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Original Article

Physiological and Histological Changes in Pancreatic Gland Associated with Ageing in Local Rabbits in Iraq

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Abstract

The pancreas is a pear-shaped flat organ resembling the letter L, and yellowish to pink in color. This organ is of medical significance since it is associated with two life-threatening diseases including diabetes mellitus and pancreatic cancer. This study was conducted on male rabbits which were assigned into 3 age groups (6-month-old, 1-year-old, and 3-year-old rabbits). Physiological and histological changes of the pancreas were studied in the adopted age groups. The physiological aspect and the histological structure of the pancreas were also studied by the analysis of the level of pancreatic gland hormones and hormonal changes. Based on the results, there were significant differences in the concentration of pancreatic gland hormones. Insulin level in the second study group was more than that in the first and third groups, while the highest concentration of blood sugar (glucose) was observed in the third group, compared to the first and second. Although the basic structure of the pancreas was similar in all samples, changes were observed in the tissue structure of the pancreas throughout the process of aging. By the increase of age (from 1 to 3 years old), Langerhans islets increased in size, contained alpha and beta cells that were surrounded by a loose connective tissue in the third stage. Moreover, no significant difference was observed in the diameters of cells that produced enzymes at all stages of life. Physiological and histological changes indicated that age plays a role in the function and structure of the pancreas gland during different stages of life. In addition, this study indicated that the hormonal variability of the pancreas is closely related to the histological composition of gland components. Therefore, further studies on the role of factors, such as gender, different breeds, or environmental conditions seem to be necessary and may provide more information on factors that may affect the effectiveness and activity of the pancreas gland.

Keywords: Age Progress, Langerhans Islets Diameters, Pancreas, Rabbit's Pancreatic Gland

1. Introduction

Rabbit, a small mammal from the Leporidae family, is adapted to live in different environments in the world and feeds on plants (1). This animal has many characteristics that make it desirable for breeding, such as a beautiful shape, good taste, usefulness, rapid reproduction, and the quality of its furs (2, 3). Most rabbits are 25-29 cm in length, and their colors range from white to black, gray and spotted, and are about four to seven months old (4). Rabbits are favored by many researchers in the laboratories due to some reasons including their short lifespan and rapid reproduction that allows scientists to monitor the effectiveness of treatment regimens on several generations of rabbits in a relatively short period of time (3). Rabbits are temperate animals and fast-adapting organisms. They are not affected by the environment in which they live (in contrast to many living organisms that are affected by the changing environment and require a habitat similar to their original environment to survive and reproduce). Therefore, experiments on rabbits are often not affected by any behavioral or genetic changes that may occur in other animals as a result of transferring to an environment different from their natural habitat (5).

The pancreas is a flat pear-shaped organ resembling the letter L. It is almost yellowish to pink in color and of medical significance, since it is associated with life-threatening diseases, including diabetes mellitus and pancreatic cancer (6). The pancreas is located horizontally behind the stomach deep in the upper left posterior of the abdomen against the spine at the level of the first and second lumbar vertebrae. It is a mixed exocrine and endocrine gland that contains two types of cells, including acinar cells and Langerhans islets. Acinar cells constitute more than 95% of the pancreas mass, are relatively large and less dense than the second type cells. Acinar cells secrete enzymes that help digest proteins, fats, and carbohydrates, such as amylase, lipase, and protease. Proteases are excreted as inactive zymogens, such as trypsinogen, which is activated by the duodenum to trypsin, which in turn activates other proteases, such as chymotrypsinogen to chymotrypsin. These enzymes are carried through a special channel that flows into the duodenum. The second type of pancreas cells is named Langerhans islets, which constitute from 1 to 2% of pancreas mass including lumps of elliptical or spherical forms with an endocrine secretion that are embedded within the acinar cells and secretes several hormones directly into the blood. These consist of several types of cells, most important of which are alpha (α) cells the α-cells are situated in the islets of Langerhans, which constitute the endocrine part of the pancreas. The most centrally located beta cells (β) are insulin-releasing and are able to lower the level of glucose in the blood by promoting its entry into muscles and other body tissues as a source of energy, as well as, storing it in form of glycogen in the liver to be used when needed. Delta cells (δ), which are scattered and the least in number, secrete somatostatin that inhibits the secretion of growth hormone and thyroid-stimulating hormone (TSH) from the anterior lobe of the pituitary and inhibit secretion of HCL from gastric parietal cells as well. Secondary cells secrete pancreatic polypeptides which activate chief cells in the stomach, inhibit secretion of bile and bicarbonate, and inhibit intestinal motility (7-10).

