



Comparative Histological, Histochemical and Ultrastructure Studies on the Exocrine Pancreas of Japanese Quail (*Coturnix coturnix japonica*) and Cattle Egret (*Bubulcus ibis*)

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ABSTRACT

The current study targeted to compare between the pancreas (exocrine part) of Japanese quail and Cattle egret. The pancreas was investigated in eight apparent healthy mature female Japanese quail and eight Cattle egret. Histological, histochemical, and ultrastructure studies were carried out. In both birds, the pancreas is located on the right side of the abdomen between the ascending and descending loops of the duodenum. The pancreas of the Japanese quail composed of dorsal, ventral, third, and splenic lobes, while in Cattle egret the pancreas is not lobulated. The Exocrine portion consists of serous acini and intercalated duct, intralobular duct, interlobular duct, and main duct. The duct system was similar in both birds with minor differences. The intralobular duct in the Cattle egret is characterized by the presence of serous glands within its wall. Under electron microscopy, the acinar cells were of two types, electron-dense, and electron-lucent acinar cells. Histochemically, the interlobular duct of the Cattle egret positively reacted to alcian blue (pH1) and aldehyde fuchsin stains but the interlobular duct of the Japanese quail was negatively reacted.

Key words: Japanese quail, Cattle egret, Histology, Ultrastructure and Histochemistry.

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INTRODUCTION

The Japanese quail (*Coturnix coturnix japonica*) is a member of the family Phasianidae, order Galliformes, class Aves. It is a granivorous bird feeding on grass seeds (Gross et al. 2020). It is distributed in Africa the in area of the Nile River Valley extending from Kenya to Egypt (Pappas 2013). Quails are utilized as a valuable source of meat and eggs with a belief in their medicinal properties (Nasar et al. 2016). The rapid increase of the economy and the growth of the population with rising health issues accelerate the global need for poultry meat. The growth period of Japanese quail life is a crucial time in appreciating the long-term high performance (Elnesr et al. 2019). They had shorter generation intervals which provide a gainful investment (Sreeranjini et al. 2010). Japanese quail had been used in different research fields as a laboratory animal (Ainsworth et al. 2010).

However, the Cattle egret (*Bubulcus ibis*) is a species of egrets or heron (family Ardeidae) lived in aquatic habitats worldwide (Kushlan and Hancock 2005). It is an omnivorous bird, feeds on insect-preys, spiders, earthworms, and other invertebrates, and is locally distributed in the Nile delta and Valley (Meese 2012).

Moreover, Goutner et al. (2001) observed that cattle egret commonly breeds in the Nile delta in the Mediterranean region. The cattle egret is considered economically important for farmers as it plays an important role in controlling pests of land and parasites of cattle (Khalifa 2014). However, the overgrowth of their population may compete with and predate the native species (Marcio et al. 2018). They consider free-living birds to act as a reservoir for many diseases (Hussein and Rezk 2016).

The pancreas of a bird has a double function as an organ with both exocrine and endocrine cell types as in mammals. The exocrine tissue consists of the bulk of the pancreatic mass; it secretes digestive enzymes that pass through the duct system to be delivered to the duodenum (Gülmez et al. 2003). Unique cell types of acini and islets secrete enzymes with a digestive role and hormones with a metabolic activity (Cigliola et al. 2015).

None of the available literature was dealing with the Cattle egret pancreas. Moreover, there was a paucity of studies on the Japanese quail pancreas. Therefore, the objective of this investigation is to investigate the histological, histochemical, and ultrastructural features of the exocrine pancreas of Japanese quail as a model of a granivorous bird and Cattle egret as an omnivorous bird.

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MATERIALS AND METHODS

Collection of Samples

This work was carried on the pancreas of eight healthy mature female Cattle egret from El Mansoria and Abu Roash and eight healthy mature female Japanese quail from the farm of Agriculture of Cairo University.

