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Histomorphological Developmental Study of Advanced Postnatal of the Pancreas of Local Rabbit

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Abstract: Current research aimed to investigate the developmental changes of the pancreatic tissues of the local rabbits during different postnatal periods (one, twenty, forty, sixty and eighty days of age). Histological sections prepared from the specimens of their pancreas were stained with general and special stains then photographed with Dino-eye piece camera and analyzed with its image software. Macroscopic examination showed that the pancreas of the rabbit was structured of three lobes that were head, body and tail lobes. The pancreas of rabbit appeared as a diffused type because the lobes were diffusely invested within the mesenteric tissue. The data showed the existence of the accessory pancreatic duct and absence of the major duct. Microscopic findings revealed immature exocrine portion at one day aged rabbits due to the morphological changes established during subsequent ages. The endocrine portion reside primarily in the islet's of Langerhans which were associated well with extra-blood vessels and exocrine ducts. Microscopically, each islet was structured of several cells (α, β and δ) which were different in size, shape and color. Islets were not fully developed at birth because their densities, cellular content and sizes were changed during different postnatal ages. In conclusion, dramatic critical morphological changes in the exocrine portion at twenty and forty days of age as well as the gradual changes at sixty and eighty days of ages were observed. In addition to that, histological changes in the islet of Langerhans such as their densities, sizes and cellular content indicated their immaturity at birth.

Key words: Pancreas, Islet's of Langerhans, mesenteric pancreas, rabbit, exocrine acini

INTRODUCTION

Rabbits are small mammals in the family Leporidae found in several parts of the world. There are eight different genera in this family classified as rabbits, including the European rabbit, Cottontail rabbits and the Amami rabbit. There are many other species of rabbit and these, along with pikas and hares make up the order Lagomorphs. The rabbit is an herbivore or more specifically a folivore, designed to exist on a diet of succulent green vegetation (Davies and Davies, 2003). Production of rabbits has a potential in developing countries to supply cheap and high quality animal protein (Mehreze and Mousa, 2011).

Pancreas is an organ of special interest from a medical viewpoint as it is the target of two major diseases that are diabetes mellitus and pancreatic cancer. It is to be hoped that a better understanding of the development of this organ will eventually contribute to the development of novel therapies for the treatment of either or both of the above diseases (Seymour et al., 2004). According to 2006 United States Departments of Agriculture (USDA) statistics, there are approximately 240000 rabbits used annually in research within the U.S. (USDA, 2007). In fact, laboratory animals have been a direct object of a great number of experiments that cannot be carried out in vitro. Experiments conducted variability on laboratory animals provided at varying extent the knowledge which can be applied to humans and domestic animals (Mazensky et al., 2009).

Rabbits were considered good experimental animal model in clinico-anatomical researches of diverse morphological anomalies and diseases in both human and animals. They have been used as an experimental model in many diseases and for the study of toxicology, pharmacology and surgery at many universities (Abidu-Figueiredo et al., 2008; Gwarzo et al., 2010). Accordingly, microscopic structure of many organs such as the pancreas in this animal is in need for depth description and exploration (Dimitrov, 2012).

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The growth pattern of the pancreas was investigated in several species. Findings hypothesized that the pancreas is not fully developed at the time of birth therefore, the complete differentiation of its exocrine and endocrine parts takes place during postnatal life (Cabrera-Vasquez *et al.*, 2009). Recently, Yebra *et al.* (2011) considered pancreas a valuable model system to study organogenesis as it is an epithelial tissue containing endocrine and exocrine cells, a ductal system, a vascular network, sensory and sympathetic innervation and a stromal component. At present, pancreatic islet transplantation was considered one of the most attractive strategies for the treatment of type I diabetes (Morini *et al.*, 2006). Actually, understanding how the pancreas develops is essential to understand the pathogenesis of congenital pancreatic anomalies (Tadokoro *et al.*, 2011).

In reality, morphogenesis of the endocrine pancreas appeared following similar progression in all mammals although the precise stage of gestation at which the specific events occur may vary amongst species. The pancreas develops from two diverticula of the primitive gut which fuse during early embryonic growth to form both the exocrine and endocrine pancreas. After birth, further re-organization of the pancreatic endocrine tissue could be occurs with changes in islet size and topography in most species. The duration of this postnatal period of islet remodeling depends on species and varies from 4 weeks in the rat to 4 years or more in the human infant (Fowden and Hill, 2001). Subsequently, Buddington *et al.* (2003) postulated that the pancreas of most mammalian animals is morphologically but not functionally mature at birth.

In general, functional maturity of the pancreas in animals occurs at or sometime after weaning (Walthall *et al.*, 2005). De Barros Reis *et al.* (2008) hypothesized that rat, the late fetal and early postnatal durations are critical periods for the pancreatic islet ontogeny. There are imperative roles of the exocrine and endocrine portions of the pancreas to secret many important digestive enzymes as well as some important hormones play a role in blood sugar metabolism. Therefore, disorders that afflict the pancreas can occur in both portions and research into the development of the pancreas could provide much insight into the etiology of many of these diseases (Mastracci and Sussel, 2012).

In contrast to the huge number of studies on the pancreas of humans, few studies on the ontogeny and developmental changes of the rabbit pancreas are available. Most investigations on the pre and post-natal development of the pancreas were carried out

on guinea pigs, mice and rats (Bock *et al.*, 1997; Malatesta *et al.*, 2002; De Assis *et al.*, 2003; Yashpal *et al.*, 2004; Yoshizawa *et al.*, 2005; Morini *et al.*, 2006; De Barros Reis *et al.*, 2008). For a lesser extent, in the veterinary practice few studies were carried on the pancreas of pigs, dog, cat and sheep (Bogdani *et al.*, 2005; Constatinescu *et al.*, 2006; Newman *et al.*, 2006; Arciszewski and Zacharko-Siembida, 2007; Ford *et al.*, 2009).

There is paucity of study on the ontogeny of the pancreas in the rabbit and to our knowledge, there is no research conduct on the pancreas of the local rabbits in Iraq up to date. So, as the present study intended to add knowledge on the pancreas of this important experimental animal model. The knowledge obtained could be constructing a basis for other veterinary or medical fields such as pathology and physiology. In view of the above, the present study was planned:

- To investigate the developmental changes in the histology of both exocrine and endocrine parts of the local rabbit pancreas, as the study try to identify the structural changes during postnatal periods that are one, twenty, forty, sixty and eighty days aged rabbits
- To explore the morphometric changes in the structure of the pancreatic lobes during the above periods too

MATERIALS AND METHODS

Through the experimental periods, the animals were housed individually in wire bottomed cages and fed *ad-libitum*. Research approved by the ethic committee for animal use and care and conducted under the order no. 167 of the council of the Veterinary Medicine College/Baghdad University on 22/January/2013.

Rabbits preparation and study design: To obtain rabbits with different ages, 15 doe and 5 bucks were bought from local supplier at Baghdad province. Clinically healthy animals were housed together in the animal house of the department of anatomy, histology and embryology and feed greenish food, commercial pellet and water *ad libitum*. Subsequently, all pregnant does were caged singly and backs were isolated. Thereafter, ten offspring (5 males and 5 females) on days 1, 20, 40, 60 and 80 post partum were selected to conduct this study.

