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Original Research Article

Anatomy of the pancreas of mizoram local Pig (Zovawk)

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ABSTRACT

Background: This study was conducted to get the data and information about the target organ that might help in creating better understating of the physiological mechanism of this organ in Zovawk.**Materials and Methods:** For this experiment 6 apparently healthy pigs were selected. Different physical parameters of the collected samples were measured. To know the cellular architecture collected pancreas samples were first fixed with 10% NBF and were subsequently processed with standard tissue processing technique. For ultra-structural studies tissues were stained with Lead citrate and uranyl acetate.**Result:** B cells were more numerous than the A cells in the islets of Langerhans which is characterized by the spherical nucleus and located almost all over the islets. Whereas A cells were characterized by the oval nucleus and distributed mainly in the center of the islets. Numerous zymogen granules were present in the acinar cells of zovawk pancreas which are thought to be the reason for the higher enzymatic properties in the zovawk pancreas.This is an Open Access (OA) journal, and articles are distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License](https://creativecommons.org/licenses/by-nc-sa/4.0/), which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.For reprints contact: reprint@ipinnovative.com

1. Introduction

Zovawk or Mizoram local pigs are locally available pigs dispersed in different portion of Mizoram (a state on the north-east region of India). These small, observant animals recently got recognized as one of the pig breeds of Mizoram. Average body weights of these small animals are around 4.5Kg (Debroy et al.,¹ 2021). As one of the most important gland of digestive system, pancreas is consists of both endocrine and exocrine portion (mixed gland). The exocrine portion of the pancreas is responsible for the secretion of different pancreatic enzymes with high digestive value. Whereas 3 important hormones such as Glucagon, Insulin and Somatostatin secretes from the endocrine portion of the pancreas, known as islets of Langerhans (Nickel et al.,² 1979). Geographical distribution of this animal makes it difficult to excess the animal by

various researchers, leads to negligible numbers of data and information regarding various organs this breed. This study was conducted to get the data and information about the target organ that might help in creating better understating of the physiological mechanism of this organ in Zovawk.

2. Materials and Methods

For this experiment 6 apparently healthy pig were selected, irrespective of sex. Collected pancreas from these animals were measured and observed right at the spot where topographical study was done with naked eyes. Different morphometric data i.e. weight of the pancreas, length of the pancreas, width of the pancreas and thickness of the pancreas were measured with the help of a physical balance and a vernier caliper. To know the cellular architecture collected pancreas samples were first fixed with 10% NBF and were subsequently processed with standard tissue processing technique. A 6µm section of the processed

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pancreas tissue was cut and was stained with Harris haematoxylin and eosin stain (Harris,³ 1900). For ultra structural examination tissue were processed with following manner

1. **Fixation:** Tissue samples for TEM studies were fixed in Karnovsky's fixative (2.5% glutaraldehyde in Sodium cacodylate (0.1M) or Phosphate buffer at pH 7.2) for 2-4 hours at 4°C. After washing in 0.1M buffer (3 changes of 15 minutes each), the samples were transported to SAIF, NEHU, Shillong in the same buffer at 4 °C for further processing.
2. **Washing:** The fixed tissues were then washed in phosphate buffer saline (0.1 M, pH 7.4) for three times of 15 minutes duration each at 4°C.
3. **Post-fixation:** The post fixations of the aforesaid washed tissues were done in 1% osmium tetroxide for 2 hours at 4°C.
4. **Washing:** The osmium tetroxide fixed tissues were again washed in phosphate buffer saline (0.1 M, pH 7.4) for three times of 15 minutes duration each at 4°C.
5. **Dehydration:** The washed tissues were dehydrated in graded acetone (viz. 30, 50, 70, 80, 90% and dry acetone) for 30 minutes each at 4°C followed by dehydration in dry acetone once again for 30 minutes at room temperature.
6. **Clearing:** Those dehydrated tissues were cleared in toluene-I and toluene-II for 30 minutes each. 30
7. **Infiltration:** The infiltration of the cleared tissues was carried out as follows:
 - (a) Part Embedding Medium and 3 parts of Toluene – 12 hours.
 - (b) Parts Embedding Medium and 2 parts of toluene – 12 hours.
 - (c) Parts Embedding Medium and 1 parts of Toluene – 12 hours (under vacuum).

