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A micromorphometric study on the common bile duct and major duodenal papilla of the domestic swine

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Abstract: The aim of the current study was to perform a comparative micromorphometric study on the thickness of mucosa, fibromuscular layer, glandular zone, serosa, and subserous layer, as well as of the entire wall of extra- and intramural parts of the porcine common bile duct in immature and mature animals. This micromorphometric study also evaluated the height and width of the lining epithelium, glandular epithelium, and goblet cells in 2-month, 6-month, and 3-year old male pigs. It was found that significant age-dependent differences in the studied parameters exist. Some differences in the studied parameters were also detected between different parts of the common bile duct, as well as between the common bile duct and major duodenal papilla in pigs of the same age. Immunohistochemical staining with smooth muscle actin, and Van Gieson and Orcein staining were performed to distinguish the smooth muscle cells and collagenous and elastic fibers. In conclusion, age dependent differences in the studied parameters in the porcine common bile duct and major duodenal papilla were detected.

Key words: Common bile duct, major duodenal papilla, morphometry, glands, goblet cells, swine

1. Introduction

Physiological in vivo animal models using pigs have been widely available to study bile secretion [1]. An enormous amount of experimental and clinical data has been reported concerning the gallbladder. Much less attention has been paid to the extrahepatic bile ducts, and a detailed description of the histological features is not available. The increasing frequency of operations for removal of diseased human gallbladders [2,3] and of diseases of the biliary tract define the importance of the ducts [4–7]. Zhang et al. [8] described a technique of laparoscopic cholecystostomy, comparing open cholecystostomy for clinical outcome and surgical stress response, which emphasizes the importance of swine as a suitable experimental model. Lee et al. [9] pointed out the importance of swine as a suitable experimental model for studying bile duct function.

Burden's study [4] was undertaken to determine the histologic structure of the human extrahepatic bile ducts, not including the sphincter of Oddi and to determine the nature of the pathologic processes that occur in the ducts.

Many investigations have been carried out on the musculature of the gall bladder and biliary pathways, mainly in order to understand its neural and hormonal control in several species, including humans but not swine [10–15]. The structure of the musculature has been studied in a few species but without significant details. Most of the studies were performed on the sphincter of Oddi in cats, dogs, and humans [12,16–20]. Boyden [21,22] carried out comparative studies on guinea pigs, possums, cats, and dogs and described the sphincter in the terminal part of the bile duct. Detailed data on the musculature of the gall bladder and biliary pathways in guinea pigs were also reported by Cai and Gabella [14]. The authors also presented information about the thickness of the other layers of the wall and the thickness of the entire wall of the gallbladder, hepatic duct, and common bile duct in this species. Ahmed and Abdalla [15] described the structure of extrahepatic bile duct and hepatopancreatic duct in camels, where the gallbladder and major duodenal papilla are not present.

However, detailed micromorphometric studies on the common bile ducts in swine are absent. This motivated us to undertake the current study.

The aim of our investigation was to perform a comparative micromorphometric study on the thickness of mucosa, the fibromuscular layer, serosa, and the subserous layer, as well as the entire wall of the extra- and intramural parts of the porcine common bile duct in animals of different age groups.

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2. Materials and methods

2.1. Animals

The present study was performed on 18 male pigs (crossbred Landras × Danube White) which were divided into 3 age groups: 2-month old (26–33 kg), 6-month old (92–100 kg), and 3-year old specimens (280–300 kg) linked to scientific project number 13/2017, Medical Faculty, Trakia University, Stara Zagora, Bulgaria. A total of 18 porcine livers, together with the initial part of the duodenum, were collected from various slaughterhouses. Specimens from different parts of common bile ducts and of major duodenal papilla were normal and were taken as soon as possible following the slaughtering of the animal. The common bile duct was subdivided into 2 parts: extra- and intramural. The specimens were taken from 3 segments of the extramural part: the initial segment near the terminal part of cystic duct, the middle segment, and the terminal segment close to the duodenal wall. The intramural (intraduodenal) part of common bile duct passes through the duodenal wall and opens in major duodenal papilla.

2.2. Histochemical study for detection of collagenous and elastic fibers

Staining with the Van Gieson technique was performed on the serial histological sections in order to differentiate between collagen (in red) and smooth muscle cell bundles (in yellow). Additional orcein staining was used to selectively visualize intensely elastic fibers (in dark brown).

2.3. Immunohistochemical study for detection of smooth muscle actin (SMA) in the muscle layer and myoepithelial cells of the glands

Tissue sections were washed in 0.1M PBS and placed in 1.2% hydrogen peroxide and methanol for 30 min. Antigen recovery in buffer (pH 9.0) continued for 20 min. Between steps, the sections were washed with an EnVisionFlex Wash Buffer, then incubated in a humidified chamber overnight at 4 °C with primary antibody monoclonal mouse antihuman smooth muscle actin clone 1A4 at 1:50 dilution. After washing with PBS, the sections were incubated with the EnVision detection system (DAKO) for 24 h at 4 °C. The immune reaction was visualized with diaminobenzidine. PBS, which replaced the primary antibody, was used as a negative control. The slices were dehydrated, washed, and coated with glass slides and photographed with a research microscope (LEIKA DM1000, Leica Camera AG, Wetzlar, Germany) equipped with a digital camera (LEIKA DFC 290).

2.4. Morphometric study

In terms of the microscopical study, tissue samples of 18 pigs (6 animals from each age group) were taken from different parts of the bile duct and from the major duodenal papilla. The tissues were fixed in 10% buffered formalin. After fixation, the materials were dehydrated, cleared, and embedded in paraffin wax. Paraffin serial histological cross and longitudinal sections were cut at 5–6 μm and stained with Hematoxylin and Eosin (H & E) in order to study the histological structure of the tissue.

For each section stained with H & E, 5 measurements were performed on the thickness of the surface, glandular epithelium, and number of glands (×100 with an area of 0.65 mm^2). The number of goblet cells was estimated per field (×200 with an area of 0.16 mm^2). The measurements were done with the research microscope mentioned above and software (LAS V4. 10.0, 2016).

2.5. Statistical analysis

The data were processed by GraphPad Prism 6 for Windows (GraphPad Software, Inc., USA) via one-way analysis of variance (one-way ANOVA) followed by Tukey–Kramer's post-hoc test and were presented as mean ± SD. P-values smaller than 0.05 were considered statistically significant. The terminology was consistent with the Nomina Histologica Veterinaria (NHV) [23].

