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Comparative light microscopic study of splenic Stroma in male and female local domestic pig (*Sus scrofa domesticus*)

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Abstract

The present study was carried out to investigate light microscopic structure and differences of splenic stroma in male and female healthy adult pigs. The capsule was outermost layer of uneven thickness at dorsal, middle and ventral aspect and showed no statistical variations in male and female pig spleen. The capsule was found to be composed of two layers namely outer fibrous layer and inner muscular layer. In both sex of pigs, it was noticed that outer fibrous layer of splenic capsule in male and female pig was composed of uneven distribution of intermingled collagen, elastic and reticular fibres. The collagen fibres were found to be predominant over the elastic and reticular fibres in outer fibrous layer of splenic capsule. The inner muscular layer of splenic capsule was composed of one to four layers of smooth muscle fibres with different orientations at different places in both male and female pig. In both sex of pig, it was observed that the branching connective tissue trabeculae extended from inner muscular layer of capsule into the splenic pulp and supported the blood vessels. The trabeculae were found to be mainly composed of smooth muscle fibres. The wavy reticular fibres were found to be predominant over other fibres types in the connective tissue trabeculae. The elastic fibres in the connective tissue trabeculae were longer and oriented parallel to the direction of smooth muscle fibres of trabeculae. The components of splenic stroma in both male and female pig were found similar.

Keywords: Domestic pig, spleen, splenic stroma, splenic capsule, splenic trabeculae

1. Introduction

In India, presently pig farming providing livelihood to poor belonging to the lowest socio-economic strata. Apart from providing meat, it is also source of bristles, manure. The other advantages of pig farming are like shorter generation interval, utilizes wide variety of feed stuff, higher dressing percentage, good demand for domestic and export market for its by-products such as bacon, ham, sausages etc. Pig farming, therefore, has a considerable potential to fulfil the requirement of meat as well as employment opportunities.

One of the lymphoid organs is a spleen which is considered as largest lymphoid organ. It is also the primary site for immune cell proliferation and differentiation and is a specialized organ to perform filtration of blood. The splenic parenchyma plays a crucial role in immune responses such as exposure to blood born antigen^[14]. Spleen also acts as erythropoietic organ besides lymphopoiesis during embryonic stage. The organ is demanding constant attention from the anatomical, immunological and clinical point of view^[2]. Detailed information about the splenic cellular composition is important for the understanding of its immunological role and for the analysis of several diseases^[15]. The splenic stroma constitutes of capsule and trabeculae that gives outer and inner supportive framework to splenic parenchyma. The components and arrangement of splenic stromal tissue reflects the contractile role in altering the blood volume of spleen to expel the erythrocytes in general circulation.

The microscopic structure of spleen of many animals is mentioned in many standard text and literature. However, it was observed that no study has been conducted on spleen of local domestic pig. Hence, the present study had been carried out with the aim to investigate light microscopic structure and differences of splenic stroma in male and female local domestic pig and compare the findings with those reported earlier in other species.

2. Materials and Methods

The present study was carried out on the spleen collected from 6 male and 6 female healthy adult local domestic pigs. Samples were collected immediately after slaughter from local pig Slaughter house.

The collected samples were washed with normal saline and small tissue pieces were fixed in 10% neutral buffered formalin, 10% formal saline and Bouin's fluid. Then the tissues were processed for routine paraffin embedding method [8]. The tissue sections of 5 µm thickness were processed for following staining procedures for histomorphological studies.

1. Haematoxylin and Eosin for normal cytoarchitectural study [17].
2. Van Gieson's method for collagen and muscle fibres [8].
3. Verhoeff's elastic stain for elastic fibres [17].
4. Gomori's reticulin method for reticular fibres [17].

In addition micrometrical studies were carried out using calibrated oculometer for recording different quantitative parameters. Data generated was analyzed statistically as per the methods suggested by Panse and Sukhatme [19].

