

# Expression of Insulin, Glucagon, Somatostatin, and Pancreatic Polypeptide in the Pancreas of the Eurasian Moorhen (*Gallinula chloropus*)

## Key words

pancreas;  
islet;  
insulin;  
glucagon;  
somatostatin;  
polypeptide;  
diabetes

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**Abstract:** The pancreas is a complex gland that possesses both endocrine and exocrine functions. The present study investigated the gross anatomy, histochemical features, and immunohistochemical expression of insulin, glucagon, somatostatin, and pancreatic polypeptide in the pancreas of the Eurasian moorhen. Grossly, the pancreas consisted of three duodenal lobes and unpaired splenic and gastric lobes. The pancreatic capsule appeared thin with no distinct lobulation pattern. Three islet types were observed namely alpha, beta, and mixed types. The alpha-type islets formed mainly of glucagon- and somatostatin-expressing cells. The beta-type islets appeared relatively smaller than the two other islet types and formed predominately of insulin-expressing cells with a limited number of other endocrine cells. The mixed islets were formed by almost equal proportions of insulin-, glucagon- and somatostatin-expressing cells. A higher number of alpha-type islets was observed in the splenic lobe than in other pancreatic lobes. Unlike other pancreatic endocrine cells which appeared oval or triangular in shape, somatostatin-expressing cells appeared with irregular outlines with cytoplasmic processes contacting each other's forming a meshwork within the islet. The results of this study revealed species-specific features of the endocrine and exocrine pancreas in the Eurasian moorhen and could suggest pancreatic functional differences depending on feeding habits.

Received: 21 October 2022  
Accepted: 10 July 2023

## Introduction

The pancreas is a glandular organ associated with the gastrointestinal tract with both digestive (exocrine) and hormone-secreting (endocrine) functions. The avian pancreas consists of 2-3 duodenal lobes and a single splenic lobe, all located mainly inside the U-shaped coil formed by the duodenum in these species (1). The exocrine pancreas is formed by pancreatic acini and ducts. The latter structures are presented in a progressive order and act mainly to drain the secretion of the pancreatic acini into the duodenum. The endocrine pancreas is made up of clusters of endocrine cells termed the pancreatic islets (of Langerhans). The latter structures are considered as micro-organs and produce several hormones including insulin (beta cells), glucagon (alpha cells), somatostatin (delta cells) and pancreatic polypeptide, pancreatic polypeptide (PP cells) (2).

In contrast to the mammalian pancreas which contains only a mixed type of islets, the avian pancreas contains more than one islet type. The three main types of islets reported in the avian species are alpha islets (formed predominately of alpha cells), beta islets (formed predominately of beta cells), and mixed type islets (contains equal proportions of alpha and beta cells) (3). Different types of pancreatic islets present in the avian pancreas act cooperatively to orchestrate the blood glucose levels, a major source of bird energy. Failure of the endocrine pancreas to maintain sufficient levels of pancreatic hormones in blood is associated with incidence of diabetes mellitus. Indeed, diabetes mellitus with an absence of insulin expression has been reported in pet birds (4, 5). The avian pancreas has been reported to be a target for pathogenic viruses, including avian influenza

and Newcastle disease viruses, that commonly infect birds (6, 7).

The Eurasian moorhen (*Gallinula chloropus* - Linnaeus, 1758), also called the marsh hen or the black gallinule, is a principal member of the family Rallidae that has been shown to withstand harsh environmental conditions. It is the most popular member of the family Rallidae around the world (8). Moorhens are distributed across several areas of the world including the Middle East, South and Central Asia, Europe, and North and South America (9, 10). Their distribution is regularly affected by seasonal changes as they usually migrate during the winter towards the southern parts of their range (11). The meat of moorhens is also famous for its great taste and is often consumed by people in their vicinity.

Understanding the functional anatomy of the pancreas is essential for interpreting interspecies-specific differences in diet and lifestyle. In the present study, the anatomy, microstructure, and distribution of different types of pancreatic islets in various regions of the moorhen pancreas were investigated for the first time. Furthermore, the relative proportions of hormone-expressing cells were compared among the pancreatic islets, the exocrine pancreas, and the pancreatic ducts.

## Materials and methods

### Animals and sampling

Ten adult black gallinules (*Gallinula chloropus*) from both sexes collected from vegetated areas near the Nile Delta of Dakahliya Governorate (Egypt) were obtained from a local distributor. The birds were transported in suitable cages to the Anatomy Laboratory at the Faculty of Veterinary Medicine at Mansoura University. The animal study protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine at Mansoura University (Code no. R.41), agreed with the ARRIVE guidelines, and was done in accordance with the National Institutes of Health guide for use of animals in research (NIH Publications No. 8023, revised

1978). All birds were euthanized by cervical disarticulation followed immediately by complete blood exsanguination. Seven birds were utilized for identifying different pancreatic lobes and characterizing the histological and histochemical features of the pancreas. The whole pancreas of the three remaining birds was extracted and different lobes were processed for serial sectioning and subsequent screening of islet composition and distribution within the whole organ using immunohistochemistry (12). Pancreases were collected as soon as the birds became completely immobile, trimmed from surrounding tissues, and fixed in 10% neutral buffered formalin. Following their fixation, pancreatic tissue samples were dehydrated, cleared, and embedded in paraffin.

### Histological and histochemical analyses

Paraffin sections of 4- $\mu$ m thickness were cut and allowed to dry at 56 °C for 1 hour. After that, the sections were dewaxed, rehydrated, and the visualizations of the exocrine and endocrine components of the pancreas were enhanced using the following stains: hematoxylin and eosin (H&E), Masson's trichrome, alcian blue-PAS (AB-PAS), and AB-aldehyde fuchsin following the protocols adopted by Suvarna et al. 2018 (13). AB-aldehyde fuchsin staining was employed to better differentiate the pancreatic islets from the surrounding exocrine tissue (14).