Each selected rabbit was euthanized prior to its dissection with an intraperitoneal injection of 500 mg kg^{-1} sodium phenobarbital (Eifler *et al.*, 2009). After that, the animal was dissected on a dissecting board. The abdominal wall opened to view the abdominal viscera, then the pancreas lobes (head, body and tail) were pointed out and their location and relationship with other

Table 1: Means of the body weights, lengths and widths of the three different lobes of the rabbit's pancreas. It showed different measurements at different postnatal periods

Measurements	1 day**	20 days***	40 days#	60 days	80 days
Weight (gram) ¥	57.51±1.30SE	150.42±1.81SE	227.16±2.35SE	309.16±2.12SE	408.47±18.7SE
Head *lobe					
Length (mm)	21.60±0.42SE	25.60±0.40SE	32.90±0.54SE	36.30±0.30SE	43.00±0.42SE
Width (mm)	6.00±0.21SE	8.30±0.33SE	15.20±0.48SE	19.20±0.46SE	23.10±0.37SE
Body lobe					
Length (mm)	15.40±0.37SE	22.80±0.53SE	26.20±0.57SE	30.40±0.33SE	35.40±0.33SE
Width (mm)	11.70±0.34SE	16.00±0.36SE	21.60±0.47SE	24.50±0.42SE	29.80±0.2SE
Tail lobe					
Length (mm)	15.90±0.27SE	22.90±0.36SE	28.20±0.51SE	32.90±0.34SE	37.10±0.46SE
Width (mm)	2.60±0.16SE	4.10±0.23SE	6.20±0.22SE SE	7.10±0.23 SE	9.30±0.24SE

*Significant differences between the length of the head lobe compared to those of body and tail lobes (p<0.05) within each age, **Significant differences between the measurements of one day of age compared to those of other ages (p<0.05), ***Significant differences between the measurements of twenty days of age compared to those of other ages (p<0.05), ¥: Significant differences between the body weights between different ages (p<0.05), #: Non significant differences between the measurements of forty days of age compared to those of sixty and eighty ages (p<0.05), SE: Standard error of the mean

digestive organs was photographed *in situ*. The topography and shape of the pancreas were studied. The results were documented with digital camera. Macroscopic linear measurements of the pancreas lobes were studied after their extirpiration. The collected specimens were measured for length, width in millimeters by using the Vernier scale. The data on various macromorphometric characters viz., length and width of the pancreatic lobes and weight of the rabbits were presented in Table 1.

The specimens (whole head, body and tail lobe) of the pancreas from each dissected animal were collected and fixed in either Bouin's solution or 10% neutral buffered formalin. After well fixation (24 h) the specimens were dehydrated by passing them through a series of ascending ethanol alcohol (70, 80, 90 and 100%) each for two hours and then specimens were cleared in xylene for one hour after that embedded in paraffin wax and then the blocks were sectioned serially at 6 μm thickness and stained with either one of the following stains, Hematoxylin and Eosin as routine stain for general features identification, Masson trichrome stain for the staining of the cellular and extracellular connective tissue of the exocrine part, Gomori's chrome alum hematoxylin and phloxine for α, β and δ cells of the islets of Langerhans and periodic acid Schiff stain for both exocrine and endocrine parts of pancreas (Luna, 1968).

Micromorphometric measurements: The tissue sections were analyzed using Olympus light microscope at a magnification of X40, X100 and X400. Sections were photographed and analyzed by Dino-eye piece camera provided with image software. For each section of pancreas, the following were determined as percentage of acinar units area per 1 mm^2 of each lobe and per 1 mm^2 whole pancreatic lobes, mean of density of islets per 1 mm^2 of each lobe and per 1 mm^2 of whole pancreatic lobes. The following variables were measured in each islet, ratio of β to α cells in each islet, ratio of δ cells to the total number of both β and α cells. Islets measured less than 50 μm in diameter were considered small in size,

whereas, those between 75-100 μm diameter and those more than 125 μm diameter were considered medium and large sized, respectively.

Statistical analysis: Statistical calculations were carried out with the SPSS 15.0 for windows software package. All numerical values were expressed as the Mean±Standard Error (SE). For comparisons of developmental parametric changes for all ages, the statistical significance was assessed by ANOVA. The significance level was set at p<0.05.

RESULTS

Anatomical results

Lobes of pancreas: The gross examination after abdominal dissection of the one, twenty, forty, sixty and eighty days old local rabbits revealed that their pancreas divided into three irregular parts or lobes which were positioned within the mesentery folds. According to the positions of these parts inside the abdominal cavity and their locations and relationships with the other digestive organs were divided into head, body and tail lobes. the pancreas of the rabbit appeared irregular in shape and there were no lines of demarcations between its lobes. So, that the lobes were named according to their locations and their approximate to the duodenum, hepatic portal vein and spleen. Current examination showed that the head lobe was located between the descending and ascending limbs of the duodenal loop. This part of the pancreas was identified as disseminated glandular structures lies within the mesoduodenum and located nearly15 mm away from the transverse limb of the duodenum at one day old rabbits. The distance increased correspondingly with increased ages, i.e. 20, 40.60 and 80 days rabbits into 20, 35, 38, 46 and 48 mm, respectively.

The glandular tissue of this lobe appeared at one day-aged rabbits as a thin foggy structure within the glistening transparent mesentery. It was distributed around the pancreatico-duodenal blood vessels and in close relationship to the ascending limb of the duodenal
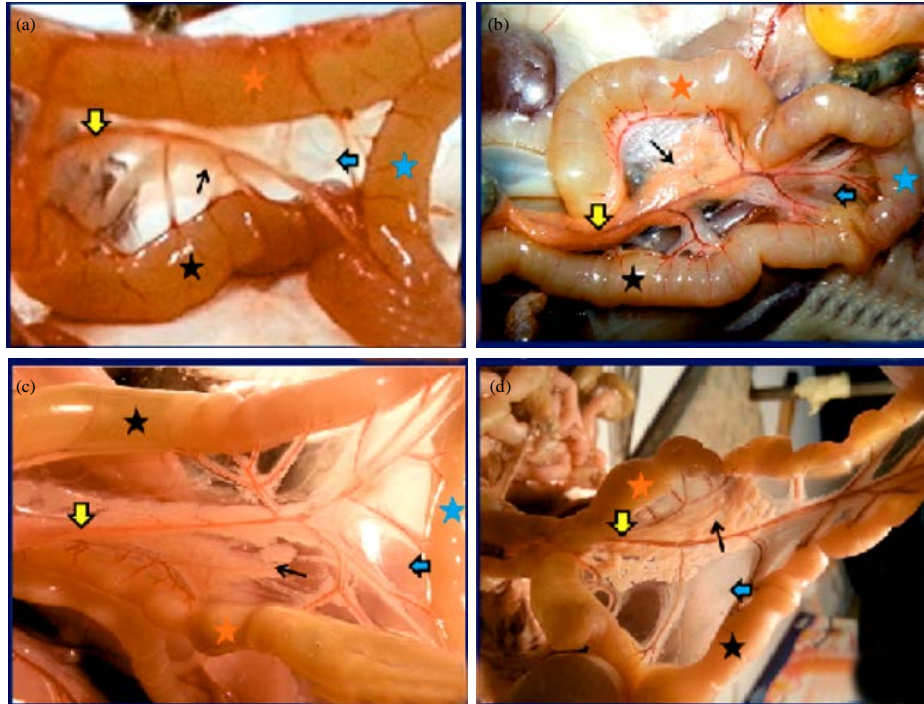
Fig. 1(a-d): Head lobe of the rabbit' pancreas at different postnatal periods, (a) 1, (b) 40, (c) 60 and (d) 80 days. It showed the glandular structure (black arrow) diffused within the glistening mesentery (blue arrow) and surrounding the pancreatico-duodenal blood vessels (yellow arrow). The glandular tissue closely associated with the ascending duodenum (red star) and away from the descending (black star) and transverse duodenum (blue star)