The pancreas was removed from the duodenum rapidly after slaughtering of the bird under an ethical protocol approved by the animal experimental local ethics committee at Cairo University. Protocol No. Vet Cu20022020142. The specimens were fixed in 10% neutral buffered formalin for 48hrs. The fixed samples were dehydrated in an ascending series of alcohol cleared in xylene, and embedded in paraffin wax overnight, and then 4-5µm paraffin sections were obtained by a rotatory microtome.

a- Light Microscopic Examination

The specimens were stained by Hematoxylin and Eosin (H & E) stain for histological structure, Masson's trichrome stain for collagen and smooth muscle fibers, Gomori's reticulin stain for demonstration of reticular fibers (Bancroft and Gamble 2013).

b- Transmission Electron Microscopy (TEM) Examination

Small pieces from the pancreas were fixed in 3% glutaraldehyde. Then examined with a JEOL 1010 transmission electron microscope at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University.

c- Histochemical Examination

Pancreas slides were stained with periodic acid Schiff (PAS) for the detection of neutral mucopolysaccharides, modified aldehyde fuchsin, and Alcian blue (pH1) to detect sulfated mucopolysaccharides (Bancroft and Gamble 2013).

RESULTS

Macroscopic Appearance

The pancreas of both Cattle egret and Japanese quail was located in the right part of the abdomen; between the two loops of the duodenum. In the Cattle egret, the pancreas appeared as an elongated large mass (Fig.1A). While in the Japanese quail, it consisted of four lobes: two main lobes; dorsal and ventral lobes, the ventral one gave the third lobe and small splenic lobe (Fig. 1B&C).

Light Microscopic Examination

The pancreas was covered with a delicate connective tissue capsule consisted of collagen and reticular fibers in both birds. This capsule appeared to be thicker and contained abundant collagen fibers in Cattle egret than in Japanese quail (Fig. 2 A&B). The capsule sent septa dividing the pancreas into lobes and lobules.

The parenchyma consisted of exocrine and endocrine parts (islets of Langerhans) supported by fine reticular stroma. The exocrine unit was comprised of pancreatic acini (tubuloacinar glands) and ducts of different orders between the lobes and lobules. The acini appeared as spherical to oval units composed of a single layer of serous cells which were pyramidal to columnar shaped cells rest on a basal lamina with a narrow lumen. The acinar cells had spherical basally situated lightly stained nuclei with prominent nucleoli. They had basal basophilic cytoplasm and apical acidophilic cytoplasm (Fig. 2 C&D).

The duct system in both birds is arranged as intercalated ducts, intralobular ducts, interlobular ducts, and the dorsal and ventral main pancreatic ducts that extend to the duodenum.

In both birds, the intercalated duct had an intra-acinar portion. This portion was lined with Centro-acinar cells which were relatively smaller cells, had a pale cytoplasm so they appeared lightly stained than the acini. They were flattened in shape and situated in the center of the lumina

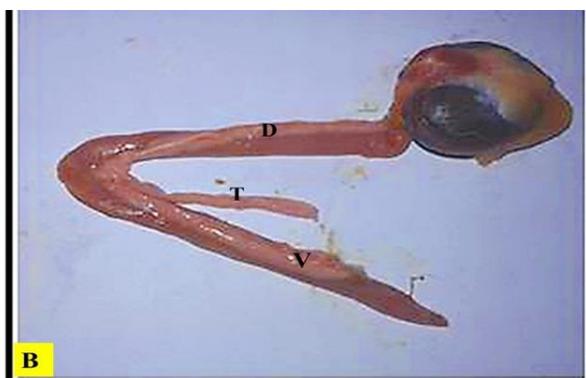


Fig.1: A photograph of the cattle egret non-lobulated pancreas (arrow) between the duodenal loops. **B:** showing Japanese quail pancreas lobes; the ventral (V), dorsal (D) and third (T). **C:** showing splenic lobe (S).

