*Bulgarian Journal of Veterinary Medicine*, 2020, **23**, No 2, 160–169
ISSN 1311-1477; DOI: 10.15547/bjvm.2228

*Original article*

# MORPHOMETRIC ANALYSIS AND MUCIN HISTOCHEMISTRY OF GALLBLADDER SURFACE AND GLANDULAR EPITHELIUM IN SWINE

I. S. STEFANOV

Department of Anatomy, Medical Faculty, Trakia University,
Stara Zagora, Bulgaria

**Summary**

Stefanov, I. S., 2020. Morphometric analysis and mucin histochemistry of gallbladder surface and glandular epithelium in swine. *Bulg. J. Vet. Med.,* **23**, No 2, 160–169.

The aim of the study was to perform morphometric analysis and to distinguish histochemically the variety of mucins in surface and glandular gallbladder epithelium in swine at different ages. The histochemical analyses by using alcian blue staining at different pH and alcian blue-PAS technique allowed distinguishing neutral and acidic mucin expression in the surface and glandular epithelium of gallbladder's fundus, body and neck where age- and site-specific localisation of mucin content was defined. Goblet cells also expressed both neutral and acidic mucins. Morphometric analysis allowed estimating the height of the surface and glandular epithelium, diameter of glands and crypts, and comparing these parameters between the three age groups and three gallbladder parts. The goblet cells were observed in the gallbladder neck only. The morphometric and histochemical analyses extend the knowledge on structural and topographic features of the studied gallbladder components that could be useful as reference data for variety of experiments on this organ.

**Key words**: epithelium, gallbladder, goblet cells, histochemistry, morphometry, mucin, swine

## INTRODUCTION

Domestic pigs are widely used as experimental animals in veterinary and human medicine. They are among the most used animals for improvement of surgical methods regarding cholecystectomy and liver transplantation in humans and in order to reveal new diagnostic and therapy methods of mucosal inflammation or lesions associated with cholelithiasis or gallbladder cancer (Griffith *et al.,* 1989; Park *et al.,* 2005; *Ai et al.,* 2007; Meyerholz *et al.,* 2010; Swindle *et al.,* 2012). The function of the surface single columnar epithelium of the allbladder (*vesica biliaris/vesica fellea*) in animals and humans is to compact and regulate bile content and together with mucous glands (*glandulae mucosae vesicae biliaris*) to secrete mucus which defines the normal and pathologic conditions of the organ

(Griffith *et al.,* 1989; Meyerholz *et al.,* 2010; Swindle *et al.,* 2012). In this respect, the similarities and differences in structure and histochemical properties of gallbladder in animals and humans need more attention in order to be clarified. For example, gallbladder glands show species-specific structure, topography and histochemical features (Pearson *et al.,* 1982; Schaller, 2007; Prozorowska & Jackowiak, 2015). In ruminants, the glands are reported to be situated in the wall of the gallbladder body (*corpus vesicae biliaris*) (Jurisch, 1909; Schaller, 2007). In humans, guinea pigs, carnivores, swine, the tubulo-alveolar glands are concentrated predominantly in the region of the neck (*collum vesicae biliaris*), whereas, they are localised across the mucosa of gallbladder (*tunica mucosa vesicae biliaris*) (Laito & Nevalainen, 1975; Lee, 1980; Schaller, 2007). However, Prozorowska & Jackowiak (2015) reported the presence of glands in the body of porcine gallbladder. In rabbits, no glands are established in the gallbladder wall (Jackowiak & Lametschwwandtner, 2005).

Jüngst *et al.* (2001) suggest that the biliary viscosity depends mainly on the concentration of mucin, a high molecular weight glycosylated protein produced by gallbladder epithelium. An increased secretion of mucin by the gallbladder epithelium might inhibit gallbladder emptying and thus, promote the formation of gallstones (Jüngst *et al.,* 2001). The fact that pig gallbladder bile mucin is very similar to human bile mucin was used by some authors to develop a useful methodology for the isolation of mucoprotein complexes (Pearson *et al.,* 1982).