loop. Whereas, at twenty, forty and sixty days-aged rabbits, the pancreatic tissue appeared as small patches, light pinkish in color. At eighty days-aged rabbits, the pancreatic tissue appeared as pinkish nodular, glistening structures with prominent convexity (Fig. 1). The duodenal loop appeared distinctly very large at all studied ages, however, the pancreatic tissue which was present within this loop was scanty. Measurements such as length and width of this lobe of pancreas were listed in Table 1. The means of lengths and widths of one, twenty, forty, sixty and eighty days old rabbits were 21.60±0.42, 6.00±0.21, 25.60±0.40, 8.30±0.33, 32.9±0.54, 15.20±0.48, 36.30±0.30, 19.20±0.46 and 43.00±0.42 and 23.10±0.37 mm, respectively.

The body lobe of the pancreas also appeared as a disseminated glandular structure in the area adjacent to the cranial part of the duodenum just below the duodeno-pyloric junction. The lobe located at the right side of the abdominal cavity and extended toward the left side till the ventral extremity of the spleen. It was bounded by the lesser curvature of the stomach, cranial part of the duodenum, descending colon and cecum. The structure of this lobe appeared slightly condensed compared to that of the head lobe of the pancreas. The tissue condensation appeared prominent at sixty and eighty days-aged rabbits (Fig. 2). The means of lengths and widths of one, twenty, forty, sixty and eighty days - old rabbits were 15.40±0.37, 11.70±0.34, 22.80±0.53, 16.00±0.36, 26.20±0.57, 21.60±0.47, 30.40±0.33, 24.50±0.42, 35.40±0.33 and 29.80±0.20 mm, respectively (Table 1).

The tail lobe appeared compact glandular structure and it can be easily recognized as a separated part from the surrounding organs such as spleen and stomach. It extended as a thin, flattened ribbon between the two extremities of the spleen, running along the gastric surface of this organ and cross over the visceral surface of the stomach (Fig. 2 and 3). The means of lengths, widths of one, twenty, forty, sixty and eighty days old rabbits were 15.90±0.27, 2.60±0.16, 22.90±0.36, 4.10±0.23, 28.20±0.51, 6.20±0.22, 32.90±0.34, 7.10±0.23, 37.10±0.46 and 9.30±0.24 mm, respectively (Table 1).
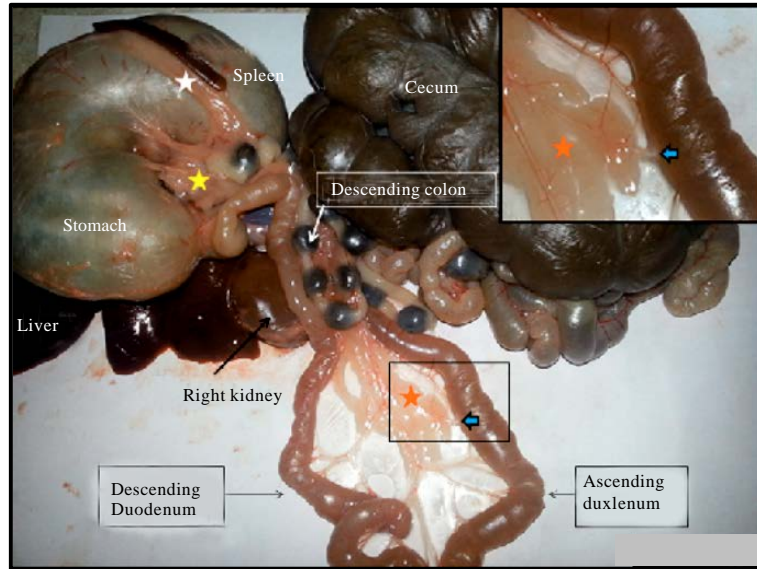
Fig. 2: Sixty days old rabbit showing *in situ* three different lobes of the pancreas. The tail lobe located at the gastric surface of the spleen (white star) and the body lobe (yellow star) and head lobe (red star). Second accessory duct (blue arrow) traversed the ascending duodenum
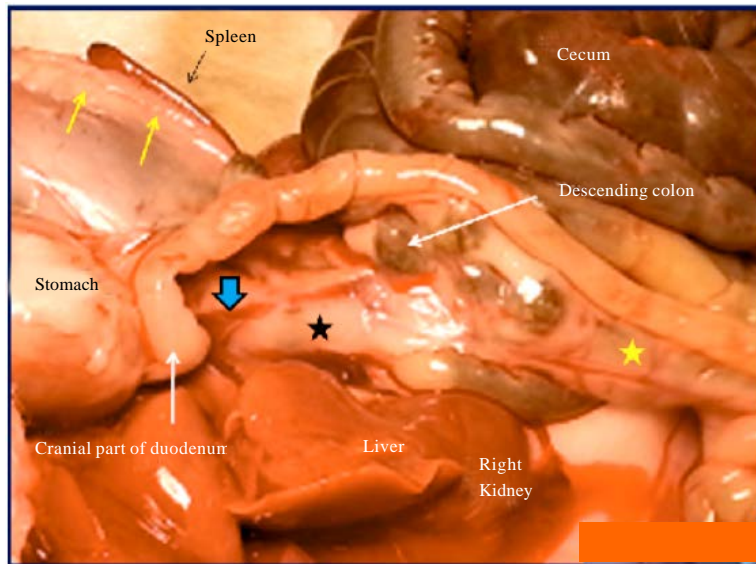

Fig. 3: Sixty days old rabbit showing *in situ* the three lobes of the pancreas. These lobes were head (yellow star), body (black stars) and tail (yellow arrows). The body lobe is passed by the portal vein (blue arrow)

