomic tools and nutritional interventions show promise in improving heart health and boosting productivity in small ruminants (Jabeen et al., 2022; Zhou et al., 2024). In conclusion, studying the histological and histochemical structures of the goat heart is vital for advancing animal health management practices and ensuring sustainable goat production in diverse environments. Such research contributes to a better understanding of the relationship between heart morphology, physiological function, and environmental stressors. Future studies that integrate histological findings with genetic and physiological data will offer invaluable insights into optimizing cardiovascular health and enhancing the productivity of goats, particularly in challenging agricultural systems (Mahmood et al., 2023; Qureshi et al., 2022).

2. MATERIALS AND METHODS

The study was conducted during July, 2020 and October, 2020 at the Department of Veterinary Anatomy and Histology, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India. Tissue samples from the hearts of 18 prenatally normal, healthy goats (*Capra hircus*) were collected, with fetuses harvested from gravid uteri procured from small animal slaughterhouses in and around Jabalpur. The fetuses were divided into three groups, each consisting of six animals, categorized based on their crown-rump length (CRL), as per the method described by Harvey (1959). The approximate age of the fetuses was determined using the CRL-based formula developed by Soliman (1975). This method has been widely adopted in previous studies for accurately determining fetal age in small ruminants (Ahmad et al., 2022; Sharma et al., 2018). The CRL is a standard method for assessing fetal development, and the Soliman (1975) formula has been validated in several studies (Mahmood et al., 2020; Kumar et al., 2023). The division of fetuses into groups based on CRL allows for the comparative analysis of heart development at different stages of gestation. Such studies provide crucial insights into prenatal cardiovascular development, as demonstrated in various histological and histochemical research (Sharma et al., 2018; Alam et al., 2021). Understanding these developmental stages is critical for improving veterinary medical practices, particularly in managing gestational health and fetal development in goats (Khan et al., 2021).

Y (days) 28.66+4.496×CRL (<20 cm)

Y (days)=73.544+2.256×CRL (>20 cm), Where Y age of fetuses in days.

CRL=Measured from occipital atlantal joint to base of first coccygeal vertebra.

Table 1: Number of foetuses in each group with their crown rump length (CRL)

Stage of development	Group	Age/CRL (crown-rump length of fetuses)	No. of animals
Pre natal	I	Foetuses upto 10 cm CRL (approx. early stage of gestation)	06
	II	Foetuses from 10+to 20 cm CRL (approx. mid-stage of gestation)	06
	III	Foetuses from 20+cm CRL (approx. late stage of gestation)	06

Tissue samples collected from various regions of the heart were fixed in 10% buffered formalin and processed using an acetone-benzene sequence to prepare paraffin blocks. Paraffin sections, 5–7 µm thick, were cut with a rotatory microtome. These sections were stained using Ehrlich's hematoxylin and eosin for general histological observations, Van Gieson's stain for connective tissue, Weigert's elastic stain for elastic fibers, and Gomori's silver impregnation stain for reticular fibers, following the techniques described by Drury and Wallington (1980). For histochemical analysis, Periodic Acid-Schiff (PAS) and Mowry's colloidal iron stains were used to detect carbohydrates and acidic mucopolysaccharides, respectively, as per the protocol established by Pearse (1980). For detailed histological investigation, the heart was divided into specific regions: right and left atrium, right and left ventricle, interventricular septum, sinoatrial (SA) node, and atrioventricular (AV) node. These regions were selected to observe the histological architecture and cellular composition across different functional zones of the heart, allowing for a comprehensive study of both myocardial and connective tissues (Ahmad et al., 2022; Mahmood et al., 2020). The combination of histological and histochemical techniques enabled the identification of various tissue components, including muscle fibers, connective tissue, collagen, and elastic fibers. The use of multiple staining methods helped in differentiating the structural elements of the heart, such as cardiac muscle cells, blood vessels, and the fibrous skeleton (Khan et al., 2021; Sharma et al., 2018). These methods provide essential insights into the organization and development of cardiac tissues in prenatal goats, contributing to the broader understanding of cardiovascular development in ruminants (Kumar et al., 2023).