3. Results

The wall of the common bile duct (CBD) consists of 3 main layers: mucosa (*Tunica mucosa*), represented by both a single layer of epithelial cells (*Lamina epithelialis mucosae*) and propria (*Lamina propria mucosae*), the fibromuscular layer (*Tunica muscularis*), and a serous coat (*Tunica serosa*), together with a subserous layer (*Telasubserosa*) (Table 1). Tubuloalveolar glands occupy both the propria and fibromuscular layer (Figure 1).

The bile duct was lined with simple columnar epithelium (cholangiocytes) with oval nuclei located at the bases of the cells (Figure 1). Goblet cells (*exocrinocyticaliciformes*) were also present and, together with columnar cells, are attached to the basement membrane forming *Lamina epithelialis mucosae*. Both *Lamina epithelialis mucosae* and underlying *Lamina propria mucosae* form the mucosa of the CBD. The submucusal connective tissue layer and *Lamina muscularis mucosae* were not defined. In both the CBD and papilla duodeni major (PDM), the thickness of *Tunica mucosa* increased with age (Table 1).

The wall of PDM is made of mucosa containing *Lamina epithelialis mucosae* and *Lamina propria mucosae. Lamina epithelialismucosae* is presented by simple columnar epithelium (cholangiocytes) and goblet cells over the basement membrane. The connective tissue layer (*Lamina propria mucosae)* is filled with tubuloalveolar glands (Figure 2).

The height of the columnar cells varied in all parts of the CBD [initial (original) segment of the extramural part of *Ductus choledochus*–DCHO, middle segment of the extramural part of *Ductus choledochus*–DCHM, terminal segment of the extramural part of *Ductus choledochus*–

Table 1. Thickness (in μm, mean ± SD) of *Tunica mucosa* (TMuc), *Lamina propria* (LP), *Tunica muscularis* (TM) thickness, *Tunica serosa* (TS) and the entire wall without serosa (EW) of the initial (DCHO), middle (DCHM), and terminal (DCHT) segments of the extramural part and in the intramural part (DCHI) of the CBD, as well as of *Tunica mucosa, Lamina propria*, and the entire wall of PDM in pigs at different ages.

Thickness	DCHO	DCHM	DCHT	DCHI	PDM
TMuc 3 year-old Mean±SD	239.0 ± 26.48	211.1 ± 23.21	240.5 ± 11.37	**** 1660.0 ± 166.3	C4 1369 ± 85.35
6 month-old mean ± SD	268.6 ± 40.42	235.1 ± 11.93	317.0 ± 20.29	**** 1367.0 ± 107.0	A4,C4 2595.0 ± 257.0
2 month-old Mean ± SD	184.1 ± 10.61	A1 119.1 ± 3.293	A4,B1 121.7 ± 1.822	**** 1215.0 ± 81.38	A4, B4,C2 1079 ± 75.51
LP 3 year-old Mean ± SD	207.6 ± 26.27	178.7 ± 23.45	208.3 ± 11.85	**** 1621.0 ± 166.2	C4 1330 ± 85.07
6 month-old mean ± SD	237.3 ± 40.28	203.3 ± 11.82	285.1 ± 19.99	**** 1328.0 ± 107.0	C4 2557.0 ± 257.0
2 month-old Mean ± SD	161.0 ± 10.42	A2 96.24 ± 3.058	A4,B1 99.05 ± 1.779	**** 1192.0 ± 81.19	C3 1051.0 ± 75.41
TM 3 year-old Mean ± SD	530.2 ± 38.02	475.1 ± 41.02	516.8 ± 42.52	**** 996.9 ± 71.59	**** 25.80 ± 5.84
6 month-old mean ± SD	** 477.8 ± 76.36	**A1 413.9 ± 76.27	** 483.1 ± 46.60	****A4 640.9 ± 50.11	**** 24.96 ± 5.60
2 month-old Mean ± SD	*** A4,B4 371.3 ± 39.52	A4,B4 299.0 ± 17.76	****B3 439.8 ± 57.89	****A2,B4 573.3 ± 43.77	**** A4,B4 14.86 ± 4.22
TS 3 year-old Mean ± SD	**** 6374.0 ± 407.0	14873.0 ± 565.1	14740.0 ± 387.4	**** 1260.0 ± 64.47	-
6 month-old mean ± SD	****A4 3871.0 ± 483.6	A4 7716.00 ± 753.1	A4 7719.0 ± 386.6	**** 1095.0 ± 27.71	-
2 month-old Mean ± SD	A4,B4 1541.0 ± 216.4	A4,B4 1919.00 ± 55.87	**** A4,B4 2649.00 ± 64.37	**** 989.4 ± 17.37	-
EW 3 year-old Mean ± SD	631.9 ± 26.85	606.0 ± 12.92	665.9 ± 42.13	**** 2255.0 ± 78.10	C4 1788.0 ± 60.05
6 month-old mean ± SD	534.9 ± 30.56	672.3 ± 90.74	771.4 ± 90.80	**** 2167.0 ± 33.11	2408 ± 239.4
2 month-old Mean ± SD	471.5 ± 59.78	514.9 ± 29.24	509.7 ± 47.11	***A4,B4 1113.0 ± 49.52	A4,B1 1296.0 ± 191.8

(-): the layer is not present.
(A1–4) indicates the statistical significant difference (with $P < 0.01–0.0001$, respectively) between the 2-month old and 6-month old pigs, as well as between 6-month old and 3-year old animals.
(B1–4) indicates statistical significant difference (with $P < 0.01–0.0001$, respectively) between the 2-month old and 3-year old pigs.
(C1–4) indicates statistical significant difference (with $P < 0.01–0.0001$, respectively) between the PDM and intra- or extramural part of the CBD.
*,**,***,**** indicate statistically significant difference (with $P < 0.01–0.0001$, respectively) between different parts of the CBD from animals of the same age.

