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Histological and histochemical studies on the structure of pancreatic ducts of the goose (Anser anser)

Turgay DEPREM[1,*], Serap KORAL TAŞÇI[1], Seyit Ali BİNGÖL[2], Ebru KARADAĞ SARI[1], Şahin ASLAN[1], Serap İLHAN[1]

[1]Department of Histology and Embryology, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey

[2]School of Health Sciences, Kafkas University, Kars, Turkey

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Abstract: The aim of the present study was to determine the histological structure and histochemical properties of the pancreatic ducts in the goose Anser anser. Pancreatic ducts of 6 male and 6 female geese were examined. Crossman's triple stain and Masson's trichrome stain were used for histological examination, and periodic acid–Schiff, alcian blue, periodic acid–Schiff/alcian blue, and aldehyde fuchsin/alcian blue stainings were performed for histochemical examination. Dorsal and ventral pancreatic ducts were found that transfer pancreatic secretion into the duodenum of the goose, and these channels were found to have a histologically similar structure. No differences were detected between male and female geese. Duct wall was observed to consist of mucosa, muscle, and adventitial layers. In addition to dark principal, light and basal cells were observed in a simple columnar epithelium layer lining the duct. Glands, including centroacinar cells, were also detected in all layers of duct. Furthermore, lymphoid follicles were observed in the connective tissue of the mucosa. Sulfated and carboxylated mucosubstance-secreting cells were found in the duct as well as neutral, acidic, and mixed mucosubstance-secreting cells. We conclude that the results of our study may contribute to future studies.

Key words: Ductus pancreaticus, histochemical, histological, goose, Anser anser

1. Introduction

In poultry, the pancreas is located on the right side of the abdominal cavity (1). The pancreas consists of a pale, elongated gland, situated in the interduodenal area and formed by ascending and descending duodenal loops (1,2). It is associated with the digestive system and is an exocrine as well as endocrine gland. The exocrine portion secretes basic electrolytes and digestive enzymes (1), whereas the endocrine portion secretes hormones such as insulin, glucagon, somatostatin, and pancreatic polypeptides (1,3,4). Many researchers have examined the histological and histochemical properties of pancreases in different bird species such as goose (5–7), ostrich (8), turkey (9), duck (10), pigeon (11), eagle (12), falcon (13), and quail (14).

Pancreas in poultry is composed of 4 lobes: dorsal, ventral, third, and splenic (6). Pancreatic ducts empty their contents from the acinus into the duodenum in the following order: intercalated ducts, intralobular ducts, interlobular ducts, and pancreatic ducts (main ducts) (2). In birds, the main ducts consist of dorsal and ventral pancreatic ducts (15). However, the presence of an additional third duct was found in some bird species (14).

The wall of pancreatic ducts in poultry consists of 3 layers: the mucosa, muscular, and adventitial layers. The mucosa layer is composed of 2 sublayers: the lamina epithelialis, with a single layer of high, columnar, epithelial cells, and the lamina propria, consisting of connective tissue. This is surrounded by a muscular layer that is arranged longitudinally on the inside and circularly on the outside. The adventitial layer surrounds the duct externally (16). Studies on the pancreas usually concentrate on acini and islets. Less information has been provided on the pancreatic duct epithelium. Yoshizawa (17), Madden and Sarras (18), and Motta et al. (4) identified the pancreatic duct in several mammalian species. McMinn and Kugler (19) and Kendrey and Roe (20) conducted histochemical studies of pancreatic duct cells in mammalian species. Although some histological studies are available on the pancreatic duct system in poultry (5,21), detailed histological studies on the overall structure of pancreatic ducts in goose pancreas are inadequate. The aim of our study was to present the histological structure and histochemical features of the pancreatic ducts of the goose.