3. RESULTS AND DISCUSSION

3.1. *Histological studies*

The basic three-layered structure of the heart, comprising the epicardium, myocardium, and endocardium, was consistently observed in all three prenatal age groups (Figure 1), similar to the findings of Traumann and Fiebiger (1957) in domestic animals and Hodges (1974) in fowl. Small mesothelial cells lined the epicardium (Figure 2, 3, 4), while Hodges (1974) reported cuboidal mesothelial cells on the surface of the epicardium (Figure 4). The myocardium, which appeared to be the thickest layer of the heart, was consistent with observations by Bloom and Fawcett (1978), Dellman and Brown (1987), and Hodges (1974) (Figure 3). The endocardium, consisting of a single layer of endothelial cells, was the innermost layer in the present study (Figure 5), supporting observations made by Bradley and Grahme (1960) and Hodges (1974).

In the current study, the atrial wall exhibited muscular arches of various sizes and numbers across all prenatal stages. The moderator band was present in the ventricular cavities

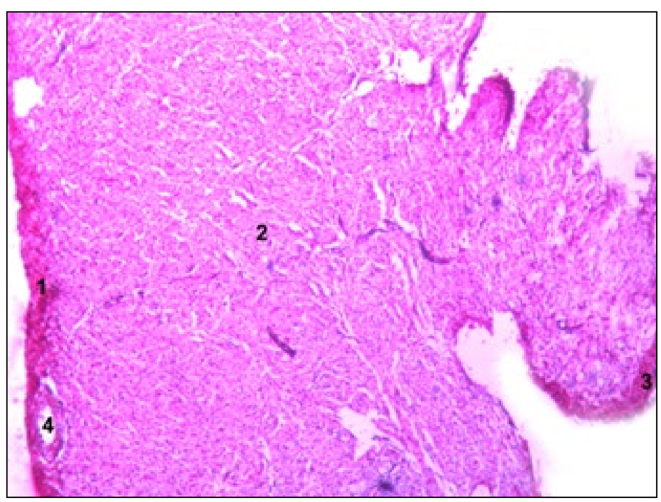


Figure 1: Photomicrograph of wall of right ventricles (Group 1) showing epicardium (1), myocardium (2), endocardium (3) and blood vessel (4). H and E×100

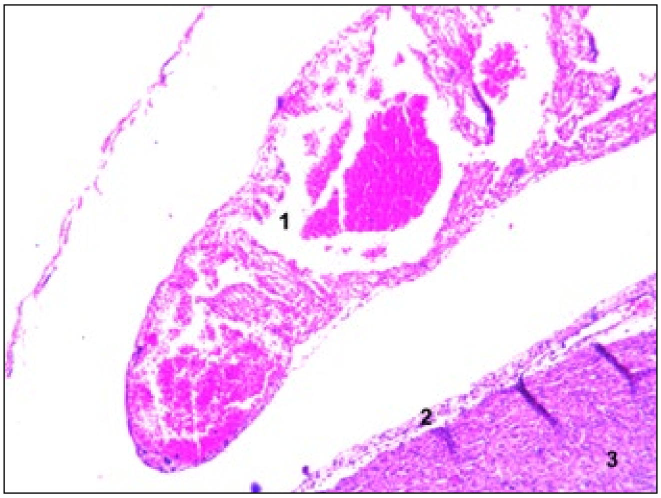


Figure 2: Photomicrograph of left atrium (Group 1) showing atria (1), epicardium (2), and wall of left ventricles (3) H and E ×40