3. Results and Discussion

In the present study histological observations were carried out on dorsal, middle and ventral aspect of the spleen of male and female local domestic pig. During the present study, it was observed that spleen of male and female local domestic pig was composed of capsule, trabeculae, white pulp and red pulp (Fig. 1 and 2).

The capsule was outermost layer ensheathed the spleen. It was uneven in thickness at dorsal, middle and ventral aspect of male and female pig spleen. The capsule was found to be composed of two layers namely outer fibrous layer and inner muscular layer (Fig. 3 and 4). In agreement with present observations Usende *et al.* [24] in Nigerian indigenous pig reported the variation in thickness of splenic capsule at different region. Waghaye *et al.* [25] in goat reported that connective tissue capsule of uneven thickness ensheathed the spleen and composed of outer fibrous and inner muscular layer. In line to this Alshamary [3] reported that splenic capsule in Iraqi camel was clearly divided into outer connective tissue fibrous layer and inner smooth muscle cell layer.

Similar observations in accordance to present findings were recorded by Maina *et al.* [18] in camel, Estacio *et al.* [10] in Philippine carabao and Raju *et al.* [20]. They reported that splenic capsule was composed of two layers. The outer layer was fibrous connective tissue layer and inner layer was composed of smooth muscle. In line with present findings Landsverk [16] stated that the capsule of spleen was composed of two layers of connective tissue and smooth muscle in domestic animals.

In both sex of pigs, it was noticed that outer fibrous layer of splenic capsule in male and female pig was composed of uneven distribution of intermingled collagen, elastic and reticular fibres. The collagen fibres were found to be predominant over the elastic and reticular fibres in outer fibrous layer of splenic capsule ((Fig. 5 and 6).

The elastic fibres in outer fibrous layer were short, wavy and intermingled with other connective tissue fibres whereas; reticular fibres were very few, wavy and intermingled (Fig. 7, 8, 9 and 10).

In agreement with the present findings regarding fibrous component of splenic capsule, Waghaye *et al.* [25] in goat reported wavy and intermingled collagen, reticular and elastic fibres in outer fibrous layer of splenic capsule. In line with present findings Das *et al.* [6] in dog, Devi *et al.* [7] in Marwari goat, Geetha *et al.* [13] in mice, rat, and guinea pig, Shringi *et al.* [22] in Large white Yorkshire pig, reported that splenic

capsule was composed of collagen, elastic and reticular fibres along with smooth muscle fibres. Firdous *et al.* [12] in fox, Reshag *et al.* [21] in chicken foetii stated that splenic capsule was composed mainly of collagen fibres and smooth muscle fibres. Khaleel [15] in Iraqi sheep and Alshamary *et al.* [3] in camel reported interwoven collagen and elastic fibres with smooth muscle fibres in splenic capsule. However, Copenhagen *et al.* [5] and Trautmann and Fiebiger [23] reported numerous elastic fibres with smooth muscle fibres in connective tissue of splenic capsule.

During the present investigations, it was observed that inner muscular layer of splenic capsule was composed of one to four layers of smooth muscle fibres with different orientations at different places in both male and female pig (Fig. 11, 12 and 13). In contrast to this Bacha and Bacha [4] in pig and Trautmann and Fiebiger [23] in domestic animals reported the interwoven layers of muscle fibres in the inner muscular layer splenic capsule. This variation may be attributed to breed difference.

It was found that, the collagen fibres were very few and interposed between muscle fibres of inner muscular layer (Fig. 5 and 6), whereas, reticular fibres were more, courser and wavy than the outer fibrous layer and interposed between muscle fibres (Fig. 9). The elastic fibres in the inner muscular layer of capsule were predominant over the other fibres. They were longer and densely arranged than the outer fibrous layer. The elastic fibres were interposed parallel to the direction of muscle fibres of inner muscular layer of splenic capsule (Fig. 7 and 8).

The result of present findings regarding predominance of elastic fibres in inner muscular layer and their arrangement parallel to direction of muscle fibres may aid in distension and contraction of organ along with smooth muscle. However the predominance of collagen fibres in outer fibrous layer of splenic capsule may be attributed to provide firmness to the organ.

