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Early View

Original Research Article

Sex-Based Differences in Gross Morphometry and Microscopy of the Pancreas of Japanese Quail (*Coturnix japonica*)

Key words

endocrine and exocrine pancreas; histomorphometry; Japanese quail; pancreatic ducts; sex differences

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Abstract: The pancreas is a very important organ in all mammals including birds, which is involved in digestion and metabolism, yet little is known about structural differences between male and female birds. Forty adult (20 males and 20 females) Japanese quails (*Coturnix coturnix japonica*) were used to analyze the morphometrical and histological differences between the pancreatic lobes. The gross anatomical results showed that both sexes have four lobes: dorsal, ventral, third, and small splenic lobe. Morphometric analysis revealed that the dorsal lobe was significantly heavier and wider in females than in males, suggesting possible sex-linked metabolic adaptations while differences in the dimensions of the ventral and third lobes were not significant. The exocrine tissue showed no differences between males and females, maintaining a consistent structure similar to other birds. However, the endocrine portion, particularly the number of islets of Langerhans, revealed sex-specific differences where females had significantly more islets in the dorsal lobe than the males, whereas males had slightly more but not significantly higher number of islets in the ventral lobe than females. Additionally, the pancreatic ductal system exhibited similar structural features in both sexes, starting from centroacinar cells to the main pancreatic duct with evidence of mucin production by the ductal epithelium observed throughout the ducts despite of the absence of the goblet cells. These findings highlight the variations in morphometry and histology of the quail pancreas between sexes, offering new perspectives on avian physiology and potential metabolic differences. This research lays the basis for further studies on the functional implications of these histomorphometrical distinctions in health and disease.

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Introduction

The avian pancreas is an elongated gland located on the right side of the abdominal cavity in all birds. It is tightly bound by mesentery and blood vessels positioned between the descending and the ascending duodenal loops (1, 2). The pancreas consists of two main lobes, the dorsal and ventral lobes however; some birds have two additional lobes namely; the third and splenic lobes. In ducks, falcons, Mynah and many other bird species, the pancreas is divided into only 3 lobes (3, 4). The pancreas of long-legged buzzards, goose, chickens and quails is composed of four lobes namely; dorsal, ventral, third and splenic lobes (5-8).

Histologically a thin layer of loose connective tissue capsule covers the avian pancreas, from which septa extend to divide the gland into lobules. Blood and lymph vessels, nerves and excretory ducts run in the connective tissue septa. The avian pancreatic parenchyma consists of integrated exocrine and endocrine tissues that are not separated by distinct histological boundaries. The exocrine portion secretes the pancreatic juice produced by the pancreatic acini and transported via the excretory ducts. The pancreatic acini have variable shapes and sizes according to the bird species. The excretory duct system begins with the centroacinar cells and extends to the intercalated, intralobular, interlobular and interlobar ducts before terminating in the main excretory duct (9-11). The endocrine portion or islets of Langerhans secretes the

hormones and can be found dispersed among the exocrine tissue with different sizes and shapes (5, 12, 13).

According to the principles of the *Nomina Anatomica Avium*, the avian pancreas exhibits a characteristic arrangement of exocrine acini interspersed with endocrine islets, reflecting its dual role in digestion and metabolic regulation (14). This conserved anatomical organization provides a structural framework for understanding species- and sex-related variations in pancreatic morphometry and histology in avian models such as the quail.

Previous studies have shown concern for the anatomical, histological and immunohistochemical structures of the pancreatic lobes. These studies reported the structure of the exocrine and endocrine as well as the duct system differences in many avian species (7, 11, 15). In addition, Parchami and Kusha reported that sex have effects on the histomorphometric properties of the islets of Langerhans in chickens (16). Up to date, there is no previous reports on the differences in gross morphometry and histology of the pancreas in male and female quails. The present study aims to provide novel insights into sex-related differences in gross morphometry, histology, histochemistry, and histomorphometry of the exocrine and endocrine pancreas in male and female quails.

Materials and methods

Experimental animals

Forty adult healthy quails (eight weeks old) of both sexes were obtained from the Quail Production Research Unit at the Faculty of Veterinary Medicine, South Valley University, Qena, Egypt. One-day-old quail chicks were reared on a chicken starter diet containing 28% crude protein of plant origin (soybean meal). After 14 days of age, the dietary protein level was reduced to 21% and maintained until sacrifice. Birds had free access to calcium in the form of limestone, as well as adequate supplies of vitamins, minerals, and antibiotics. During the first week of rearing, room temperature was maintained at 40 °C and then gradually reduced to 38 °C. Throughout the experimental period, birds were exposed to a lighting regimen of 12 h light per day from hatching until the end of the experimental period. At eight weeks post hatching, 40 birds were grouped into males and females (20 birds in each group). The birds were euthanized by using carbon dioxide (CO_2) inhalation (17). This method induced rapid loss of consciousness followed by death.

Ethical Statement

The study and sampling collection were approved and carried out in compliance with the guidelines of the Egyptian animal law and the approval of the Ethical Committee at the Faculty of Veterinary Medicine, SVU, Qena, Egypt (Approval Number: VM/SVU/24(12)-17). The ARRIVE guidelines were followed throughout all the experimental procedures.

For anatomical and morphometrical analysis

The birds were euthanized and the pancreata were removed immediately. The different lobes of the pancreas were dissected and subjected to anatomical and morphometrical measurements. The weight (g) of the birds, the whole pancreas and the pancreatic lobes was measured using Berekel balance. Length and width (mm) of the pancreatic lobes were measured using digital Vernier caliper (Vogel, Kevelaer, Germany). Measurements were analyzed by Student's t-test using the IBM SPSS software v22 (Chicago, IL, USA) and /or ANOVA one-way. Data were expressed as Mean ± SE.

For histological and histochemical analysis

For histological analysis, the pancreatic lobes were washed immediately after removal in cold normal saline solution, fixed in 4 % neutral buffered formalin (NBF) for 48 hrs, and were processed for paraffin embedding. Serial Sections (3-5 µm thick) were cut using an automated rotatory microtome (Leica RM2235, Leica Biosystems, Wetzlar, Germany) and stained with Mayer's hematoxylin and eosin (HE) or Masson's trichrome stain. Histochemical staining was performed using modified aldehyde fuchsin, periodic acid–Schiff (PAS), Alcian blue (AB) or Alcian blue-periodic acid-Schiff (AB-PAS) technique (18). Sections were examined and photographed using a Leica DMLS microscope (Leica Microsystems) equipped with a Leica ICC50 HD camera.

For semithin analysis

Pancreatic tissue samples, which were collected for semithin sections were fixed in 2.5% glutaraldehyde, post fixed in 1% osmic acid at 4°C for two hrs, dehydrated and embedded in Epon 812 resin (Electron Microscopy Sciences; USA). Resin-embedded samples were left to polymerize at 60ºC for 2-3 days. Semithin sections (0.5 µm) were cut using an ultramicrotome (Ultracut E; Reichert-Leica) and stained with toluidine blue. Sections were examined and photographed as described in the histological analysis.

For histomorphometrical analysis

Histomorphometry was performed on HE-stained sections (five representative non-overlapping fields/bird, and five birds per sex were used) using Image-J 1.46r software (National Institute of Health, Bethesda, Maryland, USA). Histomorphometric measurements included the number of islets in each pancreatic lobe. Measurements were analyzed by Student's t-test using the IBM SPSS software v22 (Chicago, IL, USA) and /or ANOVA one-way. Data were expressed as Mean ± SE.