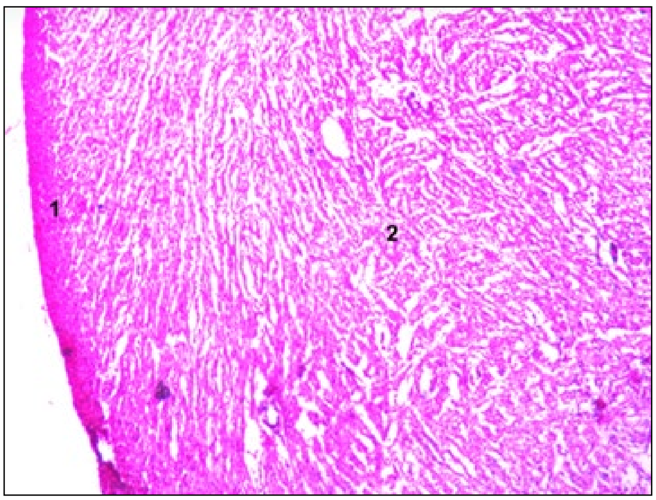
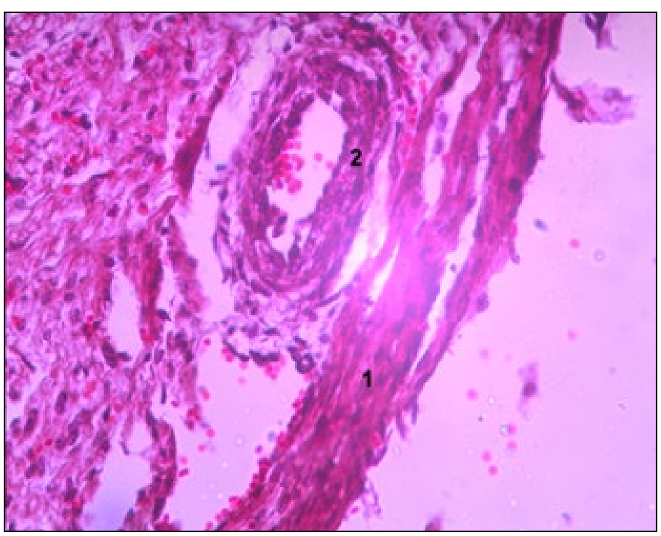


Figure 3: Photomicrograph of wall of left ventricles (Group 1) showing epicardium (1), and myocardium (2) H and E ×40



Figur 4: Photomicrograph of ventricular wall (Group 1) showing epicardium (1), and blood vessels (2) H & E × 200

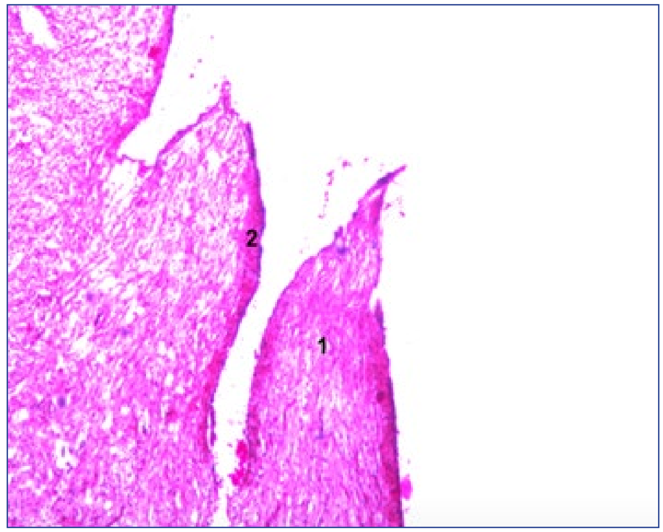


Figure 5: Photomicrograph of cavity of right ventricle (Group 1) showing trabeculae (1), endocardium (2) H and E ×40

(Figure 6), and the dorsal muscular arches of the atrium were larger than the ventral and lateral arches (Figure 2), findings consistent with Copenhaver (1964) in the atrial myocardium. The ventricular wall was found to be thicker than the atrial wall, with the left ventricle being thicker than the right and the interventricular septum in all prenatal groups (Figure 2). These results align with Bradley (1960), who noted the increased thickness of the left ventricular myocardium, which sustains higher blood pressure during systole and diastole. The ventricular myocardium consisted of three zones: superficial longitudinal, middle transverse, and inner oblique muscle fibers, corroborating Dellman's (1971) findings of the three continuous layers of muscle bundles. Additionally, trabeculae carneae of various sizes were observed projecting into the ventricular cavities,