The current study aimed to investigate physiological and histological changes in the pancreas that occur during the process of aging.

2. Material and Methods

2.1. Animals

This study was carried out on nine male rabbits which were assigned into three age groups. Group 1(n=3), Group 2 (n=3), and Group 3 (n=3) consisted of 6-month-old, one-year-old, and three-year-old male rabbits, respectively. The physiological and histological changes in the pancreatic gland at different age groups were studied on these three groups of rabbits. All instruments applied in this study were calibrated and maintained in accordance with routine quality control procedures overseen by the Quality Assurance Department of the Tikrit University, College of Science, Department of Biology, Tikrit, Iraq. Local breed rabbits were housed under controlled environmental conditions (20±2°C, 14:10h light: dark cycle) and allowed ad libitum access to food and water.

2.2. Blood Sampling and Hormonal Assays

Hormonal analysis of blood samples (i.e., physiological analysis) was performed using the enzyme-linked immunosorbent assay (ELISA) method. The blood samples were collected from the ear vein in anticoagulant Venoject tubes (BD Life Sciences, Cockeysville, Md, USA), and the concentration of somatostatin and insulin were measured using Rabbit Somatostatin ELISA Kit (ARP, Catalog# E04S0192, USA) and Rabbit Insulin ELISA Kit (Crystal Chem, Catalog #90186, USA), respectively. Moreover, the blood sugar was measured for all animals.

2.3. Tissue Processing and Histological Examination

According to the tissue processing technique, the pancreas was quickly removed and the samples were prepared after cutting a sagittal section(5×5×10 mm) from the head, body, and tail, according to routine tissue technique (11). In brief, the routine tissue techniques included fixation, washing, dehydration, clearing , infiltration, embedding, sectioning, mounting, and staining steps. The histological examination and microscopic photography with a magnification of 40×werecarried out after the conduction of the mentioned steps to study the morphological changes of both exocrine and endocrine cells. Moreover, the pancreatic acini and the islets of Langerhans in the microscopic field were counted by calculating these structures in twenty microscopic fields for each sample by the objective lens 10× (12).

2.4. Statistical Analyze

Data were statistically analyzed using correlation analysis and analysis of variance (ANOVA) to assess the variance among different age groups, the relationship between age and different variables, and the differences between mean values of age groups. The statistical data were analyzed using the SPSS (Version 1.0.0.1406) and Microsoft Excel XP software. A p-value ≤0.01wasconsidered statistically significant.

3. Results and Discussion

Statistical differences were observed among three study groups in terms of concentration of insulin, somatostatin, and blood sugar in the blood samples. Moreover, there were clear changes or differences in pancreas tissue structure by the age progress.

3.1. Hormonal Changes

Statistical analysis showed that the level of insulin and somatostatin were higher in the second group (1-year-old rabbits), compared to the first (6-months-old rabbits) and third (3-year-old rabbits) groups (P≤0.01). However, blood sugar was statistically higher in the third group, compared to the first and second groups (P≤0.01), as shown in table 1. In addition, no statistically significant difference was observed in terms of diameters of acinar cells (P≤0.01) among the three age groups (Table 2). As indicated in table 3, there were statistically significant differences between the second group with the first and third groups in terms of the diameters of Langerhans islets (P≤0.01).

