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Histomorphological Study of the Pancreas between the Iraqi Squirrel (*Sciurus anomalus*) and Syrian Hamster (*Mesocricetus auratus*)

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Abstract:

Background: Rodents are commonly used as experimental models in pancreatic research. However, there is limited data regarding the comparison of the pancreas between the Iraqi squirrel and the Syrian hamster, which restricts our understanding of their physiological differences and potential implications for research.

Aims: The study aims to investigate the histomorphological variances between the pancreas in the Iraqi squirrel and the Syrian hamster. Tissue samples were collected from the pancreas of four healthy adult Iraqi wild squirrels and four healthy adult Syrian hamsters. Pancreatic tissue was analyzed using hematoxylin and eosin, the periodic acid-Schiff, and Masson's trichrome. In addition, the areas of the 40 islet Langerhans for each species were computed and analyzed (8 pancreatic tissues, 5 samples per tissue).

Results: The anatomical results revealed a diffuse and more visible structure in the squirrel pancreas compared to the hamster. In contrast, the hamster pancreas exhibited a distinct and well-defined lobular organization. The pancreas of these two models has three main lobes. The histochemical analysis revealed pancreatic islets with a distinct structure, differentiated from the surrounding acinar cells. Furthermore, the squirrel pancreas exhibited a more pronounced organization of acinar cells and islets of Langerhans compared to the hamster. Quantitatively, no statistically significant differences in pancreatic islet area were observed within the same species. Conversely, the pancreatic islets of a squirrel have a significantly larger area than those of a hamster.

Conclusions: The findings provide an important anatomical and histological database for understanding the functional differences and similarities between the two species. Finally, this study supports the potential use of the Iraqi squirrel as a comparative model.

Keyword: Pancreas, Macroscopic anatomy, Squirrel, Hamster

P-ISSN:2410-8863
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Pages: 96-107

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Introduction:

The pancreas has two main components: exocrine and endocrine. The exocrine secretes digestive enzymes. The endocrine gland controls blood sugar levels. The exocrine is composed of acinar cells and duct cells. In contrast, the endocrine system contains the islets of Langerhans that secrete insulin and glucagon hormones (Alkhatib, 2024). Insulin is produced by beta cells to lower blood sugar levels. Glucagon is produced by alpha cells that raise blood sugar levels (Hu *et al.*, 2025). The pancreas also contains other cells that contribute to regulating digestion and metabolism, such as acinar cells that produce digestive enzymes and D cells that secrete somatostatin to modulate insulin and glucagon release (Valente *et al*., 2024). Furthermore, it helps prevent disorders such as diabetes and digestive problems (Bastidas-Ponce *et al*., 2017).

Several scientific studies investigated the basic similarities of the pancreas in various animals. In addition, qualitative differences in the anatomical structure of the pancreas were reported in several other comparative studies (Tsuchitani *et al*., 2016; Noor Assim Farhan & Ahmed Abdulla Hussein, 2024; Shaik *et al*., 2025). The differences reveal physiological adaptations related to lifestyle and diet. A comparative anatomical study revealed that rodents like mice and rats possess a diffuse structure in their pancreas. Furthermore, the pancreas in rodents is less organized compared to the human pancreas (Treuting *et al*., 2017). Conversely, some other rodents have shown a pancreatic structure similar to that of humans(Tsuchitani *et al*., 2016).

In addition, scientific efforts continue to investigate the anatomical and histological structures of the pancreas. For example, the study on the Syrian hamster (Mesocricetus auratus) revealed three main parts of the pancreas: The duodenal lobe, the gastric lobe, and the splenic lobe *(*Mohammadzadeh *et al*., 2025). Furthermore, it has been shown that the precise structure of the islets of Langerhans and the distribution of endocrine cells within the pancreas vary among species according to the metabolic needs of each animal (Steiner *et al*., 2010). Recent comparative research has indicated differences in the histological response to various stains, such as Hematoxylin and Eosin (H&E, for general structure determination), Periodic Acid-Schiff (PAS, for detecting glycogen and mucopolysaccharides), and Masson's trichromatic stain (MTC, for connective tissue differentiation). Comparative studies of the pancreas in different animal species, including rodents, help understand anatomical and histological differences. Furthermore, it also enables researchers to select the most suitable animal models for clinical research applications.