Pancreatic duct: Current findings revealed absence of major pancreatic duct and the persistence of the accessory pancreatic duct in the local rabbit. It drains the pancreatic secretion from different parts of the pancreas and runs towards the ascending limb of the duodenum. It joined and opened into duodenal lumen before the reflection of the ascending duodenum towards the left side and its subsequent joining with the jejunum (Fig. 4). This duct empties into the ascending duodenum approximately 21, 31, 36, 41 and 43 cm distal to the
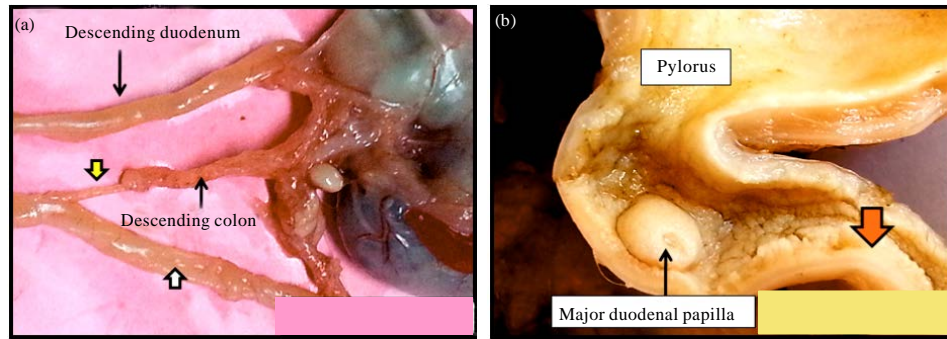
Fig. 4(a-b): (a) 20 days old rabbit showed accessory pancreatic duct (yellow arrow), empty in the lumen of ascending duodenum (white arrow) and (b) 80 days old rabbit showed the major duodenal papilla of the common bile duct at the cranial part of the descending duodenum (red arrow)

entrance of the common bile duct of the one, twenty, forty, sixty and eighty days old rabbits pancreas, respectively. Second accessory pancreatic duct was also detected (Fig. 4) which was emptied into the lumen of ascending duodenum near its initiation from the transverse limb of the duodenum. Currently, fine dissection identified absence of the major pancreatic excretory duct that connects the common bile duct which open into the proximal part of the duodenum via., the major duodenal papilla (Fig. 4).

Statistical analysis of gross results: Statistical analysis of the gross morphometric measurements revealed non significant differences in the body weights and measurements of length and width of the pancreatic lobes between male and female local rabbits implicated in this research. In another aspect, the analysis revealed distinct and significant higher lengths of the right lobes compared to those of the middle and left lobes. In another aspect, the data showed significant differences between the measurements of one day of age compared to those of other ages (p<0.05) and significant differences between the measurements of twenty days of age compared to those of other ages (p<0.05). Non significant differences observed between the measurements of forty days of age compared to those of sixty and eighty ages (p<0.05). Significant differences between the body weights between different ages (p<0.05) (Table 1). Weights of local rabbits appeared different from other breeds in other countries. The mean weights of local rabbits at birth was 57.51 g which was lower than those recently measured to the New Zealand rabbits weight (62.71 g), whereas, higher than the local Sudan rabbit which weigh 42.58 g at birth. The weight of local rabbits after weaning was 227.16 g which appeared very low compared to those of the

New Zealand rabbits (383.56 g) and local Sudan rabbits (390 g) for the same period of life (El-Rahman *et al.*, 2012; Elamin *et al.*, 2012).

Histological results
Exocrine acini, capsule, septa and ducts: Microscopic examination of one day-aged rabbits revealed ill-defined connective tissue capsule surrounding the lobes of the rabbit pancreas. The acinar units of the exocrine portion which constitute the bulk of the organ was subdivided into lobules by very fine connective tissue septa. The structure of each acinus was composed of several pyramidal-shaped cells possessing dark round peripherally located nuclei. Numerous zymogen granules were present at the apical part of the acinar cells and were extended peripherally in some of these acinar cells. Multiple centro-acinar cells were seen within the lumen of many acini. They were recognized by their locations as well as by their faintly-stained rounded large nuclei. Many myoepithelial cells were detected adjacent to the periphery of the acini which were characterized by their flattened shape and darkly stained cells. At this age the pancreas showed the development of large number of intralobular and large interlobular ducts characteristically at tail lobe that were lined with cuboidal and columnar epithelium, respectively. The accessory duct which was formed by the joining of several large interlobular ducts traversed the wall of duodenum. It collects the exocrine secretions from all lobes and drain into the lumen of the duodenum. At the site of entrance or traversing the wall of duodenum, the lumen of this duct appeared stellate in shape, lined with tall columnar epithelium surrounded with mainly collagenous fibrous connective tissue (Fig. 5). Morphometric measurements revealed up to 50.63% of acinar units area to the whole gland tissue. The data
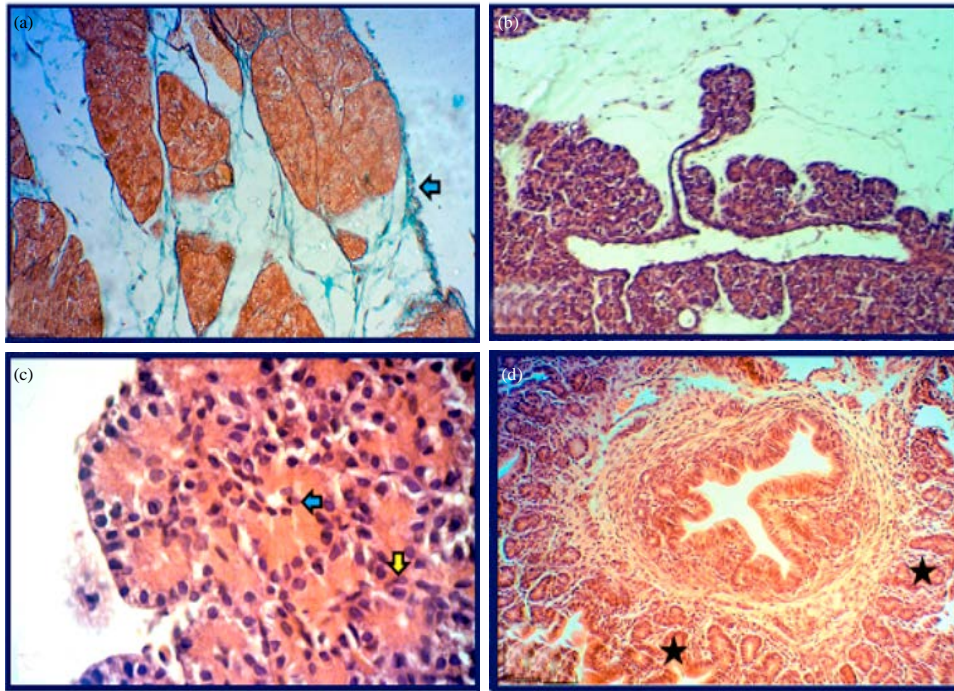
Fig. 5(a-d): (a) Tail lobe of one day-aged rabbit surrounded by thin capsule (blue arrow), Masson trichrome, X200, (b) Body lobe of one day-aged rabbit showed the large interlobular duct, H and E, X100, (c) Tail lobe of forty days-aged rabbit showed condensed pancreatic acinar units with sparse connective tissue septa. The acini showed centro-acinar cells (blue arrow) and peripherally surrounded by myoepithelial cells (yellow arrow), H and E, X 400 and (d) Accessory duct of one day aged rabbit, characterized by its stellate lumen and it traversed the wall of duodenum (black stars), H and E, X 100

Table 2: Changes in percentage of acinar units area per 1 mm^2 of each lobe and per 1 mm^2 whole pancreatic lobes at different post-natal periods

Ages	Acinar units area per 1 mm^2 of head (%)	Acinar units area per 1 mm^2 of body (%)	Acinar units area per 1 mm^2 of tail (%)	Acinar units area per 1 mm^2 of whole lobes (%)
One-day old	42.34	47.50	62.05	50.63
Twenty-days old	67.01	70.14	76.57	71.24*
Forty-days old	74.83	77.97	85.58	79.46#
Sixty-days old	78.69	83.81	89.59	84.03#
Eighty-days old	88.46	88.89	92.02	89.79#

*Significantly (p<0.05) different compared to those of one, forty, sixty and eighty days of age, #Not significant (p>0.05) differences between forty, sixty and eighty days of age, SE: Standard error of the mean

showed slightly compact acinar tissue at the tail lobe compared to those found at the head and body lobes (Table 2).