There is no information about goblet cells (*exocrinocyti caliciformes*) presence in gallbladder wall as another source of mucins. Such cells were described as a part of simple columnar epithelium in the wall of common bile duct of animals but not in the gallbladder (Seeger *et al.,* 2017).

The aim of the current study was to perform morphometric analysis and to distinguish histochemicaly the variety of mucins in gallbladder surface and glandular epithelium in pigs at different ages in order to provide new useful information about the structural and topographic features of the components of this organ.

## MATERIALS AND METHODS

### *Animals*

The present study was performed on 18 male pigs (crossbred Landrace × Danube White) divided into three groups: at the age of 2 months (26–33 kg), 6 months (92–100 kg) and 3 years (280–300 kg). The procedures were performed according to the Bulgarian laws about the animal care. Specimens from the fundus, adventitial (attached to the liver) and serosal (unattached) parts of gallbladder body and neck were processed by the classical histological methods. Single and serial histological cross and longitudinal sections of 6 μm thickness from material fixed in 10% buffered formalin, were stained with haematoxylin and eosin (H&E) (Vitanov *et al.,* 1995) and used for morphometric and histochemical study.

### *Morphometric study*

For each section stained with H&E, ten measurements of thickness of the surface and glandular epithelium, outer and inner (luminal diameter) diameter of the mucous glands, number of glands and crypts per field (×100 with an area of 0.65 $mm^2$) glandular cells, number of glandular cells per gland were performed. The number of

goblet cells was estimated per field (×200). Measurements were done on a light microscope (LEIKA DM1000) equipped with a digital camera (LEIKA DFC 290) and software (LAS V4.10.0 2016).

*Histochemical study*

Alcian blue (AB) staining with at pH 2.5 as well as combined alcian blue-PAS (AB-PAS) staining techniques were performed on the other histological serial sections in order to detect and differentiate polysaccharide content in surface and glandular epithelium. The AB-PAS staining method was used to differentiate acidic from neutral mucins and to visualise mixture of them. Alcian blue staining at pH 2.5 detected only acid mucins represented by glycosaminoglycans (Pearse, 1962; Mowry, 1963; Greiner *et al.,* 1985).

*Statistical analysis*

The number of cells and diameter of glands were estimated by light microscope (LEIKA DM1000) equipped with a digital camera (LEIKA DFC 290) and software (LAS V4.10.0 2016). The data (mean± SD) were processed by GraphPad Prism 6 for Windows (GraphPad Software, Inc., USA) via one-way analysis of variance followed by Tukey-Kramer's *post-hoc* test and. P-values <0.05 were considered statistically significant.

## RESULTS

The surface epithelium (*lamina epithelialis mucosae*) of the gallbladder is represented by a simple columnar epithelium. The morphometric study showed that the epithelium between mucosal folds in different parts of the gallbladder attained a thickness from 20.16 μm to 42.40 μm in 2-month-old animals and from 24.53 μm to 45.83 μm in 3-year-old animals. In animals of all ages the highest epithelium was found in *collum vesicae biliaris* followed by *fundus* and *corpus vesicae* ($P<0.0001$ at the age of 2 and 6 months and $P<0.01$ at 3 year-old animals). The epithelium of adventitial part was higher than that in serosal part of gallbladder body as well ($P<0.0001$). The lowest epithelium was observed in serosal part of gallbladder body and in serosal and adventitial part of the neck at the age of 2 months followed by 6-month-old and 3-years-old pigs. This epithelium was higher than the surface epithelium covering the mucosal folds varying from 19.83±1.09 to 41.71±1.73 μm. However, in the gallbladder fundus, the height of the surface epithelium between mucosal folds didn't differ significantly among the three age groups as did the height of the epithelium covering the folds. In general, the most pronounced age-dependent difference in the height of surface epithelium was estimated in the gallbladder neck with the lowest epithelium was observed in 2-month-old, and the highest one: in 3-year-old animals.