* Correspondence: turgaydeprem@hotmail.com

2. Materials and methods

This study examined the pancreas and duodenum of 6 male and 6 female geese (*Anser anser*), slaughtered for consumption by local breeders. Removed pancreatic ducts were freed from the surrounding tissue and, after being measured, were divided into approximately 4 equal parts. The resulting tissues were fixed in Bouin's fluid and 10% formalin solution. Following routine histological processes, serial paraffin sections of 6 µm in thickness were prepared. Crossman's triple staining and Masson's trichrome staining were performed for a general histological examination of duct sections. A series of histochemical tests were used to obtain the characteristics of mucins secreted by the epithelial mucous cells. Periodic acid–Schiff (PAS) staining was performed for the presence of mucin. Alcian blue (AB) at pH 1.0 and 2.5 was used to determine weak and strong mucins, respectively. Meanwhile, AB at pH 2.5 in combination with PAS staining (AB/PAS) was used for neutral and acid mucins. The combination of aldehyde (AF) and AB at pH 2.5 (AF/AB) was used to differentiate between sulfated and carboxylated mucins (22). The sections were examined under a BX 51 light microscope (Olympus, Japan).

3. Results

The goose pancreas was found between the ascending and descending duodenal loops and was composed of dorsal, ventral, third, and splenic lobes. The 2 main pancreatic ducts, dorsal and ventral, which transmit pancreatic secretion into the duodenum, were detected in the goose (Figure 1a). The length of these channels was found to be approximately 4 cm. Furthermore, duct lumen was coated with secretory material. The wall structure of the duct was composed of 3 layers. The innermost layer, the mucosa, was lined with a simple columnar epithelium. The muscular layer was composed of inner longitudinal and outer circular thick muscle. It was also enveloped with a thin adventitia (Figure 1b). The epithelial layer of the duct showed 3 types of cells: dark principal, light, and basal cells (Figure 2a). The dark principal cells, which constitute the majority of cells in the ductal epithelium of pancreatic ducts, were also found to have high columnar structure. Light cells with pale cytoplasm and large round nuclei were observed in lesser numbers. The third cell type, basal, was found to be located between epithelial cells and the basement membrane of the main duct. Basal cells were less in number and relatively small, with dense nuclei. In addition, glands were observed in mucosa, muscular, and adventitial connective tissue layers (Figure 1b). These glands were found to contain centroacinar cells (Figure 2b). As glands within the duct wall comprise centroacinar cells, the duct wall was considered as a part of the goose exocrine pancreas. These glands were observed to be present in the 3 layers (mucosa, muscular, and adventitial) of region 1 of the duct. They intermittently exited from the pancreas to the duodenum in the mucosa and muscular layers of regions 2 and 3 of the duct, and mostly in the connective tissue layer of mucosa of region 4. They were not present in the muscular and adventitial layers. Dorsal and ventral pancreatic ducts exhibited similar histological structure, and there were no differences between males and females (Figure 3 and 4). In addition, lymph follicles were observed locally in connective tissue parts of the mucosa (Figure 1b). Positive reactions were observed in duct epithelium with PAS and AB (pH 1 and pH 2.5), PAS/AB (pH 2.5), and AF/AB (pH 2.5) staining. PAS staining