During the present work average thickness of capsule in male pig spleen at dorsal, middle and ventral aspect was recorded as $12.036 \pm 0.747 \mu\text{m}$, $12.74 \pm 0.30 \mu\text{m}$ and $13.10 \pm 0.689 \mu\text{m}$ respectively, whereas, in female pig spleen it was recorded as $12.189 \pm 0.654 \mu\text{m}$, $13.699 \pm 1.435 \mu\text{m}$ and $12.413 \pm 0.768 \mu\text{m}$ at dorsal, middle, ventral aspect respectively.

The recording of present study regarding the thickness of splenic capsule showed non significant variations between dorsal, middle, and ventral aspect of spleen and also between male and female pig spleen.

In both sex of pig, it was observed that the branching connective tissue trabeculae extended from inner muscular layer of capsule into the splenic pulp and supported the blood vessels. The sections stained with Haematoxylin and Eosin, Van Gieson's stain, Gomori's reticulin stain and Verhoeff's elastic tissue stain showed that, the component of trabeculae were similar to that of the capsule.

The trabeculae were found to be mainly composed of smooth muscle fibres (Fig. 1 and 2). The wavy reticular fibres were found to be predominant over other fibres types in the connective tissue trabeculae and were mostly arranged parallel to longitudinal direction of trabeculae (Fig. 9). The connective tissue trabeculae were also observed to be rich in elastic fibres. The elastic fibres in the connective tissue trabeculae were longer and oriented parallel to the direction of smooth muscle fibres of trabeculae (Fig. 14). However, the fine collagen fibres were observed in meagre amount in trabeculae (Fig. 5).

The observations of the present study are in line with the reports made by Landsverk [16], Bacha and Bacha [4], Trautmann and Fiebiger [23] in domestic animals, Khalel [15] in Iraqi sheep, Maina *et al.* [18] in camel, Waghaye *et al.* [25] in goat, Firdous *et al.* [12] in fox, Usende *et al.* [24] in Nigerian indigenous pig, Devi *et al.* [7] in Marwari goat, Muslih and Mirhish [1] in gazelle and Reshag *et al.* [21] in chicken. In accordance with present observations, Raju *et al.* [20] in goat reported that splenic trabeculae were extensively branched with large amount of elastic and reticular fibres as compared to collagen fibres.

In agreement with the present finding, Copenhaver *et al.* [5] mentioned that predominancy of smooth muscle fibres and elastic fibres in trabeculae enable spleen to change the volume of organ by contraction to expel the erythrocytes and increase the number of these corpuscles in the general circulation.

In both male and female pig, it was noticed that, the vessels entered in the splenic pulp were supported by the connective tissue trabeculae. The largest vessel and nerves were found near the hilus. The trabecular vessels consisted of marked internal elastic lamina with few elastic fibres in the wall. This finding is in accordance with report made by Faroon and Henry [11] in sheep, Trautmann and Fiebiger [23] in domestic animals, Maina *et al.* [18] in camel, Das *et al.* [6] in dog and Ellenport [9] in pig.

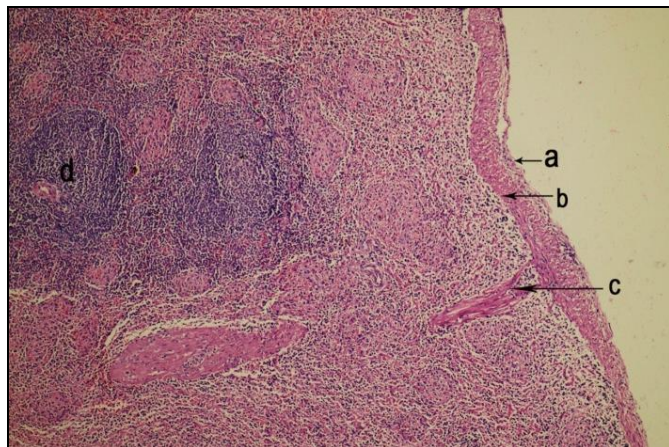


Fig 1: capsule b. Inner muscular layer splenic capsule c. Trabeculae d. White pulp (Haematoxylin and Eosin, X 100)