Table 1. Evaluation of the pancreatic parameters according to the age groups 1, 2, and 3

Group	Insulin	Somatostatin	Blood sugar
1	0.2 ± 0.011	0.03 ± 0.012	96 ± 3.469
2	0.89 ± 0.052	0.013 ± 0.017	89 ± 1.155
3	0.56 ± 0.035	0.03 ± 0.005	112 ± 1.149

Table 2. Meandiameter of acinar cells at age groups 1, 2, and 3 (micrometer/ 40× magnification)

First group	Second group	Third group
5.91 ± 0.50	5.08 ± 0.36	5 ± 0.37

Table 3. Mean diameter of Langerhans islets at groups 1, 2, and 3 (micrometer/ 40×magnification)

First group	Second group	Third group
19.90 ± 0.75	39.66 ± 3.99	26.87 ± 3.29

3.2. Histological changes

Histological examination in the early stage of life (6-month-old rabbits) showed that the pancreas was found in small separated lobules, and external secretory units consisting of a number of pyramidal cells with intercalated small ducts inside the lobules, as well as, interlobular ducts (Figures 1 and 2).In addition, internal secretory units represented by Langerhans islets were a number of hormone-secreting cells of small sizes, shapes, and diameters adjacent to the blood vessels (Figure 3). Pancreatic cells at puberty (middle age) were found to be interconnected and external secretory units, with their cells having a purple cytoplasm, dark-blue nuclei, and large multicellular Langerhans islets with abundant capillaries (Figures 4 and 5) and large-size pancreatic ducts lined with simple cuboidal cells surrounded by a medium-sized blood vessel (Figure 6). Eventually, the pancreas in the advanced age stage or perennial rabbits (3-year-old rabbits) contained lobules with external secretion comprised of pyramidal cells of the same size (in contrast to the previous two age groups). However, the intercalated ducts were difficult to show inside the lobules (Figures 7 and 8). Furthermore, adipose tissue was observed at this stage between lobules in the parenchyma of the pancreas as large adipocytes, which also appeared between external secretory units (Figures 9 and 10).

The pancreas is a mixed gland of exocrine and endocrine secretion surrounded by a capsule of very thin connective tissue that invaginates into the gland to form septa that further divide the pancreas into lobes containing many distinctive lobules (5). The pancreas is a very sensitive tissue to metabolic disorders in the body and can cause an imbalance of essential hormones secreted from cells of Langerhans islets (endocrine secretion), which are located within the structure surrounded by acinar cells (exocrine secretion) (1).

Based on the obtained results, hormonal secretion, at the first stages of life, play a role in the metabolism of glucose as indicated by normal results recorded with normal histological structure. It should be noted that better effectiveness of pancreatic hormonal activity was observed with the age progress, with the extended size of Langerhans islets. This finding indicated that the progress of age in rabbits is associated with better enzymatic and hormonal activities. This finding is consistent with those obtained by researchers in other studies (9, 13).