### Immunohistochemistry

Following their dewaxing and rehydration, tissue sections selected for the immunohistochemical procedure were prepared as previously described (15, 16). Briefly, antigenic epitopes were retrieved via boiling the sections in citrate buffer (pH = 6) using a microwave at 750 watts for 20 minutes. To minimize nonspecific binding of antibodies, tissue sections were incubated with 5% bovine serum albumin (BSA) for 1 hour before their incubation with the primary antibodies. The optimal working dilution for each primary antibody, that achieved strong positive signals with minimal background, was determined and used. Incubation of sections with primary antibodies was done for 3 hours at room temperature. Details of primary antibodies used in

**Table 1:** Primary antibodies used in the present study

Target protein	Clone	Host	Code #	Company	Dilution
Insulin	Monoclonal anti-Insulin (EPR17359)	Rabbit	ab181547	Abcam (Cambridge, MA, USA)	1:100.000
Glucagon	Monoclonal anti-Glucagon (ICACLS)	Mouse	14-9743-80	Invitrogen (Waltham, MA, USA)	1:500
Somatostatin	Monoclonal anti-Somatostatin (ICACLS)	Mouse	14-9751-80	Invitrogen (Waltham, MA, USA)	1:200
Pancreatic Polypeptide	Polyclonal anti-Pancreatic polypeptide (1973)	Goat	NB100-1793	Novus Biologicals, (Littleton, CO, USA)	1:200

the present study are shown in Table 1. For negative control sections, primary antibodies were omitted and replaced with the blocking buffer. Next, all sections were washed thoroughly in PBS containing 0.05% Tween-20 (PBST) and further incubated with corresponding biotinylated secondary antibodies (Jackson ImmunoResearch, West Grove, PA, USA) for 30 minutes.

The VECTASTAIN Elite ABC-Horseradish Peroxidase (HRP) kit (PK-6100, Vector Laboratories, Burlingame, CA, USA) was applied for an additional 30 minutes to allow binding to the secondary antibody. The latter step was preceded by covering the sections with 0.3% H<sub>2</sub>O<sub>2</sub>/PBS for 20 minutes to quench the endogenous peroxidase activity. Bound antibodies were visualized by incubating the stained sections with diaminobenzidine solution (SK-4103, Vector Laboratories) for 2-10 minutes. All sections were finally counterstained with hematoxylin, dehydrated, and cleared (17, 18).

### Photomicrography, morphometry, and cell quantification

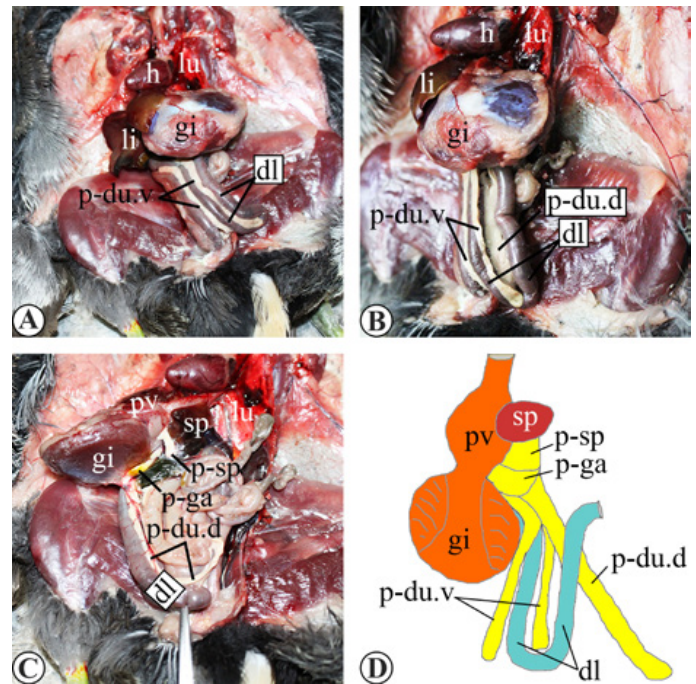
All stained sections were analyzed and photographed using a Leica DM3000 light microscope. For the histological and histochemical analyses, three pancreatic sections 500 µm apart were examined per bird. For the immunohistochemical study, 12 sections 100 µm apart spanning the whole pancreas were evaluated. Islet diameter was estimated using the measure tool of the ImageJ2 software (19). The number of cells expressing insulin, glucagon, somatostatin, and pancreatic polypeptide per every 100 cells was quantified within and outside (acinar and ductal epithelium) the pancreatic islets as described previously (15).

### Statistical analysis

Data were analyzed using the GraphPad Prism 7 (GraphPad Software, CA, USA). Differences in islet diameter and distribution of each type of the hormone-expressing cells were determined using one-way ANOVA followed by Tukey's multiple comparison test. P-values ≤0.05 were considered significant.