DCHT, and intramural part of *Ductus choledochus*–DCHI] and with age (Table 2). In mature animals, the epithelium of the extramural part of the CBD ($P < 0.0001$) was smaller than that of the DCHI and PDM. In immature pigs, the surface epithelium showed a similar height along the extra- and intramural parts of the CBD but was smaller than those of the PDM ($P < 0.0001$). The height of the epithelium of all parts of the CBD and PDM increased

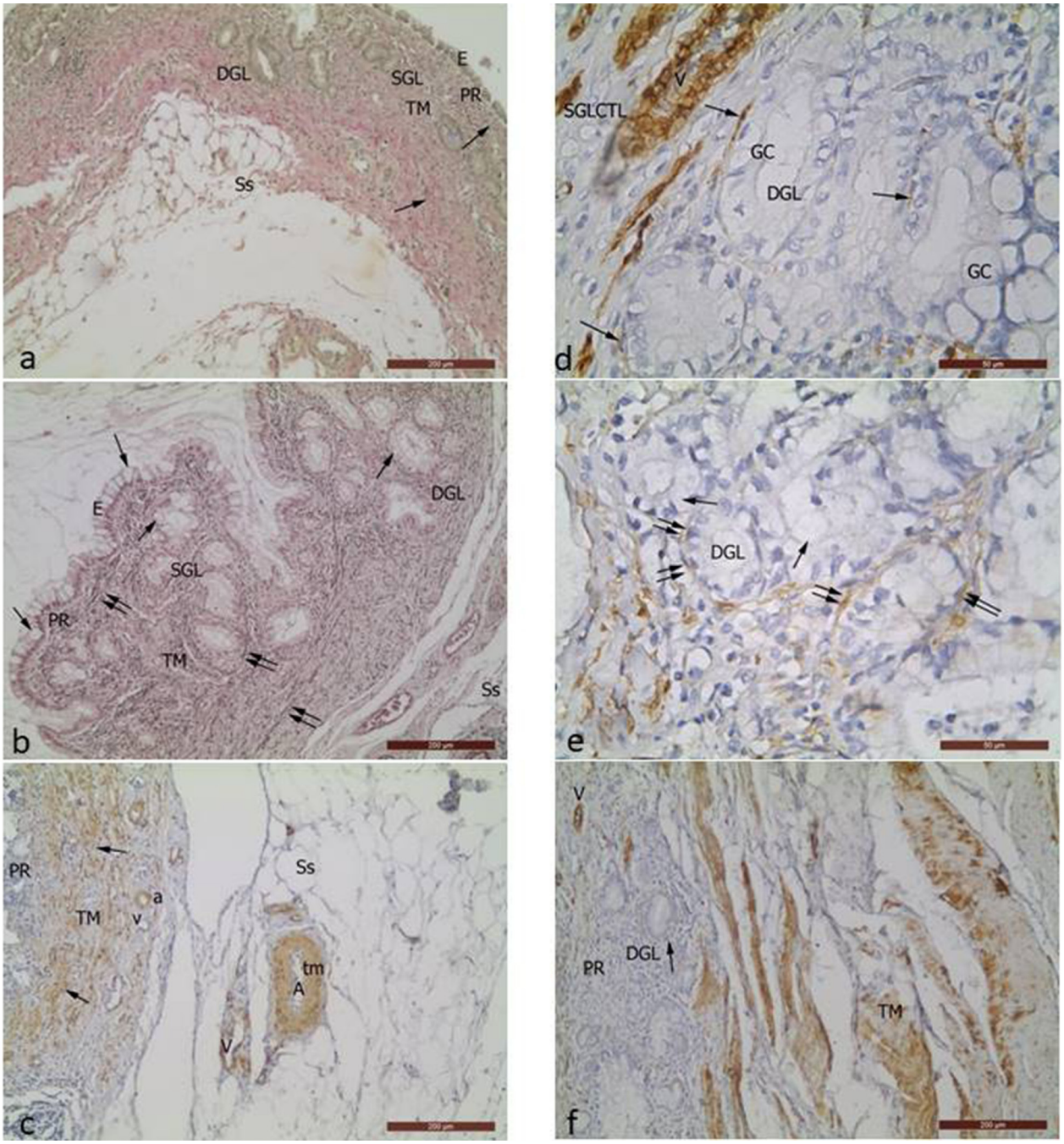
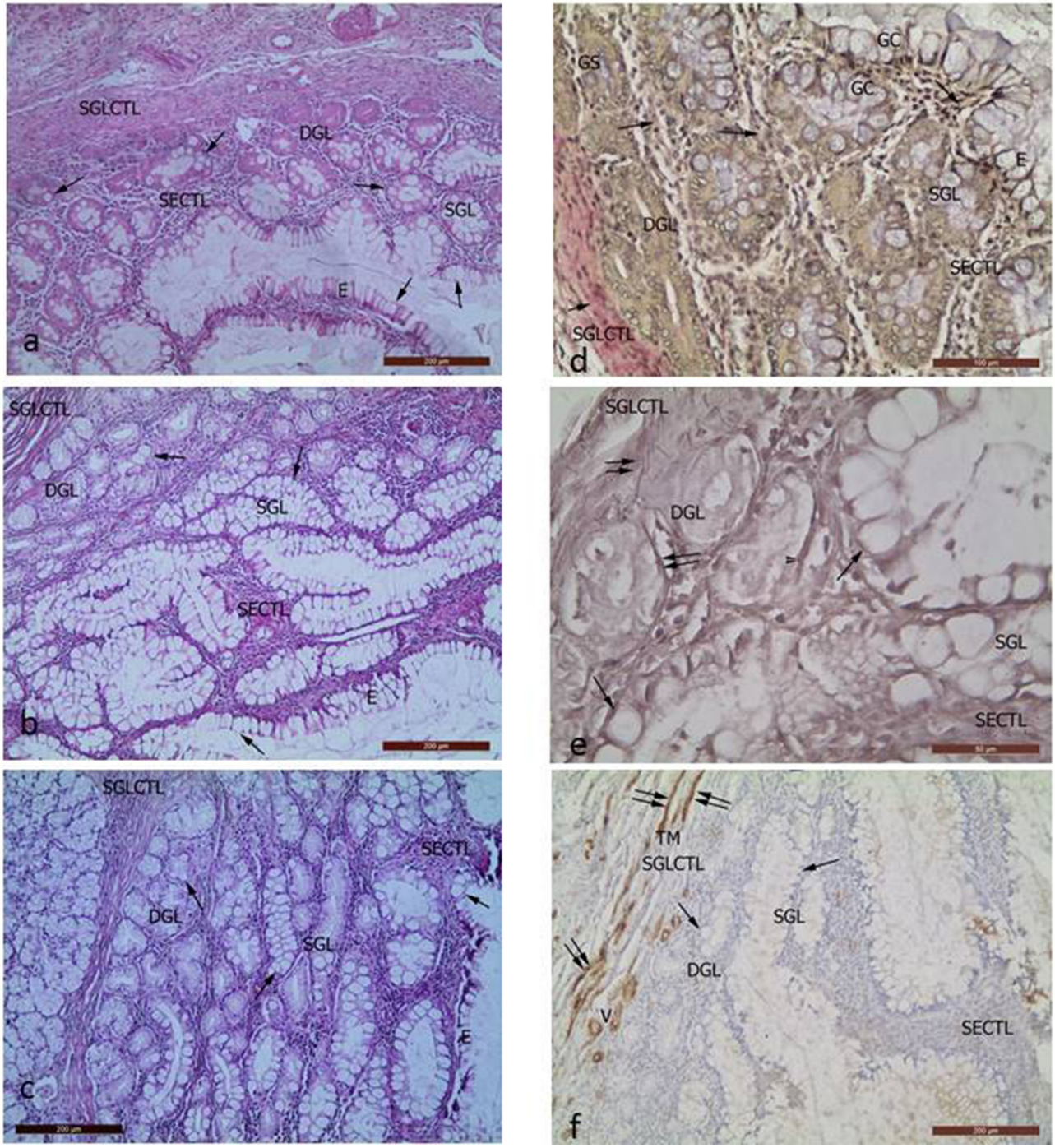