Results

Gross anatomical results

The adult Japanese quail (*Coturnix coturnix japonica*) males are characterized by a rusty brown throat and breast feathers (Fig. 1A). The testes are large, whitish and oval in shape (Fig. 1B). Histological sections of the testes revealed seminiferous tubules containing sperms in their lumens (data not shown). Light tan feathers with black speckling on the throat and upper breast characterize the adult female quails (Fig. 1C). The females began

to lay eggs 35 days after hatching and an active ovary with well-developed oviducts were visible in gross anatomy evaluation (Fig. 1D). The gross anatomical investigation showed that the quail pancreas was a long, narrow, pale pinkish gland, which located on the right side of the abdominal cavity. It has a smooth surface and was tightly bound by the mesentery and blood vessels in a horizontal position between the descending and the ascending duodenal loops. The pancreas of the Japanese quail is consisted of the dorsal, ventral, third, and a very small splenic lobe. The dorsal pancreatic lobe lies along the mesenteric border of the descending duodenum and represents the largest portion of the pancreas in between the interduodenal space. The third pancreatic lobe is smaller and situated medially between the dorsal and ventral lobes, closely associated with the pancreatic ductal system. A small splenic lobe was identified near the splenic extremity of the pancreas adjacent to the spleen. It was connected to the main pancreatic tissue by fine parenchymal strands. Due to its very small size, precise morphometric measurements of the splenic lobe could not be reliably obtained (Fig. 2).

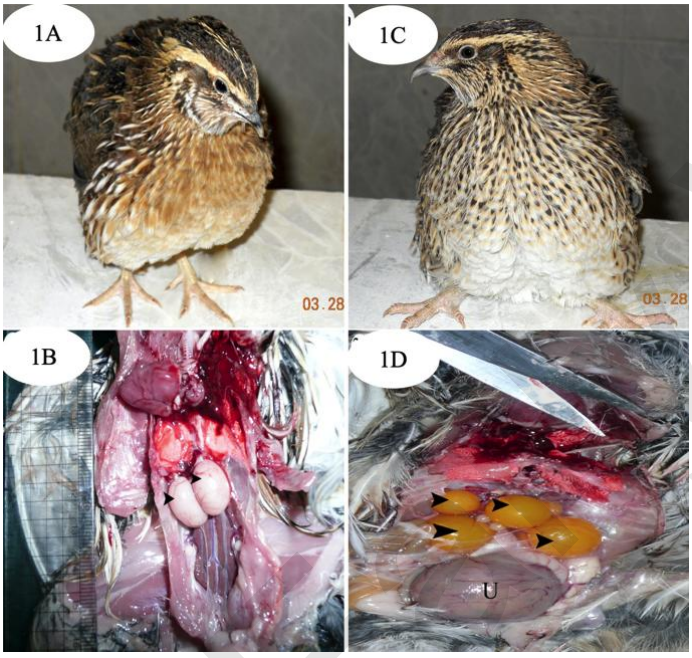

Figure 1: Sexual dimorphism and reproductive organs of adult male and female quail. 1A; Adult male quail with rusty brown throat and breast feathers. 1B; Right and left testes of the adult male quail (arrowheads). 1C; Adult female quail has light tan feathers with black speckling on the throat and upper breast. 1D; ovary of adult female quail containing various follicles (arrowheads) and the well-developed oviduct containing an egg (U)

Morphometrical Analysis Results

The morphometrical analysis revealed that the body weight of the female quails was significantly higher than that of male quails (p = 0.026; Fig. 3A). There was no difference between the weight of the pancreas in male and female (Fig. 3B) while the ratio of pancreas to body weight in female was significantly higher than in male birds (Fig. 3C). The dorsal lobe of the pancreas in female quails was significantly heavier than in males (p = 0.04; Fig. 3D) while no differences were found between the ventral and third lobes in male and female (Fig. 3D). No significant differences were found in the length of the pancreas between male and female quails (Fig. 3E). The dorsal lobe width was significantly greater in female quails than in males (p = 0.0004; Fig. 3F). The dorsal lobe in male quails was significantly wider than the third lobe (p = 0.01; Fig. 3F). The dorsal lobe in female quails was significantly wider than the ventral and third lobes (p = 0.0001 and p = 0.004, respectively; Fig. 3F).

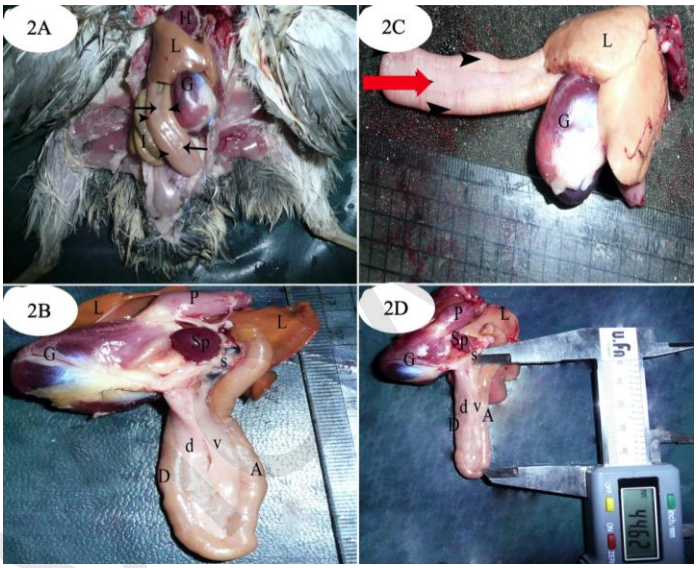

Figure 2: The anatomical position of the quail pancreas. Heart (H); liver (L); gizzard (G); intestine (I); duodenum (arrowheads); pancreas (arrows), proventriculus (P); spleen (Sp); descending duodenum (D); ascending duodenum (A); dorsal lobe of the pancreas (d); ventral lobe of the pancreas (v); splenic lobe of the pancreas (s), third lobe of the pancreas (red arrow)

Histological, Histochemical and Semithin Analysis Results:

Stroma of the quail pancreas

Photomicrographs of the quail pancreas stroma are presented in Figure 4. The stroma of the pancreas begins with a thin layer of loose connective tissue capsule covered by mesothelial cells, from which septa extend to divide the gland into incomplete lobules. Collagen fibers were observed in the capsule, interlobular, as well as interacinar connective tissue stained with Masson's trichrome or modified aldehyde fuchsin. The basement membrane was visible in the islets of Langerhans among the endocrine cells stained with PAS stain. Smooth muscle fibers were absent in the capsule.

Parenchyma of the quail pancreas

Exocrine Portion of the quail`s pancreas

The parenchyma of quail pancreas consisted of exocrine and endocrine portions, was located in the meshwork of reticular fibers. The exocrine portion presented in Figure 5 was arranged in the form of serous tubuloacinar glands and occupied a larger area of the pancreas. There were no significant differences between the histological structure of the exocrine portion of the dorsal, ventral, third (head, body and tail regions) and splenic

lobes of the pancreas in male and female quail. In addition, there were no differences in the histological structure of the exocrine portion of different regions of the dorsal lobe of the pancreas in male and female. The exocrine portion contains a variable number of secretory acini (glandular epithelium) consisted of a variable number of pyramidal to columnar cells having basophilic base and numerous apical acidophilic zymogen granules. The apical cytoplasm was filled with acidophilic secretory granules, usually called zymogen granules because they contain the precursors of the enzymes of the pancreatic juice. In contrast to salivary gland the myoepithelial cells are absent in the pancreatic acini. Parasympathetic ganglia with nerve bundles were observed among the exocrine portion of the pancreas. In semi-thin sections stained with toluidine blue, the nuclei appear vesicular with distinct nucleolus where the apical cytoplasm of the acinar cells filled with the secretory zymogen granules.