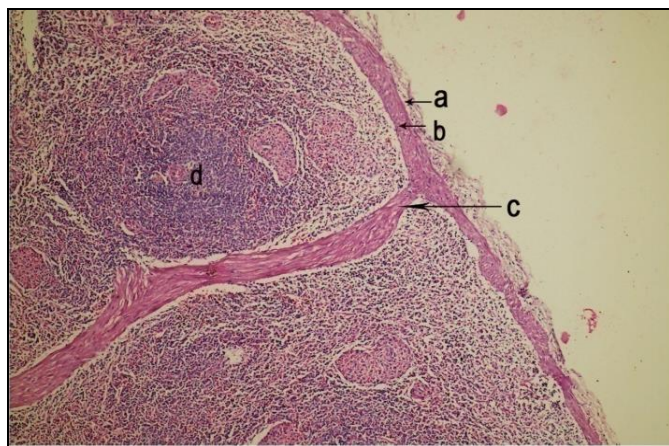


Fig 2: Photomicrograph of spleen in female a. Outer fibrous layer of splenic capsule b. Inner muscular layer splenic capsule c. Trabeculae d. White pulp (Haematoxylin and Eosin, X 100)

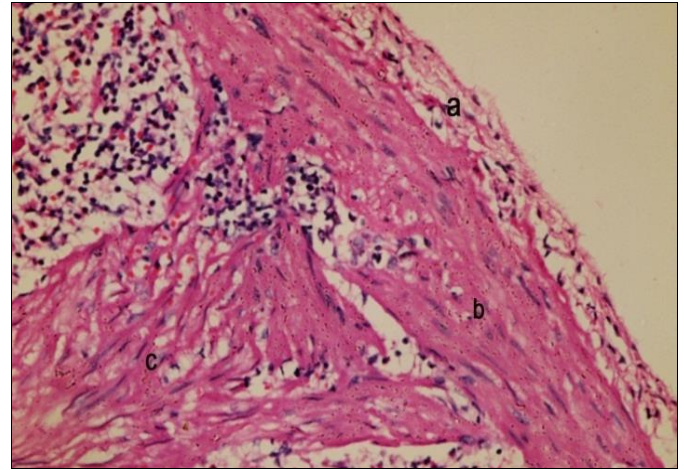


Fig 3: Photomicrograph of spleen showing two layers of splenic capsule in male a. Outer fibrous layer b. Inner muscular layer c. Trabeculae (Haematoxylin and Eosin, X 400)

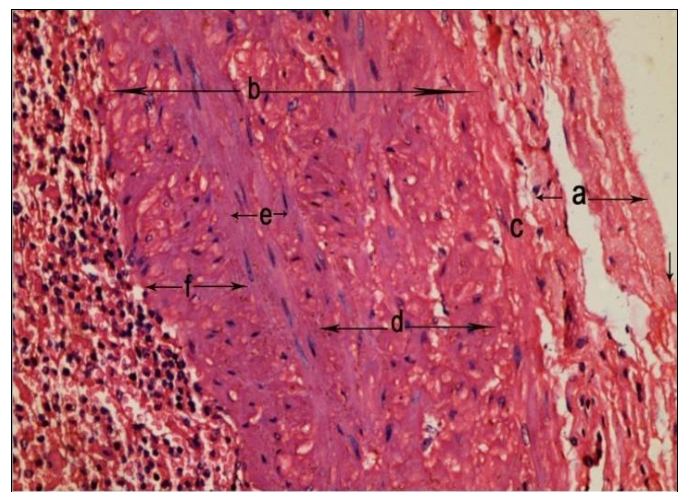


Fig 4: Photomicrograph of spleen showing two layers of splenic capsule in female a. Outer fibrous layer b. Inner muscular layer c. Circular arranged muscle fiber layer d. longitudinally arranged muscle fiber layer e. circularly arranged muscle fiber layer f. longitudinally arranged muscle fiber layer (Haematoxylin and Eosin, X 400)