Figure 1. a. Van Gieson stain. Arrows: thick collagenous bundles (intensively red) between smooth muscle bundles (yellow) in TM and thin ones (pale red) in PR of DCHO. Pig at the age of 2 months. Bar = 200 µm. **b.** Orcein stain. Elastic fibers (double arrows) are predominantly situated in TM between smooth muscle bundles and around glands (SGL and DGL) of DCHO**.** Arrows: goblet cells. Pig at the age of 6 months. Bar = 200 µm. **c.** strong SMA-immunoreactivity in the smooth muscle cells (arrows) of TM and of *Tunica media* (tm) in arteries (A); veins (V); arterioles (a) and venules (v) of DCHO. Bar = 200 µm. **d.** strong SMA-immunoreactivity in blood vessel media (V) and strong to medium SMA-immunoreactivity (arrows) in myoepithelial cells of the glands (DGL) of DCHO. Pig at the age of 6 months. Bar = 50 µm. **e.** strong to medium SMA-immunoreactivity (double arrows) in myoepithelial cells of glands (DGL) in the major duodenal papilla. Arrows: glandular goblet cells. Pig at the age of 6 months. Pig at the age of 6 months. Bar = 50 µm. **f.** strong SMA-immunoreactivity in blood vessel media (V) and strong to medium SMA-immunoreactivity in TM and in myoepithelial cells (arrows) of the glands (DGL) of DCHI. Pig at the age of 6 months. Bar = 200 µm. E: lining epithelium; PR: *Lamina propria mucosae*; TM: *Tunica muscularis*; Ss: subserosal layer; SGL: superficial glands; DGL: deep glands; GC: goblet cells; SGLCTL: subglandular connective tissue layer.



Figures 2a–2c. H & E stain. Microscopic structure of the major duodenal papilla in pigs at the age of 2 months, 6 months, and 3 years, respectively. Bar = 200 µm. **d.** Van Gieson stain. Arrows: thick collagenous bundles (intensively red) in SGLCTL and thin ones (pale red) in SECTL; GC: mucosal and glandular goblet cells, Bar = 100 µm. **e.** Orcein stain. More elastic fibers (double arrows) are localized in SGLCTL and around glands than in SECTL. Bar = 50 µm. **f.** SMA-immunohistochemistry. Strong immunoreactivity of smooth muscle cells in *Tunica muscularis* (TM, sphincter of Oddi) of PDM and in *Tunica media* of the blood vessels (V: arterioles and venules in this picture). Bar = 200 µm. E: lining epithelium of the mucosal folds; SECTL: subepithelial connective tissue layer; SGCTL: subglandular connective tissue layer; SGL: superficial glands; DGL: deeply localized glands; arrows: mucosal and glandular goblet cells.

Table 2. Height and width (in μm, mean ± SD) of the surface columnar epithelium (CE) and secretory cells of superficial (SGE) and deep glandular layer (DGE) of the initial (DCHO), middle (DCHM), and terminal (DCHT) of the extramural part and in the intramural part of the CBD, as well as of PDM in pigs at different ages.

Parameters	DCHO	DCHM	DCHT	DCHI	PDM
CE height 3 year-old Mean ± SD	31.46 ± 1.23	32.40 ± 1.08	32.25 ± 1.60	**** 38.75 ± 0.69	C4 39.19 ± 0.71
6 month-old mean ± SD	31.29 ± 1.44	31.88 ± 1.44	31.88 ± 1.37	**** 38.87 ± 0.93	C4 37.58 ± 0.83
2 month-old Mean ± SD	A4,B4 23.15 ± 0.49	A4,B4 22.91 ± 0.527	A4,B4 22.63 ± 0.34	A4,B4 23.64 ± 0.39	C4,A4,B4 27.76 ± 0.71
CE width 3 year-old Mean ± SD	5.53 ± 0.45	5.49 ± 0.37	** 6.33 ± 0.75	**** 7.262 ± 0.50	C4 7.83 ± 0.36
6 month-old mean ± SD	5.39 ± 0.52	5.24 ± 0.38	** 6.24 ± 0.74	**** 7.10 ± 0.50	C4 7.68 ± 0.36
2 month-old Mean ± SD	5.01 ± 0.45	4.99 ± 0.33	*** 6.03 ± 0.75	**** 6.89 ± 0.52	C3 7.43 ± 0.29
SGE height 3 year-old Mean ± SD	20.50 ± 1.23	20.83 ± 0.96	20.81 ± 0.89	**** 28.78 ± 0.68	C4 28.22 ± 0.89
6 month-old mean ± SD	20.23 ± 1.24	20.64 ± 0.94	20.63 ± 0.83	**** 28.62 ± 0.66	C4 28.06 ± 0.86
2 month-old Mean ± SD	A4,B4 16.51 ± 0.21	A4,B4 16.50 ± 0.29	A4,B4 16.59 ± 0.23	A4,B4**** 26.79 ± 0.57	A4,B4,C4 26.55 ± 0.57
SGE width 3 year-old Mean ± SD	8.50 ± 0.70	8.55 ± 0.69	8.57 ± 0.72	8.57 ± 0.54	8.56 ± 0.55
6 month-old mean ± SD	A3 7.37 ± 0.74	A4 7.33 ± 0.73	**** 8.66 ± 0.67	8.57 ± 0.54	8.67 ± 0.58
2 month-old Mean ± SD	B4 7.35 ± 0.80	B4 7.28 ± 0.90	**** 8.49 ± 0.78	8.52 ± 0.54	A4,B4,C4 6.49 ± 0.33
DGE height 3 year-old Mean ± SD	19.48 ± 0.63	19.79 ± 0.70	19.81 ± 0.74	**** 17.33 ± 0.20	C4 17.39 ± 0.19
6 month-old mean ± SD	19.45 ± 0.63	19.52 ± 0.65	19.58 ± 0.64	**** 17.09 ± 0.19	C4 17.16 ± 0.19
2 month-old Mean ± SD	A4,B4 16.08 ± 0.29	A4,B4 16.50 ± 0.29	A4,B4 16.47 ± 0.35	A4,B4 16.49 ± 0.37	A4,B4 16.56 ± 0.35
DGE width 3 year-old Mean ± SD	8.57 ± 0.70	8.64 ± 0.71	8.63 ± 0 .75	8.0 ± 10.54	7.95 ± 0.53
6 month-old mean ± SD	A3 7.52 ± 0.70	A3 7.42 ± 0.79	**** 8.77 ± 0.65	8.75 ± 0.51	8.78 ± 0.58
2 month-old Mean ± SD	B3 7.46 ± 0.79	B3 7.32 ± 0.81	**** 8.65 ± 0.71	8.66 ± 0.49	A4,B4,C4 4.70 ± 0.28

