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## Age related histological change in exocrine part of pancreas in chabro chicken

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### Abstract

Present study was conducted on the exocrine part of pancreas of twenty four apparently healthy chabro chickens irrespective of sex. The birds were divided into 0 day, 30 days, 60 days and 150 days age groups. The pancreas of chabro chicken was covered by very thin fibrous connective tissue capsule. The reticular and collagen fibers became coarser in the capsule with the advancement of the age of the birds. Very fine to fine reticular fibers generally encircled one third to two third part of the individual acini at the age of 0 day group but as the age advanced most of the acini were completely encircled by relatively coarser reticular fibers. The lymphatic aggregations were frequently observed in the stromal tissue and their occurrence was relatively more in the splenic lobe. The bipolar staining character became prominent and as age of the birds advanced the basophilic staining zone increased in cell cytoplasm. One to two centroacinar cell were found in the central part of acini. The intercalated ducts were lined by the simple squamous epithelium, intralobular ducts with squamous to low columnar and interlobular duct with simple cuboidal or simple columnar epithelium. The lumen of most of ducts was filled with eosinophilic secretory products.

Keywords: Pancreas, chabro chicken, histology

### Introduction

The pancreas derived its name from the Greek roots 'Pan' and 'creas' meaning "all" and 'flesh" respectively (Slack, 1995) <sup>[20]</sup>. Pancreas is an important mixed gland associated with the gastrointestinal tract. Pancreas plays major role in chemical break down of food through its exocrine secretory products. The exocrine function of pancreas is the production and secretion of fluids which help in digestion (Ensmiger, 1992) <sup>[6]</sup>. Duodenum receives digestive enzymes and bicarbonate from the pancreas. The bicarbonates counter the effect of hydrochloric acid from the proventriculus and increase the pH of intestinal contents of the later half of duodenum from strongly to weakly acidic; hence, it protects the intestinal mucosa.

### **Materials and Method**

For the present study the pancreas was collected from twenty four apparently healthy chabro chicken irrespective of sex. The birds were procured at the time of hatching and were grown up to the prescribed age. These were divided in to four age groups i.e. 0, 30, 60 and 150 days after hatching; each group contained six chickens. Small pieces of tissue from each lobes of the pancreas were fixed in10% neutral buffered formalin and were processed by a routine paraffin embedding technique. Five to six microns thick paraffin sections were obtained and were stained by following staining procedures-

- 1. Hematoxylin and Eosin stain For general histoarchitectural study
- 2. Gordon and Sweet's method for reticulin For reticular fibers
- 3. Masson's Trichrome method For collagen fibers
- 4. Verhoeff's method for elastic fibers for elastic fibers

The micrometrical studies were conducted with the help of calibrated computerized image analyzer. The data generated by the histological observations was subject to statistical analysis to one way ANOVA for various parameters and for the test of significance with age (Snedecor and Cochran, 1)

### **Results and Discussion**

The pancreas of chabro chicken was covered by very thin fibrous connective tissue capsule

