SHORT COMMUNICATION

Postnatal Development of Splenic Stroma in Indian Domestic Pig *(Sus scrofa domesticus)*

Muppidi Harshini[1]*, Nalla Rajendranath[1], Sundari Ranjith Kumar[1], Jorige Radha Krishna Rao[2]

Abstract

The study was conducted on spleen of 18 healthy pigs (6 each of 0-4 weeks, 4-10 weeks and above 10 weeks of age) to evaluate the postnatal development. The sections were processed and stained to obtain the histological details. The spleen consisted of stroma and parenchyma. The stroma was composed of capsule and trabeculae. Microscopic details obtained by staining with H&E, Van Gieson, Verhoeff, Masson trichome revealed that splenic capsule was thick with two layers, *viz.,* an outer dense irregular fibrous connective tissue layer and inner smooth muscle layer. Collagen fibers were predominant over elastic and reticular fibers. Trabeculae extended from the capsule dividing the splenic parenchyma into compartments and served as a frame work to support muscle, collagen, elastic and reticular fibers. The collagen, elastic, reticular and smooth muscle fibers increased with age. There was also a significant increase in trabecular branching, capsule thickness as the age progressed.

Key words: Pig, Postnatal development, Spleen, Stroma, Trabeculae.

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Introduction

Animals must depend on spleen for blood transfusion during natural heals of mechanical injury because the spleen specializes in immunological defense as it filters, retains and deals with the blood borne pathogens in white pulp (Rahman *et al*., 2016). The contractility of spleen connective tissue and smooth muscles in the capsule and trabeculae are prominent in ruminants, which aid in the function of spleen. The present work was undertaken with the objective of studying the micro-architecture in developing spleen for better understanding of immune function in postnatal pigs to accept environmental challenges.

Materials and Methods

The present investigation was carried out in Department of Veterinary Anatomy, College of Veterinary Science, Rajendranagar, Hyderabad (India). Eighteen fresh post-natal domestic pig spleen samples were procured immediately after slaughter at regular weekly intervals from local slaughter houses in and around Hyderabad. The samples collected were washed cleanly and transported to the laboratory. Collected spleen samples were divided into three groups, *viz.,* Group-I (0-4 weeks), Group-II (4-10 weeks) and Group-III (above 10 weeks) as per their approximate age derived by noting the dentition pattern.

For microscopic studies fresh tissue specimens were cut at desired size from dorsal, middle, ventral extremities of spleen and fixed in 10% NBF solution for a minimum of 24 h. Post-fixation the specimens were processed for routine paraffin embedding method. Paraffin sections of 6 µm thickness were cut and used for routine and special histological staining methods: Haematoxylin & Eosin (H&E) method was used to study the detailed micro-architecture, Van-Gieson's method for demonstration of collagen fibers, Masson's trichrome method to differentiate collagen and muscle fibers, Verhoeff's method for identifying elastic fibers, and Wilder's method to identify reticular fibers. Micrometric observations were taken from samples of spleen of all three groups wherever necessary using a calibrated ocular micrometer in the present study. The data collected was analyzed statistically using one way ANOVA.

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Results and Discussion

The spleen consisted of stroma and parenchyma. The stroma was composed of capsule and trabeculae.

Capsule

The spleen of pigs examined in this study was enveloped by a thick capsule made up of two connective tissue layers, *viz.,* outer dense irregular and inner smooth muscular layer

from which several trabeculae arose to enter the splenic parenchyma (Fig. 1). In the present study, outer fibrous layer was comprised of intermingled collagen and elastic fibers, of which the collagen fibers were predominant over elastic and reticular fibers (Fig. 2, 3). Elastic fibers were short and intermingled with collagen fibers and were arranged parallel to the direction of muscle fibers (Fig. 4, 5). Similar reports were made by Devi *et al.* (2016) and Chuarasia *et al.* (2022) in goats, and Shringi *et al.* (2018) and Kaur *et al.* (2020) in pigs.

In the present study, smooth muscular layer of splenic capsule was comprised of outer parallelly and inner obliquely arranged smooth muscle fibers in all three groups (Fig. 6). Contrary to our findings, Devi *et al.* (2016) in goats and Shringi *et al.* (2018) in pigs reported three layers of smooth muscle in splenic capsule. The collagen, elastic and smooth muscle fibers progressively increased with advancement of age. The thickness of capsule was uneven and there was a significant increase ($p<0.05$) with age.