Most previous studies have focused on routine histological descriptions using H&E staining. Furthermore, a few studies integrated specialized stains such as PAS and MTC stain to analyze the functional and structural components of pancreatic tissue. Although several studies have examined the pancreas of laboratory rodents like mice, rats, and hamsters, no study has investigated the Iraqi squirrel pancreas to date. Therefore, no comparative investigation exists that compares the squirrel pancreas with those of other rodent species. This limits our understanding of the qualitative differences between the species. This research gap prevents the use of the squirrel as a comparative model in

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Pages: 96-107

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anatomical and functional studies of the pancreas. Therefore, the current study aims to conduct an anatomical, histological, and numerical comparison of the pancreas between the Iraqi squirrel and Syrian hamster. The study is based on macroscopic examination and histological sections stained with H&E, PAS, and MTC.

Materials and Methods:

Ethical approval:

The Scientific Ethical Committee of the College of Veterinary Medicine, University of Diyala, Iraq, approved this study (Approval no: Vet Medicine (236); October, 2025, M and H).

Experimental design:

Four healthy adult squirrels and four healthy adult hamsters were used in this study. The average weights of squirrels and hamsters were 230 and 120 grams, respectively. Animals were obtained from local markets in Diyala governorate and the capital of Baghdad. The animals were carefully selected to ensure similarity in age and relative size to minimize the influence of individual factors on the results. The squirrels and hamster were anesthetized using a ketamine-xylazine mixture (ketamine 40-100 mg/kg + xylazine 5-12 mg/kg) and (ketamine 200 mg/kg + xylazine 10 mg/kg), respectively, for intraperitoneal injection based on standard guidelines in rat and hamster anesthesia (Paulo *et al*., 2012). This process was followed by a physical procedure to confirm death in accordance with international guidelines for euthanasia of laboratory animals (Leary & Johnson, 2020). A tissue sample of approximately 1 cm^3 of the squirrel and hamster pancreas was collected. These samples were immersed in 10% Neutral Buffered Formalin (NBF) for 48 hours (Paulo *et al*., 2012), then treated with routine histological methods, followed by staining using H&E, PAS, and Masson's stains (Al-Saffar & Hameed Nasif, 2020; Gurina & Simms, 2025). Furthermore, the area of 40 islet Langerhans samples was computed and analyzed (8 pancreatic tissues, 5 samples per tissue).

Statistical analysis:

The statistical analyses were conducted on data derived from measurements of the area of islets of Langerhans. These were utilized for the pancreatic tissues of squirrels and hamsters. The limited sample size (four groups per species, with ten islets per group) necessitated the assessment of distribution normality for each group by the Shapiro-Wilk test. The findings indicated a non-normal distribution across all groups (four groups of squirrels and four groups of hamsters). Consequently, the non-parametric Kruskal–Walli's test was employed to compare the areas of Langerhans islets among the four groups. A P-value of less than 0.05 was deemed significant for each statistical analysis.

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Pages: 96-107

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Results and Discussion:

Anatomical Findings:

The anatomical section of an Iraqi squirrel's abdomen is shown in Figure (1A). This Figure demonstrates a clear organization of the internal organs. From Figure (1A), we can observe that the squirrel pancreas is a diffuse structure. The pancreas comprises three main lobes. The first extends to the stomach (gastric lobe). The second extends to the spleen, embedded within the mesentery (splenic lobe). The last lobe is subdivided into smaller sections that extend to the duodenum via the mesentery (duodenal lobe). The squirrel pancreas tissue mass is distributed in the form of small lobules interspersed with a network of blood and lymphatic vessels. A similar diffuse structure of the pancreas was reported in rabbits (Tsuchitani *et al.*, 2016).