## Results

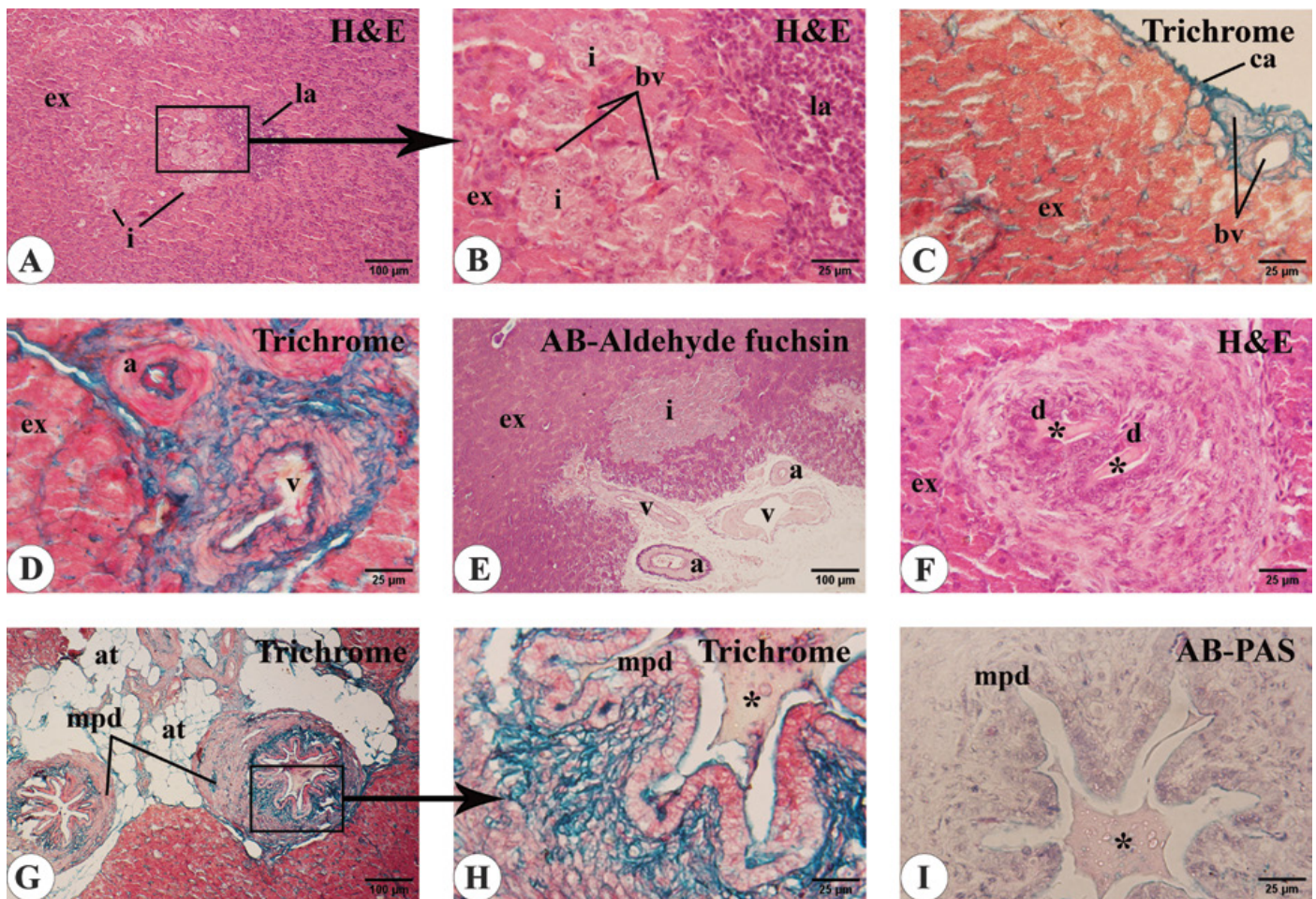
Grossly, the pancreas of the Eurasian moorhen appeared to consist of three duodenal lobes, two ventral and one dorsal, a single gastric lobe, and a single splenic lobe (Figure 1). The nomenclature of these lobes is based on their relation to the duodenal loop, the gizzard, and the spleen respectively (Figure 1A-C). The dorsal duodenal lobe is the largest of all lobes. It was located dorsal and parallel to the left ventral duodenal lobe and continued cranially with the gastric and splenic lobes (Figure 1B,C,D).



**Figure 1:** Gross anatomic appearance of the pancreas in the Eurasian moorhen. Photographs A-C) and schematic representation D) of ventral views of the celomic cavity showing different parts of the pancreas and their relations to surrounding organs. The paired ventral duodenal lobes (p-du.v) are displaced to show the single dorsal duodenal lobe (p-du.d) in B. The gizzard (gi) is also right-shifted to reveal the gastric (p-ga) and splenic (p-sp) parts of the pancreas. Abbreviations: dl, duodenal loop; h, heart; li, liver; lu, lungs; pv, proventriculus; sp, spleen

Microscopically, in both H&E- and AB-aldehyde fuchsin-stained sections, the pancreatic islets appeared as lightly stained cellular clusters permeated by a large number of blood vessels and surrounded by the acini of the exocrine pancreas (Figure 2A,B,F). Lymphoid aggregations were occasionally seen in the vicinity of a number of islets (Figure 2B). Large-sized pancreatic islets (> 200 µm in diameter) were seen within the dorsal duodenal and the splenic lobes (Figure 2E). The pancreas was invested by a thin connective tissue capsule through which pancreatic blood vessels find their way to the pancreatic parenchyma (Figure 2C-E). The lobulation of the pancreas appeared indistinct and the parenchyma of each lobe formed a single entity. The pancreatic ducts appeared of variable sizes and secretory activities based on their position within the pancreatic ductal tree. The wall of the pancreatic ducts appeared thicker, and their lumen became irregular towards the ductal exit from the pancreas (Figure 2F-I). The pancreatic secretion is collected by two main pancreatic ducts that were situated near the midparts of the three duodenal lobes (Figure 2G). These secretions showed moderate reactions for alcian blue-PAS staining (Figure 2I).