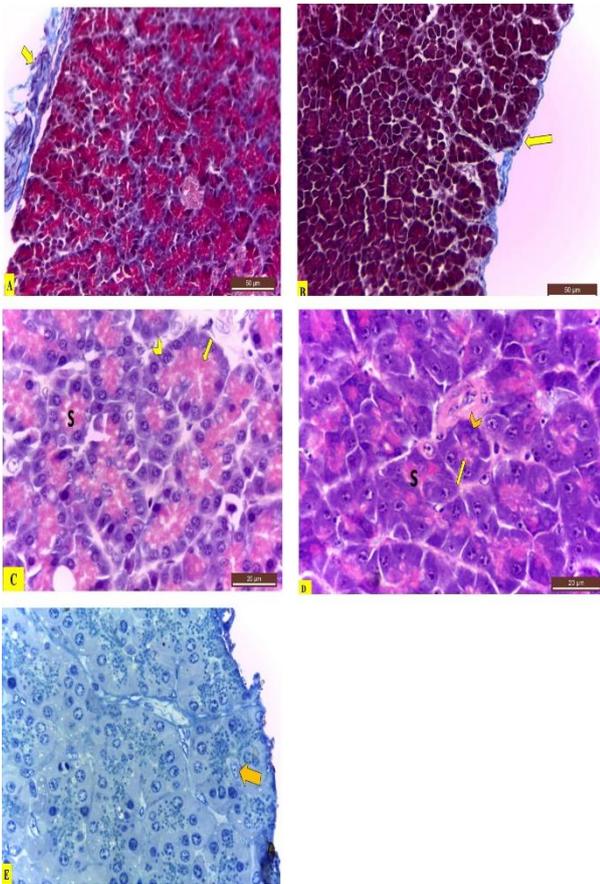


Fig. 2A: Section of cattle egret pancreas showing thick capsule (arrow). **B:** section of Japanese quail pancreas showing thin capsule (arrow). Masson's Trichrome X400. **C:** **D** sections of pancreas showing serous adenomere (S) with apical acidophilic cytoplasm (arrow) and basal basophilic cytoplasm (arrowhead). H&E X1000. (C) in egret and (D) in Japanese quail. **E:** Semithin section of cattle egret pancreas showing Centro-acinar cell (arrow). Toluidine blue X1000.

of the acini in front of the apical acidophilic zones (Fig. 2E). They are lined by simple squamous epithelium (Fig. 3 A&B). This duct continued as a larger intralobular duct; lined by simple cuboidal cells (Fig. 3 C&D); rested on collagen and reticular fibers (Fig. 3 E&F).

The interlobular ducts are lined by simple cuboidal epithelium in both birds. Furthermore, in Cattle egret the wall of the interlobular duct contained serous acini (Fig. 4 A&B).

In Cattle egret, the main excretory duct was lined by simple columnar epithelium (Fig.4 C) rested on collagen and reticular fibers contained smooth muscle fibers (Fig.4 D&E). In Japanese quail, the main duct had the same structure, but the epithelium rested mainly on reticular fibers (Fig. 4F&G).

Ultrastructure Examination

The ultrastructural features of pancreatic acini of both birds appeared lined by two types of cells, an electron-dense (dark acinar cells) and electron-lucent (light acinar cells) (Fig.5 A&B). In Cattle egret, the dark cells had spherical nuclei with peripheral clumps of heterochromatin in addition to the presence of numerous spherical and elongated mitochondria with irregular cristae. On the other hand, in Japanese quail, the dark acinar cells contained