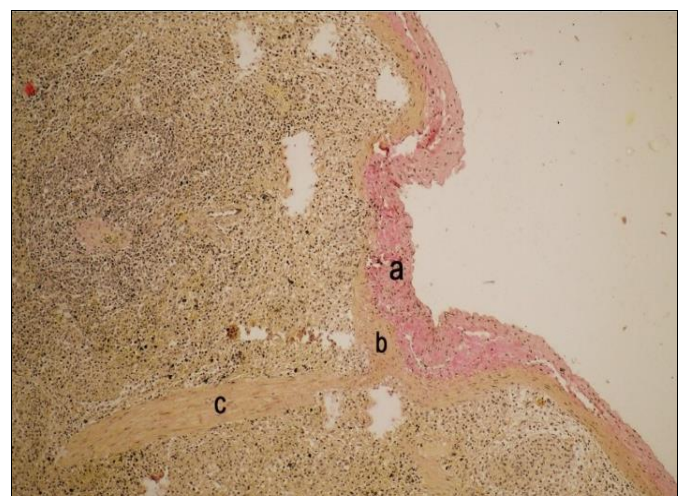


Fig 5: Photomicrograph of showing collagen fibers at dorsal aspect of splenic capsule in male a. Outer fibrous layer b. Inner muscular layer c. Trabeculae (Van Gieson's, X 100)

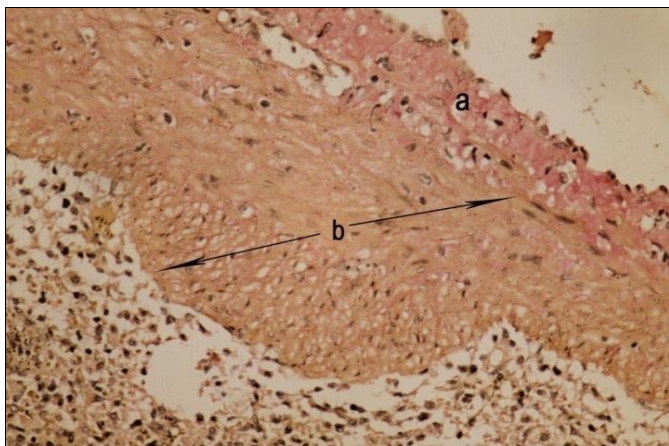


Fig 6: Photomicrograph of showing collagen fibers at middle aspect of splenic capsule in female a. Outer fibrous layer b. Inner muscular layer (Van Gieson's, X 400)

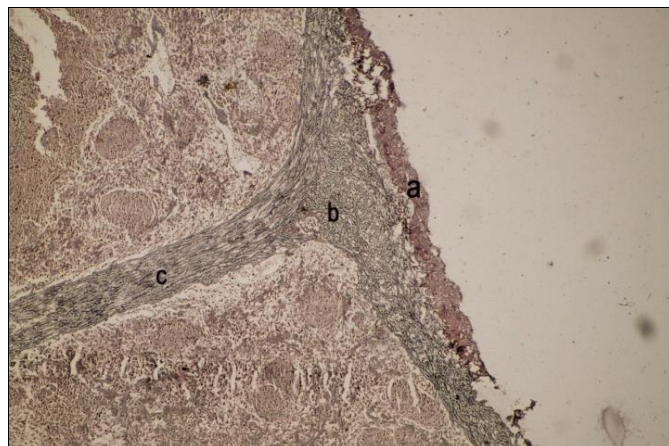


Fig 9: Photomicrograph of showing reticular fibers at ventral aspect of splenic capsule in male a. Outer fibrous layer b. Inner muscular layer c. Trabeculae (Gomori's reticulin, X 100)