(A1–4) indicates statistical significant difference (with $P < 0.01–0.0001$, respectively) between the 2-month old and 6-month old pigs, as well as between 6-month old and 3-year old animals.
(B1–4) indicates statistical significant difference (with $P < 0.01–0.0001$, respectively) between the 2-month old and 3-year old pigs.
(C1–4) indicates statistical significant difference (with $P < 0.01–0.0001$, respectively) between the PDM and intra- or extramural part of the CBD.
*,**,***,**** show statistically significant difference (with $P < 0.01–0.0001$, respectively) between different parts of the CBD from animals of the same age.

with age. In immature animals, it was significantly smaller than in mature ones.

There were no age differences in the lining epithelium width of the CBD and PDM. However, the lining epithelium width was larger in the DCHT, compared to DCHO and DCHM. The epithelium was the highest in DCHI and similar to those of PDM.

The height and width of the mucosal goblet cells (MGC) and glandular goblet cells (GGC) varied with age (Table 3).

In the epithelial layer of the CBD, the height of the MGC increased with age. It was smallest in immature animals but in the 6-month and 3-year old animals, it was the same. In 2-month old pigs, DCHO, DCHM, DCHT, and DCHI had an MGC at similar heights but were smaller than in PDM ($P < 0.001$). In mature animals, the height of the goblet cells (GC) increased in DCHT, and the highest cells were detected in DCHI and in PDM ($P < 0.001$). In immature animals, the MGC were of similar width in the initial, middle, terminal, and intramural parts of the CBD but were wider than in the PDM ($P < 0.001$). In mature animals, the above-mentioned parts of the CBD possess MGC with similar width but that are narrower than in the PDM. In immature pigs, the MGC in DCHO, DCHM, DCHT, and DCHI were wider than in mature ones but in the PDM the width increased with age; in mature pigs, they were wider than in immature ones.

GGC were localized in the superficial (GCSGL) and deep glandular layers (GCDGL) (Figures 1–2).

GCSGL height increased with age. In 2-month old animals, the height was the same in all parts of the CBD but was smaller than in mature animals ($P < 0.001$). In 6-month and 3-year old animals, the goblet cell height in DCHI was higher than in the extramural part. There were no significant differences between 6-month- and 3-year old animals. The height of GCSGL in the PDM increased with age. The smallest cells were found in 2-month old pigs. In 6-month- and 3-year old animals, the height was similar. In the PDM of 2-month old animals, compared to CBD, the GCSGL were smaller. There were no such differences in 6-month and 3-year old animals.

The width of GCSGL in the DCHT in 2-month old pigs was larger than the DCHO and DCHM but similar to DCHI. In the other 2 age groups, no differences were observed in all segments of the CBD. The width of GCSGL in the PDM increased with age. In the PDM of 2-month old pigs, GCSGL were narrower than in the DCHI, while in mature animals the GCSGL of the PDM were wider than those of the CBD.

In 2-month old pigs, GCDGL were present in the DCHI only and were smaller than in mature animals but larger than in the PDM. In mature animals, GCDGL of the DCHI were larger than the extramural parts but similar to those of the PDM. The height of GCDGL of the PDM increased with age in 2-month old pigs, and GCDGL were the smallest.

In 2-month old pigs, GCDGL of the CBD were wider than in mature animals, as well as wider than those of the PDM. In mature animals, GCDGL of all parts of the CBD had the same width but were narrower than those of the PDM.

The width of GCDGL of the PDM increased with age, and GCDGL were the narrowest in 2-month old pigs.

The propria consists of loose connective tissue represented predominantly by elastic fibers and small amount of collagenous fibers and thin collagenous bundles. It fills the core of the mucosal folds and extends to the fibromuscular layer. The base of the folds contains much more elastic fibers than its tip. The submocosal layer was not defined, and *Lamina muscularismucosae* was absent. In the extramural part of CBD, propria thickness increased with age and, in 6-month old animals, it was the highest. However, in the DCHI, this layer was thickest in 3-year old pigs, followed by 6-month and 2-month old animals.

In the PDM, the propria was filled with glands surrounded by many elastic fibers (Figure 2). Propria thickness also increased with age, and it was the highest in 6-month old pigs. In 6-month old pigs, this layer of the PDM was thicker than those of the DCHI. In the other 2 age groups, the propria of the PDM was thinner than the DCHI.

The mucosal layer of the CBD and PDM forms folds in which height varied with age (Figures 1 and 2). The height of the folds was similar in the 3 parts of the extramural portion of the CBD in 2-month old pigs (DCHO: 84.71 ± 3.09 μm; DCHM: 81.34 ± 1.37 μm; DCHT: 81.09 ± 0.89 μm), but in the DCHO (103.8 ± 9.94 μm in 6-month old animals and 102.4 ± 2.98 μm in 3-year old animals) and DCHT (131.6 ± 7.88 μm in 6 month and 96.32 ± 4.241 μm in 3-year old animals) of the CBD of mature animals, they were higher than in DCHM (88.60 ± 2.01 μm in 6-month old animals and 84.42 ± 2.97 μm in 3-year old animals), as well as higher than the same parts in 2-month old pigs. In the intramural part of the CBD, the height of the folds increased with age: 219.3 ± 9.92 μm in 2-month old, 651.6 ± 27.25 μm in 6-month old, and 699.2 ± 61.41 μm in 3-year old animals.

The folds in the PDM were highest in 6-month old (2360.00 ± 255.8 μm), followed by 3-year old (1017.00 ± 33.64 μm) and 2-month old (578.4 ± 89.71 μm) animals. .

The mucous glands (*Glandulae ductus choledochi*) were lined with cuboidal cells whose cytoplasm was stained light red, and the nuclei were round and situated close to the basal lamina. In some cells, the nuclei were flat. The glandular cuboidal cells were smaller in height but wider than the lining columnar epithelium (Table 2).