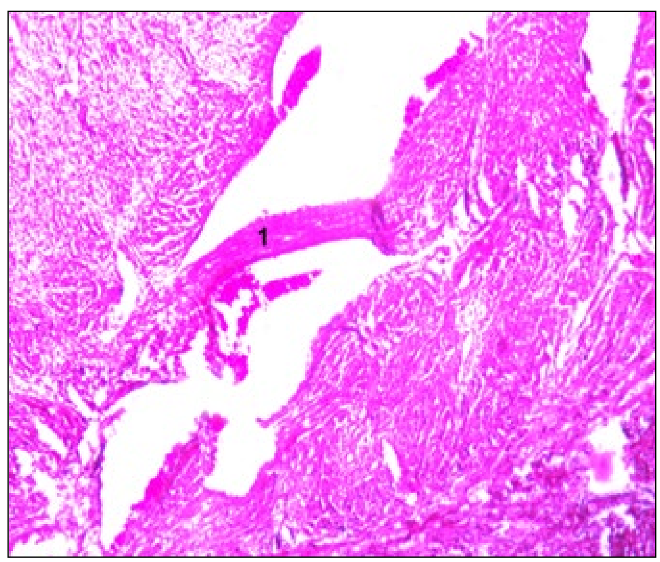


Figure 6: Photomicrograph of left ventricle (Group 1) showing moderator band (1) in cavity. H and E ×40

supporting Wieman's (1949) findings that trabeculae carneae develop in the myocardium and extend into the ventricular cavities.

In all prenatal groups, the cardiac valves were folds of the endocardium in the atria and ventricles, with a core of connective tissue (Figure 7, 8, 9), which is consistent with the observations of Carleton and Short (1954) and Blood and Fawcett (1978), who reported that the cardiac valves are formed by the endocardium and strengthened by fibrous tissue. Numerous elastic fibers were identified in the cardiac valves, a finding that agrees with Cormack (1987), who

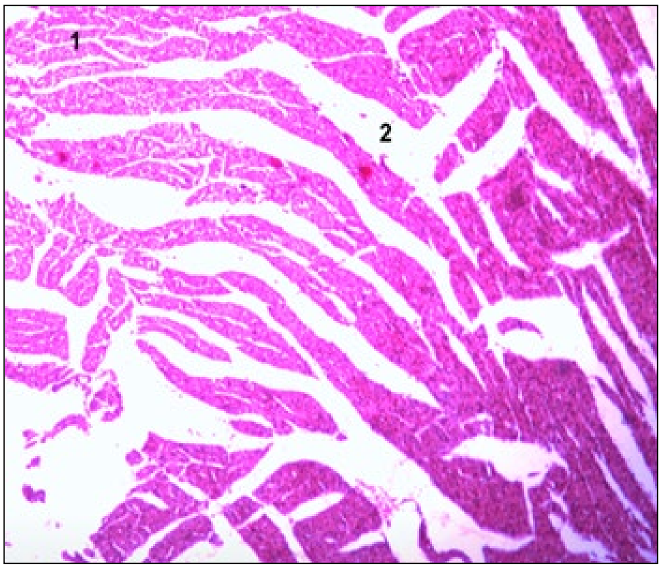


Figure 7: Photomicrograph of ventricular wall (Group III) showing transverse muscle fibers (1) of myocardium and interfasicular spaces (2). H and E ×40

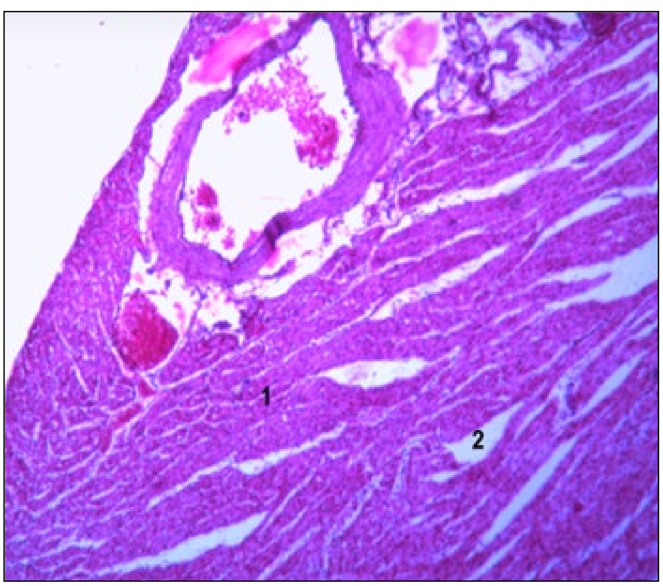


Figure 8: Photomicrograph of ventricular wall (Group III) showing oblique muscle fibers (1) of myocardium and interfasicular spaces (2). H and E ×40