Compound tubulo-alveolar glands (*glandulae mucosae vesicae biliaris*) in the body of the gallbladder had predominantly oval secretory units smaller than in those in the gallbladder neck and rarely elongated tubulo-alveolar secretory end-pieces (Fig. 1–4). In the neck, glands reached the muscular *(tunica mucosae*) and even subserosal layer (*tela subserosa*), whereas in the gallbladder body, the glands were predominantly situated in *lamina propria mucosae* between the muscular layer and surface epithelium. Their excretory duct opened on the surface epithelium between base of the folds (*plicae mucosae*) or in gallbladder crypts. The crypts were significantly larger than

tubulo-alveolar glands and were more often situated in the serosal compared to the advential site of gallbladder (Table 1). In serosal site, their number decreased with age but no age dependent changes were identified in adventitial site. Crypts' diameter was larger in the gallbladder neck than in the body and also increased with age.

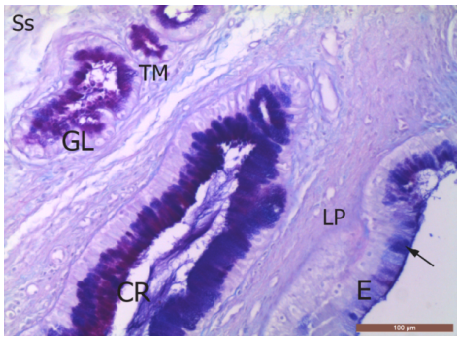


**Fig. 1**. Mucous expression of surface epithelium (E), crypts' epithelium (CR) and epithelium of glands (GL) in *collum vesicae biliaris* of 2-month-old pigs. The amount of neutral mucins increased towards glands especially in their deeper portions. Arrow – goblet cell; LP – *lamina propria mucosae;* TM – *tunica muscularis;* Ss – *tela subserosa;* AB-PAS staining bar = 100 μm.

The glands were more numerous in the neck (*glandulae colli vesicae biliaris*) (from 4.56±0.77 to 14.67±2.30) than in the body (from 4.50±1.72 to 7.66±0.75) (Table 1, Fig. 2 and 3). Moreover, the highest density of glands was detected in the serosal site of the neck than in its adventitial site, but in contrast, the number of glands in adventitial site of the body wall was higher than that in the serosal site. Different types and size of secretory endpieces of mucous glands were also detected. In the neck, both small single glands (with highest diameter in animals at the age of 3 years, followed by 6-month-old and 2-month-old) and complex

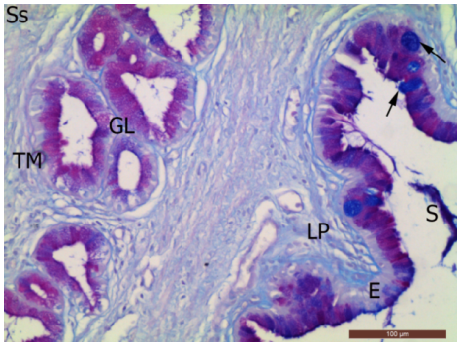


**Fig. 2.** Mucous expression of luminal secretion (S), surface epithelium (E) and epithelium of glands (GL) in *collum vesicae biliaris* of 6-month-old pigs. The amount of neutral mucins increases towards glands. Arrows – goblet cells; LP – *lamina propria mucosae*; TM – *tunica muscularis*; Ss – *tela subserosa;* AB-PAS staining, bar = 100 μm..

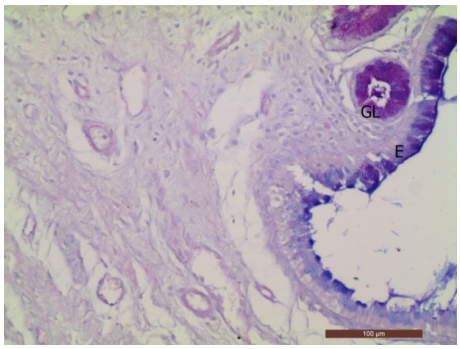


**Fig. 3.** Mucous expression of surface epithelium (E), and epithelium of glands (GL) in *corpus vesicae biliaris* of 6-month-old pigs. The glands are smaller than those in gallbladder neck. Goblet cells are missing; AB-PAS staining bar = 100 μm..

glands of the tubulo-alveolar secretory units showing the same age-dependent change in their diameter, was observed. The values of gland diameter correlated with the number and height of gland mucous cells (*exocrinocytus mucosus*) increasing with age (Table 1). The lowest significant difference was detected bet-

ween diameter of glands in body and neck in the gallbladder of 2 month-old pigs.