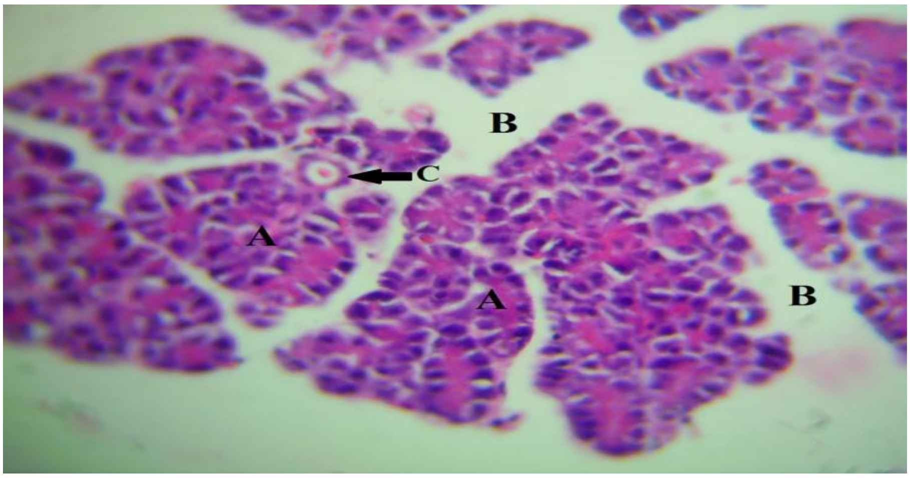

Figure 1. (6-month-old rabbits): Lobules of the pancreas are small in size and separated from each other (A), Fibrous septa (B), Duct between lobules (C) (H&E. 40×).

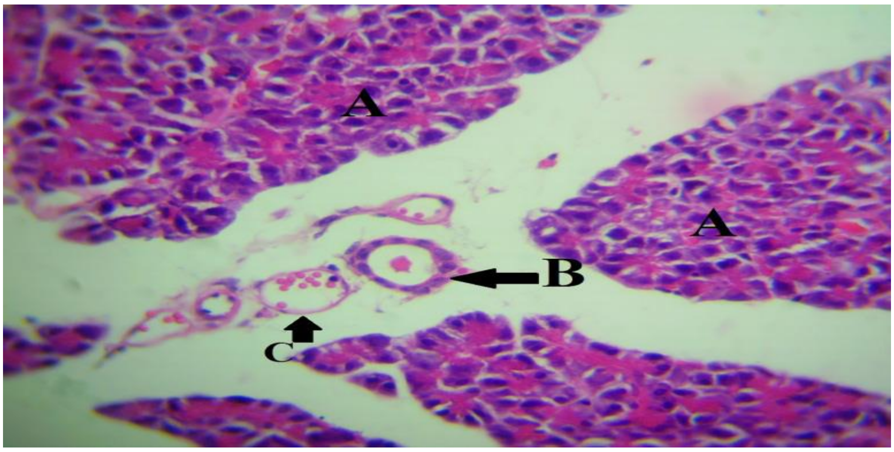

Figure 2. (6-month-old rabbits): Pancreatic secretory units (A), Duct between lobules (B), Blood vessels (C) (H&E. 40×).

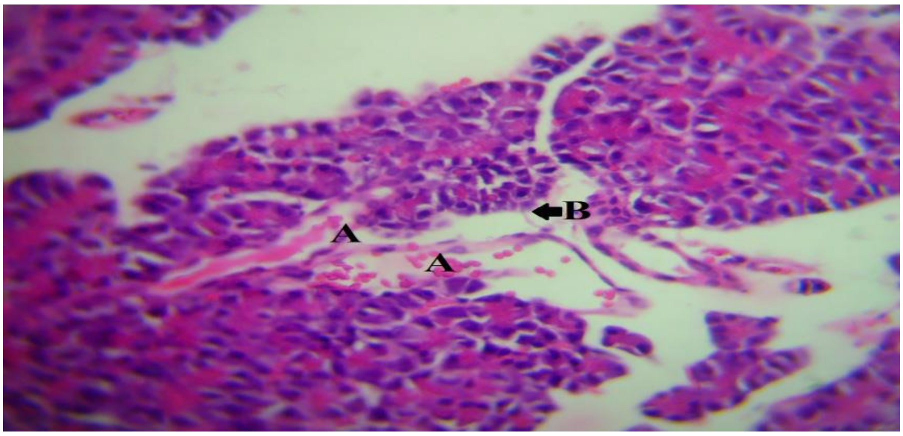

Figure 3. (6-month-old rabbits): Capillaries between pancreatic lobules (A), Islet of Langerhans with endocrine cells (B) (H&E. 40×).