Table 3. The height and width (in µm, mean ± SD) of the mucosal goblet cells (MGC) and of the goblet cells in superficial (GCSGL) and deep glandular layers (GCDGL) of the initial (DCHO), middle (DCHM), and terminal (DCHT) segments of the extramural part and in the intramural part (DCHI) of the CBD, as well as of PDM in pigs at different ages.

Parameters	DCHO	DCHM	DCHT	DCHI	PDM
MGC height 3 year-old Mean ± SD	31.66 ± 1.38	31.9 ± 1.44	32.09 ± 1.61	**** 38.84 ± 0.76	37.79 ± 0.51
6 month-old mean ± SD	31.39 ± 1.44	31.85 ± 1.42	31.96 ± 1.39	**** 38.99 ± 0.93	37.67 ± 0.74
2 month-old Mean ± SD	A4,B4 23.23 ± 0.51	A4,B4 22.99 ± 0.54	A4,B4 22.75 ± 0.32	A4,B4 23.72 ± 0.38	A4,B4,C4 27.83 ± 0.69
MGC width 3 year-old Mean ± SD	16.72 ± 0.88	16.53 ± 0.94	16.97 ± 1.12	16.86 ± 0.98	C4 19.44 ± 0.43
6 month-old mean ± SD	16.55 ± 0.87	16.76 ± 0.85	16.87 ± 0.72	16.85 ± 0.91	C4 18.66 ± 0.62
2 month-old Mean ± SD	A4,B4 18.08 ± 0.41	A3,B4 17.98 ± 0.45	A3,B2 18.13 ± 0.56	A1,B1 17.80 ± 0.76	A4,B4,C4 16.34 ± 0.70
GCSGL height 3 year-old Mean ± SD	25.39 ± 1.89	25.88 ± 2.06	25.79 ± 1.94	**** 28.70 ± 2.29	27.68 ± 1.68
6 month-old mean ± SD	25.47 ± 1.84	26.43 ± 1.90	26.01 ± 1.87	**** 28.95 ± 2.16	27.80 ± 1.62
2 month-old Mean ± SD	A4,B4 21.57 ± 0.67	A4,B4 21.79 ± 0.90	A4,B4 21.87 ± 0.86	A4,B4 22.89 ± 0.62	A4 ,B4, C1 19.71 ± 0.57
GCSGL width 3 year-old Mean ± SD	18.94 ± 0.86	19.00 ± 0.79	18.81 ± 0.87	19.43 ± 1.04	C4 21.80 ± 0.87
6 month-old mean ± SD Min-Max	18.78 ± 0.93	18.60 ± 0.75	18.56 ± 0.86	19.37 ± 1.18	C4 21.66 ± 0.84
2 month-old Mean ± SD	A2,B2 17.25 ± 1.70	A3,B3 17.16 ± 1.92	** 18.66 ± 1.85	19.82 ± 1.26	A4, B4, C4 17.51 ± 0.75
GCDGL height 3 year-old Mean ± SD Min-Max	20.14 ± 1.04	19.93 ± 1.24	20.14 ± 1.04	**** 22.07 ± 0.66	C4 22.32 ± 1.19
6 month-old mean ± SD	20.00 ± 1.00	19.80 ± 1.25	20.01 ± 1.053	**** 21.96 ± 0.69	22.09 ± 1.10
2 month-old Mean ± SD	-	-	-	A4,B4 20.40 ± 0.82	A4,B4 16.61 ± 0.77
GCDGL width 3 year-old Mean ± SD	16.59 ± 0.46	16.59 ± 0.40	16.73 ± 0.45	16.51 ± 0.54	C4 17.97 ± 0.71
6 month-old mean ± SD	16.31 ± 0.51	16.35 ± 0.49	16.44 ± 0.48	16.32 ± 0.54	C4 17.7 ± 7 0.85
2 month-old Mean ± SD	-	-	-	A4,B4 18.07 ± 0.84	C4,A4,B4 13.85 0.56

(A1–4) indicates statistical significant difference (with $P < 0.01–0.0001$, respectively) between the 2-month old and 6-month old pigs, as well as between 6-month old and 3-year old animals.
(B1–4) indicates statistical significant difference (with $P < 0.01–0.0001$, respectively) between the 2-month old and 3-year old pigs.
(C1–4) indicates statistical significant difference (with $P < 0.01–0.0001$, respectively) between the PDM and the intra- or extramural part of the CBD.
(*/**/***/****) indicates statistical significant difference (with $P < 0.01–0.0001$, respectively) between different parts of the CBD from animals of the same age.

SMA-immunohistochemistry allowed for identifying myoepithelial cells localized between the cuboidal glandular cells and the basement membrane (Figure 1).

In 6-month old and 3-year old animals, the height of the glandular epithelium of the superficial glandular layer (SGE) of the CBD was the same but higher than in the 2-month old animals. In the intramural part of the CBD, the SGE was the highest (Table 2). The width of the SGE of DCHO and DCHM in 2-month and 6-month old animals showed similar values but was smaller than in 3-year old animals and of other parts of the CBD.

The deep glandular layer of the CBD is represented by alveoli-containing cells (DGE), whose height increased with age. In 6-month and 3-year old animals, the height of secretory cells in the DCHI was the smallest, but in 2-month old pigs, the cells have the same height in all parts of the CBD. The width of DGE in DCHO and DCHM of the extramural part of the CBD in 2-month and 6-month old pigs was the same but smaller than in 3-year old animals. Other parts of the CBD showed no age differences.

The SGE and DGE of PDM showed similar values to the DCHI in 6-month and 3-year old animals, but they were the smallest in 2-month pigs. In 6-month and 3-year old animals, SGE was higher than DGE and had a similar width. However, in 2-month old pigs, SGE was higher and wider than DGE.

Tubuloalveolar-mucous glands were arranged in superficial and deep groups, forming a tick common glandular layer (CGL) (Table 4). The glandular layer occupied the propria and fibromuscular layers of the CBD and propria of the PDM only (Figures 1 and 2). The total thickness of the CGL increased to the DCHT and is similar in 2-month and 6-month old pigs. The thickness of this layer in the DCHT increased with age; it was the highest in 3-year old animals. The CGL is represented by both superficial (SGL) and deep glandular sublayers (DGL). The thickness of the SGL is approximately 60%, but the thickness of the DGL made up 40% of the total thickness of the CGL.

In 2-month old pigs, the CGL of the PDM was thinner than in 6-month and 3-year old animals. The thickness of the SGL is 70%, 60%, and 67% of the total thickness of the CGL in 3-year old, 6-month old, and 2-month old animals, respectively.

In all age groups, the number of alveoli in the SGL was higher than in the DGL of the CBD. However, the number of alveoli in the SGL of the PDM was smaller than in the DGL.