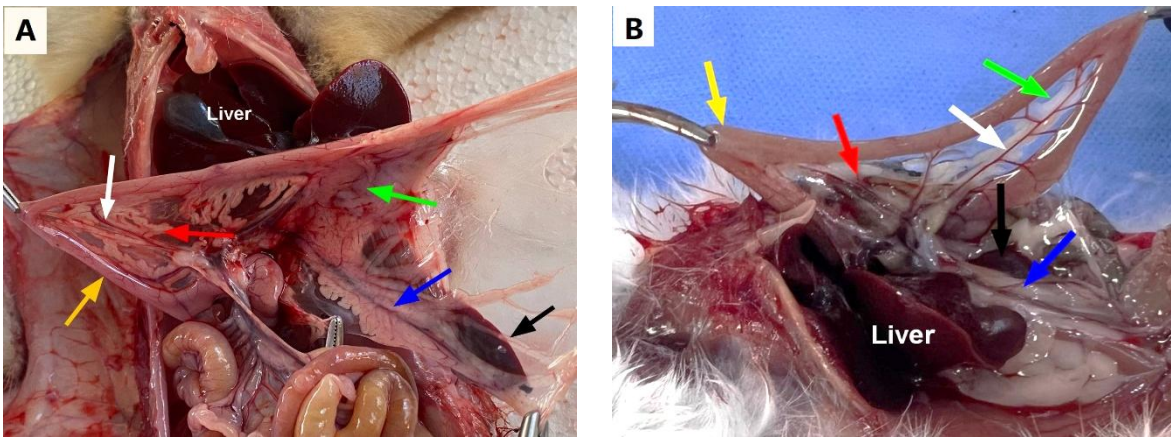

Figure 1: Photographic images for the anatomical dissection of the pancreas in squirrels (A) and hamsters (B) show the pancreatic gastric lobe (green arrows), pancreatic duodenal lobe (red arrows), pancreatic splenic lobe (blue arrows), spleen (black arrows), duodenum (yellow arrows), and blood vessels (white arrows).

In contrast, as shown in Figure (1B), the Syrian hamster pancreas showed as a semi-transparent lobular structure and was connected to the digestive tract by the mesentery. The pancreas consists of a head and three main lobes: the duodenal lobe, the gastric lobe, and the splenic lobe. The duodenal lobe is the smallest and is located along the duodenum, while the gastric lobe extends toward the stomach. The splenic lobe is the longest and extends towards the spleen. These results are consistent with another study that showed that the hamster pancreas consists of three main lobes. These three lobes form a λ-shaped organ (Pour *et al.*, 1977). Furthermore, a fatty tissue connects the lobes surrounds the tail of the pancreas was also observed.

The current study revealed some similarities and differences in the anatomical organization of the pancreas between the squirrel and hamster: The general shape of the pancreas in both species appears similar. The size and relative organization of lobules appear more visible in squirrels than in hamsters. It can be noticed from Figure (1A) that the squirrel pancreas is characterized by a close connection with the duodenum. This region is the most extensive site of lobule distribution. This feature is similar

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Pages: 96-107

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to that found in mice and ferrets (Wieczorek *et al*., 1998). In contrast, the hamster pancreas had a more clearly anatomically lobular organization, consisting of three main lobes, giving the organ an appearance of λ-shaped (Pour *et al*., 1977). These differences in anatomical organization and lobule distribution may reflect evolutionary adaptations related to pancreatic function in each species. The proximity of the pancreas to the duodenum may influence the efficiency of pancreatic secretion into the gastrointestinal tract. Therefore, studying these anatomical differences among various rodents contributes to a better understanding of the structural and functional variability of the pancreas.

Histological Finding:

The pancreas of the squirrel demonstrates a distinct lobular structure (Figure 2A). It appears as a highly lobulated gland that consists of closely spaced lobules separated by thin septa of connective tissue. The lobules were separated by areas of interlobular connective tissue containing quantities of adipose tissue. Each lobe consisted of tightly packed serous acini, separated by fine connective fibers and small blood vessels (Figure 2A). The exocrine and endocrine secretory portions were clearly distinguished in H&E sections. The islets of Langerhans appeared as pale-stained clusters of cells scattered among the acini. There are polygonal cells with dark, round nuclei distributed irregularly within the cell cords, and a rich capillary network is also visible within the islets (Figure 2A). Similar observations were recorded in other species, such as rats (Riccillo *et al*., 2004).