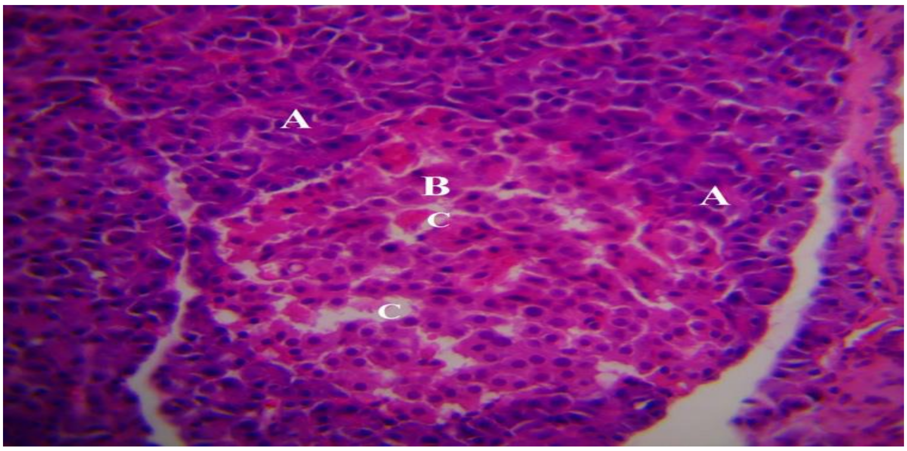

Figure 4. (12-month-old rabbits): Crowding of external pancreatic units (A), Large size and diameter of Langerhans islets with the abundance of their endocrine cells (B), Capillaries in islets of Langerhans (C). (H&E. 40X×).

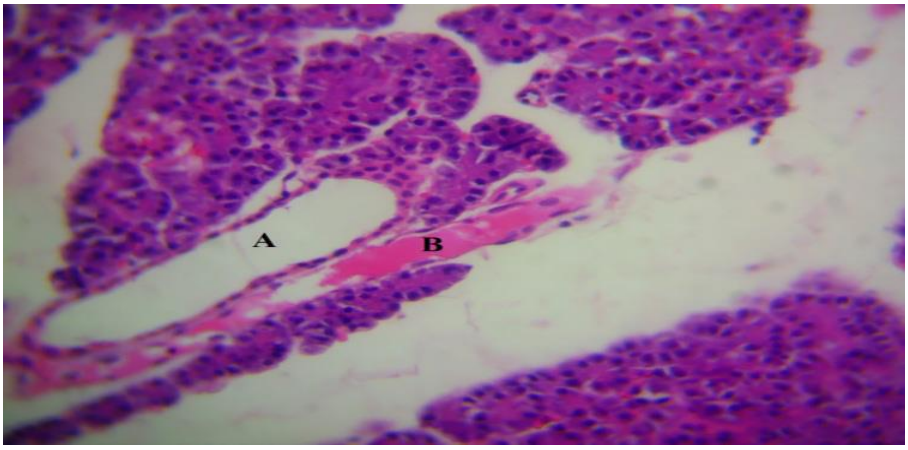

Figure 5. (12-month-old rabbits): Large duct between lobules (A), Blood vessel congestion (B) (H&E. 40×).

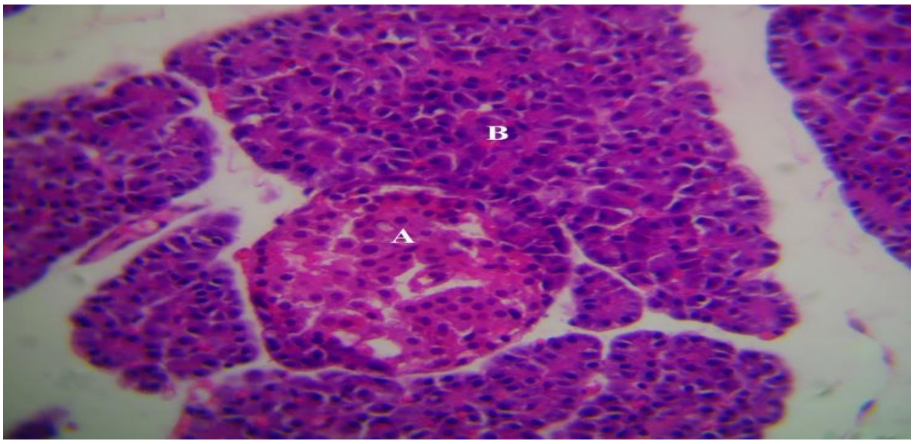

Figure 6. (12-month-old rabbits): Large Langerhans islet with its endocrine cells (A), External excretory units (B) (H&E. 40×).