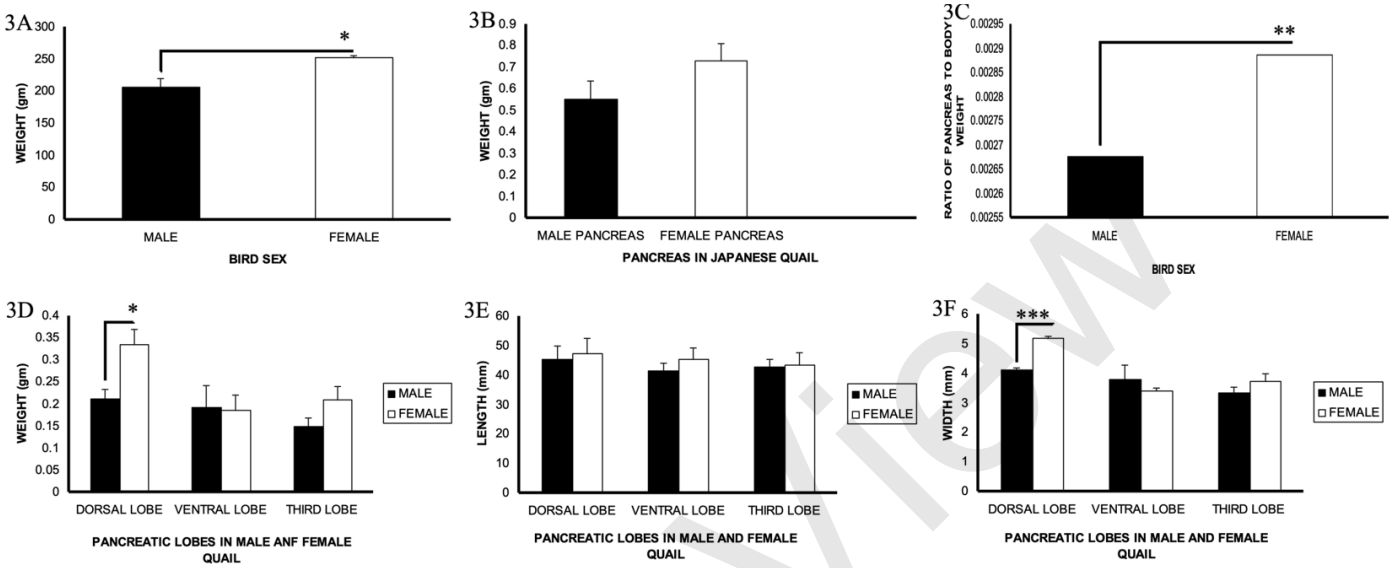

Figure 3: The results of morphometrical measurements of male and female quail pancreas. 3A; Body weight of the female and male quails (*p*-value = 0.026). 3B; Weight of the pancreas in female and male quails (*p*-value = 0.21). 3C; ratio of the pancreas weight to the body weight of the female and male quail. 3D; Weight, 3E; Length and 3F; Width of the pancreatic lobes in male and female quail

Pancreatic ducts of the quail pancreas:

Photomicrographs of the quail pancreas ducts (main, intercalated, intralobular and interlobular) are presented in Figures 6 and 7. There were no differences in the histological features of the ducts of the pancreatic lobes in both male and female quail. Small centroacinar cells without granules were rarely observed in the central lumen as the beginning of the duct system which then continued as the intercalated ducts. The Intercalated ducts were lined with simple squamous or low cuboidal epithelium that rest on a very thin layer of connective tissue. The intercalated ducts reach intralobular ducts lined with simple cuboidal epithelium. The lumen of the acini and ducts showed PAS-positive secretory materials. A thin layer of collagenous fibers appears blue in color underlying ductal epithelium stained with modified aldehyde fucshin or Masson's trichrome stain. The intralobular ducts were lined with a single layer of simple cuboidal epithelium, which became low columnar epithelium at the interlobular ducts level. The highly cellular connective tissue lamina propria formed of collagenous connective tissue surrounding the ducts and stained blue with modified aldehyde fucshin stain or Masson's trichrome technique. The mucosa of the interlobular ducts were folded and lined with simple columnar epithelium where the lumen was filled with eosinophilic secretory material. The lamina propria was formed of collagenous connective tissue surrounded by thin muscular layers. Basophilic staining on the apical surface of the interlobular ducts, which indicates possible secretory functions of the epithelium lining the ducts. Interestingly, compound tubulo-acinar glands similar to the exocrine portion of the pancreas were found intermittently inside the connective tissue of the ducts starting from the interlobular ducts to the point where the pancreas emptied its contents into the duodenum. A layer of mucous covered the apical surface of columnar epithelial cells stained with modified aldehyde fuchsin stain (Fig. 6).

The main excretory ducts composed of three layers; mucosa, muscularis and adventitia. The mucosa forms primary and secondary folds, which are lined with simple to stratified columnar epithelium. The lamina propria formed of a highly cellular connective tissue. The muscular layer is thicker in the main duct than the interlobar duct. It is formed of smooth muscle fibers that are arranged in two layers: inner longitudinal and outer circular. The adventitia is composed of loose connective tissue that surrounds the ducts and connects them to other surrounding tissues. The ductal epithelium had apical secretory granules are stained with Alcian-PAS, both secretory granules and apical surface coats of luminal border of these cells are stained with purple color (AB-PAS-positive). The glands inside the wall of the

ducts within the lamina propria are structurally similar to the exocrine part of the quail pancreas and no endocrine islets were noticed among those glands. Parasympathetic ganglia with nerve bundles were observed also in the lamina propria of the interlobular and main ducts. Sections stained with modified aldehyde fucshin stain and Masson's trichrome technique showed collagenous connective tissue around the ductal epithelium and within the muscular layer (Fig. 7).

At least three types of cells were recognized lining the epithelium of the pancreatic ducts: the dark principal cells, light cells and the basal cells. The principal cells make up the majority of the ductal cells. These cells increased gradually from flattened or low cuboidal in the intercalated duct to cuboidal in the intralobular duct, while they became high columnar cells in the interlobular and main ducts. The light cells are rarely seen between the principal cells, but appeared more frequently in the main and interlobular ducts, and seemed absent in other segments. The light cells had light cytoplasm and large rounded nuclei. The basal cells are the third type, and these cells are few in number with relatively small dark nuclei and are seen between the epithelial cells and the basement membrane of the large ducts. Goblet cells were not evidenced among the columnar epithelium of the excretory ducts in quails (Fig. 7).