Similar to what was observed in one day-aged rabbit, microscopic examination of twenty day-aged rabbit revealed ill-defined connective tissue capsule. However, at this age the mesenteric tissue surrounding the lobules were reduced and characteristically in the tail lobe, leaving sparse and very fine connective tissue septa between them. The reduction of mesenteric tissue corresponded with marked proliferation of the acini resulting obvious increase in the bulk of the acini. The tail lobe clearly

showed higher reduction of the mesenteric tissue and therefore, appeared highly compact compared with those of the head and body lobes. The findings showed narrowing of the connective tissue septa with the reduction of the centroacinar cells. Correspondingly, intralobular ducts were less in number compared with those observed at one day aged rabbit pancreas and that some of them contained secretory material. The accessory pancreatic duct was lined with tall columnar epithelium with few goblet cells (Fig. 6). Morphometric measurements showed that the percentage of acinar units area to the bulk of the
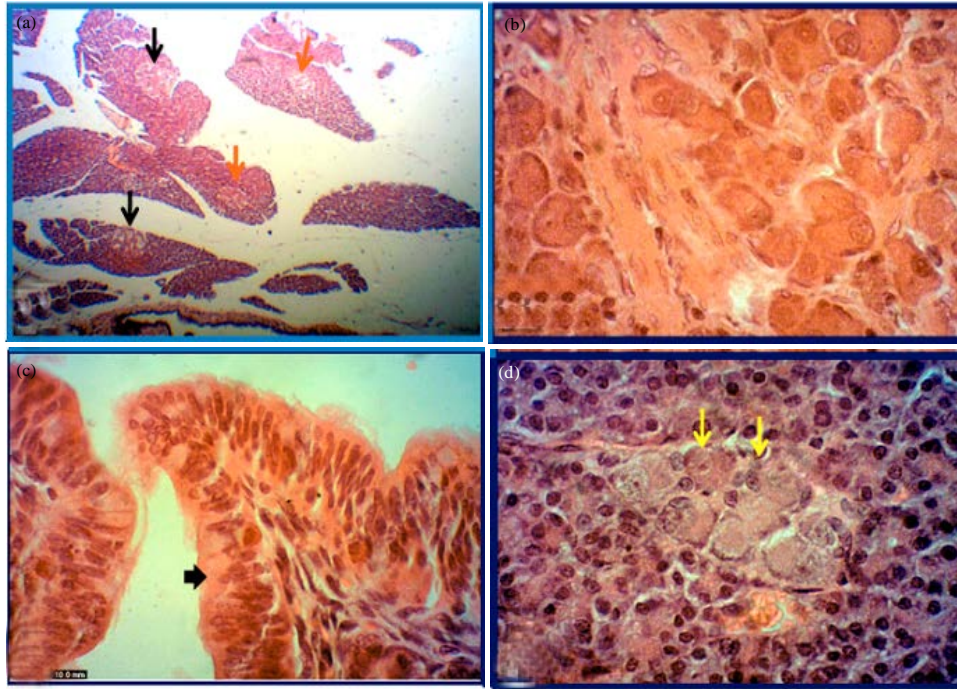
Fig. 6(a-d): (a) Twenty-days aged rabbit pancreas showed lightly stained islet's of Langerhans. The islet's appeared large (black arrows) or medium in size (red arrows) at the center or the periphery of the pancreatic lobules H and E, X100, (b) One day-aged rabbit pancreas showed large autonomic mesenteric ganglion, H and E, X400, (c) Accessory duct of forty days-aged rabbit lined with tall simple columnar epithelium with numerous goblet cells (black arrow), H and E, X400 and (d) Group of intramural ganglion cells (yellow arrows) between the acini of head lobe, H and E, X100

glands was elevated up to 71.24%, significantly, higher compared to those of one day aged rabbits (p<0.05).

At forty days-aged rabbit, the histological examination revealed that connective tissue capsule remain thin in nature with the presence of fine septa intervening between acinar units. There was obvious increase in the bulk of the acini on the expanse of the other pancreatic structures. The ratio percent of acinar area to the whole gland tissue was increased up to 79.46%, significantly (p< 0.05) different compared to those of one and twenty days-aged rabbits (Table 2). Acinar lumina were distinct and the centro-acinar cells were sparse in number. Very fine connective tissue present between the acini, but it increased at the sites of blood vessels and ducts location. Intralobular ducts and large interlobular ducts were present and most of them were filled with secretory materials. Similar to the previous ages, the accessory excretory pancreatic duct was lined with simple columnar cells with many prominent goblet cells.

Examination of the pancreas at sixty-aged rabbits revealed slight changes in the acinar units, connective tissue septae, duct system as well as their blood vessels. Intralobular and interlobular septae were prominently reduced separating the acinar units and pancreatic lobules, respectively. In fact, condensation of pancreatic tissue at this age was comparable to the gross appearance of this organ. The tail lobe showed marked cord-like exocrine tissue. Most of the small and large interlobular ducts duct were filled with secretory materials. The exocrine portion at this age of animal appeared well developed compared to previous ages and in another hands it appeared similar to what was found at the subsequent eighty days-aged rabbits. The presence of the secretory materials in most branches of the duct system gave rise to such attitude. The percentage of acinar units to the total gland tissue was increased up to 84.03%, significantly (p< 0.05) different compared to those of one and twenty days-aged rabbits. Whereas, not significantly different from those of the forty days-aged rabbit pancreas. At eighty days-aged rabbits, slight changes observed compared to the sixty days-aged rabbit pancreas. The percentage of acinar area to the total gland tissue was increased up to 89.79%,

significantly (p< 0.05) different compared to those of one day and twenty days-aged rabbits. Whereas, not significantly different from the those of the forty and sixty days-aged rabbit pancreas (Table 2).

Autonomic ganglion: Microscopic examination of different parts of the pancreas revealed the presence of large autonomic ganglion located within the mesentery nearby the body lobe of the pancreas of which that part closely related to the duodeno-pyloric junction. The ganglion found at one day-aged rabbits and the subsequent ages. In the same time, current findings revealed the presence of intramural ganglion cells in the exocrine portion at both head and tail lobes of the pancreas at one day-aged rabbits and the subsequent ages (Fig. 6).

