The Pharma Innovation Journal 2022; SP-11(7): 2330-2336

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(7): 2330-2336

www.thepharmajournal.com
Received: 26-04-2022
Accepted: 11-06-2022

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Histological studies on the rectum of pig (*Sus scrofa domesticus*)

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Abstract
The present investigation was carried out on 10 rectums from recently slaughter adult pigs. Histologically, rectal wall was composed of four layers: tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa or adventitia. Tunica mucosa was consisted of three layers: the lining epithelium, lamina propria and muscularis mucosae (lamina muscularis). Tunica submucosa layer made up of loose connective tissue with numerous small and large blood vessels. Tunica muscularis composed of inner circular muscle layer and outer longitudinal muscle layer and the outer longitudinal muscle layer contained more elastic fibres than the inner circular muscle layer. Tunica serosa or tunica adventitia was outermost layer of rectum wall consisting of loose connective tissue with many blood veins. The peritoneal portion of rectum covered by tunica serosa and retroperitoneal portion of rectum covered by tunica adventitia.

Keywords: Histology, pig, rectum, tunica

Introduction
The pigs are highly adaptable and rapidly growing species that may be attractive for small and marginal farmers for rearing. The majority of pigs are used for meat but they are also used for skin, fat and other materials. The pigs are biologically very similar to the human being. Because of this biological similarity, they are being used in medical research (Lind *et al.*, 2007) [15].
The rectum acts as a transient storage site for feces. The rectum receives fecal material from the descending colon, transmitted through routine muscle contractions called peristalsis. As the rectal walls expand due to the materials filling it from within, stretch receptors from the nervous system located in the rectal walls stimulate the desire to pass faeces, a process called defecation (Barrett, 2019) [2].
Body temperature can also be taken from the rectum. Some drugs are also administered via per rectal route due to rectal absorption results in more of the drug reaching the systemic circulation with less modification. Per rectal route is considered as more effective route for delivering medication. Rectal administration also diminishes the side effect of some drugs, such as gastric irritation, nausea and vomiting (Lowry, 2016) [16].
Due to the morphoscopy and histological method of the pig and human rectum, the structural similarity is established. On the basis of this result we determine that pigs can be used as biomodel for reconstruction the rectal pathology for biomedical researches and development of the new method of preventing diseases and treatment in proctology (Plakhotnyi *et al.*, 2021) [21]. Scarce work on the rectum of pig evoked interest to undertake the present study and to elucidate the histology of rectum of pig.

Materials and Methods
The present research was carried out on the rectum of adult pig (*Sus scrofa domesticus*). Histological studies on the research samples were conducted in the department of anatomy, CVAS, Bikaner. The 10 samples were utilized in present study on rectum. The samples of rectum were collected from freshly slaughtered adult pigs which were free from any pathological condition of digestive system, from the slaughter house, Bikaner.
Representative samples of rectum will be collected from identical sites and fixed either in 10% formalin or Bouin's fluid for 48 hours, and 18 hours, respectively followed by washing overnight in running tape water, dehydrated in ascending order of alcohol (50%, 70%, 90%

and then Absolute alcohol I, II and III), cleared in Chloroform and finally impregnated with paraffin. Paraffin blocks were prepared, numbered and stored at 4 ^{0}C in refrigerator (Luna, 1968) [17]. Five to six micron thick sections were made by using semi-automatic microtome then mounting of the sections on albuminized slides and drying of sections and then staining for general histo-morphological observations.

Table 1: Representative samples

S.N	Name of stain	Purpose	References
1.	Ehrlich's Hematoxylin and Eosin	For routine histomorphology	Singh and Sulochana, 1997 [23].
2.	Van Gieson's method	For collagen fibers	Singh and Sulochana, 1997 [23].
3.	Verhoeff's stain	For elastic fibers	Singh and Sulochana, 1997
4.	Masson's trichrome stain	For collagen fibers	Luna, 1968 [17].
5.	Gomori's method	For reticular fibers	Luna, 1968 [17].
6.	Crossman's modification of mallory's triple stain	For collagen, elastic and reticular fibers	Singh and Sulochana, 1997 [23]