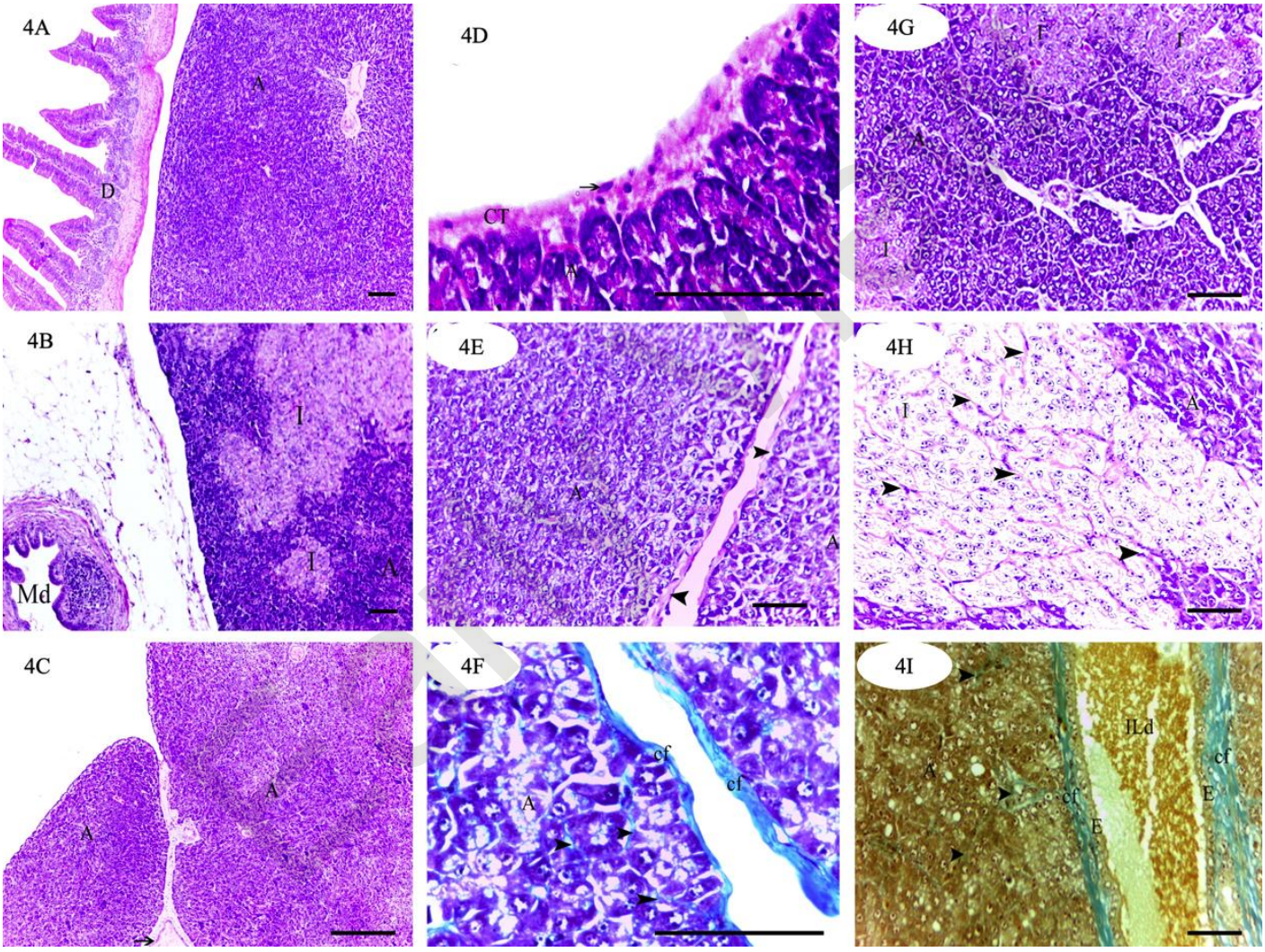

Figure 4: Photomicrograph of the stroma of the quail pancreas. 4A; pancreatic acini (A), duodenum (D). 4B; Pancreatic acini (A); small and large islets of Langerhans (I), main pancreatic duct (Md). 4C; Pancreatic acini (A), interlobular duct (arrow). A-C are Stained with H and E; Scale bars = 100 µm. 4D; Pancreatic acini (A), connective tissue capsule (CT), mesothelial cells (arrow). 4E; pancreatic acini (A), capsule (arrowheads). 4F; Pancreatic acini (A), collagenous fibers (cf), interacinar collagenous fibers (arrowheads).4D and E stained with H and E; 4F Stained with Masson's trichrome; Scale bars = 50 µm. 4G; Pancreatic acini (A), islets of Langerhans (I) stained with H and E. 4H; Pancreatic acini (A), large islet (I), basement membrane of the endocrine cells (arrowheads) stained with PAS. 4I; exocrine tissue of the pancreas (A), intralobular duct (ILd), simple cuboidal epithelium(E), collagenous fibers in lamina propria of the intralobular duct (cf), interacinar collagenous fibers (arrowhead) stained with modified aldehyde fuchsin stain. Scale bars = 50 µm