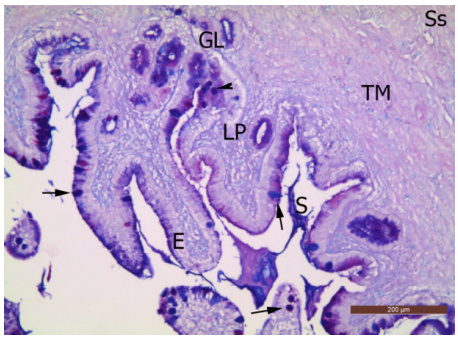


**Fig. 4.** Mucous expression of luminal secretion (S), surface epithelium (E) and epithelium of glands (GL) in *collum vesicae biliaris* of 3-year-old pigs. Goblet cells (arrows; arrowhead) are located among the cells of surface epithelium and the glandular cells, respectively. Goblet cells express a mixture of neutral and acidic mucins. LP – *lamina propria mucosae;* TM – *tunica muscularis;* Ss – *tela subserosa;* AB-PAS staining, bar = 200 µm.

The goblet cells were only observed in the gallbladder neck of all studied animals ranging from 2 to 20 in mature and from 1 to 4 in immature pigs (Fig. 1, 2 and 4). In 3-year-old animals their density was the highest, and lowest in 2 month-olds with statistical difference ($P<0.0001$). The number of these cells differed also in gallbladder neck parts. Their number was significantly higher in the dorsal part of the gallbladder neck near the opening of the *ductus cysticus* than in its ventral part. Significantly higher goblet cells density was also found in the mucosal epithelium of adventitial site of gallbladder wall attached to the visceral surface of liver than in the opposite free serosal site. Single goblet cells were also observed in some glands of the gallbladder neck.

Histochemical observations of gallbladder mucosa showed the composition of porcine mucus produced by the epithelial cells (Fig. 1–4). Positive PAS and AB staining in epithelial cells indicated the presence of mucus glycoproteins (Fig. 1–4). Red-purple, blue-purple and blue PAS-positive reaction of the apical part of the epithelium was observed and different colour intensity throughout the gallbladder was found. At 2 months of age, the highest expression of acidic and neutral mucins mixture was defined in the apical part of the surface epithelium of the gallbladder fundus and neck, but a lower one in the body. Neutral mucins were predominantly observed in the neck, followed by the body and the fundus. In the other two age groups, the highest expression of neutral mucins also was observed in the gallbladder neck, but the expression of mixture of acid and neutral mucins was similar in the three parts of the organ. The blue colour appeared rarely and indicated the presence of acidic mucins only.

The blue colour in the apical parts of the epithelial cells after AB staining only indicated secretions containing acidic glycoproteins. In the gallbladder fundus of the three groups of animals, the highest blue coloration intensity (strong reaction) in response to AB was observed in the apical part of all epithelial cells, followed by body and neck of the organ.

In the glands of gallbladder neck and body, the majority of epithelial cells expressing neutral mucins were detected in animals at the 3 years of age, and the least – at the age of 2 months. However, in the glands of gallbladder neck unlike the body, more cells expressing neutral mucins were found.

Goblet cells showed stronger reactivity to AB staining in mature animals which is related to the higher amounts of acidic mucins in the cells of mature vs immature animals expressing medium reactivity to

**Table 1.** Morphometric data (mean ± SD) of glands and glandular epithelium of the mucosal fold epithelium of gallbladder in pigs at different ages.

