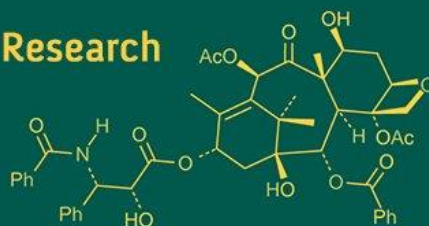


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Histomorphological and histometrical evaluation of the endocrine pancreas in Chabro chicken

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Abstract

The present investigation was carried out on the endocrine portion of the pancreas obtained from twenty-four apparently healthy Chabro chickens of both sexes. Based on age, the birds were allocated into four groups: 0 day, 30 days, 60 days, and 150 days. The islets of Langerhans exhibited considerable morphological variation and were predominantly oval or round, although irregular, quadrilateral, and elongated islets were also encountered. A relatively greater concentration of islets was consistently observed in the splenic lobe of the pancreas. In some developing islets, clusters containing both acinar and islet cells were evident, representing intermediate stages of islet development. Two distinct categories of mature islets were identified, namely beta islets and mixed islets. Beta islets were composed primarily of aggregates of beta cells exhibiting diverse cellular morphologies, including oval, triangular, irregular, elongated, and polyhedral forms. Mixed islets, consisting of both beta and alpha cells, were observed exclusively in the splenic lobe. The alpha cells were spherical, elongated, or irregular in shape and were comparatively smaller than the beta cells. Capillaries were commonly distributed throughout the islets of Langerhans, and in some instances these vascular channels formed dilated sinus-like structures.

Keywords: Histology, pancreas, islets of Langerhans, Chabro chicken

Introduction

Chabro chicken is an Indian rural meat-type breed developed through the crossbreeding of Barred Plymouth Rock and Red Cornish chickens. The pancreas performs an important endocrine function by secreting the hormones insulin and glucagon (Denbow, 2000; Frandson *et al.*, 2009) ^[5, 7]. The endocrine component of the pancreas, known as the islets of Langerhans, comprises alpha (A) and beta (B) cells that are organized into separate islets in domestic animals (Getty, 1975) ^[8]. The beta (B) cells synthesize and secrete insulin, which promotes glucose uptake by the liver, skeletal muscles, and adipose tissue, while also stimulating lipogenesis. In contrast, the alpha (A) cells produce glucagon, which induces lipolysis in adipose tissue, reduces glycogen reserves in the liver and muscles, decreases glucose utilization, enhances gluconeogenesis, and suppresses lipogenesis (Scanes, 2011) ^[17]. Together, insulin and glucagon play a pivotal role in maintaining glucose homeostasis by regulating blood glucose concentrations. A review of the available literature indicates that limited attention has been devoted to the histological characteristics of the endocrine pancreas in chickens, particularly in the Chabro breed. Therefore, the present study was undertaken to investigate the histology of the endocrine pancreas in Chabro chickens.

Materials and Methods

For the present study, the pancreas was collected from twenty-four apparently healthy Chabro chickens, irrespective of sex. The birds were procured at the time of hatching and reared until the prescribed ages. They were divided into four age groups: 0, 30, 60, and 150 days post-hatching, with six chickens in each group. Small tissue samples from each lobe of the pancreas were fixed in 10% neutral buffered formalin and processed using the routine paraffin-embedding technique (Luna, 1968) ^[12]. Paraffin sections of 5-6 µm thickness were prepared and stained using the following methods:

- Hematoxylin and Eosin stain (Luna, 1968) ^[12]-for the general histoarchitectural study.

- Gordon and Sweet's method for reticulin (Bancroft & Stevens, 1977)-for demonstrating reticular fibers.
- Masson's Trichrome method (Luna, 1968) ^[12]-for demonstrating collagen fibers.
- Verhoeff's method for elastic fibers (Bancroft & Stevens, 1977)-for demonstrating elastic fibers.
- Gomori's method for pancreatic islets (Luna, 1968) ^[12]-for identifying alpha and beta cells.

Micrometric measurements were performed using a calibrated computerized image analyzer. The data obtained from the histological observations were subjected to one-way ANOVA to evaluate age-related differences and determine the statistical significance of the various parameters (Snedecor & Cochran, 1994) ^[21].