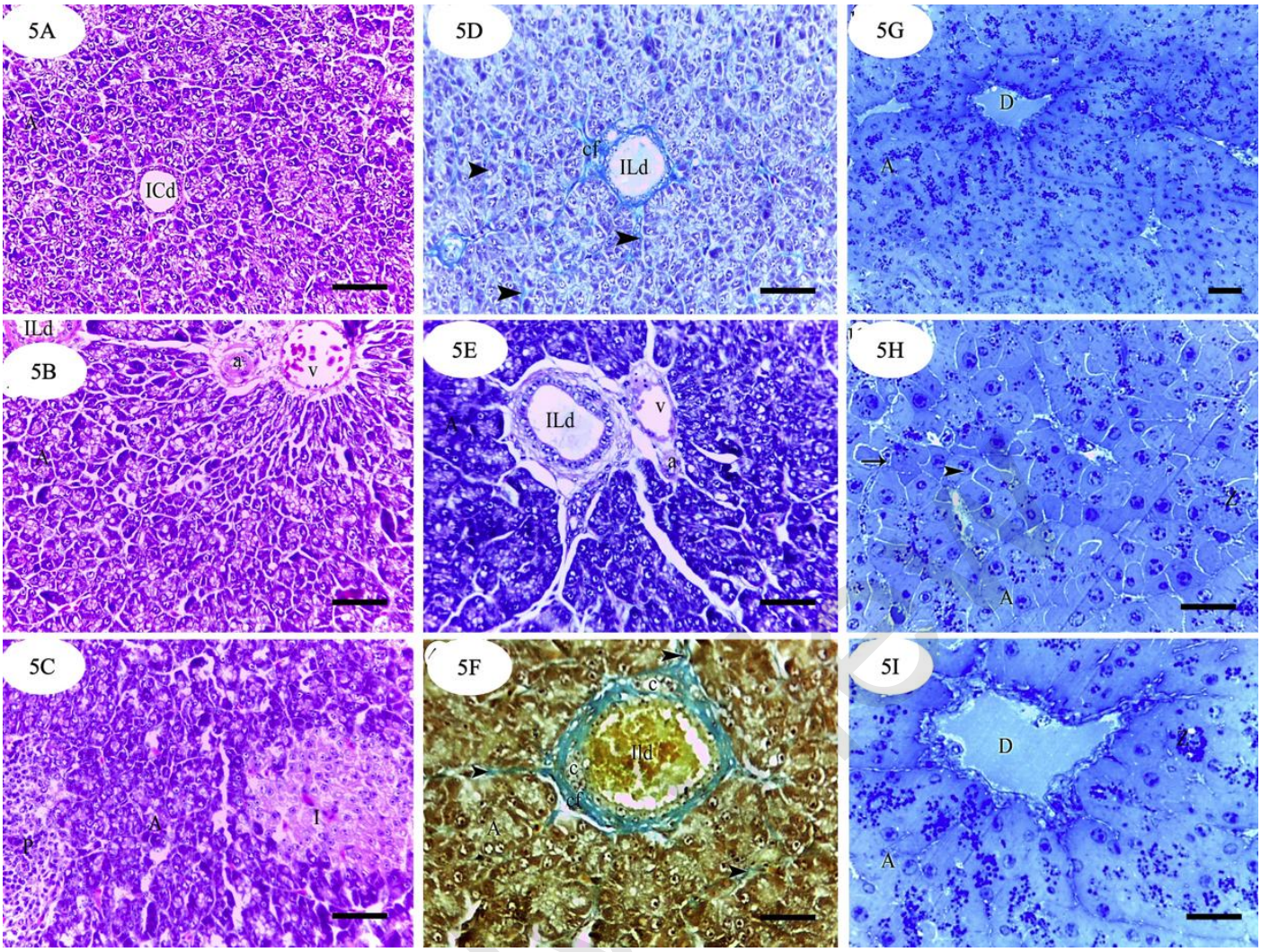

Figure 5: Photomicrograph of the exocrine portion of the quail pancreas. 5A-C; Pancreatic acini (A), intercalated duct (ICd), intralobular duct (ILd), branch of the pancreaticoduodenal artery (a), branch of the pancreaticoduodenal vein (v), parasympathetic ganglia (P), islet of Langerhans (I). 5A-C are stained with H and E; Scale bars = 50 µm. 5D; Pancreatic acini (A), intralobular duct (ILd) lined with simple cuboidal epithelium, thin collagenous connective tissue (Cf) surrounding the duct, interacinar collagenous fibers (arrowheads) stained with Masson's trichrome stain; Scale bars = 50 µm. 5E; Pancreatic acini (A), note the apical PAS-positive granules, intralobular duct (ILd) lined with simple cuboidal epithelium with apical PAS-positive secretory granules, branch of the pancreaticoduodenal artery (a), branch of the pancreaticoduodenal vein (v); stained with PAS; Scale bars = 50 µm. 5F; Pancreatic acini (A), intralobular duct (ILd) lined with simple cuboidal epithelium, thin collagenous connective tissue (Cf) surrounding the duct, crypt like glandular structure (c) in the connective tissue of the duct, interacinar collagenous fibers (arrowheads) stained with modified aldehyde fucshin, Scale bars = 50 µm. 5G, 5H, 5I; Semi-thin sections of the pancreas of the quail stained with toluidine blue showing the pancreatic acini (A) which consist of light and dark acini with apical zymogen granules and vesicular nucleus, pancreatic duct(D), apical zymogen granules (Z) and vesicular nucleus, centro-acinar cell (arrow); 5G; Scale bars = 100 µm and 5H, 5I Scale bars = 50 µm

Endocrine Portion of the quail's pancreas

The endocrine portion of the pancreas of the Japanese quail (islets of Langerhans) was found scattered within the exocrine portion of the pancreas in the form of large and small islets. The endocrine parts of the dorsal, ventral and third lobes of the quail pancreas were scattered singly or in small groups of islets of various shapes and sizes in the interstitium of the exocrine part. The islets are relatively numerous in the cranial or head regions**,** and decreased gradually in size and number of cells toward the tail regions to leave quite small islets in the tail regions. The endocrine tissue in the splenic lobe of the pancreas in male formed of mainly large islets concentrated in the center of the splenic lobe while in female the islets are mainly small and scattered in the complete splenic lobe (Fig. 8). The number of islets in the dorsal lobe of female quails was significantly higher than that of males ($p = 0.03$). The number of islets in the ventral lobe of the pancreas in male quail was higher than in female with no significant difference (p-value ≤ 0.09), while there were no significant differences in the number of islets between the third or splenic lobes in male and female (Fig. 9).

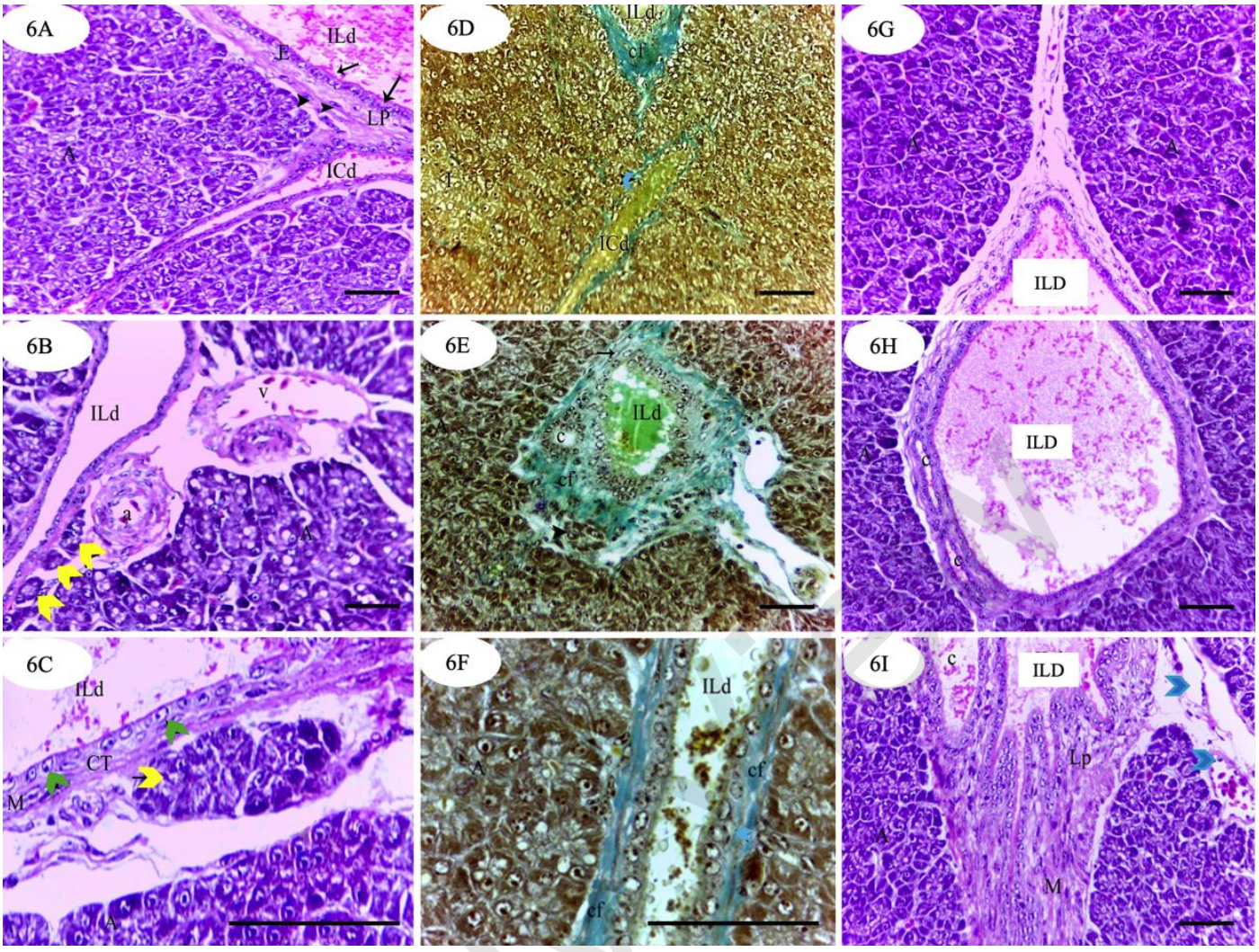

Figure 6: Photomicrograph of the intercalated, intralobular and interlobular ducts of the quail pancreas. 6A-C; Pancreatic acini (A), intralobular duct (ILd), cuboidal epithelium (E), intercalated duct (ICd), light cells (green arrowheads), lamina propria (LP), smooth muscle fibers (black arrowheads), secretory material on the luminal surface of the ductal epithelium (arrows), pancreatic acini (yellow arrowheads) within the connective tissue of lamina propria of the duct, branch of the pancreaticoduodenal artery (a), branch of the pancreaticoduodenal vein (v), connective tissue of lamina propria (CT), smooth muscle fibers (M) surrounding the duct, endocrine islet (I), collagenous fibers (cf) of lamina propria, interlobular duct (ILD), crypt-like glandular structure (c) blood vessels (blue arrowheads). 6A-C and 6G-I stained with H and E, 6D-F, stained with modified aldehyde fuchsin; scale bars = 50 µm; 6F scale bars = 25 µm