Following staining methods were used:

Results and Discussion

Histological Observations

The rectal wall was composed of tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa or adventitia (Fig.1 and Fig.2). The findings of present study was in agreement with Takahashi *et al.* (1956) [25] in cat, Dellmann and Eurell (1998) [5] in horse, cattle and carnivores, Kadam *et al*. (2007) [10] in goat, Kadam *et al*. (2008) [11] in sheep, Kadam *et al.* (2009) [12] in sheep, goat and cattle, Bello *et al*. (2015) [3] in camel and AL-Samawy *et al.* (2019) [1] in camel. The finding was in partial harmony with the findings of Plakhotnyi *et al.* (2021) [21] in pig, wall of rectum was represent with three layers: internal layer (mucosa) and submucosa, middle layer (muscular) and external layer (adventitia or peritoneum).

Tunica mucosa

Tunica mucosa was comprised of three layers; the lining epithelium, lamina propria and muscularis mucosae (lamina muscularis). This was in conformity with findings of Takahashi *et al.* (1956) [25] in cat, Jit (1974) [9] in monkey, Dellmann and Eurell (1998) [5] in horse, cattle and carnivores, Kadam *et al*. (2007) [10] in goat, Kadam *et al*. (2008) [11] in sheep, Zhaxi *et al.* (2014) [27] in double-humped camel, Bello *et al*. (2015) [3] in camel, Hussein and Rezk (2016) [7] in cattle egret, AL-Samawy *et al.* (2019) [1] in camel, Plakhotnyi *et al.* (2021) [21] in pig and Ranjan and Das (2021) [22] in rabbit.

In the tunica mucosa caudal portion of rectum were presented some mucosal folds. The findings of present study was in accordance with Takahashi *et al.* (1956) [25] in cat, Smuts *et al.* (1987) in camel, Park and Kim (2001) [18] in mud loach, Agarwal *et al.* (2002) in rabbit, Zhaxi *et al.* (2014) [27] in double humped camel, Hussein and Rezk (2016) [7] in cattle egret, Plakhotnyi *et al.* (2021) [21] in pig and Ranjan and Das (2021) [22] in rabbit.

The lining epithelium was formed by the Simple columnar epithelium with scattered goblet cells. Columnar epithelial cells presented oval nuclei that occurred at the base of the cells. The goblet cells were unicellular mucous cells with a globular shape. The findings of the present study were in close agreement with that of Dellmann and Eurell (1998) [5] in horse, cattle and carnivores, Park and Kim (2001) [18] in mud loach, Kadam *et al*. (2007) [10] in goat, Kadam *et al.* (2009) [12] in sheep, goat and cattle, Portilla *et al*. (2011) [4] in rabbit, Jarrar and Faye (2015) [8] in camel, Hussein and Rezk (2016) [7] in cattle egret, AL-Samawy *et al.* (2019) [1] in camel and Plakhotnyi *et al.* (2021) [21] in pig.

The lining epithelium of pig rectum showed absence of the villi. The present study was in contradiction with the observation Hussein and Rezk (2016) [7] stated that the mucosa of the rectum of cattle egret was thrown into long and branched leaf-like villi lined by simple columnar epithelium containing goblet cells.

The simple columnar epithelium was present in the rectum and at the anorectal junction, it was gradually changed into non-keratinized stratified squamous epithelium (Fig.8). The findings of present study was in conventionality with Dellmann and Eurell (1998) [5] in horse, cattle and carnivores, sheep and goat and Plakhotnyi *et al.* (2021) [21] in pig. The present study was in contradiction with the observation of Plakhotnyi *et al.* (2020) [20] mention that the mucous coat of pig rectum and human rectum was covered with simple cuboid epithelium, that gradually changed with stratified epithelium. The columnar zone of anal canal was covered with stratified cuboidal epithelium, but intermediate zone and anorectal line was covered with stratified squamous epithelium.

At the junction of anal canal and anus, non-keratinized stratified squamous epithelium was changed into the keratinized stratified squamous epithelium (Fig.8). This finding resembled with the reports of Dellmann and Eurell (1998) [5] in horse, cattle and carnivores, sheep and goat and Plakhotnyi *et al.* (2021) [21] in pig.