2.1. Preparation of embedding medium

Araldite cy212 - 10 ml

DDSA (dodecyl succinic anhydrite) - 10 ml

DMP (2, 4, 6 tridimethylamino methyl phenol) - 0.4 ml

Plasticizer (Dibutyl phthalate) - 1.0 ml

The above gradients were added and stirred vigorously in order to mix them thoroughly. Then the air bubbles were allowed to settle down before use.

Embedding: The infiltrated tissues were then embedded in pure embedding medium using gelatin capsules

1. **Polymerization:** The embedded blocks were kept at 50°C for 24 hours (polymerization) and then at 60 C for 48 hours.
2. **Ultra-sectioning:** Silver-to-gray (70-80 nm) ultra-thin sections were cut with diamond knives using a ultra-microtome (Leica ultra-cut) UCT and mounted on copper grids.

3. **Negative staining:** Lead citrate and uranyl acetate were used for staining the ultrathin sections which were then examined under a Transmission Electron Microscope, JEOL, JEM-2100, Japan which was operated at 80 KV.

4. **Photography:** The photography was taken with the help of the digital camera which was inbuilt with the microscope.

5. **Interpretation:** Some of the interpretations were noted while viewing the tissues in the TEM and rest were done with the help of photomicrographs.

3. Results and Discussion

3.1. Gross examination

3.1.1. Position

Pancreas was located in the dorsal part of the abdominal cavity in a close relationship to the proximal part of the duodenum as reported by Ozdemir,⁴ (2005) in porcupine.

3.1.2. Color and shape

The pancreas of zovawk was grayish pink in color in the fresh state. This observation was in conformity to that mentioned by Sultan⁵ (1999) in the camel and by Sisson⁶ (1975) in the horse. But Dhoolappa et al.⁷ (2004) reported that the color of the pancreas of the Indian donkey was grayish pink to pale brown. It is covered by great amount of fat. Zovawk pancreas has no definite shape (Figure 1). This findings were contrary to the findings of May, (1970) and Dhoolappa et al.,⁷ (2004). A triangular shape of the pancreas has previously been mentioned in the horse by May⁸ (1970), Indian donkey and sheep A totally different shape of the pancreas, a V- shape, was observed in the dog Miller et al.,⁹ (1964).

3.1.3. Lobation

Pancreas is consists of two lobes, i.e. left lobe and right lobe and a body. Left lobe is way more large and thick than the right lobe. Between these two lobes there is a centrally placed body of the pancreas. Body of the pancreas is thick and bigger than the right lobe (Figure 1). This observation is similar to what had already been described by Mustafa et al.¹⁰ (1983), Smuts and Bezuidenhout¹¹ (1987), Taha¹² and Abdel (1998) and Sultan⁵ (1999) in the camel. However, Dhoolappa et al.⁷ (2004) have a different notion that in the pancreas of the Indian donkey the right lobe is longer than the left lobe. The presence of an accessory lobe, which was ascribed to the pancreas of the camel by Hegazi¹³ (1945), Mustafa et al.¹⁰ (1983) and Sultan⁵ (1999), was not seen during the present study.

3.1.4. Measurements

3.1.5. Weight

The weight of zovawk pancreas was ranged from 198.79g to 235g on an average of 216.02g (Table 1). While Mustafa et al.¹⁰ (1983) reported that average weight of pancreas of camel was 300 g and 95 g in the results of Dhoolappa et al. (2004) in the Indian donkey.

3.1.6. Length

Length of the left lobe of pancreas was ranged from 13 cm to 15.34 cm on an average 14.6 cm. Length of the body of the pancreas was ranged from 7.6 cm to 10.9 cm on an average of 8.65 cm. Length of the right lobe of pancreas was ranged from 3.56 cm to 5 cm on an average of 4.32 cm (Table 1). Correlation between the length of the left lobe and body weight of the animal is significant @ 0.01 levels (Table 2). Correlation between the length of the right lobe and the weight of the pancreas is significant @ 0.05 levels. Maximum and minimum length was found in left and right lobe respectively (Table 1).