Discussion

The current study described the macroscopic and microscopic features of the pancreatic lobes of male and female quail. The quail pancreas was located on the right side of the abdominal cavity surrounded by the mesentery and blood vessels and positioned between the descending and the ascending duodenal limbs, which is in agreement with the previous reports in other avian species (1, 2, 7, 17, 19). The pancreas is the longest gland in birds. In our study, the quail pancreas filled the space between the duodenal limbs in quail, which in agreement with the reports in quail (7), and in red jungle fowl (15). In contrast to our results, the pancreas does not fill the space between the two duodenal limbs in goose (6), in golden eagle (20) and in white-eared bulbul *(Pycnonotus leucotis)* (21).

Gross morphological examination showed that the quail pancreas appears whitish to pale pink in color, regardless of sex. This observation aligns with the findings in chickens (5), in ostriches (22), in quail (7), in geese (23), and in Kestrel (*Falco tinnunculus*) (24). However, it contrasts the report that described the pancreas of captive bustards as pale yellow (25) and the pancreas of white-eared bulbul as white in color (21). In the current study, the pancreas of the quail consisted of four lobes: dorsal, ventral, third, and splenic in both male and female birds. The splenic lobe of quail pancreas was small and appeared as a small extension of the ventral lobe. This anatomical arrangement agrees with the findings of (5) in chickens, in geese (6), and in both black partridge (*Melanoperdix niger*) and local crow (*Linnaeus*

corvus) (11). In contrast, several authors reported that the duck pancreas comprises only three lobes (3, 26, 27). Similarly, the pancreas of the duck (3) and sparrow hawk (26), respectively, as having only dorsal, ventral, and splenic lobes without a third lobe. The splenic lobe was observed as a small extension of the ventral lobe (28), whereas the complete absence of the splenic lobe in the mynah bird was reported (4). On the other hand, the ostrich pancreas has three main lobes dorsal, ventral and third lobes while the splenic lobe was not macroscopically visible (29) however, it was detectable in microscopic sections. Furthermore, the pancreas of white-eared bulbul was formed only of two lobes; the ventral and the dorsal lobe (21).

The pancreas of the Japanese quail exhibits a completely smooth external surface and lacks the distinct lobulation characteristic of mammalian pancreata. This morphological feature is consistent with the general pattern described for avian species. In the present study, three pancreatic lobes were consistently identified: the dorsal, ventral, and third lobe. The dorsal and ventral lobes occupied the interduodenal space, while the smaller third lobe was positioned medially and closely associated with the main pancreatic ducts. Similar anatomotopographical arrangements have been reported in other birds including chicken and quail (14). The smooth surface and lobulated organization of the avian pancreas are considered adaptive features that facilitate its accommodation within the duodenal loop and support efficient exocrine and endocrine functions (14).

In the present study, the pancreas of females was 0.176 g heavier compared to males, which indicates a sex-based difference in the ratio of the pancreatic weight to the body weight of the bird, which might be linked to differences in metabolism. The pancreas weight in black partridge and local crow was 0.699 ± 0.33 and 0.705 ± 0.42 g, respectively (11). Furthermore, the mean weight of white-eared bulbul and whole pancreas weight was 27.6 ± 3.04 g and 0.07 ± 0.022 g, respectively, the mean weight of dorsal and ventral lobes was 0.034 ± 0.005 g and 0.036 ± 0.0054 g, respectively, which indicate a direct correlation between the size of the bird and the size of its pancreas (21).