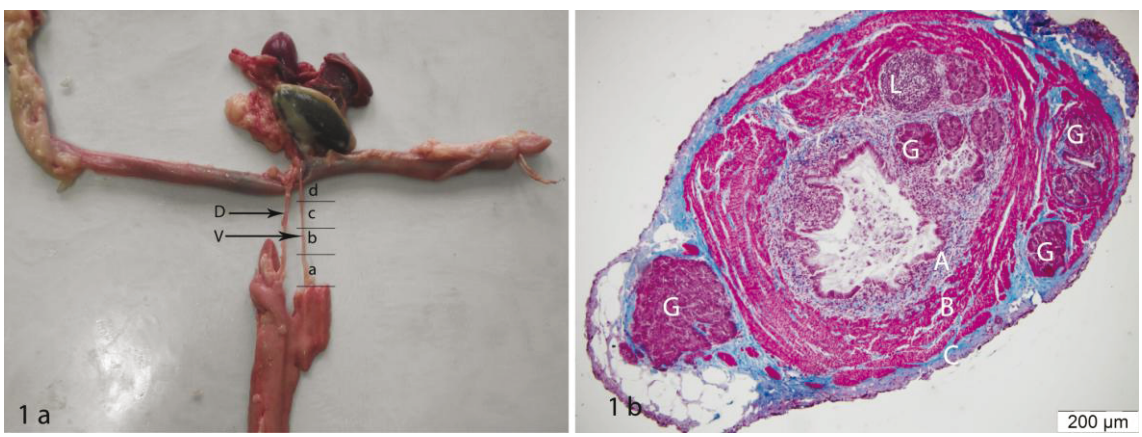

Figure 1. a) Macroscopic appearance of dorsal and ventral pancreatic ducts in goose. D: Dorsal pancreatic duct, V: ventral pancreatic duct, a: region 1, b: region 2, c: region 3, d: region 4. **b)** Histological appearance of dorsal pancreatic duct in male goose (region 1). A: Mucosa layer, B: muscular layer, C: adventitia, G: glands, L: lymph follicle. Masson's trichrome.

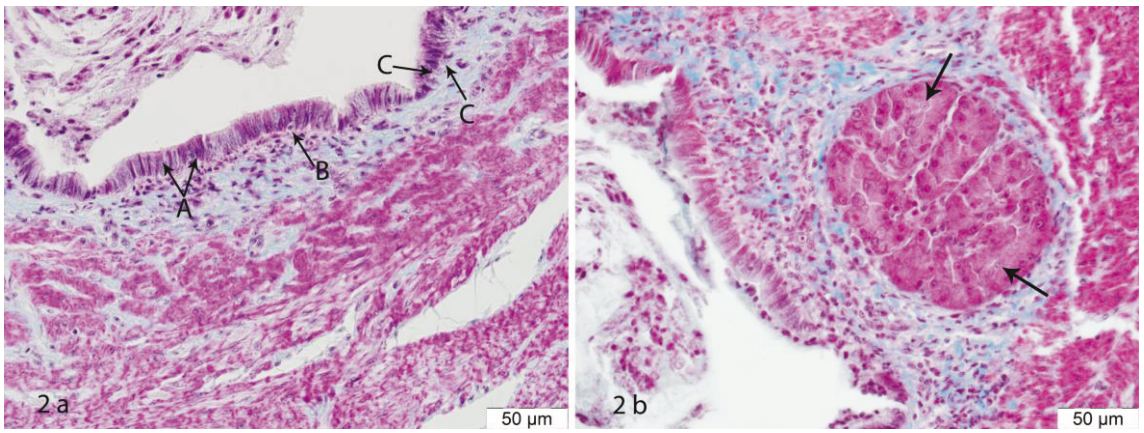

Figure 2. a) Epithelial layer of ventral pancreatic duct in female goose (region 3). A: Principal cells, B: light cells, C: basal cells. **b)** Centroacinar cell (arrow) in pancreatic duct. Crossman's triple stain.