Endocrine portion (Islet's of Langerhans): Histologically the endocrine portion reside primarily in the islet's of Langerhans which were distributed throughout the three lobes of the pancreas. On hematoxylin and eosin stained sections, the islets were easily identified as as lighter colored aggregated cells distributed between darkerly stained pancreatic acini (Fig. 6). The individual islets were variable in shape but were usually rounded or ovoid structures of pale irregular anastomosing cellular cords separated by fairly scant connective tissue and numerous blood capillaries. They were associated well with extra-blood vessels and exocrine ducts. Microscopically, each islet was structured of several cells which were different in size, shape and color. Less frequently, single endocrine cells were present within the exocrine pancreatic tissue (Fig. 7). A significant difference was
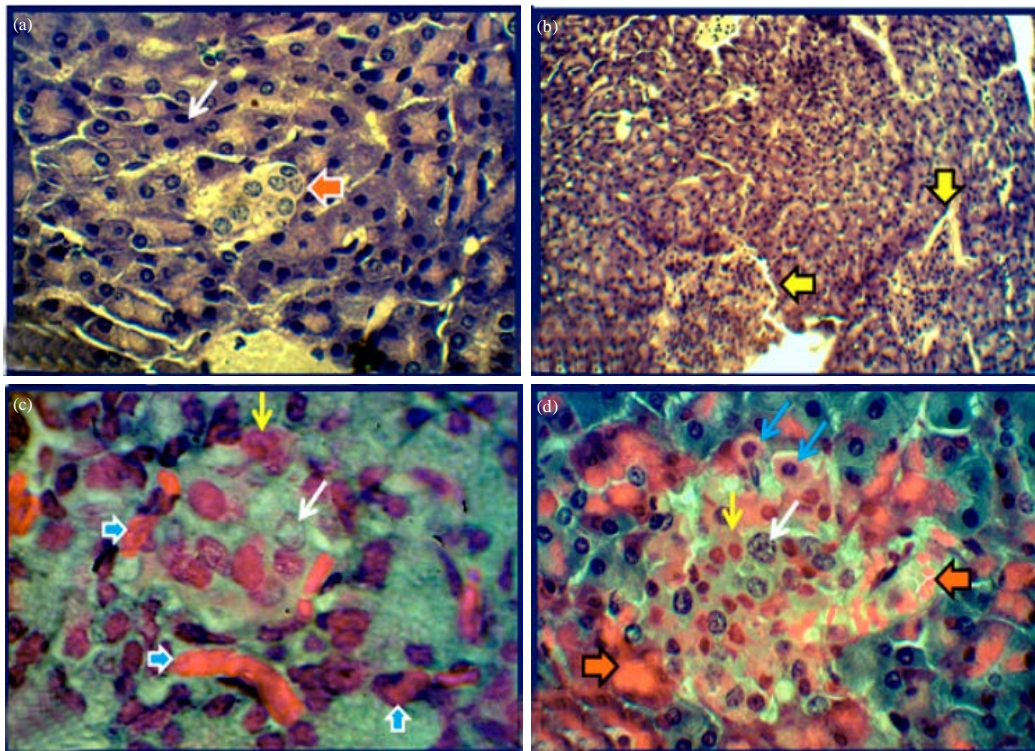

Fig. 7(a-d): (a) Twenty- days aged rabbit pancreas showed lightly stained islet's of Langerhans. The islet's appeared small in size (red arrows) embedded in the pancreatic tissue (white arrow) as a small group of cells, PAS, X400, (b) Forty-days aged rabbit pancreas showed lightly stained islet's of Langerhans (yellow arrows) embedded within the acinar units, PAS, X100, (c) Islet's of Langerhans at one-day aged rabbit pancreas showed red colored α cells (yellow arrow) and faintly stained blue β cells (white arrows) supplied with abundant blood capillaries (blue arrows), Gomori's Trichrome, X1000 and (d) Islet's in twenty-days aged rabbit pancreas showed red colored α cells (yellow arrow), blue stained β cells (white arrow) and δ cell (blue arrows). The islet supplied well by blood capillaries (red arrows), Gomori's Trichrome, X400

Table 3: Mean of density of islets per each mm² of pancreatic lobe and per each mm² of the whole pancreatic lobes at different post-natal periods

Ages (days)	Mean of islets number mm⁻²			
	Head lobe	Body lobe	Tail lobe	Whole lobes
One	0.37±0.11	0.45±0.04	1.30±0.33*	2.12±0.02
Twenty**	0.40±0.03	0.47±0.23	2.12±0.65*	2.99±0.10
Forty#	0.39±0.01	0.49±0.06	2.32±0.02*	3.20±0.51
Sixty#	0.40±0.34	0.48±0.01	2.34±0.05*	3.22±0.43
Eighty#	0.42±0.06	0.49±0.02	2.37±0.67*	3.28±0.06

*Significantly (p>0.05) higher density compared with the body and head lobes within each age, **Significantly (p>0.05) higher density compared with the one day of age, #: Densities at forty, sixty and eighty were significantly (p>0.05) higher compared with that of one day of age but not significantly different compared with the twenty day of age

Table 4: Changes in the ratios of β to α and δ to the sum of at β and α at different post-natal periods

Ages (days)	Mean number of β to α	Ratio	Mean number of δ to sum of β and α	Ratio**
One	5.8/5.3	1.09 : 1	0.1 : 11.1	0.009
Twenty	16.5/9.2	1.79 : 1*	1.4/25.7	0.054
Forty	28.2/12.3	2.29 #	1.8/40.5	0.044
Sixty	37.1/16.1	2.30 #	2.1/53.2	0.039
Eighty	49.4/21.3	2.32 #	2.2/70.7	0/031

*Significantly (p>0.05) higher ratio of β to α compared with the other ages, #: Not significant (p>0.05) differences between forty, sixty and eighty days of age, **Remain in lowest ratio compared to β to α ratio during post-natal periods

observed between the head, body lobes and tail lobe in the mean islet density and mean islet diameters. Most of islets present at the tail lobe were small in size, whereas, islets at both right and middle lobes were situated at the periphery or the centers of the pancreatic lobules. These lobes composed of medium islets and the head one showed frequently large islets too (Fig. 6). In the rabbit, islets were usually occupied a central position in each pancreatic lobule. The density or the number of these islets was highest characteristically at the tail lobe of the rabbit pancreas, whereas, lowest number of them counted at head and body lobes of which both have nearly similar densities (Table 3).

Sections of pancreas prepared and stained with Gomori's Trichrome from one aged rabbits revealed mainly two types of cells of approximately equal number constituting each islet. The first type was stained faintly blue represented the β cells (insulin producing cells), whereas, the second was red in color represented α cells (glucagon producing cells). The ratio of β cells to α cells was 1.09-1 (Table 4). Some islets were showed single δ cells and its ratio to the sum of β and α cells was 0.009-1. Many blood capillaries were present inside and outside the islets (Fig. 7).

On twenty days of age, the picture of islet's of Langerhans was obviously changed compared to those of one day aged rabbit pancreas. The number of β cells was increased and the ratio of β cells to α cells was elevated into 1.79-1 and few cells of δ type with a ratio of this type to the sum of both β and α which was 0.054-1 (Table 4). The cells of δ were detected singly at the periphery of the islets which were larger than the α cells and faintly pinkish in color (Fig. 7).