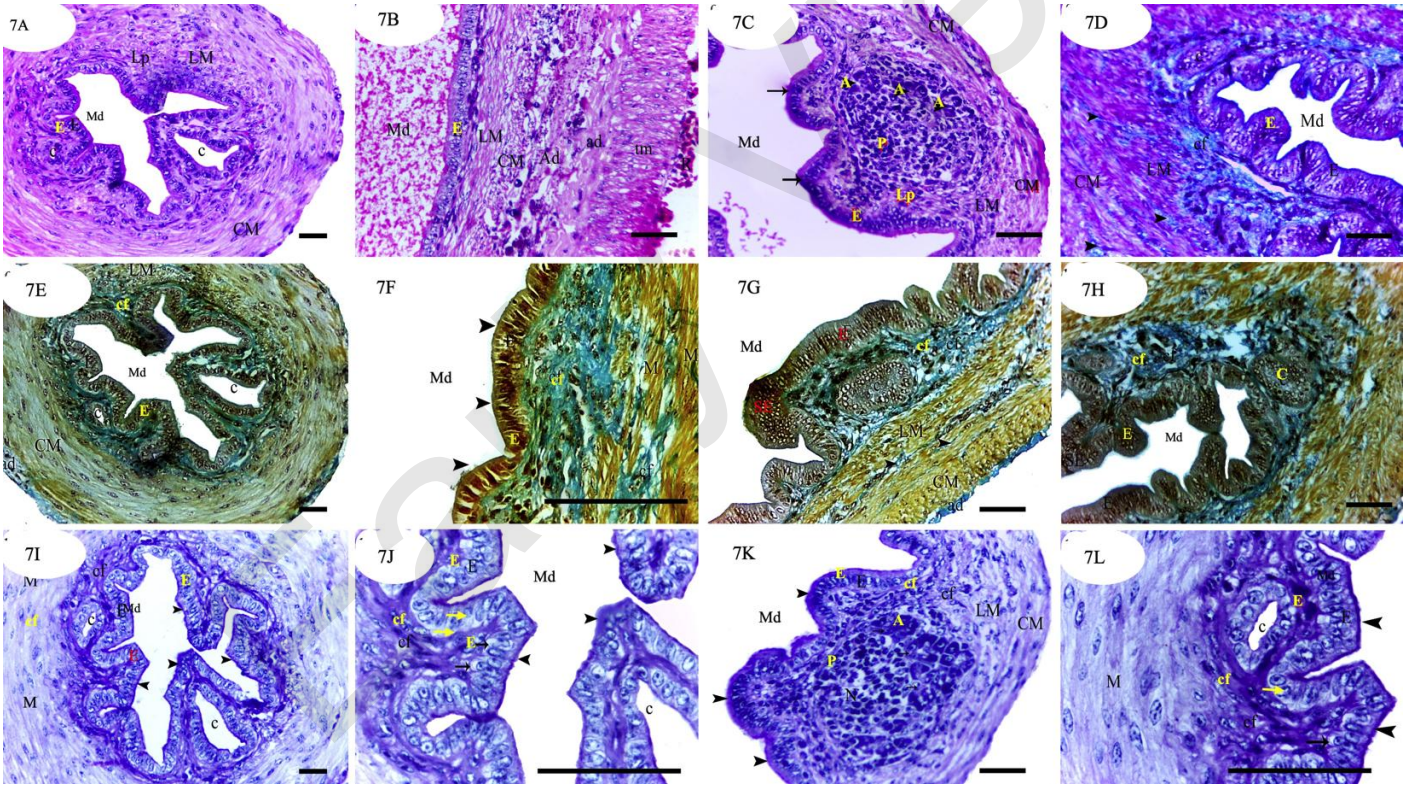

Figure 7: Photomicrograph of the main duct of the quail pancreas. 7A; Main pancreatic duct (Md), with small and large folds, columnar epithelium (E), lamina propria (Lp), crypt-like glandular structure (c), longitudinal layer of smooth muscle fibers (LM), circular layer of smooth muscle fibers (CM), adventitia of the main pancreatic duct (Ad), adventitia of the adjacent blood vessel (ad), tunica media of the blood vessel (tm), nucleated red blood cells (R), exocrine glands within the lamina propria (A), parasympathetic ganglia (P) within the lamina propria, secretory material (arrows) on the surface of epithelium, collagenous fibers (cf), collagenous fibers (arrowheads) among the muscle fibers, stratified columnar epithelium (SE), crypt like glandular structure (c), light cells (arrows), PAS-positive secretory material (arrowheads) coats the surface of the lining epithelium, parasympathetic ganglia (P) within the lamina propria. 7A-C stained with H and E; 7D stained with Masson's trichrome; 7E-H stained with modified aldehyde fuchsin stain. 7I-L stained with PAS. 7A, E and I scale bars = 100 µm while the rest of figure 7 scale bars = 50 µm

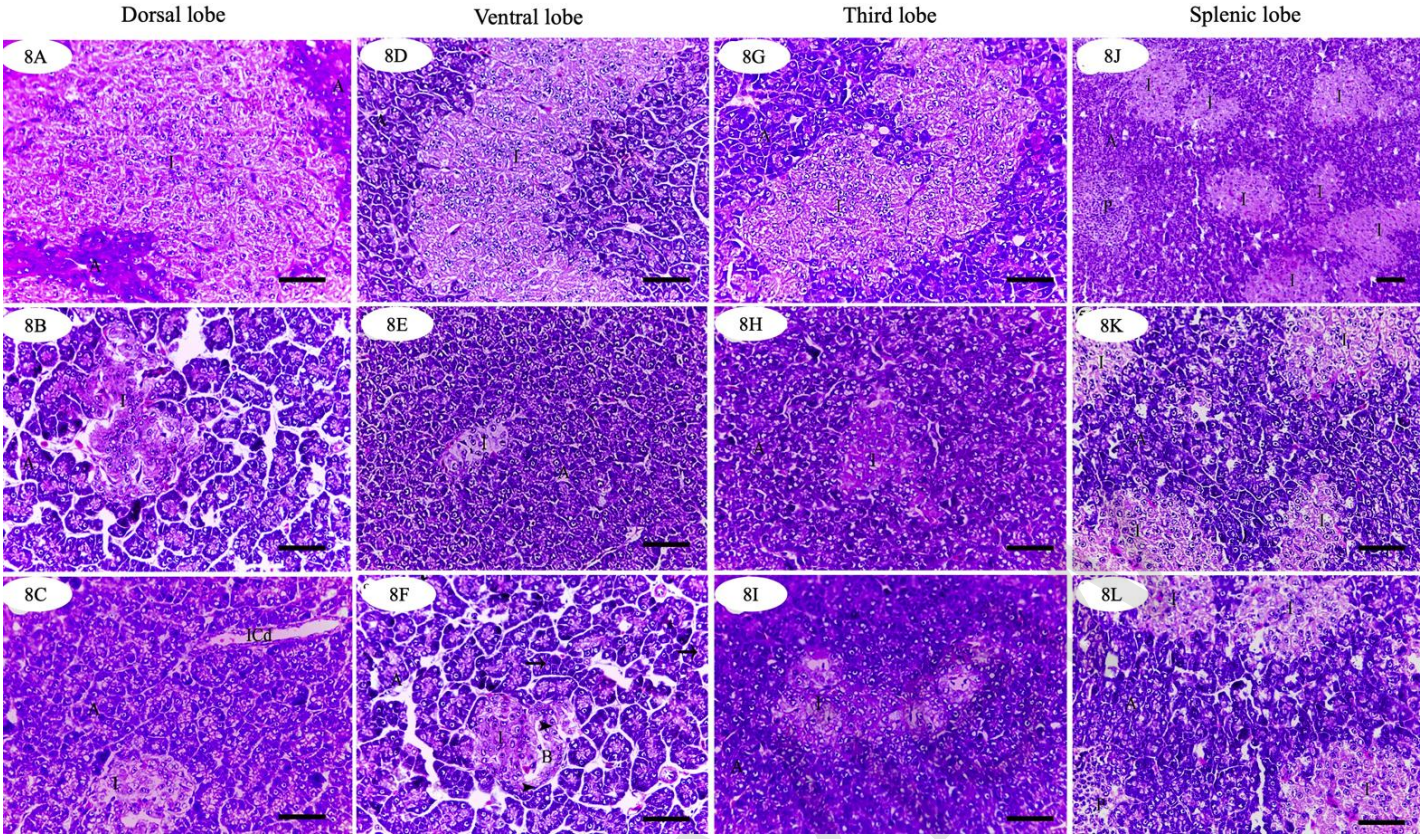

Figure 8: Photomicrograph of the endocrine portion of the quail pancreas. 8A-C, 8D-F and 8G-I represent the head, body and tail regions of the dorsal, ventral and third lobe of the pancreas respectively. 8J-L represent the splenic lobe of the pancreas of the quail. Pancreatic acini (A), islets of Langerhans (I) and intercalated duct (ICd), blood capillary (B), endocrine cells (arrowheads), centroacinar cells (arrows), parasympathetic ganglia (P). 8A-L stained with H and E stain; scale bars = 50 µm except 8I scale bar = 100 µm.

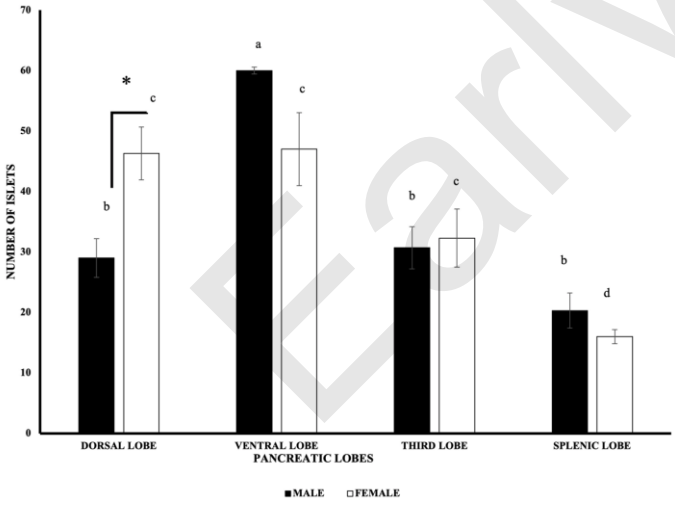

Figure 9: Number of islets in different lobes in male and female quails. * indicates significant in the same lobe between male and female. a, b indicate significant among the pancreatic lobes of the male quail. c, d indicate significant among the pancreatic lobes of the female quail. a

In contrast to our findings, the duck's pancreatic lobes varied in length measuring 6.83 ± 0.76, 4.72 ± 0.34, and 3.97 ± 0.63 cm for the dorsal, ventral and third lobe, respectively (27). In the current study, the pancreatic lobes was slightly higher but with no significant differences (47.26 ± 5.42, 45.26 ± 3.99, and 43.39 ± 4.17 mm for the dorsal, ventral and third lobe in male and female, respectively). The mean length of dorsal and ventral lobe was as small as 1.3 ± 0.05, 0.88 ± 0.03 cm respectively, in the white-eared bulbul (21). The whole pancreas of turkey has a total length of 9.82 ± 0.28 cm, width of 2.17 ± 0.10 cm and thickness of 1.12 ± 0.08 cm (30). The width of the pancreatic lobes was significantly higher in female quail than in male. The dorsal lobe of female pancreas was also wider than the dorsal and ventral lobes.