Thin connective tissue capsule around the parenchyma of pancreas was observed by Faris (2012) [7] in pigeon, Hamodi et al. (2013)<sup>[9]</sup> in common gull and guinea fowl, Sivakumar et al. (2000) <sup>[19]</sup> in Japanese quail and Basir and Abi (2015) <sup>[3]</sup> in Caspian gull. The capsule chiefly consisted of reticular and collagen fibers along with blood vessels and nerves which were often oriented parallel to the mesothelial lining; the nerves were usually present in apposition to the underlying parenchyma. With the advancement of the age of the birds the reticular and collagen fibers became fine to coarser and the undifferentiated mesenchymal cells were replaced by the fibroblasts. Fine to coarse reticular and collagen fibers were observed in the capsule of pancreas by Mobini (2013) <sup>[15]</sup> in pigeon, Hamodi et al. (2013)<sup>[9]</sup> in common gull and guinea fowl and Al-Sharoot (2016)<sup>[2]</sup> in early hatched goose. The occurrence of collagen fibers was also reported in the pancreatic capsule Basir and Abi (2015)<sup>[3]</sup> in Caspian gull and Beheiry et al. (2018)<sup>[4]</sup> in goose. In chabro chicken at all the places the external surface of the capsule had mesothelial lining formed by a layer of squamous cells. This was in accordance with the findings of Basir and Abi (2015)<sup>[3]</sup> in Caspian gull and Al-Sharoot (2016)<sup>[2]</sup> in early hatched goose. Septae divided the parenchyma into many lobules and sublobules. The septae varied considerably in thickness and were formed by fine to coarse reticular and collagen fibers along with the fibroblasts; the collagen fibers were usually oriented in the direction of the length of the septae. The elastic fibers and smooth muscles were observed only in the wall of septial blood vessels. As age of the chabro chicken advanced the reticular and collagen fibers gradually increased in occurrence and became coarser. Sivakumar et al. (2000) [19] in Japanese quail, Al-Agele and Mohammed (2012) [1] in golden eagle, Faris (2012)<sup>[7]</sup> and Mobini (2013)<sup>[15]</sup> in pigeon stated that septae from the connective tissue capsule penetrated into the pancreas and divided the parenchyma into indistinct lobes and lobules. In group - 1 the interstitial tissue had very fine to fine reticular fibers which generally encircled one third to two third part of the individual acini but as the age advanced most of the acini were completely encircled by relatively coaser reticular fibers. Stromal tissue lymphatic aggregations were observed in all the lobes of the pancreas of chabro chicken, however, their occurrence was relatively more in the splenic lobe than the other lobes. In group - 1 these were in the form of diffusely arranged lymphocytic aggregations in septial and subscapular zone but with the advancement of age dense lymphatic aggregation along with ill developed lymphatic nodules were observed in interacinar interstitial tissue. Sivakumar et al. (2000) [19] in Japanese quail and Jain (2009) <sup>[10]</sup> in vanaraja and cari shyama reported the presence of lymphocytes in the form of nodules in exocrine part of pancreas. Not uncommonly the peripheral part of the exocrine parenchyma was relatively densely packed than the central part. In the present work the exocrine part consisted of numerous secretory acini, arranged in lobules and sublobules, and the duct system; latter consisted intercalated, intralobular and interlobular ducts. The interlobular ducts were observed between the lobules and sublobules whereas, other two type of ducts were found inside the lobules and sublobules. Similar observations were found in Japanese quail (Sivakumar et al., 2000)<sup>[19]</sup>. The exocrine part of parenchyma in the pancreas of chabro chicken was formed by numerous serous tubuloacinar secretory acini. In group - 1 it consisted of developed as well as developing acini. Many developing acini were in the form

of cluster of irregularly arranged pyramidal cells, whereas, in many others a part of the acini was formed by cluster of irregularly arranged cells. As the age of the chicken advanced almost all the acini were fully developed and were usually densly packed in the parenchyma, particularly in its peripheral part; some of these were present in the radiating manner around the intralobular ducts and some others were partially or fully encircled by the capillaries. Faris (2012) [7] in pigeon, Mobini, (2013) <sup>[15]</sup> in pigeon and Al Sharoot, (2016) <sup>[2]</sup> in early hatched goose reported that the exocrine part of pancreas consisted of serous tubular acini. The shape of acini varied considerably and was elongated, oval and globoid which was in full agreement with the work of Hamodi et al. (2013)<sup>[9]</sup> in guinea fowl. In pigeon (Saadatfar *et al.*, 2010)<sup>[12]</sup> and Caspian Gull (Basir and Abi, 2015)<sup>[3]</sup> the exocrine acini were round to oval in shape. The pancreatic acini were chiefly lined with single layer of pyramidal and columnar shaped cells which rested on the basement membrane around the lumen. The pyramidal shaped cells were observed in the pancreatic acini of pigeon (Faris, 2012) [7], golden eagle (Al-Agele and Mohammed, 2012)<sup>[1]</sup> and goose (Beheiry et al., 2018) <sup>[4]</sup>. Acinar epithelial cells in fowl and pigeon were polygonal Nickel et al. (1977) [16], tall columnar in goose (Gulmez, 2003)<sup>[8]</sup>, pyramidal to tall columnar and/or quadrate in common gull and guinea fowl (Hamodi et al., 2013)<sup>[9]</sup>. The nuclei of the acinar cells were spherical and oval in shape and were usually located in the basal half of the cells. Most of these were vesicular but some cells had dark basophilic nuclei. Hamodi et al., (2013)<sup>[9]</sup> stated that each acinar cell contained a round and large nucleus in common gull and guinea fowl which was basally to centrally and basally located in the acinar cell of common gull and guinea fowl, respectively.