Analysis of insulin-expressing cells within the Eurasian moorhen pancreas revealed the presence of three islet types: alpha, beta, and mixed (Figure 3 A-L). The alpha-type pancreatic islets lacked insulin expression, though few cells could be observed in a number of serially sectioned islets (Figure 3 A). These islets consisted mainly of glucagon-,



**Figure 2:** Microscopic architecture of the pancreas in the Eurasian moorhen. Pancreatic islets (i) appeared as lightly stained cellular clusters surrounded by the acini of the exocrine pancreas ("ex" in A,B,E,F). The connective tissue fibers are distributed mainly within the capsule (C) and the wall of the blood vessels (D) and large pancreatic ducts (G,H). Note the secretion within the pancreatic ducts ("\*" in F, G-I). Abbreviations: a, artery; at, adipose tissue; bv, blood vessel; ca, capsule; d, duct; la, lymphoid aggregation; mpd, main pancreatic duct; v, vein

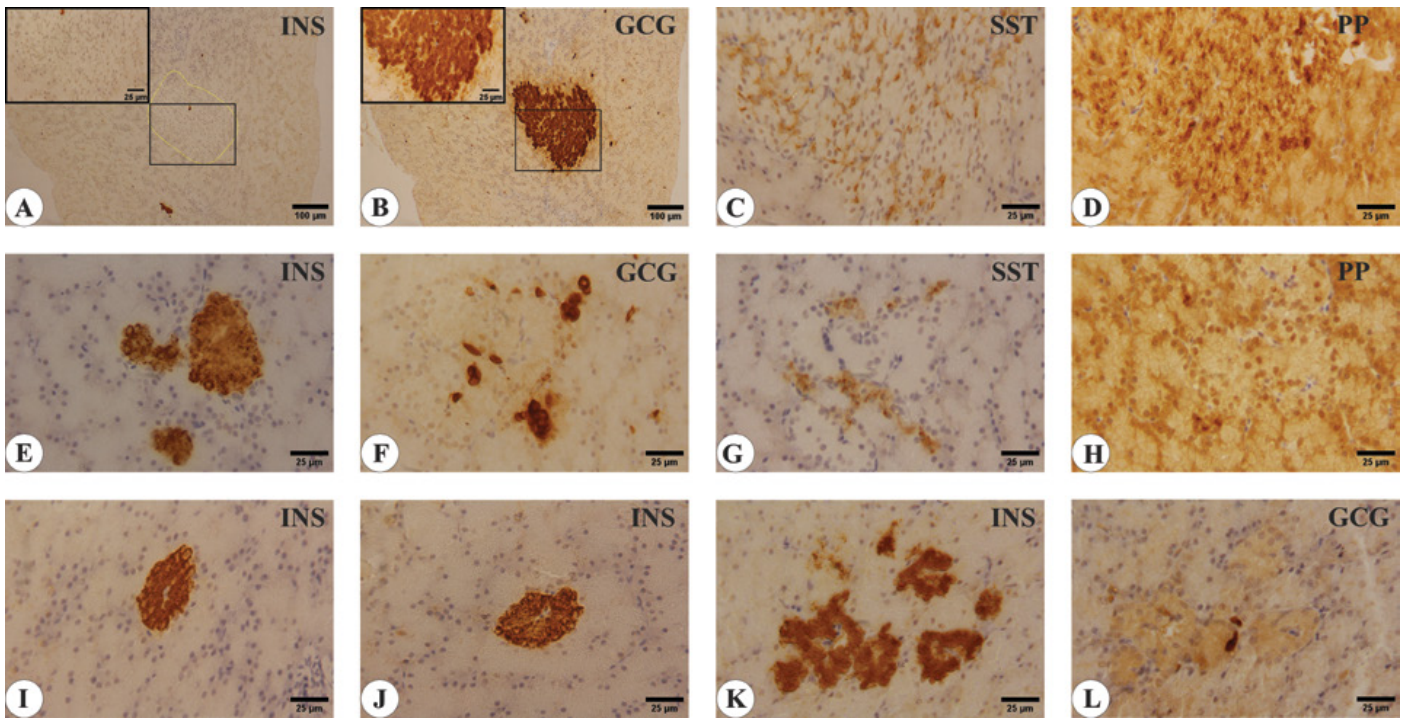
somatostatin-, and PP-immunoreactive cells of decreasing proportions (Figure 3 B-D, Table 2). The mixed-type islets contained almost equal proportions of the insulin- and glucagon-expressing cells in which the former type of cells tended to have a centric position within the islet (Figure 3 E,F). The beta-type pancreatic islets appeared of smaller diameter ( $38 \pm 2.9 \mu\text{m}$ ) compared to the alpha- ( $183 \pm 55.4 \mu\text{m}$ ,  $P < 0.001$ ) and mixed-type ( $72 \pm 15.6 \mu\text{m}$ ,  $P < 0.05$ ) islets and formed predominately of insulin-expressing cells (Figure 3I-L). Although the majority of insulin-expressing cells were found within the islets especially those of beta and mixed types (Figure 4 A-C), a fraction of these cells was also detected within or near the wall of pancreatic ducts especially those having small size (Figure 4 D, E). The specificity of these immunoreactions was confirmed by their absence in pancreatic sections incubated with no primary antibody (Figure 4 F).

Regarding the glucagon-expressing cells within the Eurasian moorhen pancreas, they revealed widespread expression within the pancreatic islets (Figure 5 A, B), the exocrine pancreatic acini (Figure 5C), and the pancreatic ducts (Figure 5 D, E). Their numbers markedly exceeded those of