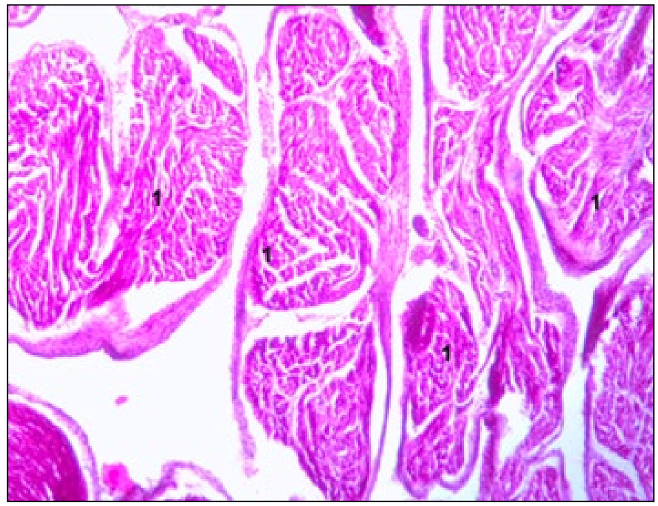


Figure 9: Photomicrograph of left atrium (Group III) showing muscular arches (1) of myocardium. H and E ×40

noted the presence of elastic fibers in the middle layer of valves in mammals. Furthermore, the cardiac valves were connected to the myocardium via the chordae tendineae, consistent with the findings of Cormack (1987). The sinoatrial (SA) node was located in the subepicardium of the right atrium at the junction of the anterior vena cava, while the atrioventricular (AV) node was found in the subendocardium of the medial wall of the right atrium, just above the tricuspid valve. Large, thick, modified Purkinje fibers were present in the nodal areas, supporting previous findings by Copenhaver (1978), Prasad (1984), and Sinha (1984) in the buffalo heart.

3.2. Histochemical studies

In the present study, pink to red collagen fibers were localized in the subepicardium and subendocardium of the prenatal hearts of goats. This distribution pattern of collagen fibers in the subepicardium and subendocardium aligns with the earlier descriptions by Dellmann (1971), Hodges (1974), and Bradbury (1975). The localization of collagen fibers between the atrial arches in all prenatal goat age groups was consistent with the reports of Hodges (1974), as shown in (Figure 10). A greater concentration of collagen fibers was observed in the perivascular pathway of the ventricular wall, as noted by Hodges (1974), who reported similar findings in the ventricular myocardium. The diffuse distribution of collagen fibers in bundles likely sustains the tensile strength of the heart. Collagen concentration in the atrium was higher than in the ventricle, possibly due to the lesser reactivity of atrial musculature, which supports Mitomo et al.'s (1969) observations.

In the prenatal goat heart, elastic fibers were diffusely arranged in the subepicardium and subendocardium. Carleton and Short (1954) and Bradbury (1977) also reported elastin in these layers. Elastic fibers were observed

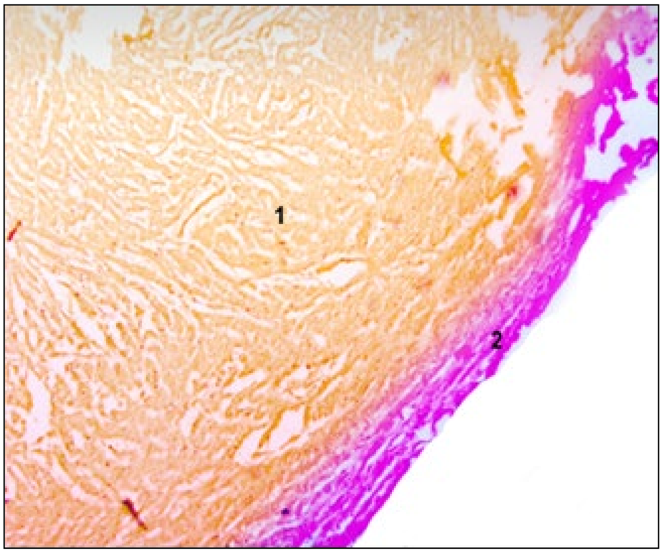


Figure 10: Photomicrograph of ventricular wall (Group III) showing muscular bundles (1) of myocardium and collagen bibersnin epicardium (2). Van Geison's stain ×40