The lamina propria was composed of loosely interwoven collagen fibers, reticular fibers and elastic fibers along with connective tissue cells (Fig.3). The crypts of lieberkuhns (intestinal glands) were present deep and contained goblet cells. Crypts of lieberkuhns were simple tubular glands. This was in uniformity with the findings of Jit (1974) [9] in monkey, Dellmann and Eurell (1998) [5] in horse, cattle and carnivores, Park and Kim (2001) [18] in mud loach, Kadam *et al.* (2008) [11] in sheep, Zhaxi *et al*. (2014) [27] in double humped camel, Hussein and Rezk (2016) [5] in cattle egret, AL-Samawy *et al.* (2019) [1] in camel , Urmila *et al.* (2019) [26] in pig and Plakhotnyi *et al.* (2020) [20] in pig. The finding was in partial harmony with the findings of Plakhotnyi *et al.* (2021) [21] in pig and Ranjan and Das (2021) [22] in rabbit.

From the cranial to the caudal region of the rectum, these intestinal glands were reduced.

Minor aggregations of lymphatic nodules were found in the tunica mucosa (Fig.1 and Fig.8). Which was in close agreement with the reports of Jit (1974) [9] in monkey, Kadam *et al*. (2007) [10] in goat, Kadam *et al*. (2008) [11] in sheep, AL-Samawy *et al.* (2019) [1] in camel, Urmila *et al.* (2019) [26] in pig and Plakhotnyi *et al.* (2020) [20] in pig. The finding was in partial harmony with the findings of Takahashi *et al.* (1956) [25] in cat submucosa, they found considerably large sized solitary lymph follicles, each of which often reached deep into

the basis of the submucosa and was surrounded by a connective tissue capsule. The tops of these follicles protruded into the propria through the muscularis, so that the crypts there were partially in a state of collapse, losing perceptibly in height and sometimes lymphocyte were found migrated out into the epithelium, Jit (1974) [9] in monkey lymphoid tissue, including lymph follicles, was seen in the mucosal membrane and submucosa, Liebler *et al*. (1988) [14] in cattle calf, Aleksandersen *et al.* (1991) in lambs and Zhaxi *et al*. (2014) [27] in double humped camel, lymphoid tissue in the mucosa was poorly developed. This was in disagreement with the findings of, Kapoor and Singh (2017) [13] studied in newborn calves lymphoid tissue mostly below tunica mucosa (submucosa) in the terminal part of rectum.

From the cranial to the caudal section of the rectum, the number of lymphatic nodules increased (Fig.8). This was in conformity with findings of Sturgess *et al.* (2001) [24] in cat.

Muscularis mucosae (lamina muscularis) was a thin inner layer of tunica mucosa. It was continuous layer and composed of smooth muscle fibers. Muscularis mucosae separated the tunica mucosa from the submucosa. The findings of the present study was in conformity with the reports of Urmila *et al.* (2019) [26] in pig. The finding was in partial harmony with the findings of Kadam *et al*. (2008) [11] in sheep, Jarrar and Faye (2015) [8] in camel and Hussein and Rezk (2016) [7] in cattle egret. The present study was in contradiction with the observation of Takahashi *et al.*(1956) [25] in cat, muscularis mucosae consisted of distinct inner circular and outer longitudinal layers, Jit (1974) [9] in monkey the two layers of muscularis mucosae were evident throughout the rectum and the upper part of the anal canal, AL-Samawy*et al.* (2019) [1] in camel, the muscularis mucosa of the rectum was comprised of inner (circular) layer and outer (longitudinal) layer of smooth muscle bundles, Plakhotnyi *et al.* (2020) [20] in pig, muscular lamina of mucous coat was made up of two layers: internal formed with circularly located myocytes and solid external layer and Ranjan and Das (2021) [22] noted that the lamina muscularis of rabbit was thick and appeared as multilayered.