Histologically, in both male and female quail, the pancreas was encapsulated with a thin layer, which made up mostly of collagen fibers and covered with a single layer of mesothelial cells. Septa extend from the capsule to divide each lobe into lobules. This result is in agreement with the findings in quails (7 and 31), in duck (11, 27, 32, 33), in goose (13, 15, 19, 23), in red jungle fowl (16), in golden eagle (20), in Kestrel (24) and in white-eared bulbul (21). In contrast, in turkey (10, 28 and 34) and in ostrich (9, 29, 35) the capsule of the pancreas was thick.

The parenchyma of the pancreas in male and female quail consisted of exocrine and endocrine tissues. The exocrine portion composed of the pancreatic acini and the duct system. The pancreatic acini of the pancreas of the quail was pyramidal in shape with basal basophilic and apical acidophilic staining. The bizonal character of the acinar cells could be attributed to the presence of ergastoplasm and zymogen granules in basal and

apical portion, respectively. The results of the current study are in agreement with the previous results in most birds (9, 11, 19, 21, 27 and 28). The pancreatic acini in birds varies in shape from triangular to tall columnar in duck (6 and 31), columnar in goose (6, 13), spherical mass in goose and in golden eagle (13, 19, 20). Other reports stated that acinar cells were pyramidal to tall columnar in shape in turkey (29), rounded, oval, or tubular in ducks, mainly tubular in turkey where the acini size and the height were bigger (10), pyramidal in adult Kestrel (24). These variations in shape may be due to the plane sections of the tissue. Semi-thin sections of the pancreas stained with toluidine blue showed the acinar cells have zymogen granules in the apical portion with deep blue color, and basal nuclei. Blood capillary and rounded centroacinar cells without granules were identified in the central lumen of the acinus. These results are similar to the findings in albino rat (36), goose (19), duck and pigeon (33). In quail (7) and in duck (19) the presence of the dark and light pancreatic acini were reported, which could not be detected in our study.

Several lymphoid aggregations and parasympathetic ganglia were found adjacent to the islets of Langerhans, pancreatic ducts and blood vessels in the parenchyma of the pancreas in male and female quail. These results are similar to what have been reported in duck (13 and 31) and in goose (19).

The centroacinar cells were rarely observed in the central lumen of the acinus, which is in agreement with the previous studies in goose (6) and in duck (31). In agreement with the current findings in the pancreas of male and female quail, the presence of centroacinar cells was previously reported in duck (10 and 37), red jungle fowl (15), dove (38), pigeon (1) goose (6 and 19), ostrich (9) turkey (10) and in black partridge (11). In contrast, no centroacinar cells were observed in crow (11) where the duct system started from the intercalated duct.

The intercalated duct was lined with squamous to low cuboidal epithelium. The intercalated duct extended as intralobular duct, which is lined by a single layer of simple cuboidal epithelium, thin layer of connective tissue surrounds the wall of this duct which is similar to all previous research in goose (32) and golden eagle (19). Three types of cells were detected within the lining epithelium of the interlobar ducts: principal, light and basal cells, which is in agreement with the results in duck and goose (14 and 31). The mucosa of this duct had several folds and invaginations; this is in line with the previous reports in goose (2 and 32). The interlobar duct was surrounded with a thick layer of connective tissue that had glands like crypts, this result is in line with the previous findings in goose (2 and 32) and in contrast in red jungle fowl (15). The main excretory duct of the pancreas in male and female quail consisted of three layers, mucosa, muscularis and adventitia, which is similar to the results in other birds (7, 11, 20, 32 and 39). The mucosa of the main duct was folded and line simple to stratified columnar epithelium. In agreement with our study, in duck (3) and ostrich (28) studies reported that the pancreatic duct contains accessory pancreatic tissue in the lamina propria. Neither goblet cells nor ductal glands were found in the pancreatic ducts of the pancreas in male and female quail while in other studies there were numerous goblet cells in the interlobar duct (32 and 37). The apical neutral and sulfomucin reaction in our results indicated involvement of the lining epithelium and the extra-epithelial ductular glands (crypt like glandular structure) of the pancreatic duct with secretion, which agrees with the previous reports (6 and 32). These results suggest that the lining epithelium of the pancreatic ducts have a dual action to facilitate the transport of the pancreatic juice and as a protective barrier for protection from autodigestion.

In the current study, the exact border between the endocrine part and exocrine part of the quail pancreas was not distinguished in the quail pancreas, which is similar to the findings in other avian species (6 and 21). The four lobes contain a variable amount of the endocrine tissue where the splenic lobe contains the highest proportion in comparison to its size. The number of islets of Langerhans in the dorsal lobe of the pancreas in female quail was significantly higher than in male. The highest count of endocrine tissue, which was formed of small and large islets, were found in ventral lobe of the pancreas in male while the lowest count was in the splenic lobe of the pancreas in female quail. This result is in contrast with the report in the third lobe has more endocrine islets than the dorsal and ventral lobes (40). In agreement with our results, in the pancreas of both sexes of chicken, there were significantly distinctive regional differences in the volume of islet and the numerical density of cells among some lobes (41). Distinct sexual dimorphism in the chicken pancreas, which depends on the morphometrical features of the islets in each lobe. In both sexes of chicken, the islets were more frequently found in the splenic and the third lobes, whereas they were more scarcely observed in the ventral and the dorsal lobes (16), which is opposite to our results. The differences between the number of endocrine islets in females and males may be due to their differences in their metabolic activities.

Conclusions

In conclusion, the pancreas of the Japanese quail exhibits characteristic avian anatomical features, including a smooth, non-lobulated surface and a consistent lobar arrangement, with significant sex-related differences observed in the pancreatic morphometry and histological organization, reflecting species-specific structural adaptation and sexual dimorphism. Further studies are required to explore the sex-based differences in the structure and function of the pancreas in different animal models for better understanding of the related diseases.

Study limitations

Despite providing a comprehensive macroscopic and microscopic characterization of the pancreas in male and female Japanese quail, the present study has certain limitations. Quantitative histomorphometric analysis of pancreatic tissue components including volume, density and size was not performed. As a result, the proportional contributions of stromal, exocrine, and endocrine components could not be expressed as percentages.

Additionally, due to the extremely small and delicate nature of the splenic lobe, reliable morphometric measurements of this lobe could not be obtained and were therefore excluded from quantitative analysis. Furthermore, although detailed morphometric comparisons were conducted between sexes, direct absolute comparisons with larger avian species is required to study the relation between the body size and metabolic scale. Future studies employing stereological techniques, quantitative image analysis, and comparative approaches in relation to body weight or tissue proportion are recommended to overcome these limitations and further elucidate structural and functional variations of the avian pancreas.

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