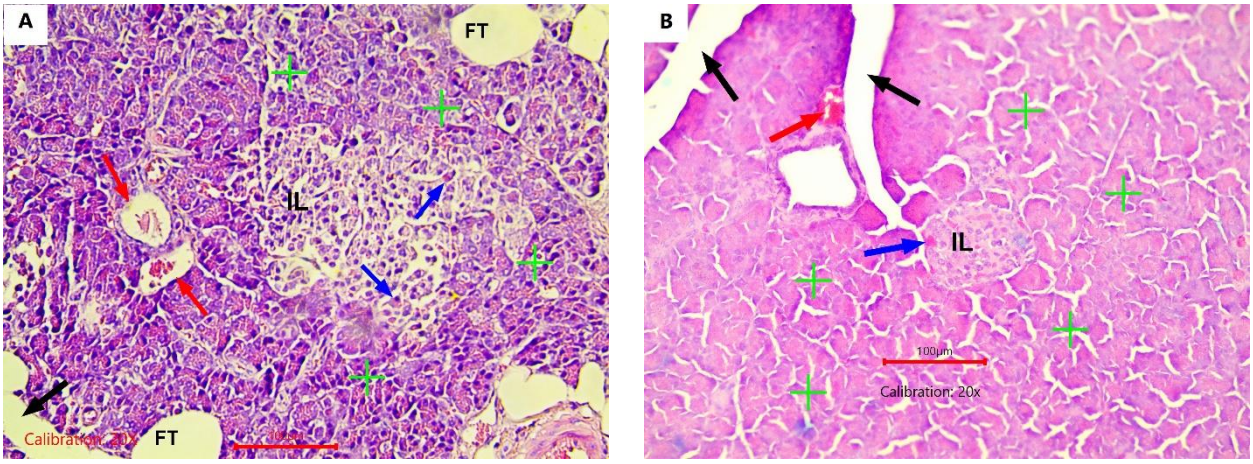

Figure 2: Photomicrographs of H&E-stained sections of the pancreas in squirrel (A) and hamster (B) showing acinar cells (green cross), interlobular septa (black arrows), blood vessels of acinar cells (red arrows), blood vessels of endocrine cells (blue arrows). FT and IL represent fatty tissues and the islet of Langerhans, respectively. H&E stain (X20)

In contrast, the hamster pancreas exhibited serous acinar cells, which are similar in structure. These acinar cells appear denser and relatively smaller than those of the squirrel pancreas (Figure 2B). A more pronounced convergence of secretory units is observed. Nuclei were often central or near the base, with less variation in cytoplasmic staining intensity. The pancreatic ducts appear less distinct in some sections and are surrounded by cuboidal to flattened epithelial cells, distinguish a denser appearance. The interlobular connective tissue appears less visible with this staining. Notably, the

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Pages: 96-107

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islets of Langerhans are less prominent and smaller than those in Squirrel, and their borders are less clearly defined. Histological findings using H&E staining indicate a general similarity between the pancreas of the squirrel and hamster. This finding is consistent with previous studies on the pancreas of the mouse (Houbracken & Bouwens, 2017). However, this study recorded some qualitative differences in acinar cell size, degree of aggregation, and prominence of the islets of Langerhans. The clear organization of acinar cells and islets in the squirrel pancreas can be explained by physiological or nutritional differences.

The intensity of secretory activity and the size of secretory units are related to diet and metabolic requirements. In contrast, the high degree of acinar cell aggregation in the hamster pancreas suggests a different histological adaptation reflecting a different functional pattern.

Enzymatic Activity of Pancreatic Parenchyma

As shown in Figure (3A), the pancreas of the squirrel showed a positive reaction to the PAS stain that may reflect the abundance of carbohydrate components within the acinar cells, which is related to the nature of their secretory activity (Uhlig *et al.*, 2022). These compounds contribute to the formation and secretion of proteinaceous and enzymatic substances in the exocrine part of the pancreas (Figure 3A), as stated by Khaitan *et al.* (Khaitan *et al.*, 2021). The islets of Langerhans were less stained than the surrounding acinar tissue. This result may be attributed to lower carbohydrate content within these cells. These results confirm that the histological organization of the PAS-stained pancreas is similar to that reported in the guinea pig (Al-Saffar & Hameed Nasif, 2020).

The PAS staining supports the highlighting of islet boundaries and the identification of endocrine cell clusters. This protocol allows for the differentiation of structural boundaries between different tissue units. A recent laboratory study on mice and rats confirmed the functional maturity of the acinar secretory system (Muratore *et al*., 2021).