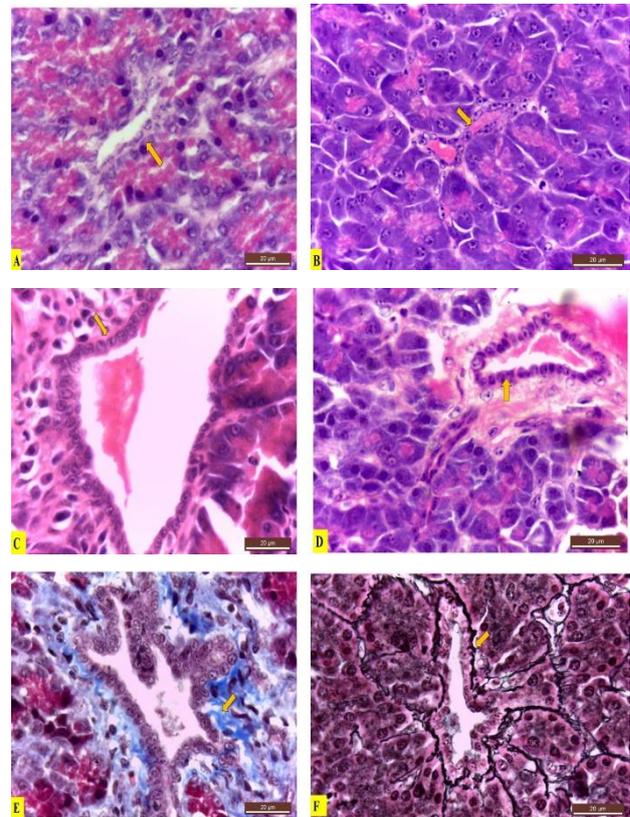


Fig. 3 A: Section of pancreas showing intercalated duct lined by squamous cells (arrow) in cattle egret and **B:** in Japanese quail. H&E X1000. **C:** Section of pancreas showing intralobular duct (arrow) lined by cuboidal cells in cattle egret and **D:** Japanese quail. H&E X1000. **E:** Section of cattle egret pancreas showing intralobular duct supported with collagen fibers (arrow) and **F:** supported by reticular fibers (arrow). Masson's trichrome X1000 and Gomori's reticulin X1000.

irregular and basally situated nuclei with prominent nucleoli. Moreover, there were numerous mitochondria which appeared mainly spherical in shape. In both birds, there were well-developed laminated cisternae of the rough endoplasmic reticulum (rER) which mainly fill the cytoplasm peri and intranuclear. Numerous zymogen granules of different sizes with electron-dense homogenous content were accumulated in the supranuclear cytoplasm (Fig. 5 C&D).

The light cells were rarer than the dark cells. In Cattle egret, the light acinar cells had large spherical nuclei with clumped peripheral heterochromatin and smooth nuclear membrane. Their lucent cytoplasm contained intranuclear rER and numerous zymogen granules of variable size. Also, there were numerous large-sized polymorphic mitochondria. In Japanese quail, there was a euchromatic nucleus with prominent nucleoli, in addition to spherical mitochondria and well-developed cisternae of rER (Fig. 5 E&F).

In both birds, Centro-acinar cells were observed between the acinar cells (Fig.5 B) which differed from the acinar cells by devoid of zymogen granules, their smaller size and being less electron-dense. These cells appeared elongated with scarce cytoplasmic organelles and flattened euchromatic nuclei with heterochromatin masses under the nuclear envelope (Fig. 5G&H).