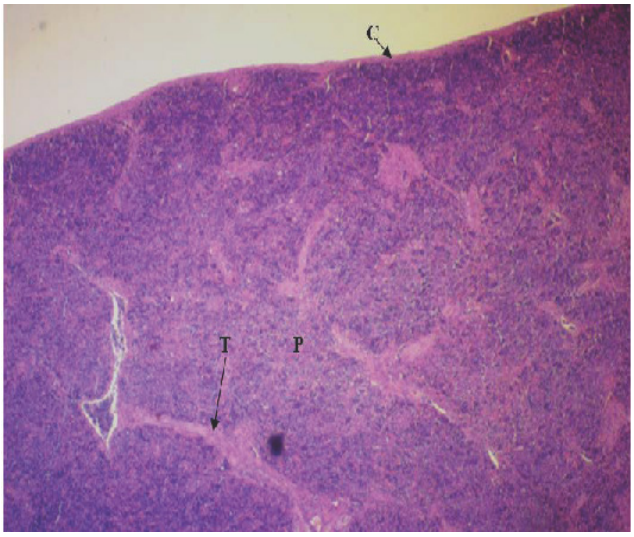

Fig.1: C/S Photomicrograph of 0-day piglet spleen (Group-I) showing capsule (C), trabeculae (T), parenchyma (P) with undifferentiated white pulp. H&E, 40X

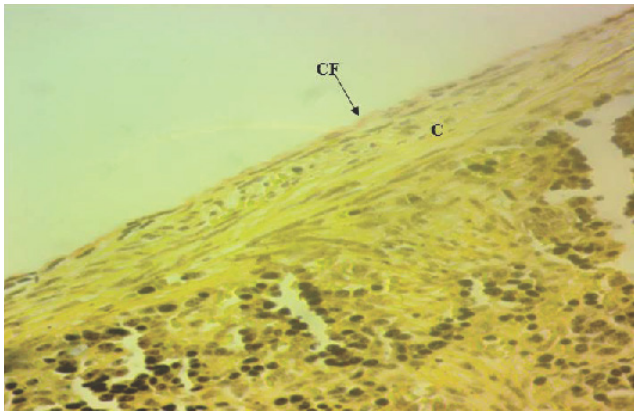

Fig. 2: C/S Photomicrograph of 0 day piglet spleen (Group-I) showing collagen fibers (CF) in capsule (C), Van Gieson, 40X

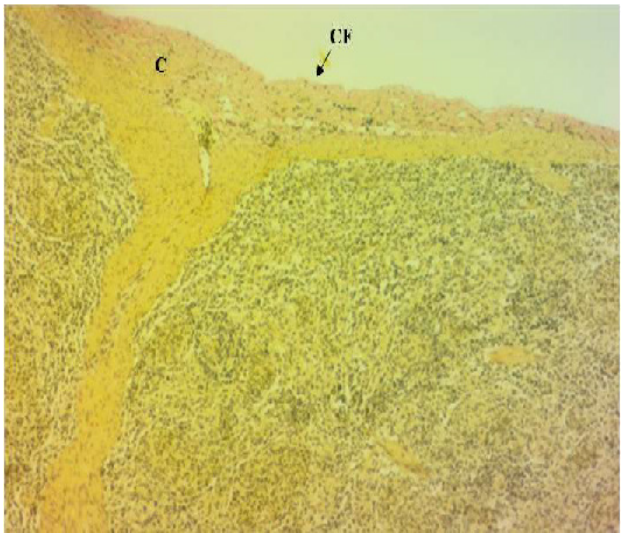

Fig. 3: C/S Photomicrograph of 21 day piglet spleen (Group-I) showing collagen fibers (CF) in capsule (C), Van Gieson, 10X