Tunica submucosa

The tunica submucosa layer was made up of a loose connective tissue with numerous small and large blood vessels (Fig.3). The findings of present study was in conformity with the reports of Takahashi *et al*. (1956) [25] in cat, Park and Kim (2001) [18] in in mud loach, AL-Samawy *et al.* (2019) [1] in camel, Urmila *et al.* (2019) [26] in pig, Heryani *et al.* (2020) in bali cattle and Plakhotnyi *et al.* (2021) [21] in pig. The finding was in partial harmony with the findings of Plakhotnyi *et al.* (2020) [20] in pig. This was in disagreement with the findings of Ranjan and Das (2021) [22] in rabbit tunica submucosa comprising of densely packed collagen and elastic fibers.

Loose connective tissue was consisted of abundantly dispersed adipose tissue. The finding was in partial harmony with the findings of Kadam *et al.* (2009) [12] in cattle, sheep and goat and Urmila *et al.* (2019) [26] in pig.

The loosely arranged collagen, reticular connective fibers and few elastic fibres were present in the blood vessels of tunica submucosa (Fig.3). This was in conformity with findings of Dellmann and Eurell (1998) [5] in horse, cattle, sheep, goat and carnivores, AL-Samawy *et al.* (2019) [1] in camel, Urmila *et al.* (2019) [26] in pig and Ranjan and Das (2021) in rabbit. The finding was in partial harmony with the findings of Park and Kim (2001) [18] in mud loach.

Tunica muscularis

Tunica muscularis was made up of two layers of muscle fibers: inner circular muscle layer and outer longitudinal muscle layer (Fig.1, 2, 4, 6 and 7). The findings of present study was in accordance with Takahashi *et al.* (1956) [25] in cat, Dellmann and Eurell (1998) [5] in horse, cattle, sheep, goat and carnivores, Bello *et al*. (2015) [3] in camel, Jarrar and Faye (2015) [8] in camel, Hussein and Rezk (2016) [7] in cattle egret, AL-Samawy*et al.* (2019) [1] in camel, Heryani *et al.* (2020) in bali cattle, Plakhotnyi *et al.* (2020) [20] in pig and Plakhotnyi *et al.* (2021) [21] in pig.

The outer and inner smooth muscle layers were separated by connective tissue layer mainly composed of collagen (Fig.4 and 6) and reticular fibers (Fig.7). Between two layers, few blood vessels were also seen (Fig.4). The outer longitudinal muscle layer contained more elastic fibres than the inner circular muscle layer (Fig.6). The findings of the present study were in close agreement with that of Dellmann and Eurell (1998) [5] in horse, cattle, sheep, goat and carnivores.

Tunica serosa and Tunica adventitia

Tunica serosa or tunica adventitia was outermost layer of rectum wall consisting of loose connective tissue with many blood veins. The peritoneal portion of rectum covered by tunica serosa and retroperitoneal portion of rectum covered by tunica adventitia. The findings of present study was in accordance with the reports of Takahashi *et al.* (1956) [25] in cat, Dellmann and Eurell (1998) [5] in horse, cattle, sheep, goat and carnivores, AL-Samawy *et al.* (2019) [1] in camel and Ranjan and Das (2021) [22] in rabbit.

Collagen fibres (Fig.5 and Fig.6), reticular fibres (Fig.7) and elastic fibres were found in tunica serosa. In the tunica serosa, elastic fibres were mostly found along with blood vessels. The finding was in partial harmony with the findings of Dellmann and Eurell (1998) [5] in horse, cattle, sheep, goat and carnivores, Ranjan and Das (2021) [22] in rabbit.

Tunica adventitia was blends with the pelvic fascia, muscle and fat. The finding was in partial harmony with the findings of Dellmann and Eurell (1998) [5] in horse, cattle, sheep, goat and carnivores and AL-Samawy *et al.* (2019) [1] in camel.