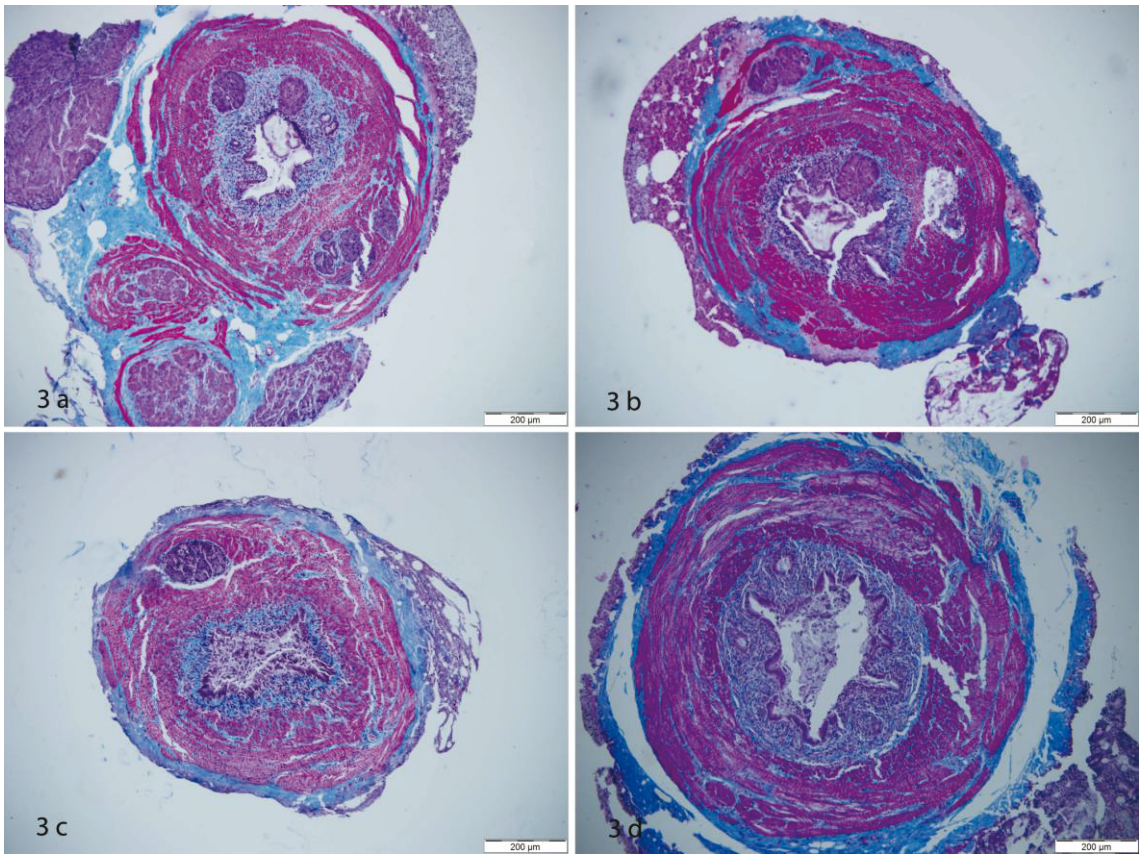

Figure 3. Regional histological appearance of ventral pancreatic duct in male goose. **a)** Region 1 (Crossman's triple stain) , **b)** region 2 (Masson's trichrome), **c)** region 3 (Masson's trichrome), **d)** region 4 (Crossman's triple stain).

revealed PAS-positive structures in the mucosal epithelial cells of the duct and the secretory material in the duct lumen. The secretion was found to fill the cytoplasm of the epithelial cells in the exit zone (region 1) of the duct from the pancreas, and was present in the apical section towards the duodenum (regions 2 and 3) and in the surface facing the lumen (region 4) (Figures 5a and 5b). With AB (pH 1 and pH 2.5) staining, weak (Figure 6a) and strong blue-stained (Figure 6b) acid mucins were determined in the cells of the duct epithelium and luminal surfaces, respectively. PAS/AB (pH 2.5) staining showed that several cells in the duct epithelium contained neutral mucopolysaccharides