At forty days of age and subsequent ages, the number of β cells increased gradually and the ratios were increased up to 2.29-1, 2.30-1 and 2.32-1 at forty, sixty and eighty days of age, respectively. Similarly, the ratio of δ to sum of both β and α was slightly changed into 0.044, 0.039 and 0.031 at the same above periods (Fig. 8) (Table 4). At these latter ages, the number of the β cells appeared higher than those of α cells as well as higher blood vascularization at both the interior of islets and the region surrounding them.

Current findings in rabbits islet of Langerhans showed that the β cells were variable in size and possessed less dense cytoplasm compared to the α cells which were usually found at the periphery of the islets. The size of β cells appeared larger compared to α cells but this finding was conflicting because some of them appeared smaller. In general, β cells resume the center of islets and those α cells at the periphery but this picture was not constant. Some of α cells distributed at the center and some of the β cells at the periphery. There were no definite zonation or demarcation between them. In conclusion, there were inconstant pattern of cells distribution within the islets in the rabbit pancreas. Currently, the size of islets was increased due to the gradual increase of their cells number, prominently the β cells (Table 4). Morphometric data of one-day aged rabbits revealed that each 1 mm³ of pancreatic parenchyma contained approximately 2.12±0.01SE islets of Langerhans. The number was the mean of those counted at head, body and tail lobes. The number of islets was increased up to 2.99±0.05SE, 3.20±0.03SE, 3.22±0.06SE and 3.28±0.10SE at twenty, forty, sixty and eighty days of age, respectively (Table 3).
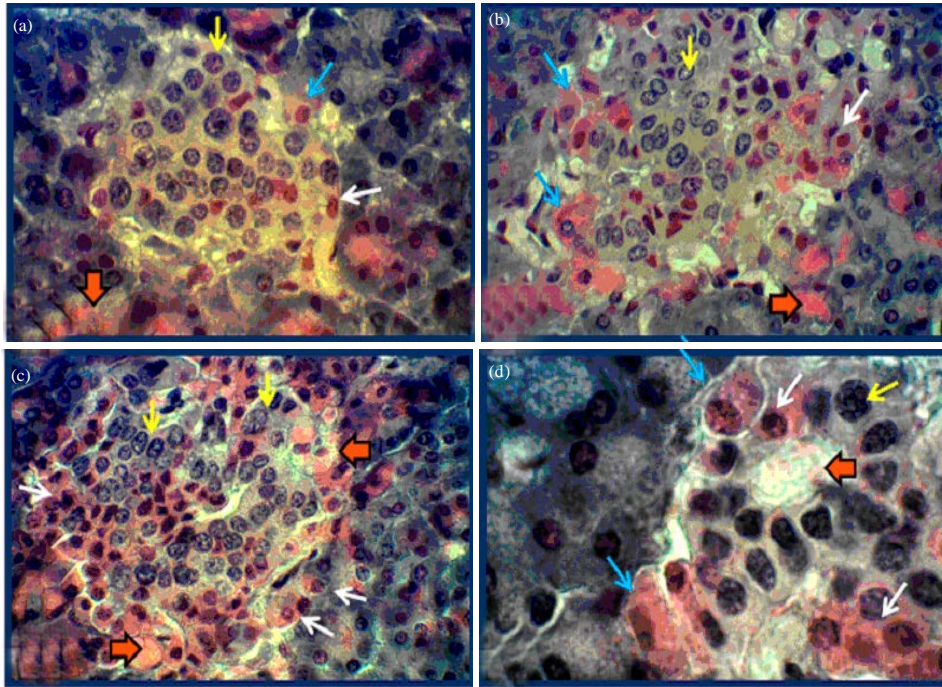
Fig. 8(a-d): Islet's of Langerhans in (a) Forty, (b) Sixty (c, d) Eighty days aged rabbit pancreas. It showed α cells (white arrows), β cells (yellow arrows) and δ cell (blue arrows). The islet supplied well by blood capillaries (red arrows), Gomori's Trichrome, X400 (A-C), X1000

DISCUSSION

The classification lobes of rabbit pancreas in this study was similar to that of Catala *et al.* (1987) whom they classified and applied the same lobes in the rabbit too. Whereas, it was different from that described the pancreas of the rat by having 4 regions, that were lower duodenal, upper duodenal, gastric and spleenic regions (Elayat *et al.*, 1995). On the same time, Slack (1995) described differently the human pancreas by having head, neck, body and tail and that the organ is connected to the duodenum by ampulla of Vater, where the main pancreatic duct join with the common bile duct. In fact, among laboratory animals the pancreas is more variable macroscopically than in its microscopic appearances and so that two basic anatomical patterns had been recognized to this organ that were mesentric and compact types. The mesenteric type, as was found in the current study in the local rabbit was diffusely distributed in the mesentery of the small intestine and such observation was in consistence with those of Eifler *et al.* (2009) and in the mouse and rat, the pancreas was also of mesenteric type but fairly close-packed in form. Whereas, the more compact type was recognized previously in hamster,

dogs, monkeys and humans (Treuting *et al.*, 2012). Current observations coincides previous findings of Yi *et al.* (2005) whom postulated that the gross anatomy of the rabbit pancreas, unlike that of other commonly used laboratory and animal species such as rat, mouse, guinea pig, cat and dog by exhibiting no clearly defined right, middle and left lobes or regions. In fact, the pancreas of rabbits was disseminated structure and often difficult to differentiated from the surrounding mesentery (Davies and Davies, 2003; Eifler *et al.*, 2009).

The body lobe of the pancreas was well characterized by the passage of the portal vein through it which was considered in the previous researches as a good vessel marker to this part of the pancreas (Brewer, 2006; Abidu-Figueiredo *et al.*, 2008). The main pancreatic duct, which was absent in the local rabbit was differently found in the other laboratory animal species such as mice (Mooren *et al.*, 2003), rats (Kara, 2005), hamster (Murray, 2012) and guinea pigs (Suckow *et al.*, 2012). The presence of accessory pancreatic duct in the local rabbit was similar to what was found in pigs and ox and dissimilar to those found in sheep and goat (Witchtel, 2002), as in the latter species the accessory duct was regressed and that the pancreatic duct was

persist and opened with common bile duct into the duodenum at the major duodenal papilla. In most dogs, two pancreatic ducts were present that are the pancreatic duct (mainly) and the accessory pancreatic present in 24% of examined cases (Sherman and Lindenmuth, 1969; Mix and Jones, 2006), whereas, oppositely, cats have the accessory pancreatic duct (mainly) and the main pancreatic duct present in 20% of examined cases (Nickel *et al.*, 1979).

The exocrine tissue development was continued throughout the postnatal periods selected in the current study. General morphology of the acinar units in the newly born rabbits at day one appeared similar to those well developed at the subsequent eighty days of age but their gradual morphological changes at twenty, forty and sixty days of age indicated that they were not fully developed at birth. The above remarks were similar to previous investigations conducted on the development of the pancreas and found in many species such as the mouse (Debray *et al.*, 2003), rats (Desouky *et al.*, 1996), guinea pigs (De Assis *et al.*, 2003) and rabbits (Aboul Mahasen and Abou, 1999) that their pancreas is not fully developed at birth.