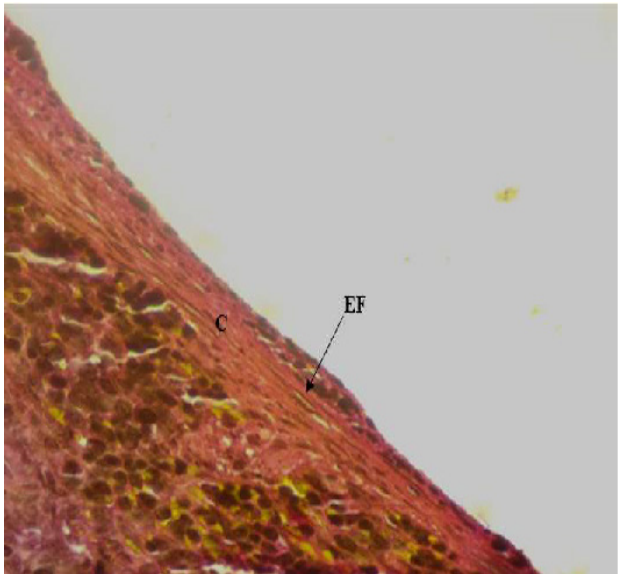

Fig. 4: C/S Photomicrograph of 0 day piglet spleen (Group-I) showing elastic fibers (EF) in outer fibrous layer of capsule (C), Verhoeff, 40X

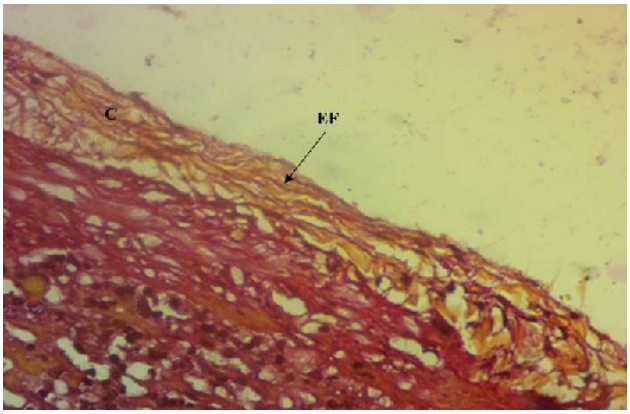

Fig. 5: C/S Photomicrograph of 240 day pig spleen (Group-III) showing elastic fiber (EF) in outer fibrous layer of capsule (C), Verhoeff, 40X

Trabeculae

Trabeculae extended from inner muscular layer of capsule into splenic parenchyma (Fig. 7), which was akin to reports in goats (Devi *et al.,* 2016; Gnanadevi *et al.,* 2019), and in pigs (Shringi *et al.,* 2018). Smooth muscle was more than connective tissue fibers. Patches of trabeculae were scattered throughout the splenic tissue and divided it into smaller compartments (Fig. 8), which was similar to the observations made in human spleen by Alex *et al.* (2015) and in pigs by Kaur *et al.* (2020). Branching of trabeculae increased with age (Fig. 8) noticed in this study was also reported by Waghaye *et al.* (2017) and Chaurasia *et al.* (2022) in goats.

In this study, splenic trabeculae were made up of muscles, collagen and reticular fibers, which formed a rigid framework in parenchyma. The collagen fibers surrounded the trabecular artery (Fig. 9). Reticular fibers were predominant and arranged parallel to the smooth

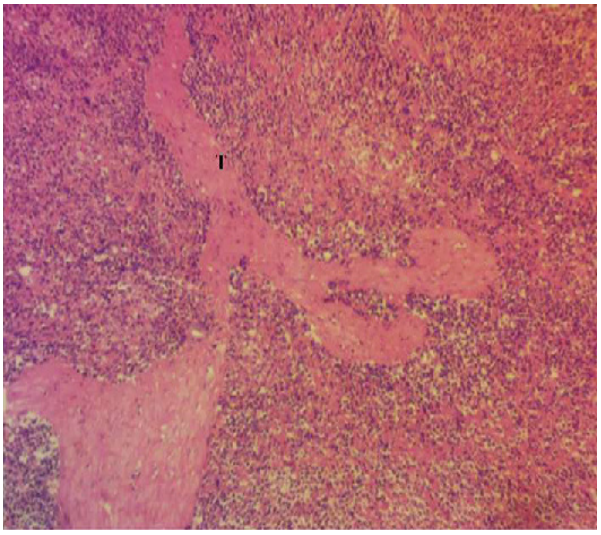

Fig. 8: C/S Photomicrograph of 240 day pig spleen (Group-III) showing branching trabeculae (T), H&E, 10X