Fig 7: Photomicrograph of showing elastic fibers at dorsal aspect of splenic capsule in male a. Outer fibrous layer b. Inner muscular layer (Verhoeffs elastic stain, X 100)

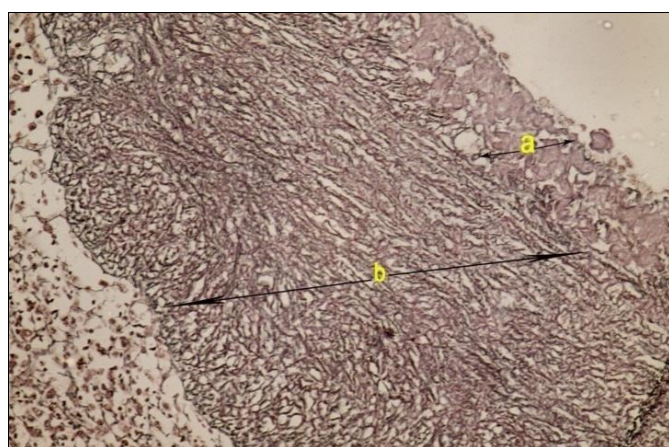


Fig 10: Photomicrograph of showing reticular fibers at middle aspect of splenic capsule in female a. Outer fibrous layer b. Inner muscular layer (Gomori's reticulin, X 400)

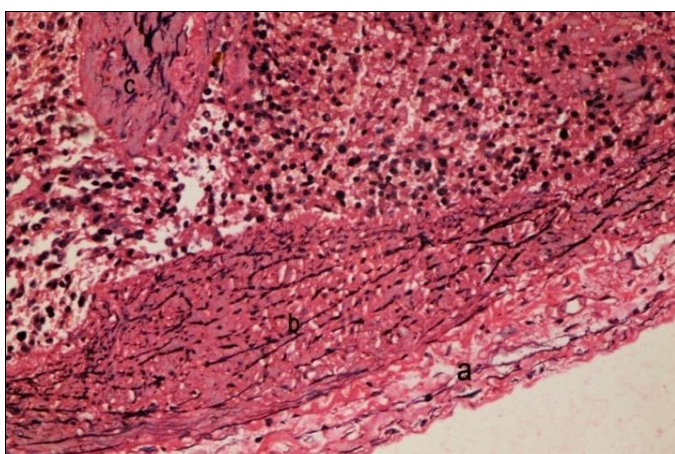


Fig 8: Photomicrograph of showing elastic fibers at dorsal aspect of splenic capsule in female a. Outer fibrous layer b. Inner muscular layer c. Trabeculae (Verhoeffs elastic stain, X 400)

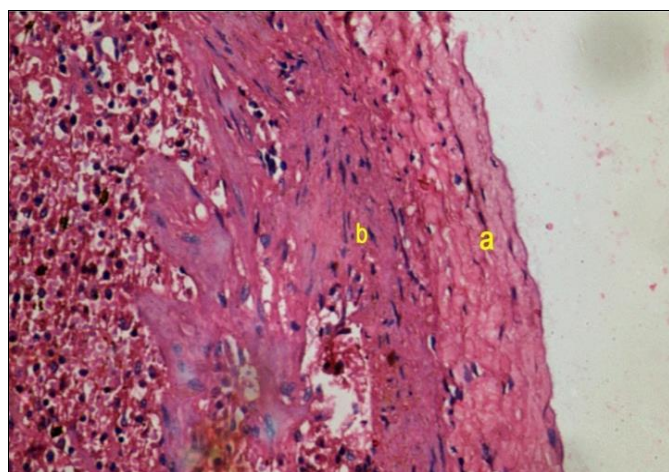


Fig 11: Photomicrograph of splenic capsule in female t. Outer fibrous layer b. Inner circularly arranged single smooth muscular layer Haematoxylin and Eosin, X 400)