In contrast, the acinar cells of the hamster pancreas showed a lower reaction to PAS staining compared to the squirrel pancreas. The apical granules appeared less stained, while the basement membranes showed mild to moderate staining with weaker cytoplasmic reaction (Figure 3B). The islets of Langerhans often showed weak or negative reaction. This reaction may be attributed to structural or functional differences in endocrine cells. Notably, the basement membrane in the hamster pancreas is less visible with PAS staining. Weak reaction at the thin fibrous septa between lobules was also recorded (Figure 3B). In contrast, another study demonstrated a strong interaction of PAS stain in the basement membrane of tubular acini and lining epithelium of the ducts in Japanese quail (AYehia *et al.*, 2021).

P-ISSN:2410-8863
E-ISSN:2958-6178
https://djvs.uodiyala.edu.iq
Pages: 96-107

Diyala Journal for Veterinary Sciences
Vol. 4 No.2 June (2026)

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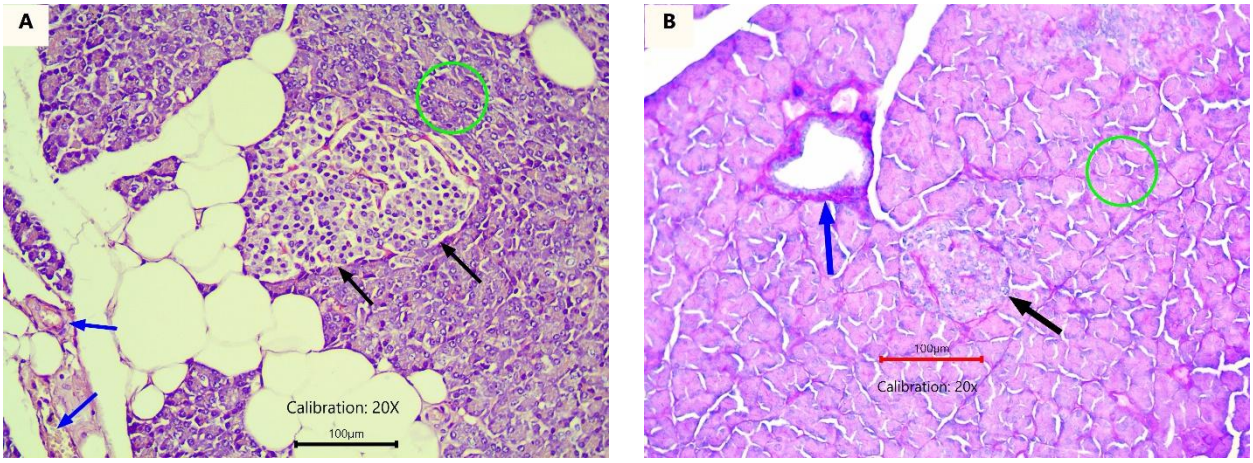

Figure 3: Photomicrographs of PAS-stained sections of the pancreas in squirrel (A), and hamster (B). Secretory units (green circle), intralobular duct (blue arrows), and islet boundary (black arrows). PAS stain, X20.

PAS staining results suggest qualitative differences in carbohydrate and glycoside content between the pancreas of the squirrel and hamster. These differences were most pronounced in the exocrine portion (Uhlig *et al.*, 2022). The positive reaction in the acinar cells of the squirrel pancreas indicates a greater abundance of glycoproteins associated with digestive enzymes, which may reflect a difference in secretory activity or dietary patterns between the two species (Khaitan *et al.*, 2021).

Connective Tissue Organization in the Pancreas

The squirrel pancreas presents a clear MTC stain reaction in its interlobular connective tissue (Figure 4A). Collagen fibers appear blue, forming distinct fibrous septa. Fine connective tissue is observed that separates the acini within the glandular tissue. Collagen fibers within the lobules were clearly visible in the blue-stained sections due to the use of aniline blue in Mason's stain. In contrast, connective tissue appeared denser around the ducts within the lobules and around the blood vessels (Figure 4C). Additionally, single or small groups of adipose tissue were observed within the pancreatic structure. These findings are consistent with previous histological studies that reported the ability of the MTC stain for visualizing the fine cellular organization of mixed glands, such as the pancreas (Mihoc *et al.*, 2024)