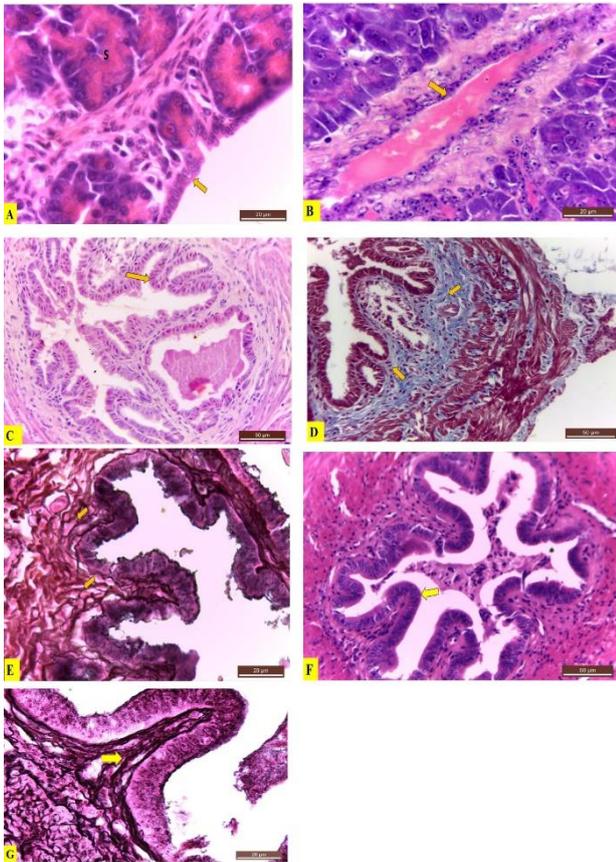


Fig. 4 A: Section of cattle egret pancreas showing interlobular duct lined by cuboidal cells (arrow) with serous acini (SD) within the wall. H&E X1000. **B:** Section of Japanese quail pancreas showing interlobular duct lined by cuboidal cells (arrow). H&E X1000. **C:** Section of cattle egret pancreas showing the main duct with mucosal folding lined by simple columnar epithelium (arrow) **D:** supported by collagen fibers and **E:** reticular fibers (arrow). H&E X400; Masson's trichrome X400 and Gomori's reticulin X1000. **F:** Section of Japanese quail pancreas showing the main duct with mucosal folding lined by simple columnar epithelium (arrow) and **G:** supported by reticular fibers (arrow). H&E X400 and Gomori's reticulin X1000.

Histochemical Examination

The apical surface of the epithelial cells lining the interlobular duct of the Cattle egret was positively reacted to alcian blue (pH1) and aldehyde fuchsin stain; so the lumen contained secretory material while the interlobular duct of the Japanese quail was negatively reacted (Fig.6 A&B). The cells of the main duct in the Cattle egret was PAS-positive while that of the Japanese quail was negatively reacted (Fig. 6 C).

DISCUSSION

In Japanese quail and Cattle egret, the pancreas is situated in the right of the abdomen between the two loops of duodenal limbs as reported in common quail (Al-Hathry 2000; Fatlawi 2018), Japanese quail (Şimşek and Alabay 2008), pigeon (Faris 2012). Our results revealed that the pancreas of Cattle egret is non-lobulated while, in Japanese quail, the pancreas is lobulated and consisted of four lobes: dorsal, ventral, third, and splenic lobes. Similar findings were reported by Ku et al. (2000), Şimşek and Alabay (2008) and Faris (2012) in chicken, Japanese quail, and

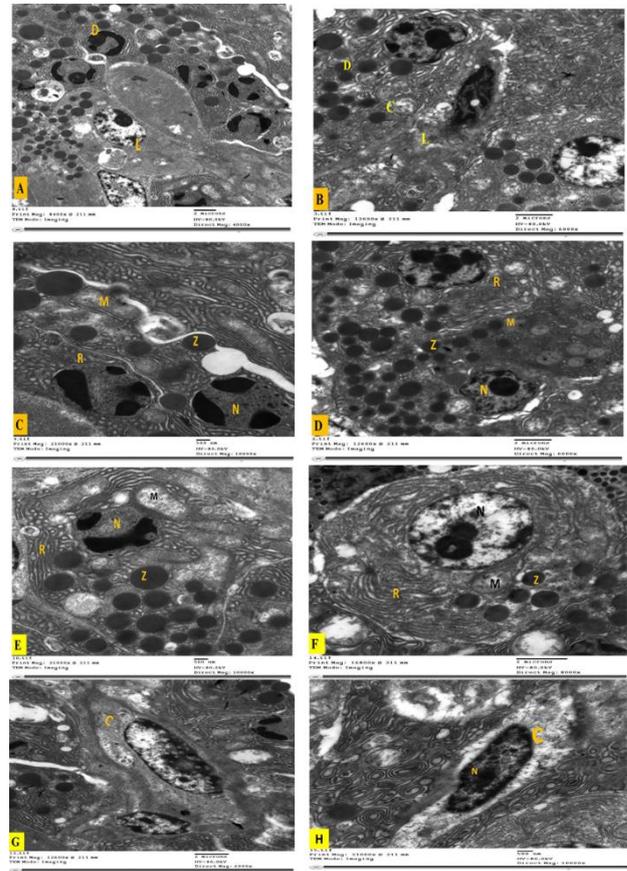


Fig. 5: Transmission electron micrograph showing light (L) and dark acinar cells (D) in **A:** Cattle egret and **B:** Japanese quail pancreas. X4000. **C:** dark acinar cells in cattle egret pancreas showing elongated mitochondria (M), zymogen granules (Z), rough endoplasmic reticulum (R) and spherical nucleus (N). X10000. **D:** dark acinar cells of Japanese quail pancreas showing spherical mitochondria (M), zymogen granules (Z), rough endoplasmic reticulum (R) and irregular nucleus (N). 4000X. **E:** light acinar cells in cattle egret pancreas: mitochondria (M), zymogen granules (Z), rough endoplasmic reticulum, nucleus (N). 10000X. **F:** light acinar cells in Japanese quail pancreas: mitochondria (M), zymogen granules (Z), rough endoplasmic reticulum, nucleus (N). 10000X. **G:** Centro-acinar cell (C) in cattle egret pancreas 6000X. **H:** Centro-acinar cell (C) with flattened nucleus (N) in Japanese quail pancreas 10000X.