The number of alveoli in both the SGL and DGL of the CBD increased with the age; the smallest was in 2-month old pigs, but in 6-month old and 3-year old pigs, the alveoli number was the same. In all age groups, the alveoli in the DCHI were more numerous than in the extramural segments. In contrast, the alveoli number in the PDM decreased with age.

The immunohistochemical staining with SMA and Van Gieson and Orcein staining showed that the muscle (fibromuscular) layer of the extramural part of the CBD was formed not by a uniform muscle sheet but by muscle bundles surrounded by dense connective tissue rich in thick collagenous bundles and delicate elastic fibers. However, well-developed SMA-positive inner circular and outer longitudinal muscle layers forming *musculus sphincter ductus choledochi* were observed in the DCHI. The *Tunica muscularis* in all parts of the CBD increased in thickness with age. In the DCHO and DCHT of the extramural part of the CBD, this layer was thicker than in the DCHM. The *Tunica muscularis* of the DCHI was significantly thicker than those of the extramural part.

In the PDM, the muscle layer with a strong SMA immunoreactivity was represented by delicate smooth muscle bundles at its base only (Figure 2f). This muscle layer was significantly thinner than those of the CBD (Table 1). In the tip of the PDM, these muscle bundles disappear and only the SMA-positive muscle cells in the wall of blood vessels were present.

The serosa (Table 1) consists of mesothelium covering the subserosa, which is represented by loose connective tissue rich in adipose tissue. The thickness of serosa, together with the subserosal layer (SSubs) in the extramural parts, was higher than in the DCHI. In the extramural part, the thinnest SSubs was present in the DCHO, followed by the DCHM and DCHT in all age groups. In 2-month old animals, the SSubs of the extramural part of the CBD and DCHI was the thinnest, followed by 6-month old pigs and it was thickest in 3-year old animals. The thickness of SSubs in the PDM showed increasing values with age but without statistical significance.

In the 3 age groups, the wall thickness of the DCHI, without serosa, was significantly higher than the extramural part and increased with age. In each age group, the thickness of the wall of the DCHO, DCHM and DCHT was almost the same. The wall of both the CBD and PDM in 2-month old pigs was thinner than in mature specimens.

4. Discussion

In this study, we presented original data about the detailed micromorphometric features of the structural components of the extramural and intramural part of the CBD, as well as of the PDM in swine at different ages. The extramural part of the CBD was subdivided into 3 segments: the DCHO, DCHM, and DCHT.

Our results showed that the wall of the common bile duct contains 3 main layers, which confirms the data reported in NHV [23] and by other authors [24]. These layers are as follows: *tunica mucosa ductus choledochi* consists of

Table 4. The thickness (in μm, mean ± SD) of the entire glandular layer (EGL), superficial glandular sublayer (SGL), deep glandular sublayer (DGL), and number (per microscopic field ×100) of secretory alveoli in superficial (ASGL) and deep glandular layers (ADGL) of the initial (DCHO), middle (DCHM), and terminal (DCHT) segments of the extramural part and the intramural part (DCHI) of the CBD, as well as of PDM in pigs at different ages.

Parameters	DCHO	DCHM	DCHT	DCHI	PDM
EGL thickness3 year-oldMean ± SD	590.2 ± 59.02	550.1 ± 36.12	**** 717.7 ± 80.34	**** 582.7 ± 34.90	C4 675.5 ± 65.17
6 month-oldmean ± SD	A4, **** 428.4 ± 29.10	**** 523.8 ± 9.466	A4, **** 611.1 ± 27.87	612.8 ± 18.64	A4, C4 763.7 ± 71.09
2 month-oldMean ± SD	B4 **** 399.4 ± 19.48	**** 505.0 ± 26.57	A4, B4 528.8 ± 15.94	**** 599.4 ± 31.79	A4, B4, C3 465.4 ± 45.74
SGL thickness 3 year-oldMean ± SD	**** 385.9 ± 48.80	**** 350.9 ± 36.24	**** 480.8 ± 51.29	** 433.5 ± 25.31	C4 489.3 ± 47.67
6 month-oldmean ± SD	A4 297.3 ± 9.63	330.9 ± 6.85	A4, **** 419.1 ± 14.27	444.5 ± 17.59	465.5 ± 50.00
2 month-oldMean ± SD	A2, B4, **** 252.2 ± 20.52	**** 313.9 ± 8.87	A4, B4 331.4 ± 6.05	**** 409.3 ± 25.52	A4, B4, C4 312.0 ± 35.27
DGL thickness 3 year-oldMean ± SD	194.5 ± 29.92	199.1 ± 8.94	** 236.9 ± 46.01	**** 145.9 ± 25.48	C2 186.2 ± 71.84
6 month-oldmean ± SD	A4, **** 131.1 ± 9.255	192.9 ± 6.23	A 3192.1 ± 23.09	168.3 ± 6.58	C4 298.2 ± 52.74
2 month-oldMean ± SD	B4, *** 147.3 ± 8.74	191.1 ± 27.15	B2 197.4 ± 15.01	B3 190.6 ± 15.08	A4, B1, C2 153.4 ± 22.84
Number ASGL 3 year-oldMean ± SD	18.94 ± 0.99	19.44 ± 0.98	18.67 ± 1.08	* 20.28 ± 1.32	C4 9.44 ± 0.51
6 month-oldmean ± SD	18.50 ± 1.20	19.33 ± 0.97	18.61 ± 1.14	* 20.56 ± 1.15	C4 11.56 ± 1.15
2 month-oldMean ± SD	A4, B4 13.33 ± 0.68	A4, B4 13.17 ± 0.70	A4 ,B4 12.67 ± 0.97	A4, B4, **** 15.83 ± 0.70	A4, B4, C4 13.50 ± 1.01
Number of ADGL 3 year-oldMean ± SD	13.06 ± 0.80	13.11 ± 1.02	12.50 ± 1.72	**** 15.44 ± 1.38	C4 19.39 ± 1.97
6 month-oldmean ± SD	13.28 ± 0.75	13.67 ± 0.76	12.66 ± 1.19	*** 14.94 ± 1.26	C4 21.28 ± 1.32
2 month-oldMean ± SD	A4, B4 9.00 ± 0.68	A4, B4 8.88 ±0.75	A4, B4 8.72± 0.66	A4, B4, ****10.83± 0.78	A1, B4, C4 22.67 ±1.188

(A1–4) indicates statistical significant difference (with P < 0.01–0.0001, respectively) between 2-month old and 6-month old pigs, as well as between 6-month-old and 3-year old animals.
(B1–4) indicates statistical significant difference (with P < 0.01–0.0001, respectively) between 2-month old and 3-year old pigs.
(C1–4) indicates statistical significant difference (with P < 0.01–0.0001, respectively) between the PDM and intra- or extramural parts of the CBD.
(*/**/***/****) indicates statistical significant difference (with P < 0.01–0.0001, respectively) between different parts of the CBD from animals of the same age.