In the present study the acinar cells showed bipolar staining character which was prominent from group- 2. Kadhim et al. (2010) <sup>[11]</sup> in red jungle fowl observed that the acinar cells showed a polarized cytoplasm. Mobini (2013) [15] in pigeon and Beheiry et al. (2018) [4] in goose observed bizonal staining character in acinar cells. In chabro chicken each acinar cell had darkly eosinophilic apical zone and darkly basophilic basal zone. The apical zone was filled with numerous cytoplasmic granules and the basal zone usually had nucleus. These findings were in accordance with the results of Al-Sharoot (2016) <sup>[2]</sup> in early hatched goose and (Beheiry *et al.*, 2018) <sup>[4]</sup> in goose. The basal basophilic cellular zone increased with the age of chabro chicken and occupied almost all of the lower half of the cell cytoplasm in group- 4. Similar to the results of Faris (2012) <sup>[7]</sup> in pigeon and Beheiry et al. (2018)<sup>[4]</sup> in goose the centroacinar cells were often observed in the central part of the acini in chabro chicken. Moreover, as reported by Beheiry et al. (2018)<sup>[4]</sup> in goose some acini had two centroacinar cells. The centroacinar cells were either elongated or spherical in shape and had clear eosinophilic cytoplasm. The nuclei of these cells were elongated and oval in shape and were slightly darker than acinar cell nuclei. According to Beheiry et al. (2018)<sup>[4]</sup> in goose these cells were rounded or flattened in shape. Similar to the present study centroacinar cells without granules were observed in the lumen of the acini in red jungle fowl (Kadhim et al., 2010)<sup>[11]</sup> and in early hatched goose (Al-Sharoot, 2016) <sup>[2]</sup>. The centroaciar cells in the pancreas of chabro chicken might be the beginning of intercalated ducts or might be the termination of interlobular ducts. The pancreatic duct system

in chabro chicken was composed of intercalated, intralobular, interlobular and main execratory or interlobar ducts. Similar ducts had been reported in the pancreas of falcon (Simsek, et al., 2009) <sup>[18]</sup> and pigeon (Mobini, 2013) <sup>[15]</sup>. The intercalated ducts drained individual acini and was lined by the simple squamous epithelium. The cytoplasm of these cells was light eosinophilic; their nuclei were usually vesicular and flattened and were located in the central part of the cells. According to Mobini (2011) <sup>[14]</sup> in goose and Al- Agele and Mohammed (2012)<sup>[1]</sup> in golden eagle, the exocrine pancreatic ducts began with intercalated ducts which were lined with squamous epithelium. The lining epithelium of intralobular ducts varied according to the size of the ducts; in smaller ducts it was either simple squamous and simple cuboidal but the larger ducts were lined by single layer of cuboidal and columnar cells. The cytoplasm of epithelial cells of these ducts was dark eosinophilic. The nuclei of these epithelial cells were vesicular and their shape varied according to shape of the epithelial cell. In squamous cells these were flattened and oval. In cuboidal cells the nuclei were spherical but in columnar cells these were oval and elongated. In smaller interlobular ducts epithelium usually resembled with the epithelium of larger intralobular ducts but in the larger interlobular ducts the duct epithelium was tall columnar and

histochemical observation revealed that most epithelial cells were secreting mucous cells. According to Kadhim *et al.* (2010) <sup>[11]</sup> in red jungle fowl, Mobini (2011) <sup>[14]</sup> in goose and Al- Agele and Mohammed (2012) <sup>[1]</sup> in golden eagle, reported the intralobular ducts were lined with cuboidal epithelium and interlobular ducts had low columnar epithelium.