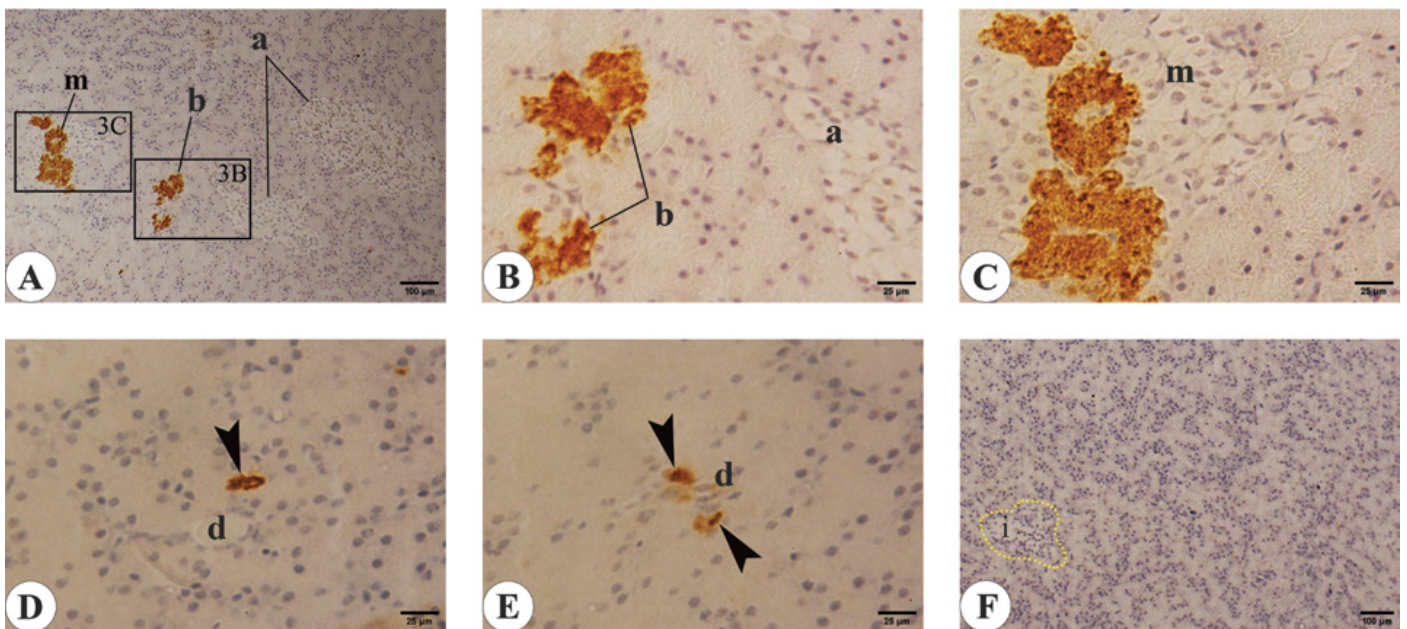
insulin-expressing cells, especially those located within the exocrine pancreas and pancreatic ducts (Table 2). They revealed the highest abundance within the alpha-type islets especially those of the dorsal duodenal and splenic lobes (Figure 5B). The extra insular glucagon-expressing cells appeared with regular outlines without any cytoplasmic branching (Figure 5 D).

The somatostatin-expressing cells revealed a branched morphology especially in the alpha-type pancreatic islets (Figures 3C, 6 A-D). The cytoplasmic processes of these cells formed an interconnected network surrounding the other types of islet cells (Figure 6 C, D). The frequency of these cells was observed to be proportionally related to that of the glucagon-expressing cells within the islet (Figure 6 A, B).

The pancreatic polypeptide-expressing cells were found mainly toward the periphery of alpha- and mixed-type islets (Figure 3 D, H). They revealed oval or triangular shapes and their frequency appeared higher within the small ( $< 50 \mu\text{m}$  in diameter) and medium-sized ( $> 50$  to  $100 \mu\text{m}$  in diameter) islets compared to the large-sized ( $> 100 \mu\text{m}$  in



**Figure 3:** Representative photomicrographs for the distribution of hormone-expressing cells within the three different types of pancreatic islets of the Eurasian moorhen pancreas. A-D) Alpha islets. E-H) Mixed islets. I-L) Beta islets. The islet perimeter is indicated by a dotted yellow line in Figure 3A. INS, insulin; GCG, glucagon; SST, somatostatin; PP, pancreatic polypeptide



**Figure 4:** Representative photomicrographs for insulin expression in the dorsal duodenal lobe of the Eurasian moorhen pancreas. A-C) Insulin-expressing cells were observed within beta (b) and mixed (m) type islets but were absent within the alpha-type islets (a). D,E) Insulin-expressing cells (arrowheads) were occasionally seen close to the wall of small pancreatic ducts (d). F) Negative control. The islet (i) perimeter is indicated by a dotted yellow line in Figure 4F

diameter) ones (Figure 7 A-C). A number of these cells was also seen within or near the lining epithelium of pancreatic ducts (Figure 7 D, E). The specificity of such immunoreactions was verified by their absence in stained pancreatic sections in which the primary antibody was omitted (Figure 7F).

A schematic representation for the distribution of various types of islets in different lobes of the Eurasian moorhen pancreas is shown in Figure 8. Based on data from complete analyses of pancreases from three adult moorhens, the splenic and gastric lobes contained a higher number of alpha-type islets and fewer beta- and mixed-type islets than the paired ventral and single dorsal duodenal lobes.

**Table 2:** Distribution of endocrine cells in the Eurasian moorhen pancreas

Part	Pancreatic islet type			Exocrine pancreas	Ductal epithelium
	Alpha	Beta	Mixed		
Insulin	+/- (1.9±0.3 <sup>Cc</sup> )	++++ (98.7±9 <sup>Aa</sup> )	+++ (45.2±5.6 <sup>Ab</sup> )	+/- (0.5±0.01 <sup>Bd</sup> )	+/- (0.3±0.07 <sup>Cd</sup> )
Glucagon	+++ (74.7±9.6 <sup>Aa</sup> )	+/- (0.5±0.0 <sup>Be</sup> )	+++ (46±13 <sup>Ab</sup> )	+ (8.6±0.55 <sup>Ac</sup> )	+ (5.2±0.60 <sup>Ad</sup> )
Somatostatin	++ (21±5.0 <sup>Ba</sup> )	+/- (0.8±0.1 <sup>Bc</sup> )	+ (7.0±1.4 <sup>Bb</sup> )	+/- (0.1±0.00 <sup>Cd</sup> )	+/- (0.2±0.05 <sup>Cd</sup> )
Pancreatic polypeptide	+ (2.4±0.7 <sup>Ca</sup> )	- (0.0±0.0 <sup>Cc</sup> )	+/- (1.8±0.9 <sup>Ca</sup> )	+/- (0.2±0.00 <sup>Cb</sup> )	+ (2.3±0.55 <sup>Ba</sup> )