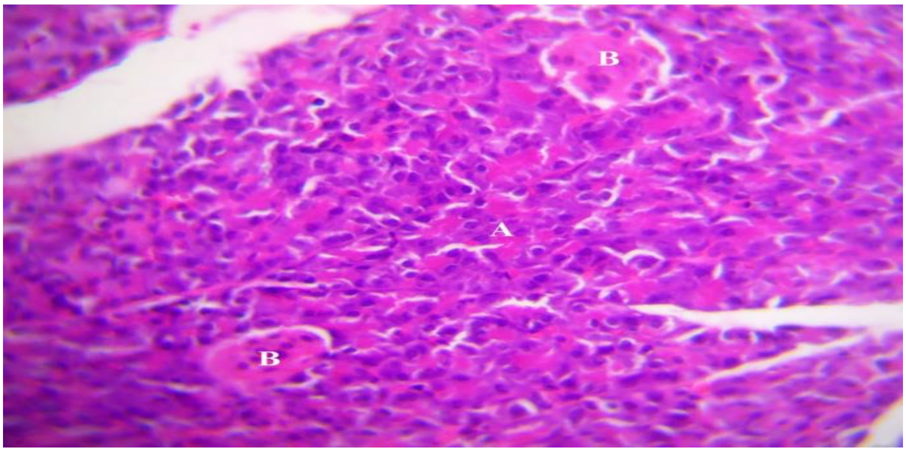

Figure 7. (36-month-old rabbits): Pancreatic lobule including the external secretory units (A), Langerhans islets (B) (H&E. 40×).

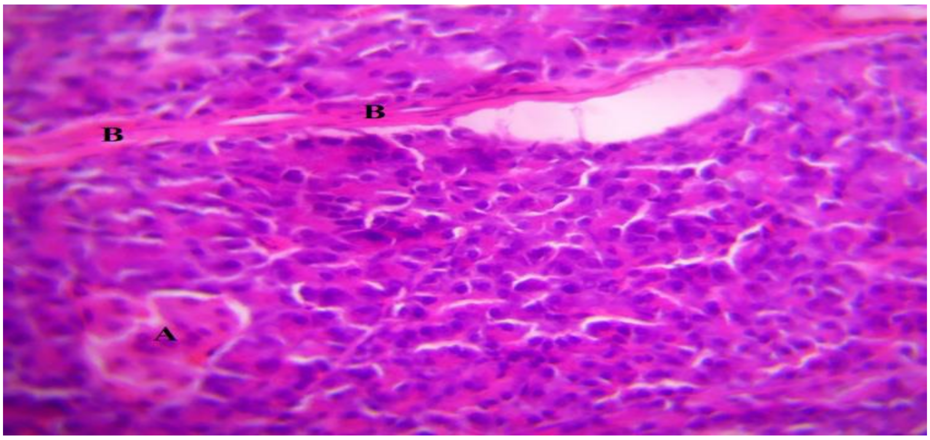

Figure 8. (36-month-old rabbits): Langerhans islets are irregular in shape (A), Fibrous septa between pancreatic lobules (B) (H&E. 40×).