In the pancreas of chabro chicken the wall of larger interlobular ducts had tunica mucosa and tunica adventitia. The mucosa constituted of folds which became larger and more pronounced with the increase in age of chabro chicken. Mobini (2013)<sup>[15]</sup> in pigeon and Kadhim et al. (2010)<sup>[11]</sup> in red jungle fowl stated that mucosal folds observed in the pancreatic duct systems. In the present study the wall of various types of ducts in group- 1 was formed only by frame work of reticular fibers but as the age advanced the more coarser reticular and collagen fibers were observed in the wall of various types of pancreatic ducts. Elastic fibers were absent in the wall of all types of ducts. As age advanced the more coarse reticular and collagen fibers were present in the wall of various types of pancreatic ducts. The lumen of most of ducts was filled with eosinophilic secretory products. These findings were similar to the work of Kadhim et al. (2010) [11] in red jungle fowl.

 Table 1: Values (Mean ± SE) of various micrometric parameters in the pancreas of chabro chicken along with the value in dorsal, ventral and splenic lobes.

Parameters	Dorsal lobe	Ventral lobe	Splenic lobe	Entire study				
Capsule thickness (in µm)	$5.98 \pm 0.48 \; (1.96 - 14.17)$	6.04 ± 0.52 (2.23 -11.30)	$5.96 \pm 0.49 \; (2.44 \;  16.65)$	$5.99 \pm 0.49 \; (1.96 - 16.65)$				
Acinar diameter (in µm)	$25.78 \pm 0.76 \; (18.82 - 37.14)$	$25.79 \pm 0.77 \; (18.17 - 35.67)$	$25.93 \pm 1.05 \; (11.20 - 38.50)$	$25.83 \pm 0.86 \; (11.20 - 38.50)$				
Epithelial height (in µm)	$12.39 \pm 0.46 \ (8.44 \ -18.98)$	$12.40 \pm 0.45 \ (7.20 \ -18.93)$	$12.53 \pm 0.47 \ (7.36 \ -19.04)$	$12.44 \pm 0.46$ ( $7.20 - 19.04$ )				
Width of Lumen (in µm)	2.83 ± 0.24 (1.21 - 5.92)	$2.83 \pm 0.26 (1.04 - 7.56)$	$2.8 \pm 0.24 \ (0.90 \ \text{-} 7.97)$	$2.82 \pm 0.24$ ( $0.90 - 7.97$ )				
Acinar cell nuclei diameter (in µm)	3.97 ± 0.97 (1.56 -7.73)	4.03 ± 0.22 (1.41 -6.90)	3.97 ± 0.17 (2.01 -6.69)	$3.99 \pm 0.45 \; (1.41 - 7.73)$				
Zymogene granules diameter (in µm)	0.853 ± .053 (0.20 -1.70)	$0.86 \pm 0.058 \ (0.46 \ -1.89)$	0.87 ± 0 .037 (0.36 -1.59)	0.86±.049 (0.20-1.89)				
Islet mass diameter (in µm)	41.42 ± 2.77 (14.76 - 77.79)	$35.89 \pm 2.44 \ (10.92 - 76.44)$	$54.87 \pm 3.31 \ (16.62 - 98.25)$	$33.05 \pm 2.84 (10.92 - 98.25)$				
Values in parentheses indicate ranges								

Table 2: Group wise values (Mean  $\pm$  SE) of various micrometric parameters in the pancreas of chabro chicken