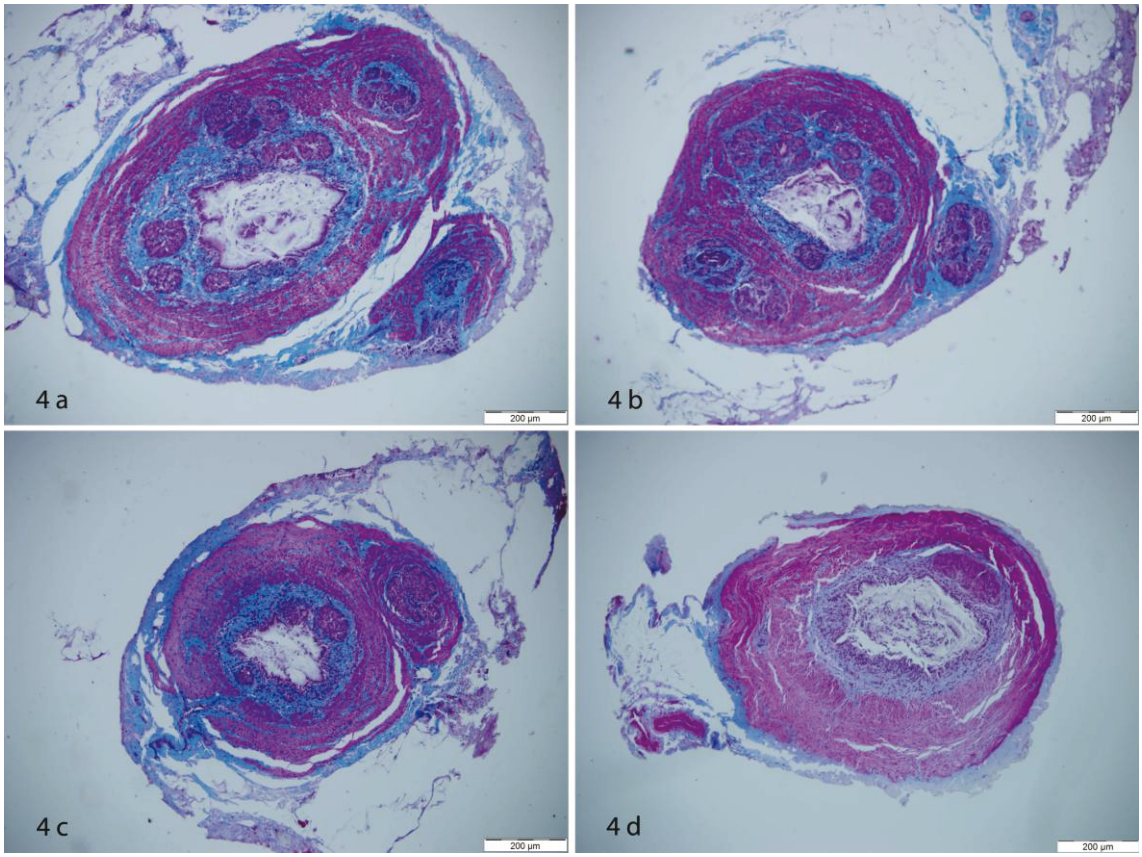

Figure 4. Regional histological appearance of dorsal pancreatic duct in female goose. **a)** Region 1, **b)** region 2, **c)** region 3, **d)** region 4 (Crossman's triple stain).

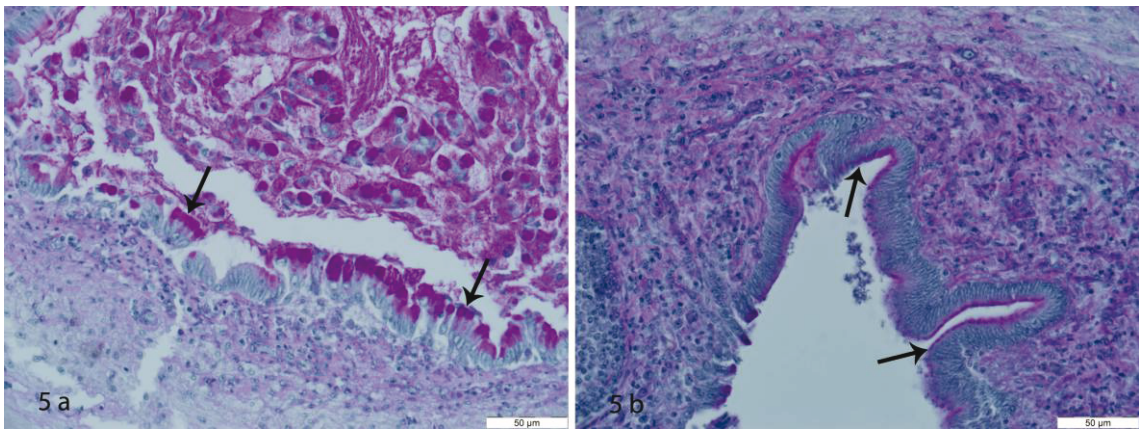

Figure 5. PAS reaction in ventral pancreatic duct of male goose. **a)** Region 1, **b)** region 4.

due to violet-colored staining, and both acidic and neutral mucopolysaccharides due to purple-colored staining (Figure 7a). In addition, AF/AB (pH 2.5) staining showed that the epithelium of the duct contained strong sulfated mucins with dark purple-colored staining, weak sulfated mucins with purple-colored staining, and carboxyl mucins with blue colored-staining (Figure 7b).