Results and Discussion

The endocrine component, or the islets of Langerhans, constituted only a small portion of the pancreatic parenchyma in the Chabro chicken. A similar observation has been reported in fowl (Bradley & Grahame, 1960) ^[3]. In the present study, the islets of Langerhans appeared as small, lightly stained, pale areas located between the pancreatic acini (Fig. 1). Most islets were present as discrete units scattered throughout the exocrine tissue, whereas some developing islets consisted of clusters of acinar and islet cells. These developing islets appeared to originate from cells detached from the exocrine acini (Fig. 3). The islets showed considerable variation in shape, including oval, round, irregular, elongated, and quadrilateral forms (Fig. 3). Similar variations in islet morphology have been reported as roughly spherical in fowl (Bradley & Grahame, 1960) ^[3], round or irregular in falcon (Simsek *et al.*, 2009) ^[18], irregular in red jungle fowl (Kadhim *et al.*, 2010) ^[11], and oval or circular in golden eagle (Al-Agele & Mohammed, 2012) ^[1]. The distribution of the islets was more abundant in the splenic lobe than in the dorsal and ventral lobes of the pancreas (Fig. 1). A higher frequency of islets in the splenic and third lobes has also been reported in coturnix quail (Smith, 1974) ^[20], chicken (Tomita *et al.*, 1985) ^[23], and native chicken (Parchami & Kusha, 2015) ^[14].

In the present study, the pancreatic islets showed considerable variation in size. The islets were generally smaller in the ventral lobe and larger in the splenic lobe. The overall average diameter of the oval and rounded islets in the pancreas of Chabro chicken was $33.05 \pm 2.84 \mu\text{m}$ (Table 2). The mean diameter of the islets in the dorsal, ventral, and splenic lobes was $41.42 \pm 2.77 \mu\text{m}$, $35.89 \pm 2.44 \mu\text{m}$, and $54.87 \pm 3.31 \mu\text{m}$, respectively (Table 2). The average diameter of the islets increased gradually with age from Group 1 to Group 4. The mean diameter of the oval and rounded islets was $33.58 \pm 3.83 \mu\text{m}$ in Group 1 and $52.02 \pm 6.25 \mu\text{m}$ in Group 4 (Table 1). A gradual increase in islet size was also observed in the dorsal, ventral, and splenic lobes from Group 1 to Group 4. Statistical analysis showed that the increase in the overall diameter of the islets among the different age groups was not significant. Likewise, the increase in islet diameter in the dorsal and ventral lobes was statistically non-significant. However, in the splenic lobe, the increase in islet diameter from Group 1 to Group 2 was statistically significant. The islets were randomly distributed throughout the exocrine tissue of all pancreatic lobes, which is consistent with the observations reported in the common gull and guinea fowl (Hamodi *et al.*, 2013) ^[10]. However, in some regions, particularly in the

splenic lobe, the islets were more concentrated in the central part than in the peripheral part of the parenchyma, where they frequently formed a large central mass of endocrine tissue.

Most of the islets of Langerhans in the pancreas of Chabro chicken were composed of clusters of lightly stained oval, triangular, irregular, elongated, and polyhedral cells. However, some islets in the splenic lobe contained both lightly and darkly stained cells (Fig. 2). Based on their cellular composition, two types of islets were identified: light islets, composed entirely of light cells, and mixed islets, containing both light and dark cells. Light islets were more numerous than mixed islets. The light (beta) islets were present in all pancreatic lobes, whereas the mixed islets were observed only in the splenic lobe. Similar observations of dark, light, and mixed islets have been reported in duck (Bradley & Grahame, 1960) ^[3] and goose (Mobini, 2011) ^[13]. The light islets observed in the present study correspond to beta islets, as described in native chicken (Parchami & Kusha, 2015) ^[14], falcon (Simsek *et al.*, 2009) ^[18], and goose (Mobini, 2011) ^[13]. A predominance of beta cell islets has also been reported in coturnix quail (Smith, 1974) ^[20], chick (Rawdon, 1998) ^[15], and goose (Gulmez, 2003) ^[9], while mixed islets have been described in Japanese quail (Sivakumar *et al.*, 2000) ^[19] and pigeon (Faris, 2012) ^[6].