The results obtained from the preliminary analysis of the hamster pancreas as shown in Figure (4A) exhibited less pronounced interlobular connective tissue. The fibrous septa appeared thinner and more dispersed, giving the pancreatic tissue a more compact appearance and less distinct interlobular separations compared to the squirrel pancreas. The intralobular connective tissue is less dense and less stained, with fewer and finer collagen fibers. This makes them difficult to distinguish in some sections. The ducts appeared to be surrounded by a thinner layer of connective tissue. The blood vessels of the hamster were less clearly defined by their surrounding fibrous border compared to the squirrel pancreas (Figure 4C, D).

P-ISSN:2410-8863
E-ISSN:2958-6178
https://djvs.uodiyala.edu.iq
Pages: 96-107

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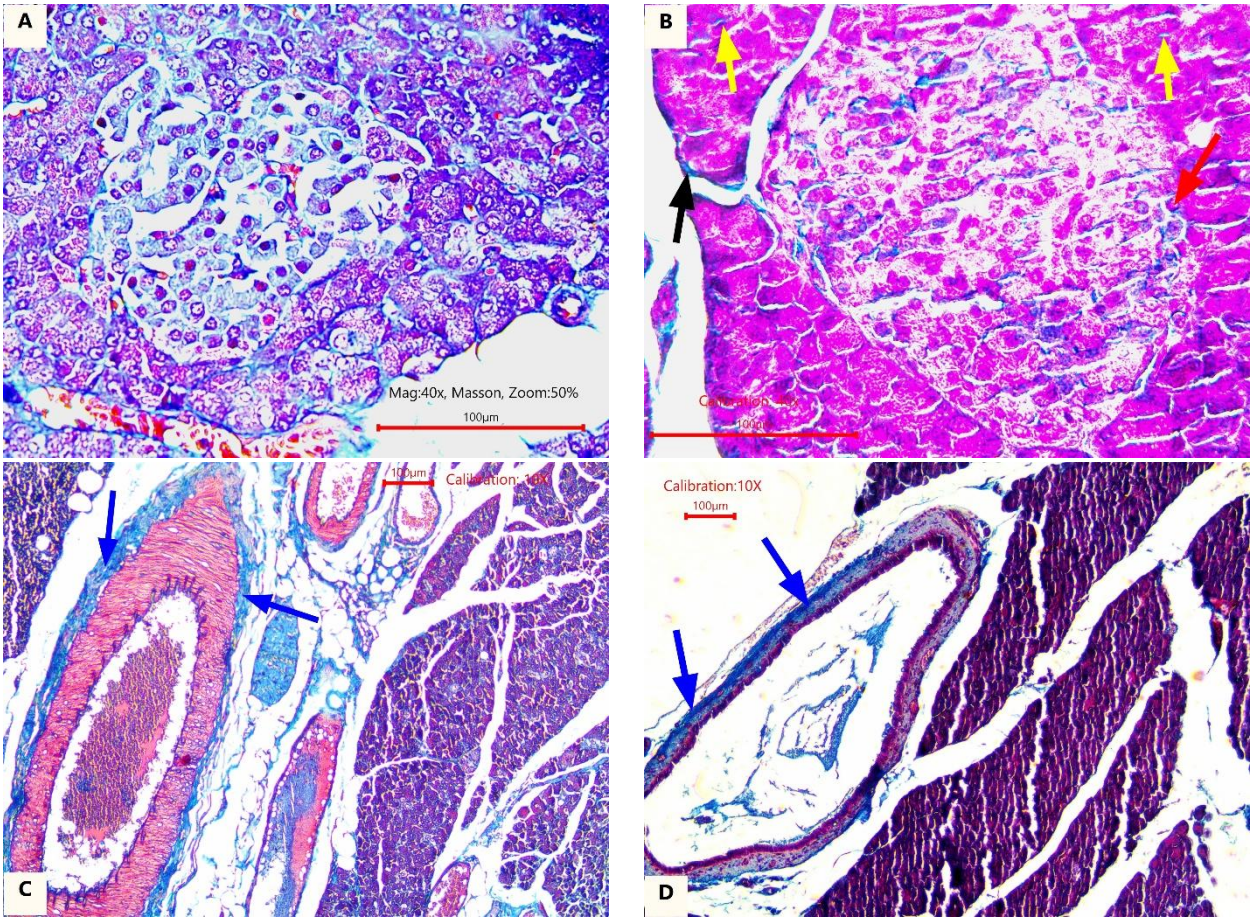