pigeon, respectively. While Saadatfar et al. (2009) and Şimşek et al. (2009) mentioned that the pancreas of Mynah and falcons composed of three lobes only.

Histologically, the exocrine pancreas of both birds was formed of serous tubulo-acinar glands and the duct system. The acinar cells of both birds are pyramidal to columnar shaped cells which in line with Mobini (2011) in goose and Al-Agele, Mohamed (2012) in golden eagle and Palaskar (2018) in Japanese Quail. These cells characterized by bi-zonal cytoplasm referred to the presence of acidophilic granules in the apical portion and rich in rER in the basal portion which in agreement with Al-Agele and Mohamed (2012) in golden eagle. However, the hydrolysis of food occurs by the action of the digestive enzymes of the pancreas and the enzymes present on the brush border of intestinal epithelium (Karasov and Hume, 1997). On the other hand, Brzęk et al. (2013) concluded that the activity of pancreatic enzymes in birds is not affected by diet composition.

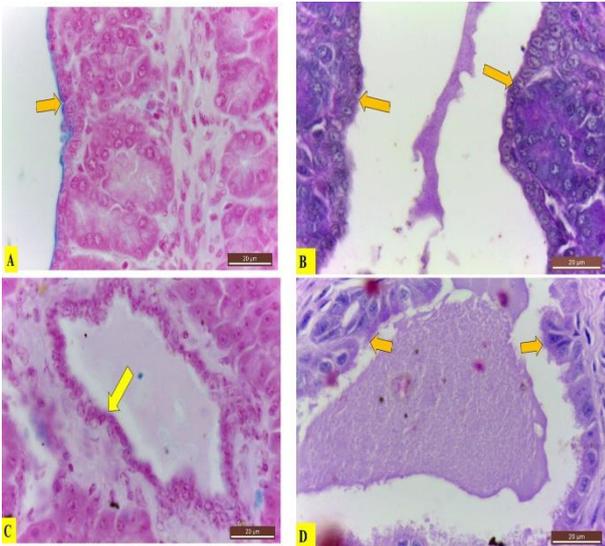


Fig. 6: **A** Section of cattle egret pancreas showing interlobular duct with Alcian blue (PH1) stain positive reaction (arrow) X1000. **B:** Section of cattle egret pancreas showing interlobular duct with aldehyde fuchsin stain positive reaction (arrow) X1000. **C:** Section of Japanese quail pancreas showing interlobular duct with Alcian blue (PH1) stain negative reaction (arrow) X1000 **D:** Section of cattle egret pancreas showing main duct with PAS stain positive reaction (arrow) X1000.

The centro-acinar cells were observed in the lumina of the acini of both birds. These cells were flattened in shape, relatively smaller and brighter without granules than the acinar cells that agreed with Gülmez (2003) in goose; Saadatfar et al. (2011) in dove; Mobini (2011) in goose, Faris (2012) in the pigeon. But the centro-acinar cell is not observed in Mynah (Saadatfar et al. 2009) and Guinea fowl and Common gull (Hamodi et al. 2013).

The intercalated ducts in both birds were similar to that described in goose (Gülmez, 2003), and pigeon (Mobini 2013). It is lined with simple squamous epithelium which disagreed with the finding of Al-Hathry (2000) in common quail, Faris (2012) in pigeon, and Mobini (2011) in goose who found that the intercalated duct was lined with simple cuboidal cells.