At day one, there were no secretory materials found inside the duct system of the pancreas. The findings correlated well with the gross observation as the digestive tracts of the one day rabbits were empty, so that their digestive systems were not active yet. At day twenty, the presence of secretory materials in some interlobular ducts may reflects the initial production of some pancreatic enzymes. Actually, previous investigator recorded the beginning of amylase production from the pancreas of rats at similar period, i.e., suckling period of the animal (Pollack *et al.*, 1986). The findings also correlated well with those of Kinouchi *et al.* (2012) whom mentioned that large molecule protein feeding during the suckling period of the rats required for the proper development of pancreatic digestive functions. The investigator believed that milk-borne large proteins which require digestion by pancreatic enzymes for intestinal absorption might be involved in the developmental processes of pancreatic digestive functions in infants. Really, the dissection of stomach of some twenty days-aged rabbits showed milky constituents whereas, others were showed greenish ingesta. The exocrine portion at the forty days-aged rabbits showed higher percentage of ratio of acinar units area/whole pancreatic lobes. Lining epithelium of the accessory duct revealed large number of goblet cells. Secretory materials were filled most of the large interlobular ducts in the pancreas. These changes reflected further production of pancreatic enzymes toward the lumen of the duodenum. The type and nature of food

ingesta which was found in the stomach of these rabbits during their dissection was dense, dark greenish in color. The latter reflects the post- weaning period of the animals at which the digestion of solid materials could be processed with the demand for the production of new enzymes such as those of the pancreas. Morphological changes in the exocrine portion being slightly progressed post forty days of age. At days sixty and eighty of age, the changes detected in the morphometrical measurements as well. The percentage of ratio of acinar units area/whole pancreatic lobes was increased and significantly different when compared to those of one and twenty days of age.

Current facts confirmed the previous considerations that the weaning is a critical period for the young mammals, since digestive processes are maturating intensively in association with a disruption of the feeding behavior (Gidenne, 2003; Montagne *et al.*, 2003; Montagne *et al.*, 2007; Gidenne *et al.*, 2007). However, previously mentioned that the development of enzymatic equipment of the rabbit's pancreas starts around days 21–24 of age, regardless of the nature of diet (Lebas *et al.*, 1971). The feeding behavior before and after weaning in the local rabbit corresponded well with the exocrine tissue maturation. Similarly, one decade before, De Assis *et al.* (2003) recorded that the morphological maturation of the pancreatic acinar tissue of the guinea pig occurred during the first month of the post-natal life significantly during the period from 2-21 days of age which was around weaning-postweaning period of the animal. The findings being in consistence with changing in food habits of the animals. Current thoughts were in agreement with those of Debray *et al.* (2003) whom believed that the development of enzymatic equipment of the rabbit's pancreas could be starts during days 21-24 of age. The investigator recorded the activities of some enzymes such as lipase and amylase which were significantly increased during the days between 25 and 42 of age, the period represent last days of suckling to post weaning in the rabbits.

Islet's of Langerhans being species dependent in its position. In the rabbit being different from those observed in the cat islets. They were usually occupied a central position in each pancreatic lobule. In laboratory animals such as the rats, the islets were quite often peripheral in the lobule. Whereas, in the mouse were eccentrically placed and so appear to be partially isolated from the exocrine pancreas (Henderson and Daniel, 1979). Higher density at the tail lobe was similarly observed in previous investigations in the pancreas of rabbits (Catala *et al.*, 1987), Wistar albino rats (Elayat *et al.*, 1995) and human

(Wang *et al.*, 2013). Whereas, the picture was different in the C57BL/6 mice in which higher density observed at the gastric lobe compared to the spleenic or left lobe (Hornblad *et al.*, 2011).

The current progressive increased ratio of β to α cells in rabbits were comparable to those observed previously in the rats pancreas too. It was found that the ratio of β cells to α cells within an islet of adult Wistar rats was over 9:1 indicating continuous elevation of this ratio during postnatal period (Berney *et al.*, 1997). The characteristic elevation of β cells in the endocrine of rabbit pancreas was in consistence with those previously observed in the fetal and post-natal rabbit pancreas that the number of cells per islet increased with the age and the ratio of β to α cells progressively changed (Beitcosme *et al.*, 1970). In addition to that the investigator recognized as low as ratio of δ to the total number of both β and α cells.

In fact, the histological changes in the endocrine tissue being obvious at twenty days of age as a pre-weaning period compared to those of one day of age. The current thoughts confirmed previous observations of Desouky *et al.* (1996) on the pancreas development of the Albino rats in, which the endocrine tissue acquired their adult form at twenty one days of age. Such maturation resulted also during the pre-weaning period of the rats.

Current findings showed no clear zonation between β and α cells in the islet's of Langerhans and appeared different to those previously observed in the rat pancreas, at which the islets revealed zonation in their distribution. The α cells spread out at the periphery and those of β cells in the centers of the islets (Berney *et al.*, 1997). Whereas, similar picture to what was observed in the rabbit islets, described previously in the mouse pancreatic islet's of Langerhans (Seymour *et al.*, 2004) and in those of both cats and dogs (Bjorneby and Kari, 2002). However in human pancreas, Bosco *et al.* (2010) observed in small islets the β cells had a core position and α cells at the periphery but the latter cells were found inside the islets of the bigger sized islets. In fact, there were diverse patterns of endocrine cell arrangement in different species. In the domestic cats, the islets are composed mainly of β cells with a few scattered α and δ cells. Some islets are found in the interlobular connective tissue. These islets have a centralized area of α cells with an equal number of surrounding β cells and a few solitary δ cells. The α, β and δ cells have also been observed in pancreatic ducts (O'Brien *et al.*, 1986). In the domestic dogs, the islets are composed of a core of β cells and are generally surrounded by peripherally located α cells. However, α cells can occasionally be found in the interior of the islet and β cells are sometimes found on the periphery (Muranishi *et al.*, 1999).

In the rabbit pancreas, islets densities and sizes were different in different regions or lobes of pancreas and in another aspect the α and β cells distribution within the interior of each was different and such differences confirmed the observations of Wieczorek *et al.* (1998). The latter and his colleagues found different regional arrangement of the islets in the pancreas of rats, dogs and minipigs. In addition to that they found characteristic arrangement of the cells producing different hormones within each islet for each species. Currently, the size of islets was increased due to the gradual increase of their cells number, prominently the β cells. The increased size of islets previously documented in the mouse by Herbach *et al.* (2011) whom believed that mean islet size increased with age or increasing demand. The thought that the β cell mass could be increased via., both β cells hyperplasia and hypertrophy characteristically at 10 days of the mouse age and continued significantly until day 90 and stayed constant thereafter. A recent study demonstrated that β cell mass could be increased by both replication and hypertrophy. The increase in the size could be caused by an increase in the mean individual cell volume as well as an increase in the number of β cell (Bogdani *et al.*, 2005). In conclusion, less organized structural islet aspect as well as changes of β cells/α cells and δ cells to the other types of cells in one day aged rabbit islets compared to the subsequent advanced ages reflected their immaturity at birth (Inuwa *et al.*, 2005).

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