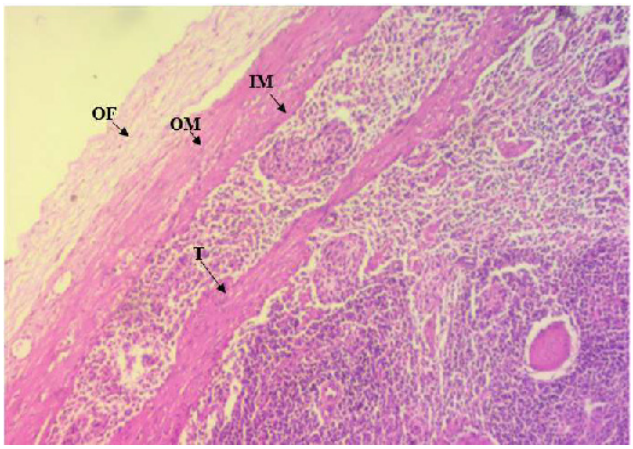

Fig. 6: L/S Photomicrograph of 40 day pig (Group-II) spleen showing outer fibrous layer (OF), outer muscular layer (OM), inner muscular layer (IM) and trabeculae (T)H&E, 10X

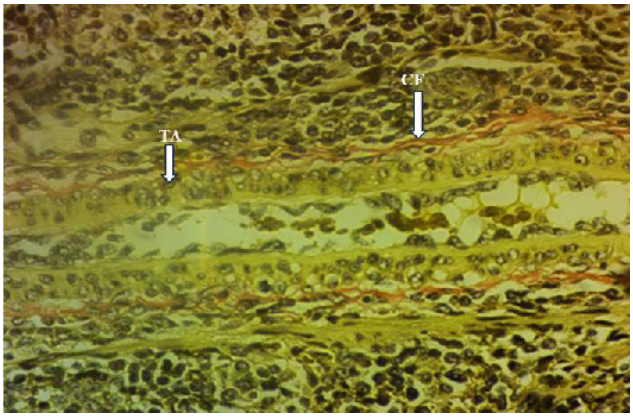

Fig. 9: C/S Photomicrograph of 6 day piglet spleen (Group-I) showing collagen fibers (CF) along the trabecular artery (TA), Van Gieson, 40X

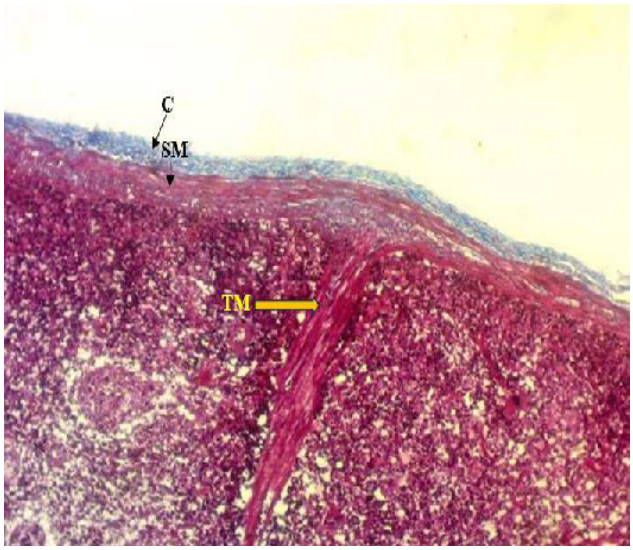

Fig. 7: C/S Photomicrograph of 40 day old pig spleen(Group-II) showing smooth muscle fibers (SM) in capsule (C)and trabeculae (TM), Masson's Trichome, 10X