in the atrial arches and between the muscle bundles of the atrial musculature (Figure 11). These findings are similar to those of Trautmann and Fiebiger (1957), Hodges (1974), and Simuelson (2007). Elastic fibers were moderate in the stroma of the ventricular musculature, supporting the findings of Blood and Fawcett (1978) in the postnatal heart of goats. Reticular fibers were observed in the atrial and ventricular walls, as well as in the muscle bundles of all prenatal goat stages (Figure 12). Trautmann and Fiebiger (1957) also noted the presence of reticular fibers in cardiac tissue. Reticular fibers increased with age, and a variable amount of reticular fibers were found in the atrial arches. This observation aligns with Prasad and Sinha's (1984)

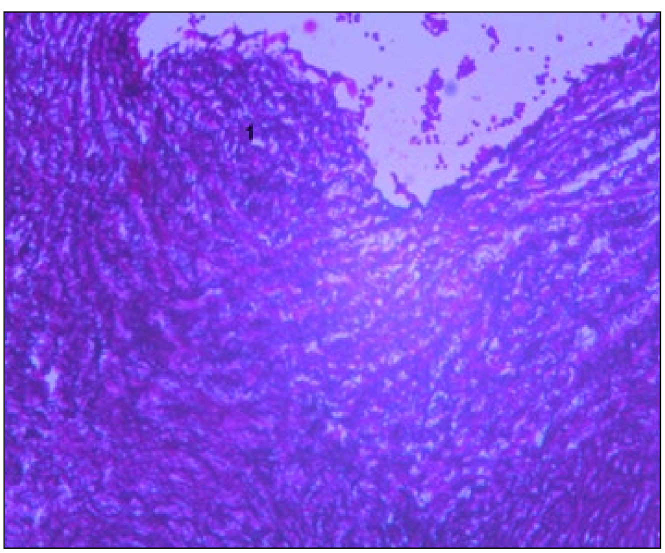


Figure 11: Photomicrograph of showing elastic fibers (1) Weigert's elastic stain ×200

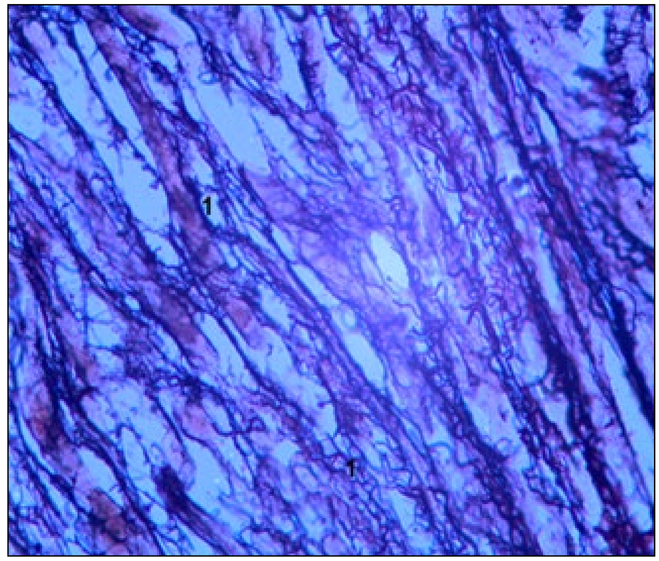


Figure 12: Photomicrograph of ventricular wall showing reticular fibers (1) Gomoris reticular stain ×200

findings in buffalo hearts. The reticular mesh formed a delicate supporting network, which Cormack (1987) similarly suggested.