Fig 1: Photomicrograph showing different layers of rectum of pig, M-Mucosa, SM-Submucosa, IC-Inner circular muscle layer, OL-Outer muscle layer, S-Serosa, BV-Blood vessel, LN-Lymphatic nodule. (H&E, 40X)

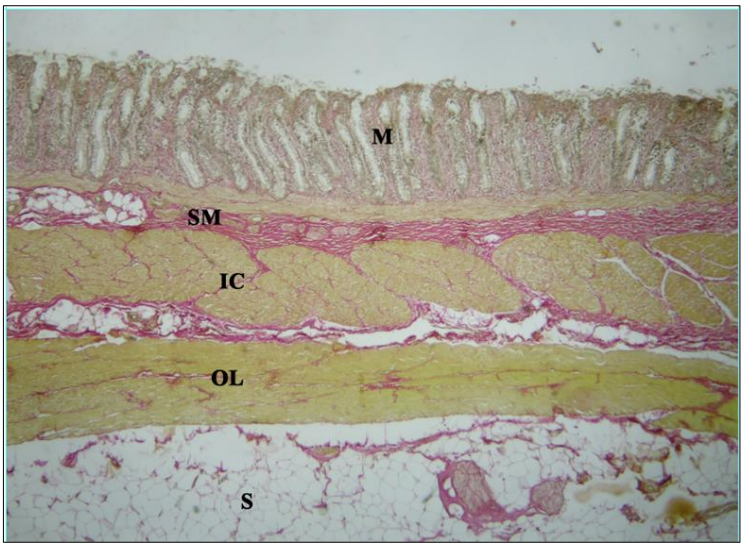

Fig 2: Photomicrograph showing different layers of rectum of pig, M-Mucosa, SM-Submucosa, IL-Inner circular muscle layer, OL-Outer longitudinal muscle layer, S-Serosa. (Verhoeff's stain, 40X)

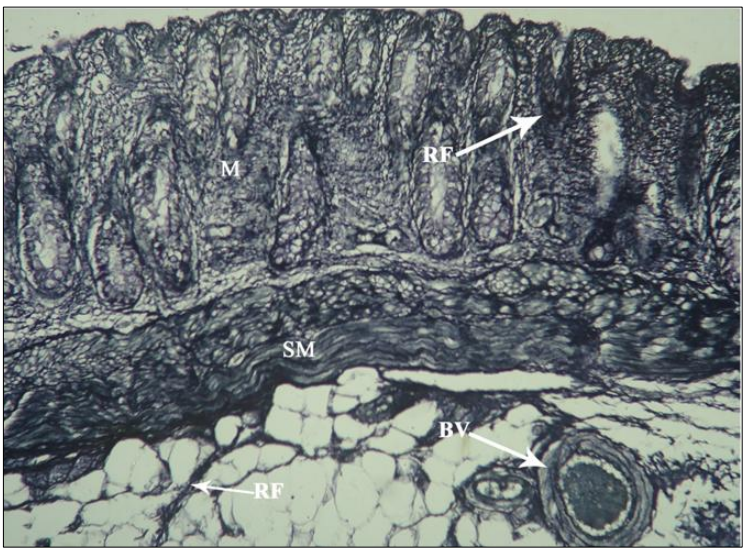

Fig 3: Photomicrograph of rectum of pig showing RF-Reticular fibers, M-Mucosa, SM - Submucosa and BV-Blood vessel. (Gomori stain, 100X)

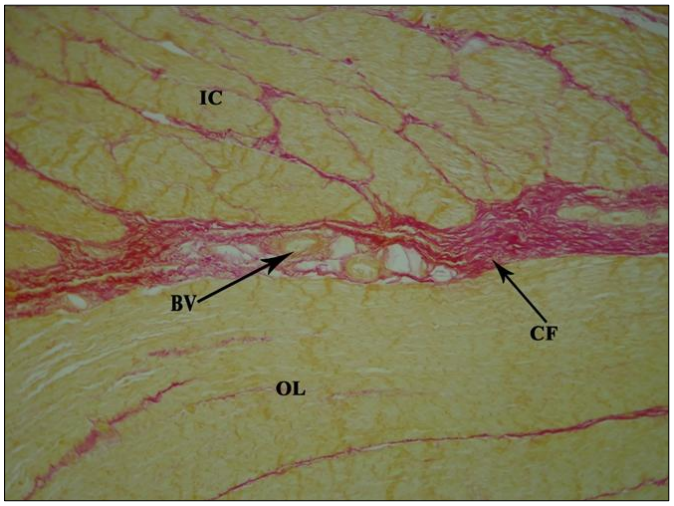

Fig 4: Photomicrograph of rectum of pig showing, IC-Inner circular muscle layer, OL-outer longitudinal muscle layer, CF-Collagen fibres, BV-Blood vessels. (Van Gieson's stain, 100X)