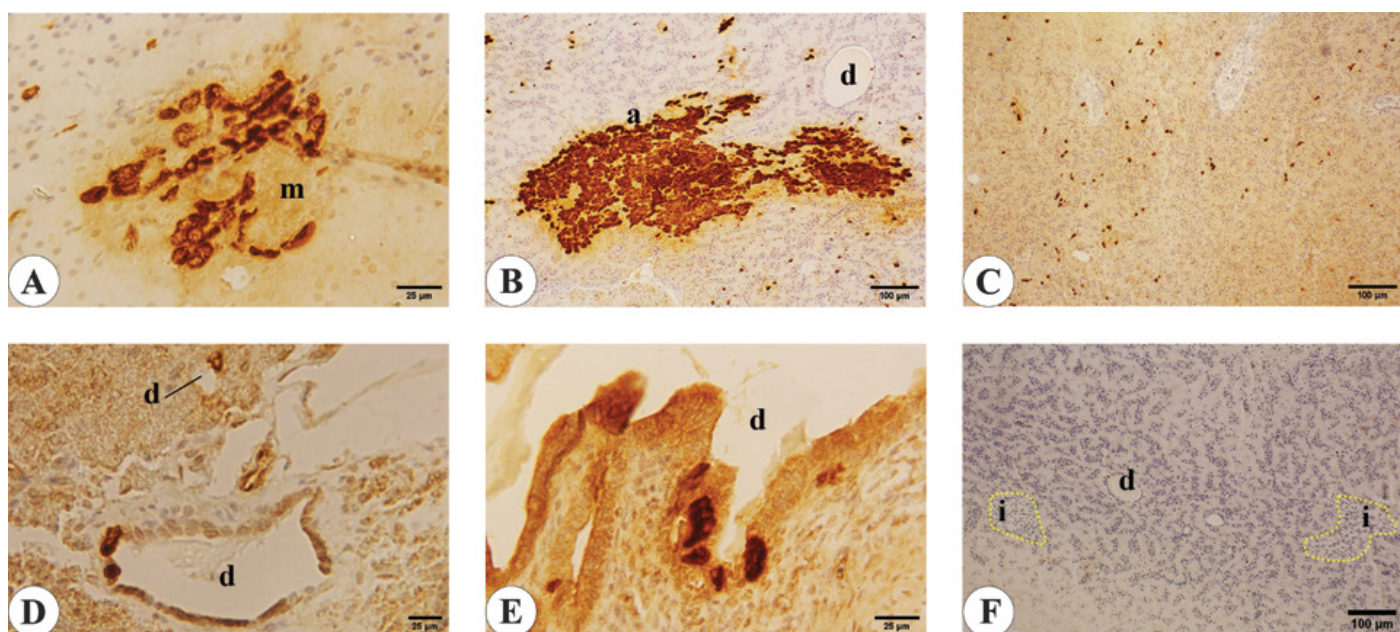
The number of hormone-expressing cells per each 100 cells was used to generate the following scheme: +/-, < 2 cells; +, 2-10; ++, 10.1-45; +++, 45.1-80; +++++, > 80. Different superscript letters (uppercases for columns; lowercases for rows) indicate statistical significance.  $P < 0.05$ .

## Discussion

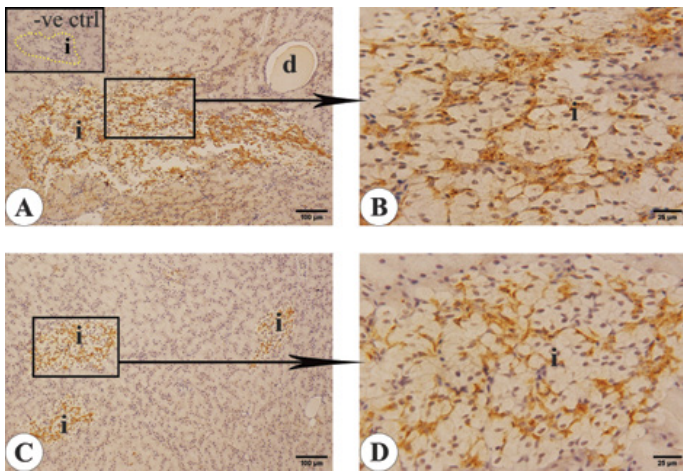
The composition of the endocrine pancreas is influenced by several physiological and pathological conditions that include fasting, and pancreatectomy. The pancreatic response to damaging insults varied according to the dietary regime. For instance, complete removal of the endocrine pancreas resulted in lethal hypoglycemia in granivorous birds, but it caused severe hyperglycemia and diabetes mellitus in carnivorous birds (20).

Differences in islet cell composition among different vertebrate species may also represent a further endocrine pancreatic adaptation. Unlike pancreatic islets of raptors which were dominated by insulin-expressing cells, the cellular composition of the three types of islets observed in the moorhen pancreas revealed a higher abundance of

glucagon-expressing cells than any other type of endocrine cells which is the case for a number of granivorous birds including chickens and ducks (21). Another similarity between the pancreas of moorhen and that of chicken is the presence of a large number of glucagon-expressing cells in the splenic lobe of the moorhen's pancreas. The presence of this large number of glucagon-secreting cells close to the spleen which is known for richness in blood possibly accelerates the delivery of the secreted glucagon to the systemic circulation. Tomita et al. noted that alpha-type islets constituted more than 75% of all islets present in the splenic lobe of chicken pancreas (22). Such enrichment of chicken pancreas with glucagon-expressing cells is usually reflected by their extremely high blood glucagon levels, ~ 10-fold more than those of mammals (20). Differences in body metabolic requirements between birds of prey and other herbivorous or omnivorous birds account for such