4. Discussion

As reported in earlier studies on poultry pancreas (1,2), the goose pancreas is located between the ascending and descending duodenal loops. Studies have also revealed that pancreas in geese (6), chickens (3), and quails (23) is composed of 4 lobes, whereas in ducks and some hawk species (13,24) it consists of 3 lobes. We also found that

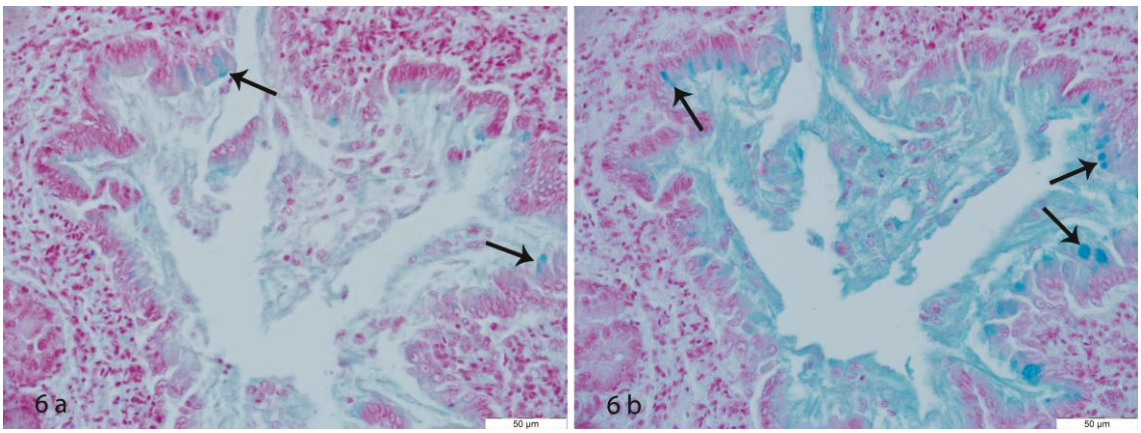

Figure 6. AB reaction in dorsal pancreatic duct of male goose (region 2). **a)** Weak mucin reaction (pH 1). **b)** Strong mucin reaction (pH 2.5).