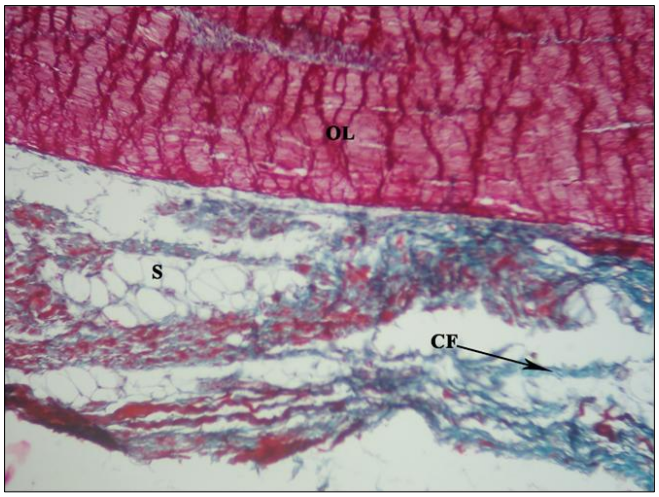

Fig 5: Photomicrograph of rectum of pig showing OL-Outer longitudinal muscle layer, S-Serosa, CF-Collagen fiber. (Masson's Trichrome stain, 100X)

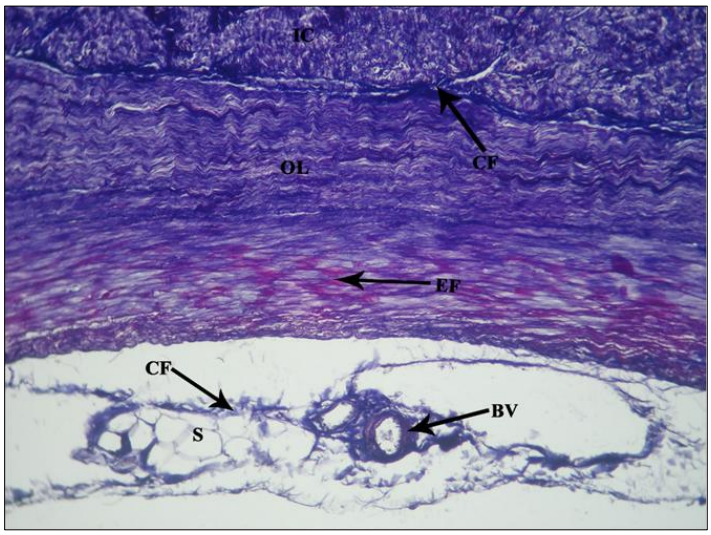

Fig 6: Photomicrograph of rectum of pig showing CF-Collagen fibers in Tunica muscularis and S-Serosa. And EF-Elastic fiber in OL-Outer longitudinal muscle layer, BV-Blood vessel, IC-Inner circular muscle layer, (Crossman's Modification of Mallory's Triple stain, 100X)