3.1.7. Width

Width of the left lobe of pancreas is ranged from 7.2 cm to 9.2 cm on an average of 8.46 cm. Width of the body of the pancreas was ranged from 7.07 cm to 7.4 cm on an average of 7.19 cm. Width of the right lobe was ranged from 4.07 cm to 4.9 cm on an average of 4.52 cm (Table 1). Similar findings were reported by McGeddy et al.,¹⁴ (2006) in domestic pig and contrary reports were reported by Dhoolappa et al. (2004) in the Indian donkey.

3.1.8. Thickness

Thickness of the Left lobe of pancreas is ranged from 3.4cm to 4.6 cm on an average of 4.20 cm (Table 1). Correlation between thickness of the left lobe and the weight of the pancreas is significant @ 0.01 levels. Correlation between the thickness of the left lobe and the length of the left lobe was significant @ 0.05 levels. Thickness of the body of the pancreas was ranged from 2.6 cm to 3.58 cm on an average of 3.05 cm. Thickness of the right lobe of the pancreas was ranged from 0.72 cm to 1.1 cm on an average of 0.9 cm. Thickest lobe of the pancreas was the left lobe whereas the thinnest lobe of the was the right lobe (Table 1). Correlation between the thickness of the right lobe and the width of the right lobe is significant @ 0.05 levels.

3.1.9. Histological examination

The zovawk pancreas was made up of exocrine as well as endocrine portion which was covered by the connective tissue capsule.

3.1.10. Capsule

The pancreas of the zovawk was covered by connective tissue capsule. Connective tissue septa extended from the

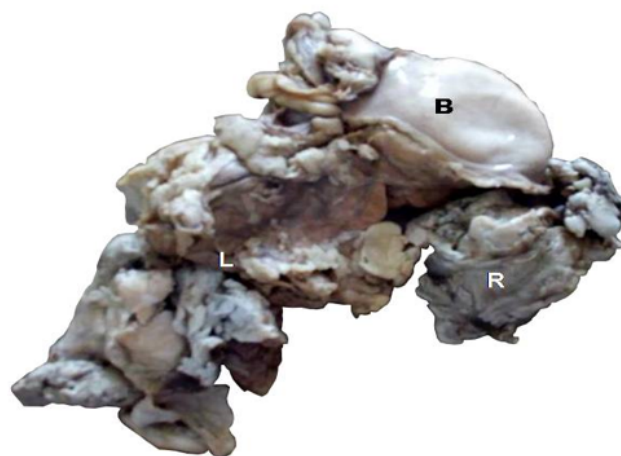


Fig. 1: Lobes of pancreas. L= Left lobe, B= Body of pancreas, R= Right lobe

capsule into the parenchyma of the pancreas dividing it into complete and incomplete lobules. This observation was similar to report of Dhoolappa (2004) in Indian donkey.

3.1.11. Exocrine portion

The exocrine portion of the pancreas was made up of secretory units and duct system (Figures 2 and 4). The excretory units were tubulo-alveolar with more acinar portion (Figure 2). They were consisted of pyramidal cells which were resting upon a basal lamina. Two types of acinar cells were identified depending upon shape and positions of their nucleus, i.e. active acinar cells and resting acinar cells. The cells located in the lumen of the acini were identified as centroacinar cells. The intralobular duct was lined by cuboidal cells supported by a basal lamina and collagen fibers. Similar findings were reported by Kalita et al.¹⁵ (2019) In Zovawk. This is slightly differ from the exocrine portion of the ruminant pancreas reported by Stinson and Calhoun, (1981) since the later showed dominance of the tubular portion.

3.1.12. Endocrine portion

The islets of Langerhans which represented the endocrine portion of the zovawk pancreas appeared as pale areas among the acini. The islets varied in shape; they were round, oval or irregular. Some islets were small others were large (Figure 3). No distinct capsule encircling the islets was observed. The islets had a rich vascular supply; some blood capillaries were enlarged forming cyst-like structure. The cells of the islets were arranged as irregular cords surrounding the blood capillaries. Mainly 2 types of prominent cells were found in the pancreatic islets, i.e. A cells or alpha cells and B cells or beta cells. B cells were found in more numerous number than the A cells in the islets of Langerhans which is characterized by the spherical