The light or beta cells were relatively larger and contained scanty, lightly eosinophilic cytoplasm (Figs. 2, 3, and 4). Their nuclei were large, vesicular, and either centrally or eccentrically located, with one or two nucleoli. Many beta cells were closely packed and frequently overlapped, giving the appearance of multinucleated cells under the light microscope. These cells were generally irregularly distributed within the islets, although in some areas they were arranged in linear rows. The dark or alpha cells were spherical, elongated, or irregular in shape and were smaller than the beta cells. They possessed dark eosinophilic cytoplasm and spherical to oval vesicular nuclei (Fig. 2). Similar morphological features of alpha and beta cells have been reported in the common gull and guinea fowl (Hamodi *et al.*, 2013) ^[10]. In pigeon, the beta cells of mixed islets were arranged as cords or irregular rows of polygonal cells (Faris, 2012) ^[6]. Likewise, smaller alpha cells and larger beta cells with prominent central nuclei have been described in red jungle fowl (Kadhim *et al.*, 2010) ^[11] and golden eagle (Al-Agele & Mohammed, 2012) ^[1]. Delta cells were not observed in the islets of Langerhans in the pancreas of Chabro chicken. The absence of delta cells has also been reported in ostrich (Stornelli *et al.*, 2006) ^[22], goose (Mobini, 2011) ^[13], and the common gull and guinea fowl (Hamodi *et al.*, 2013) ^[10]. Using Gomori's method for pancreatic islets, the beta cells stained blue, whereas the alpha cells stained pink (Fig. 6).

No distinct capsule was observed surrounding the islets of Langerhans in the pancreas of Chabro chicken. However, the reticular fibers encircling the adjacent acini formed an almost continuous boundary around the islets. Similar observations, indicating the absence of a distinct border between the endocrine and exocrine portions of the pancreas, have been reported in mynah (Saadatfar & Asadian, 2009) ^[16], pigeon (Faris, 2012) ^[6], common gull and guinea fowl (Hamodi *et al.*, 2013) ^[10], and early-hatched goose (Al-Sharoot, 2016) ^[2]. Both developing and mature islets exhibited a rich vascular supply. Numerous capillaries were present around the periphery of the islets, often

surrounding one-third to two-thirds of their circumference (Figs. 3 and 4). Capillaries were also frequently observed within the islets, where they occasionally formed dilated sinus-like structures (Fig. 4). A well-developed network of blood capillaries within the pancreatic islets has also been reported in goose (Gulmez, 2003; Mobini, 2011) [9, 13], ostrich (Stornelli *et al.*, 2006) [22], mynah (Saadatfar & Asadian, 2009) [16], pigeon (Faris, 2012) [6], and common gull and guinea fowl (Hamodi *et al.*, 2013) [10]. Furthermore, in fowl, the islet cells have been reported to be clearly separated from the surrounding serous acini by highly vascular areolar tissue, with wide blood vessels located between the anastomosing cords of endocrine cells (Bradley & Grahame, 1960) [3].

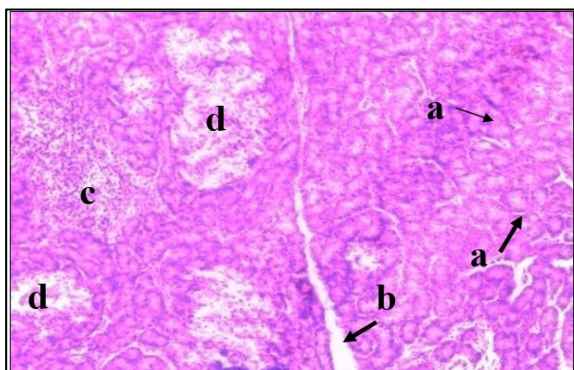


Fig 1: Photomicrograph of splenic lobe of pancreas in 60 days old chabro chicken showing acini (a), interlobular septum (b), lymphatic aggregation (c) and islets of Langerhans (d). H&E; X100

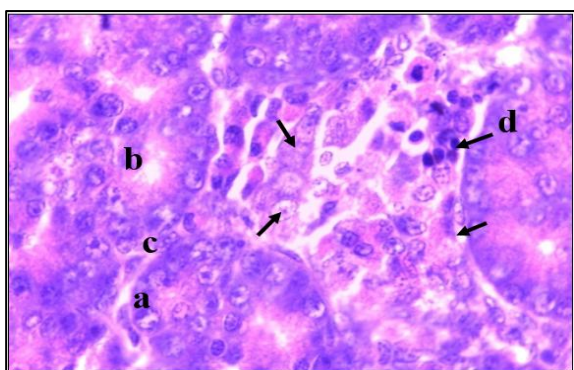


Fig 2: Photomicrograph of splenic lobe of pancreas in 0 day old chabro chicken showing acini (a), lumen of acini (b), light cells of islets of langerhans (c) and dark color cell of islets of langerhans (d). H&E; X1000