Moreover, the intralobular ducts which lined by simple cuboidal cells in both birds were found to be similar to that described by Şimşek and Alabay (2008) in Japanese quail, Al-Hathry (2000) in common quail; Al-Shaeli (2010) in duck; Faris (2012) in pigeon; Beheiry et al. (2018) in goose. While in falcon, it is lined with simple squamous epithelium (Şimşek et al. 2009) and with tall columnar epithelium in goose (Gülmez 2003).

In both birds, the interlobular ducts were lined with simple cuboidal epithelium which disagreed with the findings of Al-Hathry (2000) in common quail, Gülmez (2003) in goose, Şimşek and Alabay (2008) in Japanese quail, and Faris (2012) in the pigeon who stated that the interlobular ducts were lined with simple columnar epithelium. Moreover, Hamodi et al. (2013) in Guinea fowl mentioned that the interlobular ducts were lined by simple squamous to cuboidal cells.

In Cattle egret the wall of the interlobular duct contained serous glands in the connective tissue which come in contact with the results of Gülmez (2003) and Mobini (2011) in goose, but these glands were not observed

in the duct of Japanese quail which agreed with the finding of Şimşek and Alabay (2008) in Japanese quail and Mobini (2013) in the pigeon.

Our result for both birds showed that the main excretory ducts were lined by a simple columnar cell. In contrast, Al-Sharoot (2016) in goose described that the main duct was lined by simple to stratified columnar cells while Beheiry et al. (2018) in goose described that the main duct was lined by stratified cuboidal to stratified columnar epithelium. Moreover, the connective tissue around the main ducts contains thick layers of smooth muscle fibers which is in accordance with Deprem et al. (2015) in goose.

As reported by Şimşek and Alabay (2008) in Japanese quail on the ultrastructure of the acini, our results for both birds distinguished the acinar cells as dark cells (electron-dense) and light cells (electron-lucent). The electron-lucent acinar cells of both birds have spherical euchromatic nuclei, many ribosomes, and rER in the basal region in agreement with Şimşek and Alabay (2008) in Japanese quail. Many rod-shaped mitochondria in cattle egret while spherical in Japanese quail. Meanwhile, Beheiry et al. (2018) in goose observed numerous polymorphic mitochondria. These light cells are thought to be active cells as mentioned by (Şimşek and Alabay 2008). While the dark cells characterized by dense cytoplasm comprised different intensities of zymogen granules, mitochondria, and rER with heterochromatic nuclei. The presence of dense zymogen granules in both birds with similar secretion supports the finding of Beheiry et al. (2018) in goose.

Centro-acinar cells are less electron-dense with euchromatic flattened nuclei. These cells differ from the acinar cells by lacking zymogen granules, their smaller size, scarce cytoplasmic organelles that in line with Şimşek and Alabay (2008) in Japanese quail; Mescher (2010) and Beheiry et al. (2018) in goose.

Histochemically, the apical surface of the luminal border of interlobular ducts cells of cattle egret were aldehyde fuchsin and alcian blue (pH 1) positive. This indicates the presence of sulfated mucins which agrees with Gülmez (2003) in goose but this duct in Japanese quail showed a negative reaction.

The epithelium of the main duct in the Cattle egret is characterized by PAS-positive granules as reported by Stornelli et al. (2006) in ostrich; Şimşek et al. (2009) in falcon; Kadhim et al. (2010) in red jungle fowl and Deprem et al. (2015) in goose. The presence of neutral mucosubstances may help the protection of the epithelium and the transportation of pancreatic secretion as mentioned by Deprem et al. (2015). Zharkov et al. (1994) mentioned that the features of the epithelial mucous in the pancreatic duct are found to be different depending on the type of the digested food.

Conclusion

The quail pancreas is divided into the dorsal, ventral, third and splenic lobes while the cattle egret pancreas is non-lobulated. The structure of the pancreas in the cattle egret and the Japanese quail was found to be similar except for the interlobular duct in the cattle egret which contains a gland within its wall. Ultra-structurally, the secretory acini in both birds were formed of dark and light cells with Centro-acinar cells. Histochemically, the lining of the

interlobular duct in the cattle egret was positive for modified aldehyde fuchsin and Alcian blue (PH1). Also, the main duct was reacted positively with PAS stain. But the interlobular duct and main duct of the Japanese quail were negatively reacted to these stains.

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