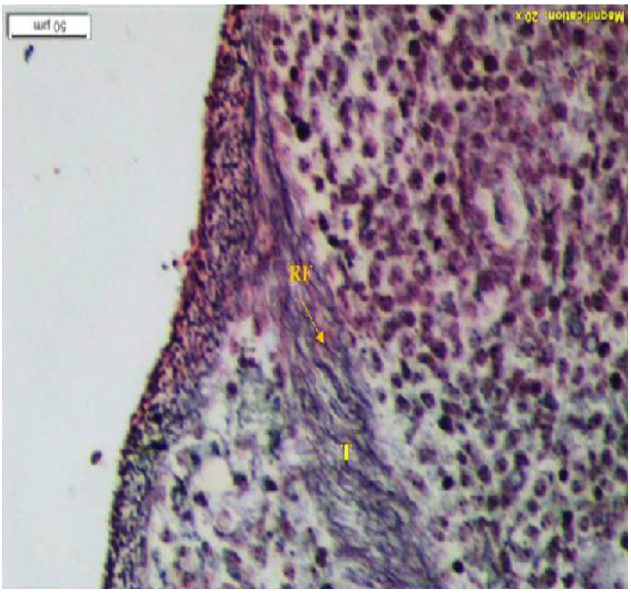

Fig. 10: C/S Photomicrograph of 6 day piglet spleen (Group-I) showing reticular fibers (RF) in trabeculae (T), Wilder's, 40X

muscle fibers (Fig. 10, 11). The reticular fibers increased with advancement of age. These observations supported the earlier reports of Eddine *et al.* (2020) in lambs, Waghaye *et al.* (2017) in goats and Kaur *et al.* (2020) in pigs. Thickness of trabeculae increased with age (Table 1), which was in accordance with the findings of Alex *et al.* (2015) in human, Rahmoun *et al.* (2019) in rabbits and Eddine *et al.* (2020) in lambs.

Fig. 11: L/S Photomicrograph of 40 day pig spleen (Group-II) showing reticular fibers (RF) in trabeculae (T), Wilder's 40X

Table 1: Micrometry of capsular and trabecular thickness in postnatal pigs of different age groups

Parameter	Group-I	Group-II	Group-III
Capsule thickness (µm)	54.08±2.17^{a}	80.62±3.94^{b}	103.36±5.50^{c}
Trabecular thickness (µm)	47.27±3.43^{a}	90.23±4.92^{b}	107.57±7.68^{b}

Values with different superscripts (a,b,c) in a row differ significantly ($p<0.001$).

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References

Alex, L., Rajan, M.L., Xavier, B., Jacob, P., Rani, K.D., & Lakshmi, G.V. (2015). Microscopic study of human spleen in different age groups. *International Journal of Research in Medical Science, 3*(7), 1701-1706.

Chaurasia, S., Menaka, R., Kalyani, I.H., & Puri, G. (2022). Gross architectural and histomorphometrical studies on the spleen of postnatal goat. *Haryana Veterinarian, 61,* 105-108.

Devi, H., Mathur, R., & Joshi, S. (2016). Histological studies on the spleen of Marwari goat (*Capra hircus*). *Veterinary Practitioner 17*(2), 190-191.

Eddine, Djallal, R., Amine, Mohamed, F., Manel, H., Khaoula, M., & Marina, L. (2020). Morpho-histological study of spleen ontogenesis in lambs during antenatal and postnatal period. *Online Journal of Animal Feed Research, 10*(1), 12-16.

Gnanadevi, R., Senthilkumar, S., Kannan, T. A., & Ramesh, G. (2019). Comparative histoarchitectural study of splenic components in sheep and goat. *International Journal of Current Microbiology and Applied Sciences, 8*(5), 1387-1394.

Kaur, H., Singh, O., & Pathak, D. (2020). Histomorphological studies on the spleen of pig (*Sus scrofa*). *International Journal of Livestock Reseaerch, 10*(12), 68-72.

Rahman, N., Tandon, R., Ghaus, F., Moinuddin, A., & Akram, W. (2016). Comparative anatomy of spleen: Histomorphometric study in human, goat, buffalo, rabbit and rat. *Academia Anatomica International 2*(1), 28-32.

Rahmoun, D.E., Fares, M.A., Bouzebda-afri, F., & Driss, K.B. (2019). An anatomical and histological study of the rabbit spleen development in the postnatal period in Algeria. *Online Journal of Animal Feed Research, 9*(2), 44-50.

Shringi, N., Mathur, R., Rohlan, K., Kumar, V., & Ganguly, S. (2018). Histological studies on the spleen of large White Yorkshire Pig (*Sus scrofa*). *Journal of Entomological and Zoologica; Studies, 6*(1), 1142-1144.

Waghaye, J.Y., Banubakode, S.B., Charjan, R., Rana, J., Nandeshwar, N.C., & Mainde, U.P. (2017). Age related histo-morphological changes of splenic connective tissue in goat (*Capra hircus*). *Indian Journal of Biotechnology, 13*(2), 1-6.