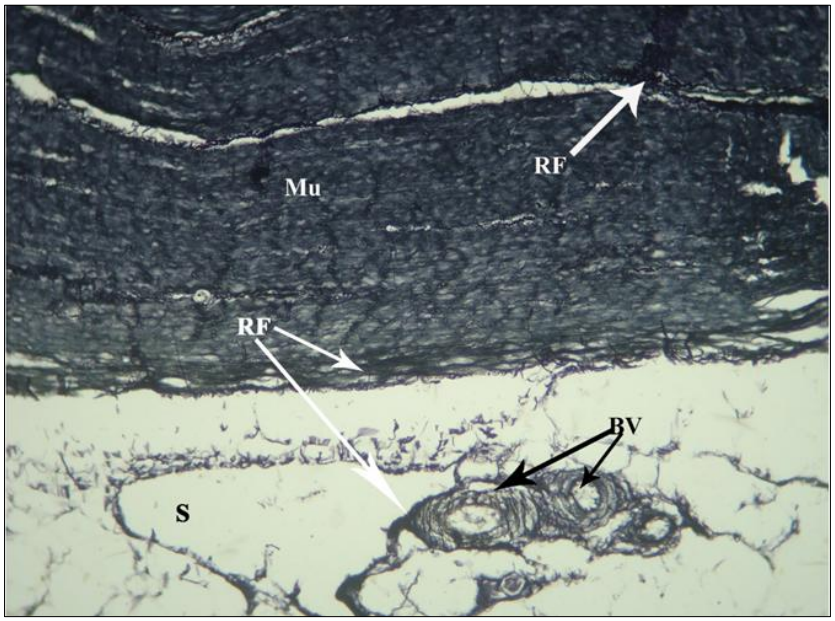

Fig 7: Photomicrograph of rectum of pig showing RF-Reticular fibers in Mu-Muscularis, S-Serosa, and BV-Blood vessels. (Gomori stain, 100X)

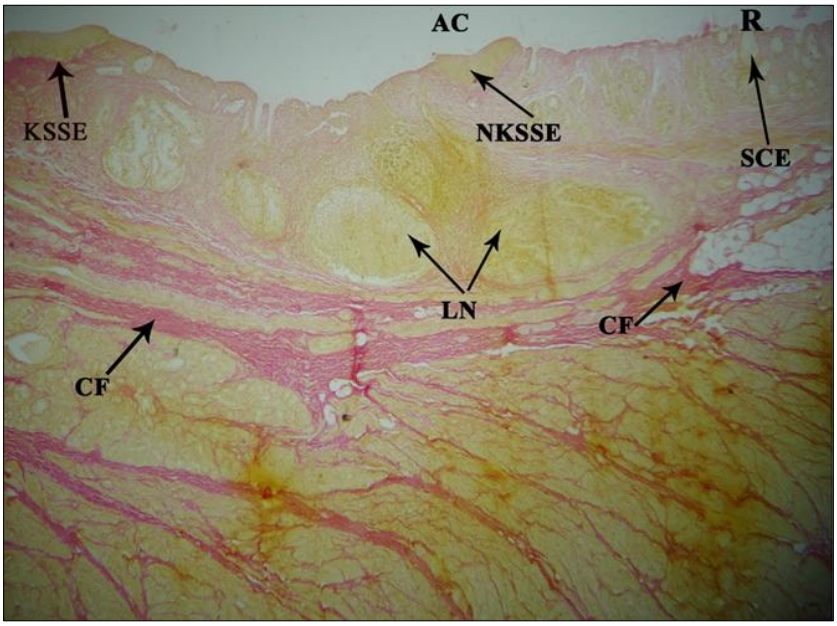

Fig 8: Photomicrograph of R-Rectum and AC-Anal canal of pig showing SCE-Simple columnar epithelium, NKSSE-Nonkeratinized stratified squamous epithelium, CF-Collagen fibers and LN-Lymphatic nodules. (Van Gieson's stain, 40X)

Conclusion

The lining epithelium was formed by the simple columnar epithelium with scattered goblet cell and it was gradually changed into non-keratinized stratified squamous epithelium at ano-rectal junction but at the junction of anal canal and anus, it was changed into the keratinized stratified squamous epithelium.

Minor aggregations of lymphatic nodules were found in the tunica mucosa. From the cranial to the caudal section of the rectum, the number of lymphatic nodules and mucosal folds increased.

Tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa or tunica adventitia were composed mainly of collagen fibers and reticular fibers but elastic fibers were seen mostly around the blood vessels in the tunica serosa layer.

Acknowledgments

The authors are highly thankful to Dean, College of Veterinary and Animal Science, Bikaner, RAJUVAS, Bikaner, Rajasthan for providing all necessary facilities to carry out research work. The authors are also thankful to all members of the Department of Anatomy for their help during the accomplishment of this work.

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