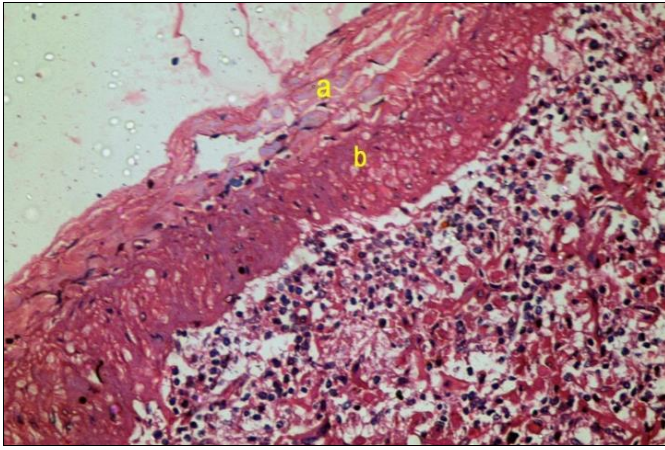


Fig 12: Photomicrograph of splenic capsule in male a. Outer fibrous layer b. Inner longitudinally ranged single smooth muscular layer (Haematoxylin and Eosin, X 400)

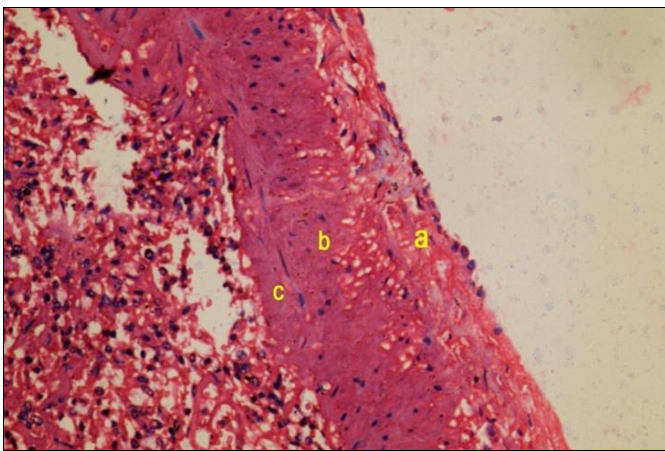


Fig 13: Photomicrograph of splenic capsule in male 1. Outer fibrous layer b. Outer longitudinally arranged single smooth muscular layer c. Inner circularly arranged single smooth muscular layer (Haematoxylin and Eosin, X 400)

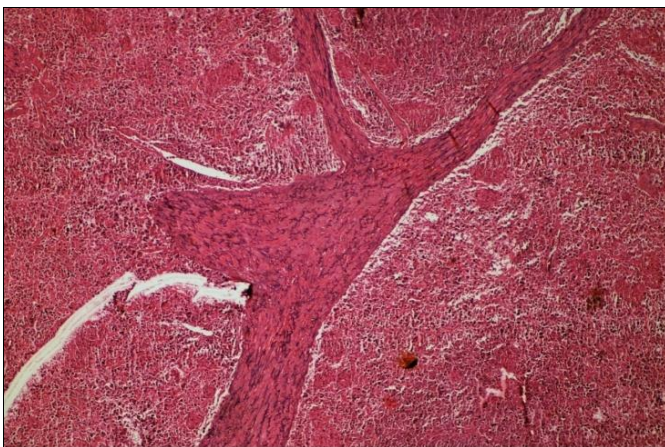


Fig 14: Photomicrograph of splenic trabeculae in female showing elastic fibers. (Verhoeff's elastic stain, X 400)

4. Conclusion

The splenic capsule was of uneven thickness at dorsal, middle and ventral aspect of spleen. The thickness of splenic capsule showed no statistical variations between male and female pig. The components and arrangement of splenic stroma in both male and female local domestic pig was found similar and also to that of the other mammalian species. The results of the present study may serve as a basis for comparative

microscopic study and future research in field of veterinary science.

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