| Parameters | 2 months of age | 6 months of age | 3 years of age |
|---|---|---|---|
| *Epithelium height of the small glandular alveoli, μm* | | | |
| Gallbladder neck | 18.43 ±1.96 | 22.42 ±3.46$^{C4}$ | 23.90 ± 2.83 $^{A1/B4}$ |
| Gallbladder body | 17.88 ± 1.69* | 20.58 ± 3.40$^{C4**}$ | 22.35 ± 2.87$^{A2/B4*}$ |
| *Number of epithelial cells in small glandular alveoli* | | | |
| Gallbladder neck | 36.28 ± 9.35* | 40.00 ± 14.34$^{C1}$ | 49.50 ± 6.76 $^{A4/B4}$ |
| Gallbladder body | 33.70 ± 7.33 | 37.83 ± 12.20$^{C1}$ | 45.05 ± 6.53$^{A2/B4}$ |
| *Diameter of small glandular alveoli, μm* | | | |
| Gallbladder neck | 64.02 ± 10.40* | 84.28 ± 20.74$^{C4}$ | 86.91 ± 20.19$^{A1/B4}$ |
| Gallbladder body | 63.61 ± 16.58 | 73.92 ± 17.28$^{C4***}$ | 79.96 ± 19.45$^{A1/B3*}$ |
| *Number of small glandular alveoli per field (×100)* | | | |
| Gallbladder neck (dorsal part) | | | |
| *Tunica adventitia* | 8.16 ± 0.69 | 10.10 ±1.05$^{G4}$ | 14.67 ± 2.30 $^{E4, F4/}$ |
| *Tunica serosa* | 19.00 ± 0.82$^{H3}$ | 21.00 ± 1.43 $^{G4H}$ | 22.67 ± 0.95 $^{E4, F4/H}$ |
| Gallbladder neck (ventral part) | | | |
| *Tunica adventitia* | 5.16±0.69$^{****}$ | 4.56± 0.77$^{G0****}$ | 8.66±0.47 $^{E4/ F4/****}$ |
| *Tunica serosa* | 6.83±0.91$^{H4****}$ | 8.66 ±1.5 $^{G4/H4****}$ | 10.50±0.50$^{E4/ F4/H4****}$ |
| Gallbladder body | | | |
| *Tunica adventitia* | 5.33 ± 0.48 | 4.50± 1.72 $^{G4}$ | 7.66± 0.75 $^{E4, F4}$ |
| *Tunica serosa* | 4.66 ± 0.75$^{H2****}$ | 4.66 ±0.75 $^{G0****}$ | 5.00±0.58$^{E0, F0/H4****}$ |
| *Epithelium height of the large glands, μm* | | | |
| Gallbladder neck | 32.48 ± 0.72 | 37.67 ± 1.52 $^{C4}$ | 39.02 ± 0.74 $^{A4/B4}$ |
| Gallbladder body | 31.73 ±0.87$^{**}$ | 36.83 ± 1.45 $^{C4***}$ | 37.78 ±1.19 $^{A4/B4****}$ |
| *Diameter of mucosal crypts* | | | |
| Gallbladder neck | 200.7 ± 104.80 | 229.5 ± 105.10 $^{C0}$ | 245.3 ± 118.40 $^{A0/B3}$ |
| Gallbladder body | 101.0 ±14.40$^{****}$ | 124.5 ±21.23$^{C0****}$ | 132.6 ± 26.23 $^{A0/B1****}$ |
| Number of mucosal crypts per field (×100); gallbladder neck | | | |
| *Tunica adventitia* | 1.0 ± 0.00 | 1.0 ± 0.00 $^{C0}$ | 1.0 ± 0.00 $^{A0/B0}$ |
| *Tunica serosa* | 2.0 ± 0.00$^{****}$ | 1.8 ± 0.40 $^{C4****}$ | 1.0 ± 0.00 $^{A4/B4}$ |