Donomotors	Groups				
r al anietel s	Group 1	Group 2	Group 3	Group 4	
Acinar diameter (in µm)	$20.58 \pm 1.08^{\rm a}$	$24.21 \pm 2.08^{b}$	$28.86 \pm 1.26^{\circ}$	30.23 ± 1.23°	
Height of acinar epithelial cells (in µm)	9.91 ± 0.44 <sup>a</sup>	$11.60 \pm 0.56^{b}$	$13.30\pm0.76^{\rm c}$	$15.03 \pm 0.96^{d}$	
width of acinar lumen (in µm)	$4.83\pm0.44^{b}$	$2.42\pm0.22^{a}$	$2.07 \pm 0.21^{a}$	$1.96 \pm 0.44^{a}$	
Acinar cell nuclei diameter (in µm)	$4.51\pm0.41^{b}$	$3.93\pm0.28^{ab}$	$3.75\pm0.33^{\mathrm{a}}$	$3.64\pm0.38^a$	
Cytoplasmic granules (in µm) diameter	$0.64 \pm 0.21^{a}$	$0.74\pm0.36^{\rm a}$	$0.95 \pm 0.09^{b}$	$1.12\pm0.10^{\rm b}$	
Diameter of islets Langerhans (in µm)	33.58±3.83 <sup>a</sup>	$42.82 \pm 5.05^{ab}$	$48.27 \pm 5.73^{b}$	$52.02 \pm 6.25^{b}$	
Thickness of capsule (in µm)	$5.55 \pm 1.06^{a}$	$5.65\pm0.77^{a}$	$5.75\pm0.76^{\rm a}$	$6.95 \pm 1.94^{a}$	

Different superscript shows significant change in values and similar superscript shows non significant change in values between the groups.



Fig 1: Higher magnification of 0 day old chabro chicken showing mesothelium (arrow) above the capsule (a) and Parenchyma having developed acini (b) and developing acini (c). H&E; X400



Fig 2: Photomicrograph of dorsal lobe of pancreas in 30 days old chabro chicken showing acini (a), inter lobular duct (b), simple cuboidal duct epithelium (c) and eosinophilic secretion in the lumen of the duct (d). H&E; X400



**Fig 3:** Photomicrograph of ventral lobe of pancreas in 150 days old chabro chicken showing developed acini (a), capillaries into interacinar space (b) and intercalated duct (c). H&E; X 200



**Fig 4:** Photomicrograph of dorsal lobe of pancreas in 150 days old chabro chicken showing parenchyma (a), interlobular ducts (b), epithelium of ducts (c) and secretory materials in duct lumen (d). H&E; X200



**Fig 5:** Photomicrograph of ventral lobe of pancreas in 60 days old chabro chicken showing loosely arranged parenchyma (a), compactly arranged parenchyma (b), islets of Langerhans (c) and acini (d).



**Fig 6:** Photomicrograph of ventral lobe of pancreas in 150 days old chabro chicken showing parenchyma (a), elastic fibers in stromal blood vessels (arrow). Verhoeff's Method for Stain; X400



**Fig 7:** Photomicrograph of splenic lobe of pancreas in 60 days old chabro chicken showing collagen fibers in capsule (a) and in interlobular septum (b). Masson's Trichrome Stain; X200



**Fig 7:** Photomicrograph of splenic lobe of pancreas in 30 days old chabro chicken showing reticular fibers in capsule (a), septae (b) and around the acini (c). Gordon and Sweet's Method; X200

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### Conclusion

The pancreas of chabro chicken was covered by thin fibrous connective tissue capsule. The interstitial tissue around the acini was formed by reticular fibers which generally encircled one third to two third part of the individual acini in group 1 and the entire acini in other groups. The lymphatic aggregations were relatively more in the stroma splenic lobe than the other lobes. Exocrine parenchyma consisted of serous tubuloacinar secretory acini and the duct system. Acini were elongated, oval and globoid. In group - 1 developed and developing acini were observed but in other groups most of acini were fully developed. The acinar epithelium consisted of single layer pyramidal cells but often columnar cells were also found. The cytoplasm of acinar cells had bipolar staining which became prominent from group- 2 onwards. The basophilic basal zone increased with the increase of age of birds. Usually one centroacinar cell was found but in some acini two cells were also observed. Duct system consisted of intercalated, intralobular and interlobular ducts. The wall of larger interlobular ducts consisted of mucosa and adventitia; the formar was projected in the lumen in the form of mucosal folds.

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