**Figure 5:** Representative photomicrographs for glucagon expression in the ventral duodenal (A, C-E) and splenic (B) lobes of the Eurasian moorhen pancreas. Glucagon-expressing cells were observed within mixed type islets (A), alpha type islets (B), exocrine pancreas (C), and pancreatic ductal epithelium (D,E) but were absent within the negative control section (F). The islet (i) perimeter is indicated by a dotted yellow line in Figure 5F. Abbreviations: a, alpha type islet; d, duct; m, mixed type islet



**Figure 6:** Representative photomicrographs for somatostatin expression in the splenic (A, B) and dorsal duodenal (C, D) lobes of the Eurasian moorhen pancreas. Somatostatin-expressing cells appeared with irregular outlines with interconnected cytoplasmic processes. The islet (i) perimeter is indicated by a dotted yellow line in the negative control (-ve ctrl) inset of Figure 6A. Abbreviations: d, duct; i, pancreatic islet

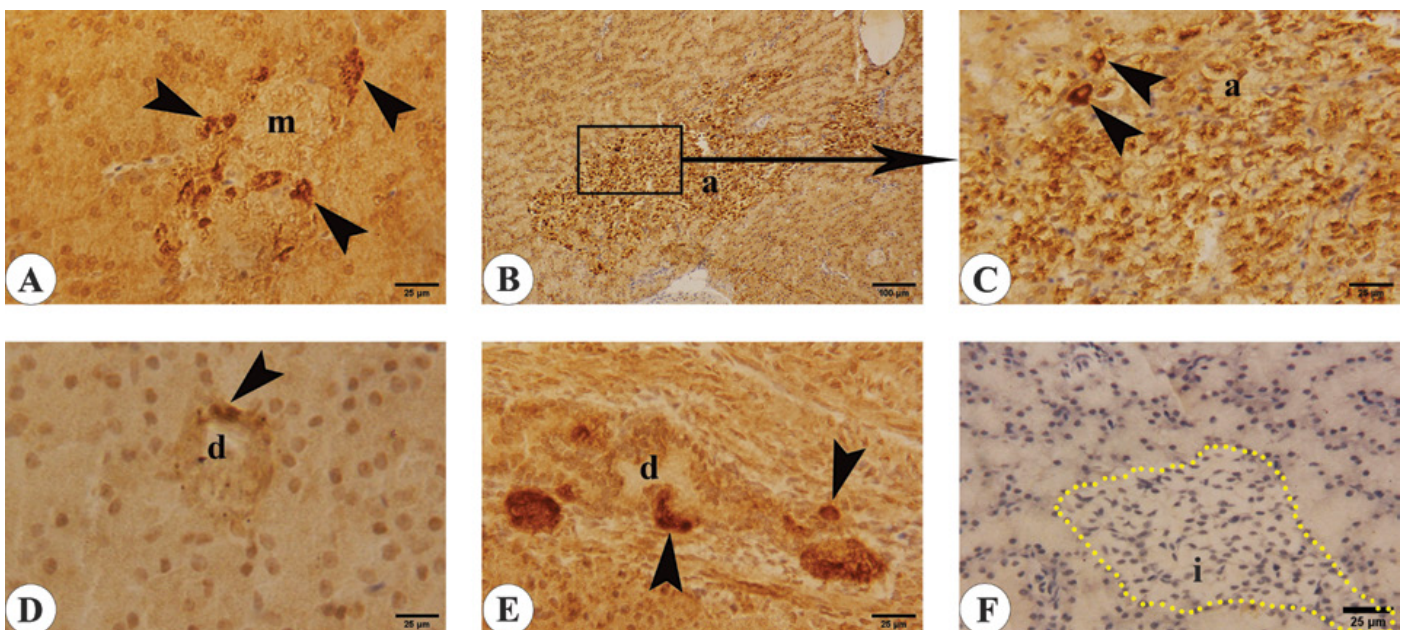
variation. Avian insulin has a strong anabolic effect; thus, it is required for building the body muscles required for hunting and grasping the prey. Conversely, glucagon is a potent catabolic hormone, and it is high levels in the herbivorous or omnivorous birds work to prevent hyperglycemic episodes via maintaining steady levels of blood glucose (23).

The pancreatic polypeptide-expressing cells appeared as the lowest abundant islet cell type within the Eurasian moorhen's pancreas. The functional roles and biological relevance of the pancreatic polypeptide-expressing cells in the avian pancreas remain poorly understood (24). The

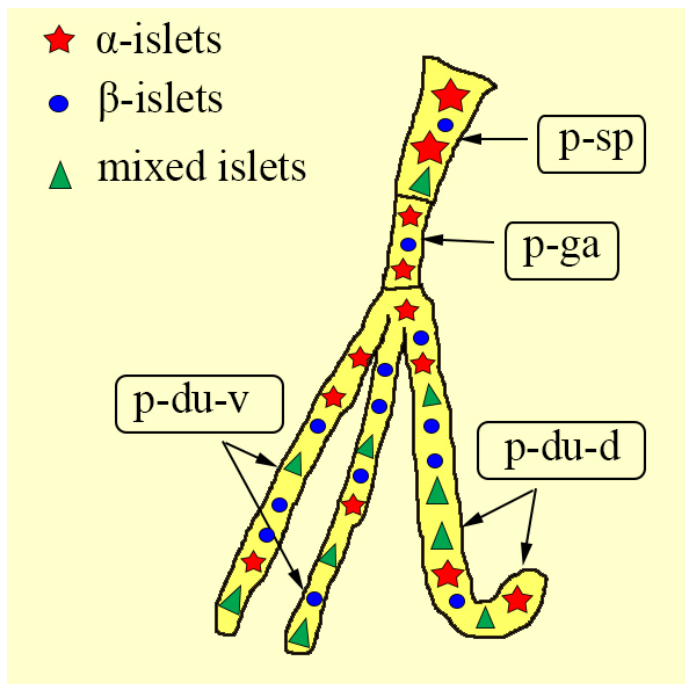
islet numbers of these cells were higher within the duodenal lobes of the pancreas than in the splenic lobes. This corresponds to their distribution pattern in the pancreas of other vertebrates including humans (25) and chickens (22).