$^{a1-4}$ statistically significant difference (from P<0.05 to P<0.0001, respectively) between the age of 3 years vs. the age of 6 months; $^{b1-4}$ statistically significant difference (from P<0.05 to P<0.0001, respectively) between the age of 3 years compared with the age of 2 months; $^{c1-4}$ statistically significant difference (from P<0.05 to P<0.0001, respectively) between the age of 6 months compared with the age of 2 months; $^{E1-4}$ statistically significant difference (from P<0.05 to P<0.0001, respectively) between gallbladder adventitial or serosal parts at the age of 3 years vs the age of 6 months; $^{F1-4}$ statistically significant difference (from P<0.05 to P<0.0001, respectively) between gallbladder adventitial or serosal parts at the age of 3 years vs the age of 2 months; $^{G1-4}$ statistically significant difference (from P<0.05 to P<0.0001, respectively) between gallbladder adventitial or serosal parts at the age of 6 months vs 2 months; $^{H1-4}$ statistically significant difference (from P<0.05 to P<0.0001, respectively) between gallbladder adventitial or serosal parts of gallbladderneck or body; */**/***/**** statistically significant difference (from P<0.05 to P<0.0001, respectively) between between the different gallbladder parts in animals at the same age.

the same staining. These cells were also observed to express a mixture of neutral and acidic mucins in AB-PAS technique.

The histochemical reaction of the surface mucous layer over the surface epithelium was similar to that of intracellular content of the epithelium (Fig. 1–4).

## DISCUSSION

According to the report published by Tang *et al.* (2013) the tunica mucosa is covered by regular simple columnar epithelium, with neither goblet cells nor glands in lamina propria mucosae. These authors claimed that the deep folds at the bottom of the mucosa, which are often cut in cross sectioning, sometimes look like glands, but without secretory activity. Later, in the Illustrated Veterinary Anatomical Nomenclature, Schaller (2007) demonstrated that the gallbladder wall contained glands (*glandulae vesicae biliaris)*, located in the neck of this organ in swine. In Nomina Histologica Veterinaria (NHV), Seeger *et al.* (2017) reported that the mucosa was lined by simple columnar epithelium represented by cholecystocytes and Brush cells. Glands of gallbladder containing mucous cells were described in *lamina propria mucosae*. According to to Seeger *et al.* (2017) the gallbladder gland and glands of its neck are existing; however, it is not clear in which part of the porcine gallbladder they were located: fundus, body or only in the neck (*collum vesicae felleae*). In the current study, we revealed that the glands existed in both gallbladder body and neck which correlates with the findings of Prozorowska & Jackowiak (2015). However, our results differ from the latter report which found out the highest number of porcine gallbladder glands in both the neck and the part of the body attached to the liver and as well as only occasional glands in the free part of the gallbladder body. We agree with the finding that glands are absent in the fundus. Unlike last mentioned research, we found out that the number of glands was higher in the neck than in the body. Moreover, the highest density of glands was detected in the serosal site of neck than in its adventitial site, but in contrast, the number of glands in adventitial site of the body wall was higher than that in serosal site. Comparably to Prozorowska & Jackowiak (2015) we detected different types and size of secretory units of mucous glands. In the neck both small, single compound glands and groups of compound glands of the coiled and alveolar secretory units, which reached the muscular and even the subserosal layer were observed. Glands in the gallbladder body possessed predominantly oval secretory units smaller than those in the gallbladder neck and elongated tubulo-alveolar secretory units. We also agree that in gallbladder body, the glands were predominantly situated in lamina propria of mucosa near the surface epithelium. In addition, we detected age-dependent differences in the diameter of glands in both porcine gallbladder and that the number of crypts in serosal site of gallbladder neck decreased with age without changes in adventitial site.

The combined AB-PAS technique is mainly used for detection and characterisation of epithelial mucosubstances (Mowry, 1963). In humans, Esterly & Spicer (1968) have reported the role of mucin nature in both normal surface and glandular epithelium of human gallbladder as well as in pathological conditions (gallbladder adenocarcinoma, obstruction and cholecystitis). The authors demonstrated clearly that the mucin in adenocarcinoma was distinctly different from that