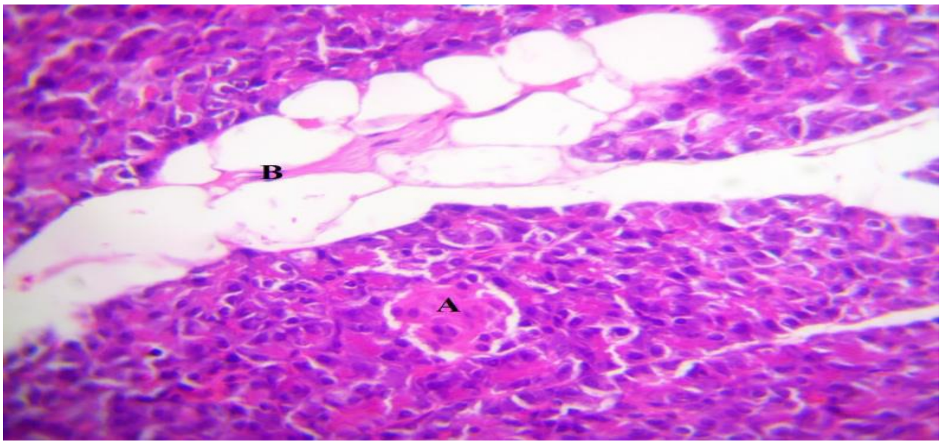

Figure 9. (36-month-old rabbits): Langerhans islets with some endocrine cells (A), Fatty tissue between the lobules (B) (H&E. 40×).

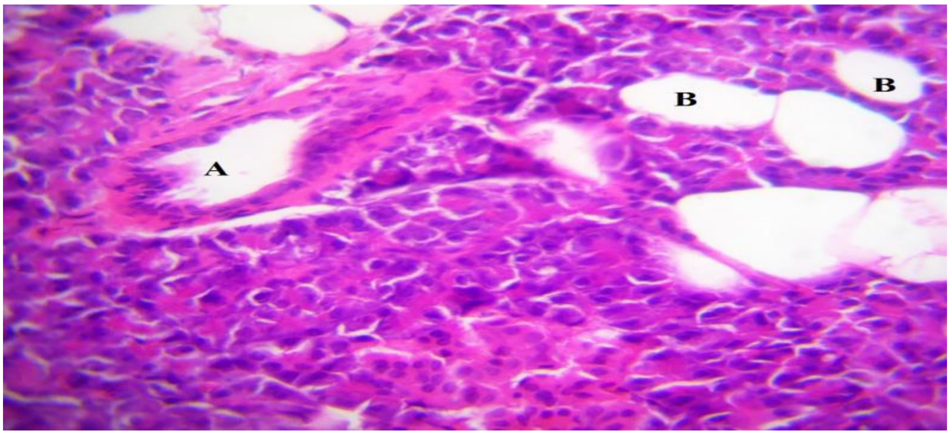

Figure 10. (36-month-old rabbits): Pancreatic duct between the lobes (A), Adipocytes between external secretory units (B) (H&E. 40×).

4. Conclusion

Based on the obtained results, it can be concluded that that middle age in the animal is the best period of its life for vital activities of the body. However, in the later stage of its life (the advanced age), a significant reduction is observed in the diameter of Langerhans islets, with an increase in fibrous and adipose tissue, which in turn reduces the ability of cells to release hormones and enzymes. This means a decline in the capacity of the pancreas to perform its metabolic function, indicating that the histological structure has a role in the metabolism of the animal's body. This result is consistent with those obtained in another study conducted by Seymour, Bennett (6). Therefore, aging has a role in the histological and functional structure of the animal, as reported by Tadokoro, Takase (14).

Authors' Contribution

Study concept and design: K. R.
Acquisition of data: I. T.
Analysis and interpretation of data: F. A. H.
Drafting of the manuscript: A. I.
Critical revision of the manuscript for important intellectual content: K. R.
Statistical analysis: I. T.
Administrative, technical, and material support: K. R.

Ethics

All instruments applied in this study were calibrated and maintained in accordance with routine quality control procedures overseen by the Quality Assurance Department of the Tikrit University, College of Science, Department of Biology, Tikrit, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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