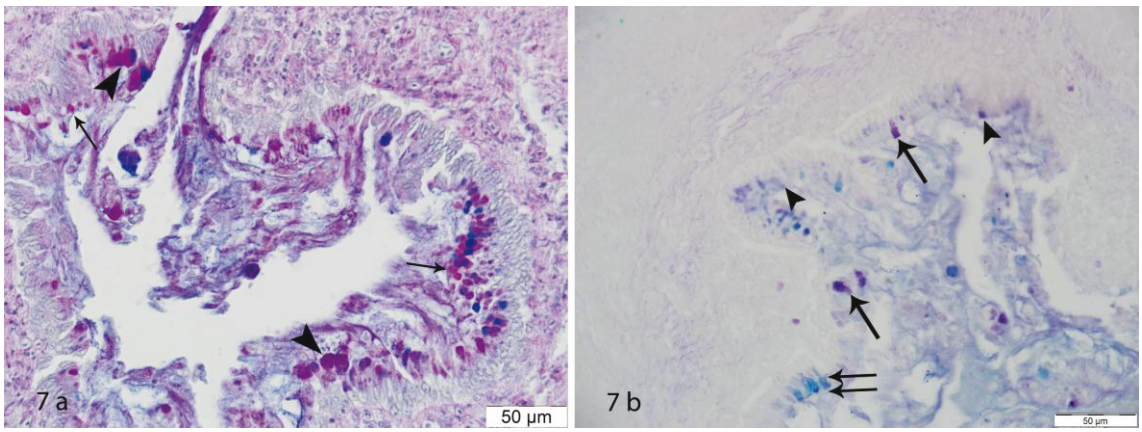

Figure 7. a) PAS/AB reaction in dorsal pancreatic duct of male goose (region 2). Arrow: Neutral mucin-containing cells. Arrowhead: Both acidic and neutral mucin-containing cells**. b)** AF/AB reaction in dorsal pancreatic duct of male goose (region 1). Arrow: Strong sulfated mucin-containing cells. Arrowhead: Weak sulfated mucin-containing cells. Double arrows: Carboxylated-mucin-containing cells.

goose pancreas is composed of 4 lobes in accordance with the presented findings (5,6).

The main pancreatic ducts in birds were found to be composed of dorsal and ventral ducts (5,15), while the presence of a third duct has been reported in some avian species (13–15,24). Our study also demonstrated that there are 2 main ducts extending from the pancreas to the duodenum. This finding is in agreement with the results of Gulmez (5) and Böck et al. (15).

As reported in previous studies (2,5,7,12,16) on the structure of pancreatic ducts, our study observed the duct to be lined with mucosa covered by high columnar epithelium. It was surrounded by muscle layer arranged longitudinally on the inside and circularly on the outside, and by externally adventitial connective tissue. In addition, the presence of glands in the lamina propria of pancreatic ducts has been reported in geese (5), cats, and humans (25). Gulmez (5) reported the presence of exocrine glands in the lamina propria and tunica muscularis of geese pancreatic ducts, but made no regional discrimination. In contrast, our study determined the presence of glands in the adventitial layer. In addition, our study observed that these glands were present especially in regions 1, 2, and 3 of the duct, and continued in the propria layer only in region 4, which is close to the duodenum. They were not present in other layers. No information was found in the literature regarding this result. In our study, the observation of glands in all 3 layers in geese suggests that this situation may be species-specific. When considering the histological structure of the exocrine glands in the pancreas, the presence of centroacinar cells in pancreatic ducts, and staining properties, it is possible to suggest that the content of secretions is the same or similar. However, the similarity between exocrine secretion of pancreatic ducts and pancreas can be better understood with future research using methods such as immunohistochemistry.

In our study, lymph follicles were observed in the lamina propria layer. The presence of lymph follicles in 4 regions of pancreatic ducts suggests that the pancreatic duct might be involved in the lymphoid system. As there is no research indicating the presence of lymph follicles in the pancreatic duct, this condition was thought to be unique to geese.

Previous studies on poultry have reported the lining of the main pancreatic duct with a single layer of columnar epithelium (5,7,12,16). Similar results were found in our study. Kodama (26) reported that pancreatic duct epithelium contains principal cells with dark and light cytoplasm. However, Böck and Geleff (27) stated that these cells were seen distortedly due to faulty fixation. On the other hand, Kadhim et al. (21) and Madden and Sarras (18) stated that there are 3 types of epithelial cells lining the pancreatic duct: principal, light, and basal cells. Our study observed 3 types of cells, consistent with the findings of Kadhim et al. (21) and Madden and Sarras (18).

Some researchers (5,21,28) have reported a variety of secretion materials in the epithelium of the pancreatic duct. In our study, positive reactions were detected by staining of duct epithelium (PAS, AB, PAS/AB, and AF/AB). We observed that our findings are compatible with studies (21,28) stating that duct epithelium contains sulfated and carboxylated mucosubstances, as well as neutral, acidic, and mixed mucosubstances. As reported by some researchers (8,21,28), we think that the presence of neutral, acidic, and mixed mucosubstances in the epithelium of ducts may help the protection of the epithelium and the transportation of pancreatic secretion.

In conclusion, we consider that duct epithelium and its wall structures should be examined in more detail because of the presence of lymph follicles in the duct, the presence of exocrine glands almost until the duodenum, and the presence of various secretions in the duct. An examination of the histological and histochemical characteristics of goose pancreatic duct may contribute to future studies.

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