PAS-positive material was identified in the subepicardium and subendocardium of the atria and ventricles across all prenatal age groups. The color intensity of the PAS-positive material varied from weak to moderate in different areas (Figure 13). In line with these findings, Mickey (1969) recorded high glycogen levels in both atrial and myocardial cells. In the present study, larger atrial arches were more deeply stained than smaller ones. Prasad and Sinha (1984) reported strong glycogen reactions in the atrial myocardium of buffaloes. PAS-positive material was also observed in the basement membrane subendothelial part of the AV cusp and the aortic cusp, corroborating Cormack's (1987) findings. Metachromasia was observed in the subepicardial layer of the

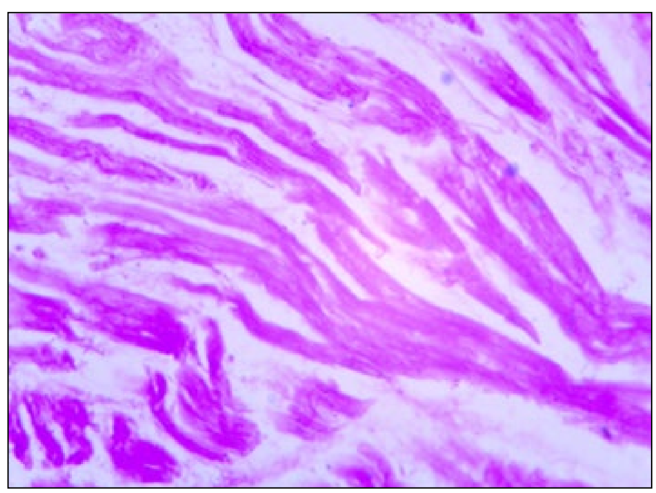


Figure 13: Photomicrograph of atrial wall showing moderates PAS positive PAS stain ×100

atrium, particularly in interfascicular connective tissue and stroma between atrial and ventricular muscle bundles. Weak metachromasia was noted, while Prasad and Sinha (1984) reported a moderate reaction for mucopolysaccharides in buffalo myocardium. The varying degrees of metachromasia in the AV cusps correspond with Mitomo et al. (1969) findings. The DNA content of the stroma in the epicardium was moderate, and chromatin granules were dark and coarse in the endocardium (Figure 14). The nuclei of atrial cardiac muscle cells appeared dark and spherical, confirming Prasad and Sinha's (1984) report. The endothelial lining of blood vessels also displayed dark magenta nuclei, consistent with the observations of McManus and Mowry (1964).

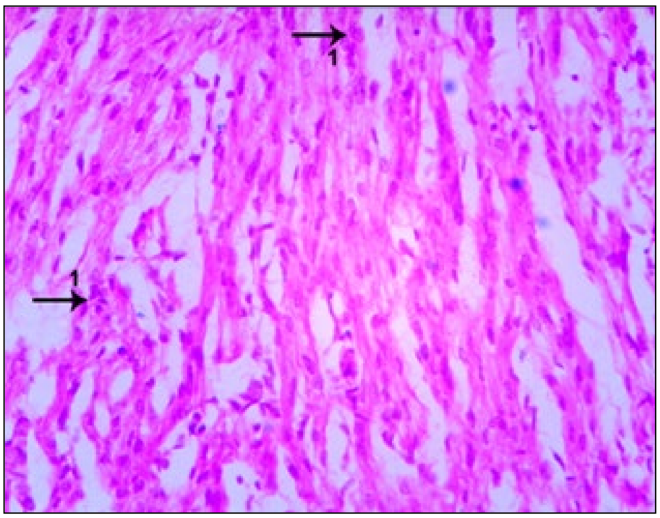


Figure 14: Photomicrograph of ventricular wall showing DNA in nucleus (1). Feulgen reaction ×200

4. CONCLUSION

The heart, located in the middle mediastinal space across all age groups, comprised three layers: epicardium, myocardium, and endocardium. Muscular arches project into the atrial cavity, with the dorsal arch being the largest. Coronary fat increased with age, and the heart's transverse diameter exceeded the sagittal diameter. Histological findings revealed three myocardial layers, with the middle being the thickest. Collagen and elastic fibers were more prominent in the atrium, with PAS-positive material, metachromasia, and moderate DNA staining observed.

5. ACKNOWLEDGEMENT

This is a part of the master thesis submitted by the first author to the Nanaji Deshmukh Veterinary Science University, Jabalpur. The authors are grateful to Dean, C.V.Sc. & A.H., NDVSU Jabalpur M.P. for providing the necessary facilities and support for the successful completion of his research work within time.

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