Somatostatin-expressing cells or delta cells produce somatostatin hormone which suppresses the secretion of both glucagon and insulin from alpha and beta cells respectively (26). These cells continuously work to balance both the anabolic and catabolic activities of birds (27). Recently, reciprocal feedback from alpha and beta cells has been suggested (28). The juxtaposition of delta cells to other types of islet cells is fundamental for the proper interplay between these cells in terms of hormonal control. In accordance with a such important role, somatostatin-expressing cells were observed in all types of islets of moorhen's pancreas with a higher presence in the alpha-type islets by the present study and others (21). In contrary to islets of humans and rodents in which somatostatin-expressing cells appear with regular outlines (29), the somatostatin-expressing cells appeared branched in moorhen's pancreas suggesting a role for cell-cell contact alongside the classical paracrine mode of signaling in the studied avian species (30).

Despite the small portion of the pancreas occupied by pancreatic ducts, their contribution to the proper functioning of both exocrine and endocrine parts of the pancreas is substantial. Pancreatic ducts convey the enzymatic secretion of the exocrine pancreas towards the duodenum. Also, their lining epithelium produces a large amount of bicarbonate for neutralizing the acidity of pancreatic juice and stomach brought chyme. In addition to this, the ductal epithelium has been suggested as a potential source for endocrine



**Figure 7:** Representative photomicrographs for pancreatic polypeptide expression in the splenic lobe of the Eurasian moorhen pancreas. Pancreatic polypeptide-expressing cells (arrowheads) were observed within mixed type islets (A), alpha type islets (B,C), and pancreatic ductal epithelium (D,E) but were absent within the negative control section (F). The islet (i) perimeter is indicated by a dotted yellow line in Figure 7F. Abbreviations: a, alpha type islet; d, duct; m, mixed type islet



**Figure 8:** Schematic representation for the distribution of various types of islets in different lobes of the pancreas in the Eurasian moorhen. Abbreviations: p-du,d, dorsal duodenal lobe; p-du,v, ventral duodenal lobes; p-ga, gastric lobe; p-sp, splenic lobe

cells during development and regeneration (31). This role is further highlighted by the lack of a definite stem cell niche within the pancreas (32). The presence of hormone-expressing cells among and close to the ductal epithelium of the moorhen pancreas confirms the role of the pancreatic ducts in the neogenesis of endocrine cells and suggests an evolutionarily conserved role for these structures.

Taken together, the results of the present study provided a complete picture of the macroscopic and microscopic anatomy of the pancreas in the Eurasian moorhen. Although the gross anatomy of the Eurasian moorhen pancreas revealed several shared similarities with other domestic birds, the immunohistochemical analysis of the expression of insulin, glucagon, somatostatin, and pancreatic polypeptide revealed a species-specific expression pattern that suggested unique endocrine signaling pathways. The large extent and the high glucagonic nature of the moorhen pancreas could represent an adaptation to its omnivorous feeding behavior via sustained and controlled release of digestive enzymes and glucose-sensing hormones. The developmental mechanisms governing the arrangements of these hormone-expressing cells into three different types of definitive islets warrant further investigations and will give insights into the adaptation of the vertebrate pancreas to various metabolic and ecological conditions.

## Acknowledgements

The author would like to thank members of the Department of Anatomy and Embriology, Faculty of Veterinary Medicine Mansoura University for their support during this work.

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## Izražanje insulina, glukagona, somatostatina in pankreatičnega polipeptida v trebušni slinavki evrazijskega zelenonoge tukalice (*Gallinula chloropus*)

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**Izvleček:** Trebušna slinavka je kompleksna žleza z endokrino in eksokrino funkcijo. V tej študiji smo preučevali splošno anatomijo, histokemične značilnosti in imunohistokemično izražanje inzulina, glukagona, somatostatina in pankreasnega polipeptida v trebušni slinavki evrazijske zelenonoge tukalice. Makroskopsko je bila trebušna slinavka sestavljena iz treh dvanajstnikovih režnjev ter neparnih vraničnega in želodčnega režnja. Kapsula trebušne slinavke je bila tanka, brez izrazitega vzorca lobulacije. Opazni so bili trije tipi otočkov - alfa, beta in mešani. Otočki tipa alfa so bili sestavljeni predvsem iz celic, ki so izražale glukagon in somatostatin. Otočki tipa beta so bili relativno manjši od drugih dveh tipov otočkov in sestavljeni pretežno iz celic, ki so izražale inzulin ter omejenega števila drugih endokrinih celic. Mešani tipi otočkov so bili sestavljeni iz enakega deleža celic, ki izražajo inzulin, glukagon in somatostatin. V vraničnem režnju je bilo opazno več otočkov tipa alfa kot v drugih režnjih trebušne slinavke. V primerjavi z drugimi endokrinimi celicami trebušne slinavke, ki so bile ovalne ali trikotne oblike, so bile celice, ki izražajo somatostatin, nepravilnih obrisov s citoplazemskimi izrastki, ki so se stikali med seboj in tvorili mrežo znotraj otočka. Rezultati te študije so razkrili vrstno specifične značilnosti endokrine in eksokrine trebušne slinavke pri evrazijski zelenonogi tukalici in bi lahko nakazovali funkcionalne razlike v delovanju trebušne slinavke glede na prehranjevalne navade.

**Ključne besede:** trebušna slinavka; otoček; inzulin; glukagon; somatostatin; polipeptid; sladkorna bolezen