of the normal, inflamed, or obstructed gallbladder. For the first time, we described in detail the number of goblet cells and mucin expression in normal porcine gallbladder in line with data about normal human gallbladder. According to Seeger *et al.* (2017), goblet cells did not exist either in the wall of gallbladder or in the cystic duct wallf. Tang *et al.* (2013) did not mention goblet cells either. We revealed that porcine gallbladder goblet cells, like those in humans, showed reactivity for both sulfomucins and carboxymucins identified in the same cell (Spicer, 1965; Esterly & Spicer, 1968). In birds, such as domestic geese, goblet cells were also observed to be PAS, AB (pH 2.5), and AB/PAS positive (Boydak & Aydin, 2009). Comparably to men (Esterly & Spicer, 1968), we assume that the presence of goblet cells in the gallbladder can be explained by both their involvement, together with the surface epithelium, in normal mucous production fulfilling protective role and perhaps, involvement in some pathologic conditions of this organ (further studies are however necessary to support that). The oligosaccharidic chains of glycoproteins in mucous cells are responsible for the viscoelastic and lubricant properties; they also modify the tertiary structure of the protein core and increase the resistance to bacterial degradation (Esterly & Spicer, 1968).

Within the cells the material was scattered throughout the cytoplasm and not restricted to the apical location characteristic of normal epithelial cells (Esterly & Spicer, 1968). According to Madrid *et al.* (1997) mucin is involved in cholesterol and pigment gallstone formation as did hormonal disturbance, modulation of liver lipid metabolism, production of cell debris.

Similarly to the human gallbladder (Esterly & Spicer, 1968), in swine, a large amount of mucus filled the gland cells but not the surface epithelial cells with their apical mucous expression. With the AB-PAS method, most of the cells stained blue-purple, indicating the presence of mixture of acidic and neutral mucosubstances, but a moderate number showed the red staining of neutral mucosaccharide, localised in the deepest glandular alveoli. In adjacent AB stained sections, some cells with weak blue or no staining corresponded to those stained red by the AB-PAS method. Neutral and acidic mucins were widely spread in glands of swine like in the human gallbladder. Mixtures of these types of mucins are also observed in individual cells.

We agree with the opinion of Prozorowska & Jackowiak (2015) who claim that the differences in size of porcine gallbladder mucous epithelial cells may depend on the secretory activity of the epithelium. In our study, we observed that the height of surface epithelium in the gallbladder fundus did not change in all age groups. So the surface epithelium of body and neck appeared to be more active in producing mucus. Unlike these authors, we also observed age-dependent differences in the height of gallbladder epithelium except for that lining the mucosal layer of the gallbladder fundus.

## CONCLUSION

This study defined the neutral and acidic mucin expression in both the surface and glandular epithelium of gallbladder fundus, body and neck as well as the anatomical knowledge about age- and site-specific differences in morphometric characteristics. Goblet cells were also established as normal structures expres-

sing both neutral and acidic mucins in the neck region. The morphometric and histochemical features of the studied structures in the gallbladder could be useful as reference data for a variety of experiments performed on this organ in swine at different age.

## REFERENCES


Ai, L. M., C. H. Peng, Y. L. Wu, L. P. Cao, H. Q. Fang, Y. B. Liu & S. Y. Peng, 2007. Orthotopic abdominal multivisceral transplantation without venovenous bypass in pigs. *Transplantation Proceedings*, **39**, 273–277.

Boydak, M. & M. F. Aydin, 2009. Histology of the Harderian gland of domestic geese (*Anser anser domesticus*). *Acta Veterinaria Brno*, **78**, 199–204.

Esterly, J. R. & S. Spicer, 1968. Mucin histochemistry of human gallbladder: Changes in adenocarcinoma, cystic fibrosis, and cholecystitis. *Journal of the National Cancer Institute,* **40**, 1–11.

Greiner, J. V., T. A. Weidmanz, D. R. Korb & M. R Allansmith, 1985. Histochemical analysis of secretory vesicles in nongoblet conjunctival epithelial cells. *Acta Ophthalmologica (Copenhagen*), **63**, 89–92.

Griffith, S. L., B. T. Burney, F. J. Fry & T. D. Jr. Franklin, 1989. A large animal model (swine) to study the diagnosis and treatment of cholelithiasis. *Investigative Radiology*, **24**, 110–114.

Jackowiak H. & A. Lametschwwandtner, 2005. Angioarchitecture of the rabbit extrahepatic bile duct and gallbladder. *The Anatomical Record*, **286**, 974–981.