Figure 4: Photomicrographs of MTC-stained sections of the pancreas in squirrel (A) and hamster (B) show thin connective tissue (yellow arrows), intralobular connective tissue (black arrows), and collagen fibers (red arrows). The pancreas blood vessels of the squirrel (C) and hamster (D) show dense connective tissue (blue arrows). MTC stain, (A, B: X40, C, D:X10)

In both species, the islets of Langerhans did not show a strong direct reaction to MTC stain. However, islets of Langerhans appear more distinct in the squirrel pancreas than in the hamster due to the contrast between the surrounding connective tissue and the islets themselves. The islet borders were less well-defined in the hamster pancreas. The difference in connective tissue distribution between the two species may be related to physiological factors, such as differences in secretion rates or the nature of the structural support.

Numerical Results:

Figure (5) shows a graph representing the distribution of islet areas in the pancreas in squirrels and hamsters. Each box demonstrates the statistical distribution of islet areas measured from four animals of each species (10 islets per animal). The squirrel results revealed a clear variation in the areas of the Langerhans islets among the four animals. In contrast, the hamster data showed limited individual variation in island areas among the four animals, with values concentrated within a relatively low range and a clear convergence in intermediate values. Overall, Kruskal–Walli's analysis did not show

P-ISSN:2410-8863
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https://djvs.uodiyala.edu.iq
Pages: 96-107

Diyala Journal for Veterinary Sciences
Vol. 4 No.2 June (2026)

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any statistically significant differences between the pancreatic islet areas inside the group members for both squirrel and hamster ($p>0.05$). These results confirm that the observed differences are due to variation within each animal rather than to real differences between animals.

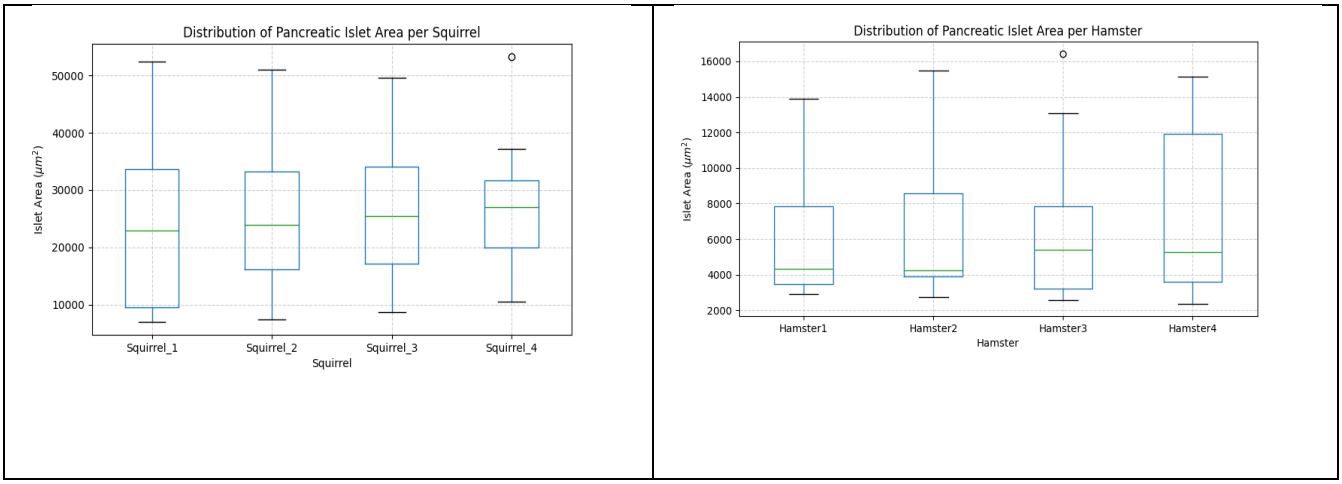

Figure 5: Distribution of pancreatic islet areas in hamster and squirrel (10 islets for each species)

Table 1 presents a numerical comparison of pancreatic indicators in the squirrel and the hamster. This table provides numerical values of the mean area, standard deviation (SD), smallest (Min), and largest (Max) area of the islets of Langerhans related to 40 islets for each species.