Lamina epithelialis mucosae, represented by columnar cholangiocytes, goblet cells and *lamina propriamucosae*, the middle layer was *tunica muscularis*, and the outer layer *Tunica serosa*. However, some differences between our findings and data reported by NHV (23) were detected. For example, we observed that the glands of the bile duct were localized not only in the propria, as mentioned by NHV [2017], but also deeply in the fibromuscular layer (*Tunica muscularis)*.

According to Burden [4], the structure of the human extrahepatic bile ducts (hepatic, cystic, and common bile ducts) did not vary (they have essentially the same

histologic appearance) with age; the youngest was 11 and the oldest was 75 years old. In contrast, we detected age-dependent differences in the micromorphometric features of the structural components of the porcine CBD and PDM wall in immature compared to mature animals. Moreover, the morphometric differences were also observed between extra- and intarmural parts of porcine CBD, as well as between the CBD and PDM in animals of the same age. Kuehnel [25] explained the function of the surface epithelium of the CBD and PDM to secrete mucins in a protective role and reabsorb water and salt.

The present investigation detected the presence of GC in the porcine CBD, which differs from the findings of Ahmed and Abdalla [15] in camels and of Mcminn and Kugler [26] in dogs, cats, and humans. According to these authors, animals are mentioned because of the absence of goblet cells, and the mucus is probably secreted by the lining epithelial cells, whose luminal borders and supranuclear regions were stained strongly with PAS. In contrast, Vankov and Ovcharov [27] reported that human CBD contains GC but detailed information is missing.

Tubuloalveolar-mucous glands of porcine CBD were arranged in SGL and DGL forming CGL. The glands secrete mucins into the lumen of the bile duct. Mucin probably provides a protective epithelial film and is added to the bile [25].

CGL occupied propria and fibromuscular layer of porcine CBD. In contrast, Ahmed and Abdalla [15] described the presence of glands only in the propria of CBD in camels. The total thickness of the CGL increased to the DCHT and is similar in 2-month and 6-month old pigs. The thickness of this layer in the DCHT increased with age. SGL thickness was about 60%, but the thickness of DGL was 40% of the total thickness of CGL.

In all age groups, the number of alveoli in SGL was higher than in DGL of the CBD. However, the number of alveoli in SGL of PDM was smaller than in DGL.

The number of alveoli in both SGL and DGL of the CBD increased with age. In all age groups, the alveoli in DCHI were more numerous than in the extramural segments. In contrast, the alveoli number in the PDM decreased with age.

In humans, Kuehnel [25] observed branched or unbranched tubular biliary glands in the CBD wall, while Mcminn and Kugler [26] claimed that the glands are of tubuloalveolar type. We found out that glands of porcine CBD are of tubuloalveolar type, which supports the findings of Mcminn and Kugler [26].

In the current study, immunohistochemical staining showed the presence of myoepithelial cells between the basement membrane and cuboidal glandular cells. Therefore, myoepithelial cells, together with elastic fibers (identified by Orcein staining) around the glands, facilitate the discharging of the secretory product from cuboidal secretory cells.

Ahmed and Abdalla [15] reported that the wall of the extrahepatic bile duct of the camel lacks muscle fibers but contains collagenous bundles and elastic fibers. This is unlike other domestic mammals in which muscle fibers are present. In the current study Van Gieson and Orcein staining showed that the fibromuscular layer contains collagenous fibers that form thicker collagenous bundles and more elastic fibers than in the propria; these fibers surround smooth muscle cell bundles. Oldham-Ott and Gilloteaux [24] stated that both circular and longitudinal muscle layers were developed in the hepatic and CBDs of mammals. They claimed that the muscle is thickest in the bovine species and thinnest in carnivores; in other species, the muscle layer is discontinuous. In some species, such as pigs, cats, and rabbits, the muscle has a spiral form [28]. In humans, the smooth muscle cells are also present [29]. The absence of muscle cells in the wall of the extrahepatic biliary ducts of the camel may suggest that the flow of the bile from the liver to the duodenum is passive, while in other species, including swine, in which the CBD possesses smooth muscle cells, the bile flow is active. Mostafa et al. [30], in a study of the camel, describe a valve-like mucosal fold at the orifice of the duct that may act as a sphincter. Radmanesh [31] assumed that the oblique course of the terminal end of the duct through the duodenum may also function as a sphincter.

In humans, well-developed smooth muscle is present in the region of the lower end of the bile duct [29]. This muscle forms the sphincter of Oddi consisting of 3 separate parts: the sphincter choledochus surrounding the lower end of the bile duct – it is always present, and its contraction is responsible for the filling of the gallbladder; the sphincter pancreaticus – a less developed muscle surrounding the terminal part of the main pancreatic duct; and the third sphincter, which surrounds the hepatopancreatic duct (or ampulla), forming a ring around the lower ends of both the bile and pancreatic ducts. This is the *sphincter ampullae*. According to Singh (29), the *sphincter ampullae* and the *sphincter pancreaticus* are often missing. In contrast, Vankov and Ovcharov [27] claimed that *Musculus sphincter hepatopancreaticae* is the only component of the sphincter of Oddi. Unlike humans, swine lack a hepatopancreatic duct; therefore, the *Musculus sphincter hepatopancreaticae* is missing [23].

Taking into account the findings of both Singh [29] and Vankov and Ovcharov [26], as well as based on the results of the current study, it was concluded that the porcine sphincter of Oddi is represented by a *Musculus sphincter*

ductus choledochi and thin smooth muscle bundles in the base of PDM. Therefore, it may be assumed that in swine the flow of bile is directed to the duodenum.

We found that in the 3 age groups of pigs that the wall thickness of DCHI, without serosa, was significantly higher than the extramural part of the CBD and increased with age. In each age group, the thickness of the wall of the DCHO, DCHM, and DCHT was almost the same. The wall of both the CBD and PDM in 2-month old pigs was thinner than in the mature specimens.

5. Conclusion

In this study, for the first time, the size of the lining epithelium, glandular secretory epithelium, mucosal and glandular goblet cells, and thickness of the layers, as well as of the whole wall of the CBD and PDM, in pigs at different ages were evaluated. The CBD's wall and the base of PDM possess smooth muscle cell bundles; therefore, we suggest that the flow of bile directed to the duodenum is active. These results may be used as reference values in performing morphological experiments with pigs.

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