Jüngst, D., A. Niemeyer, I. Müller, B. Zündt, G. Meyer, M. Wilhelmi & R. Pozo, 2001. Mucin and phospholipids determine viscosity of gallbladder bile in patients with gallstones. *World Journal of Gastroenterology*, **7**, 203–207.

Jurisch, A., 1909. Beitrage zur mikroskopischen Anatomie und Histologie der Gallenblase. *Anatomische Hefte*, **39**, 395–467.

Laito, M. & T. Nevalainen, 1975. Gland ultrastructure in human gallbladder. *Journal of Anatomy*, **120**, 105–112.

Lee, S. P., 1980. The mechanism of mucus secretion by the gallbladder epithelium *British Journal of Experimental Pathology*, **61**, 117–119.

Madrid, J. F., F. Herna & J. Ndez Ballesta, 1997. Characterization of glycoproteins in the epithelial cells of human and other mammalian gallbladder. A Review. *Microscopy Research and Technique*, **38**, 616–630.

Meyerholz, D. K., D. A. Stoltz, A. A. Pezzulo & M. J. Welsh, 2010. Pathology of gastrointestinal organs in a porcine model of cystic fibrosis. *American Journal of Pathology*, **176**, 1377–1389.

Mowry, R. W., 1963. The special value of methods that color both acidic and vicinal hydroxylgroups in the histochemical study of mucins. With revised directions for the colloidal iron stain, the use of alcian blue G8X and their combinations with the periodic acid-Schiff reaction. *Annals of the New York Academy of Sciences*, **106**, 404–423

Park, P., M. Bergström, K. Ikeda, A. Fritscher-Ravens & P. Swain, 2005. Experimental studies of transgastric gallbladder surgery: Cholecystectomy and cholecystogastric anastomosis (videos). *Gastrointestinal Endoscopy*, **61**, 601–606.

Pearse, A. G. E., 1962. Histochemistry Theoretical and Applied. 2nd edition., London, J. & A. Churchil Ltd, pp. 432–434.

Pearson, J. P., R. Kaura, W. Taylor & A. Allen, 1982. The composition and polymeric structure of mucus glycoprotein from human gallbladder bile. *Biochimica et Biophysica Acta*, **705**, 221–228.

Prozorowska, E. & H. Jackowiak, 2015. Light microscopy and scanning electron microscopy study on microstructure of gallbladder mucosa in pig. *Microscopy Research and Technique*, **78**, 220–229.

Schaller, O., 2007. Illustrated Veterinary Anatomical Nomenclature, 2nd ednn, Enke Verlag, Stuttgard, Germany, p. 173.

Seeger, J., M. Stoffel & P. Simoens, 2017. Nomina Histologica Veterinaria, submitted by The International Committee on Veterinary Histological Nomenclature (ICVHM) to the World Association of Veterinary Anatomists, pp. 30–31.

Spicer, S. S., 1965. Diamine methods for differentiating mucosubstances histochemically. *Journal of Histochemistry & Cytochemistry*, **13**, 211–234

Swindle, M. M., A. Makin, A. J. Herron, F. J. Jr. Clubb & K. S. Frazier, 2012. Swine as models in biomedical research and toxicology testing. *Veterinary Pathology*, **49**, 738.

Tang, Z., Z. Ruan & Y. Yin, 2013. Development of digestive glands in pigs. Chapter 2. In: Nutritional and Physiological Functions of Amino Acids in Pigs, eds F. Blachier et al., Springer-Verlag, Wien, pp. 25–26.

Vitanov, S., D. Dimitrov & A. Bochukov, 1995. A Manual for Exercises in Cytology and Histology, Ist edn, Zemizdat, Sofia, Bulgaria, pp. 11–12.

Paper received 31.10.2018; accepted for publication 18.01.2019

**Correspondence**:

I. S. Stefanov
Department of Anatomy, Medical Faculty,
Trakia University,
11 Armeiska Street 11,
6000 Stara Zagora, Bulgaria
tel: +359 042 664413
e-mail: iv_stefanov@uni-sz.bg;
ivstefanov@abv.bg