Table 1 shows a clear difference in the distribution of Langerhans islets between hamsters and squirrels. The mean area for hamsters was 6.94×103 µm2 compared to 26.86×103 µm2 for squirrels. The standard deviation shows a relatively high average value for both the squirrel and the hamster. This standard deviation reflects significant variation in the size of the islets of Langerhans within each animal. Such observation is a normal biological variation expected in histological studies. The lowest value of islet area of the squirrel pancreas was 7.55 ×103 µm2, while the highest pancreatic islet area was 53.22 ×103 µm2. In contrast, the minimum and maximum islet area of the hamster pancreas were 2.37×103 µm2 and 23.54×103 µm2, respectively. Our hamster results are consistent with another study that indicates a detailed quantitative characterization of islets of Langerhans(Taga et al., 1999).

The results of Table 1 show a clear difference in the distribution of the pancreatic islet areas between the hamster and the squirrel. The Mann–Whitney U test confirmed a statistically significant difference between the two groups ($p<0.05$). This result suggests a real difference in the tissue structure of the pancreas between the two animals. The increased size of the pancreatic islets in squirrel pancreas reflects a physiological adaptation to higher metabolic conditions compared to hamsters. This can be explained by differences in metabolic requirements, feeding patterns, and movement between the two species. Therefore, the evolutionary differences in the histological structure of the pancreas may lead to functional differences in the regulation of pancreatic hormone secretion.

P-ISSN:2410-8863
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https://djvs.uodiyala.edu.iq
Pages: 96-107

Diyala Journal for Veterinary Sciences
Vol. 4 No.2 June (2026)
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Pancreatic islets share the same basic function across species. However, their internal structure and cellular composition may differ. Scientific literature indicates that cell distribution within the islets varies between species, and islet size can also vary depending on the physiological and genetic characteristics of each species (Steiner *et al*., 2010).

Table (1): Quantitative comparison of pancreatic islet area in squirrel and hamster. The group size is 40 islets for each animal model.

Species	Mean Area (μm^2)	SD	Min	Max
Squirrel	26862.93	13322.63	7055.70	53226.13
Hamster	6943.83	5364.72	2370.13	23542.00

Conclusions:

This study is the first comparative anatomical and histological description of the pancreas between the Iraqi squirrel and the Syrian hamster. The study revealed clear anatomical differences between the pancreas of the squirrel and hamster. The squirrel pancreas is characterized by a diffuse structure. In contrast, the hamster pancreas exhibited a distinct and well-defined lobular organization. Histological findings show a general similarity in the basic structure of the pancreatic tissue between the two species. The differences between the two species were qualitative. These differences are related to the size and density of acinar cells and the distribution of the islets of Langerhans within the tissue. The squirrel pancreas tissue reveals a stronger reaction to PAS staining. This result suggests a qualitative increase in carbohydrate and glycoprotein content in the pancreas of squirrels compared to hamsters. The finding confirms the higher exocrine activity in the pancreas of squirrels. The squirrel pancreas demonstrates more developed and thicker connective tissue than the hamster pancreas after applying MTC stain to the test samples. This may reflect functional or adaptive differences related to metabolic activity or diet. The results confirm that combining anatomical and histological investigation using multiple stains provides a more comprehensive understanding of structural differences between animal species.

Recommendations:

It may now be of utility to use immunohistochemistry techniques to determine the distribution of alpha and beta cells in squirrels and hamsters. We also recommend a study that links the histological structure to the secretory activity of the pancreas. This includes measuring levels of digestive enzymes and blood glucose-regulating hormones. It would also be beneficial to include other commonly used rodents in future works. Furthermore, using advanced imaging techniques, such as micro-CT or electron microscopy, may clarify the three-dimensional structure of the pancreas. Finally, this study may contribute to the use of the squirrel as a potential experimental model in future studies related to pancreatic function and disease.

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Pages: 96-107

Diyala Journal for Veterinary Sciences
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