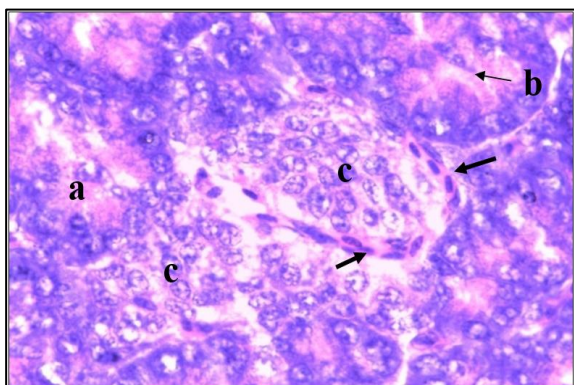


Fig 3: Photomicrograph of dorsal lobe of pancreas in 0 day old chabro chicken showing acini (a), lumen of acini (b) and islets of Langerhans (c) partially encircled by capillaries (arrow). H&E; X1000

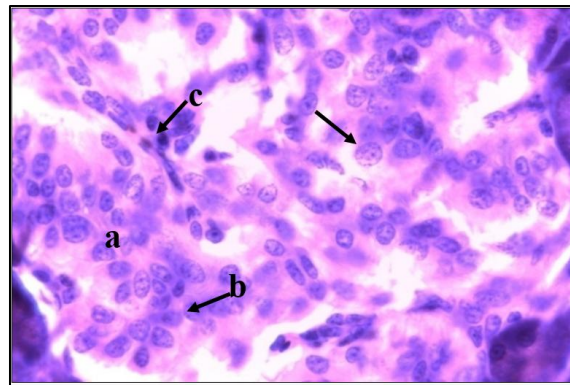


Fig 4: Photomicrograph of ventral lobe of pancreas in 60 days old chabro chicken showing light (beta) islets (a), beta cells of islets (b), nuclei of islets cells (arrow) and blood capillaries inside (c). H&E; X1000

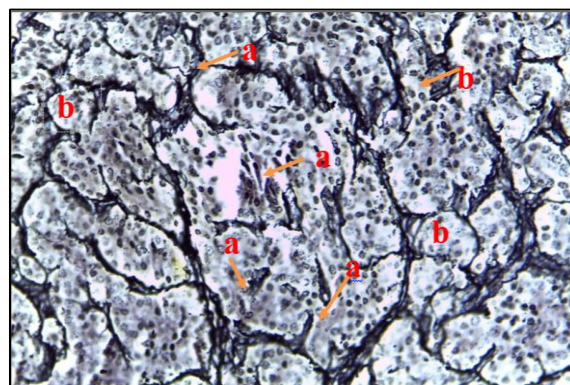


Fig 5: Photomicrograph of ventral lobe of pancreas in 60 days old chabro chicken showing reticular fibers inside islets of langerhans (a) and around the acini. (b). Gordon and Sweet's Method; X400

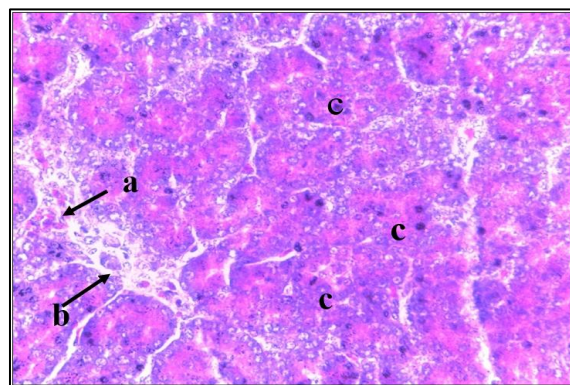


Fig 6: Photomicrograph of splenic lobe of pancreas in 0 day old chabro chicken showing pink colour alpha cells (a) and blue colour beta cells (b) in the mixed islets. Several exocrine acini (c) are also visible. Gomori's Method for Pancreatic Islets; X400

Conclusion

The islets of Langerhans in the pancreas of Chabro chicken were predominantly oval and rounded, although irregular, quadrilateral, and elongated islets were also observed. The islets were more abundant in the splenic lobe than in the dorsal and ventral lobes. Some developing islets consisted of clusters of both acinar and islet cells. The fully developed islets were classified as beta and mixed types. Mixed islets, composed of both beta and alpha cells, were observed only in the splenic lobe, whereas beta islets were present in all pancreatic lobes. The alpha cells were relatively smaller than the beta cells. A rich capillary network was present within the islets, and the capillaries occasionally formed dilated sinus-like structures.

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