Table 1: Different gross measurements of Zovawk pancreas

S.No	1	2	3	4	5	6	Average
Body weight	250 kg	250 kg	165 kg	220 kg	240 kg	250 kg	
Weight of Pancreas	233.1 g	211.25 g	198.79 g	208 g	210 g	235 g	216.02 g
Length (Left lobe)	14.98 cm	14.7 cm	13 cm	14.6 cm	15.2 cm	15.34 cm	14.6 cm
Length (Body)	8.1 cm	7.92 cm	7.6 cm	10.9 cm	8.09 cm	9.33 cm	8.65 cm
Length (Right Lobe)	3.9 cm	4.8 cm	5 cm	4.2 cm	4.5 cm	3.56 cm	4.32 cm
Width (Left lobe)	8.7 cm	8.45 cm	7.2 cm	8.31 cm	8.9 cm	9.2 cm	8.46 cm
Width (Body)	7.2 cm	7.07 cm	7.1 cm	7.31 cm	7.09 cm	7.4 cm	7.19 cm
Width (Right lobe)	4.4 cm	4.9 cm	4.9 cm	4.7 cm	4.2 cm	4.07 cm	4.52 cm
Thickness (Left Lobe)	4.6 cm	4.5 cm	3.4 cm	4.13 cm	4.12 cm	4.5 cm	4.20 cm
Thickness (Body)	3.2 cm	2.8 cm	2.6 cm	3.06 cm	3.1 cm	3.58 cm	3.05 cm
Thickness (Right Lobe)	0.72 cm	1.1 cm	1.08 cm	0.93 cm	0.78 cm	0.8 cm	0.90 cm

Table 2: Correlation between different parameters of pancreas with body weight of the animals

Parameters	Number of animals	Body weight of animal (Pearson Correlation)
Weight of Pancreas	6	0.719
Length (left lobe)	6	0.944**
Length (body of pancreas)	6	0.135
Length (right lobe)	6	0.602
Width (left lobe)	6	0.921**
Width (body of pancreas)	6	0.251
Width (right lobe)	6	0.558
Thickness(left lobe)	6	0.964**
Thickness (body of pancreas)	6	0.676
Thickness (right lobe)	6	0.547

*. Correlation is significant at the 0.01 (2-tailed).

**. Correlation is significant at the 0.1 (2-tailed).

nucleus and located almost all over the islets. Whereas A cells were characterized by the oval nucleus and distributed mainly in the center of the islets of pancreas (Figure 3). In the year 2019 Kalita et al. reported similar features in Zovawk. This also confirms the findings of Alani¹⁶ (1987) and Sultan (1999) in the camel and Mukherjee et al.,¹⁷ (1986) in sheep and by McGeddy et al.,¹⁴ (2006) in domestic pig.

3.1.13. Electron microscopic examination

The ultrastructure of the exocrine and endocrine cells (islets of Langerhans) of the zovawk pancreas had been studied. The secretion products of the exocrine pancreas appear as collections of densely stained material in the apices of these smaller acinar cells. The cytoplasm of the acinar cells is basophilic. Numerous zymogen granules were present in the acinar cells of zovawk pancreas (Figure 6). The nuclei of the pancreatic acinar cells are tiny and round with very large

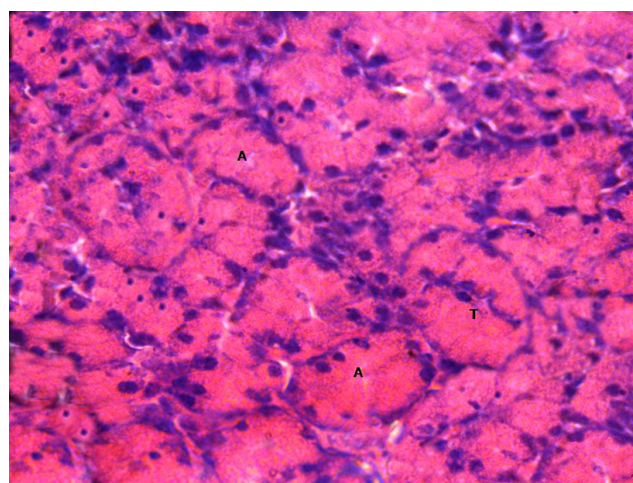


Fig. 2: Demonstration of T = Tubular and A = Acinar secretory units. H&E 40X

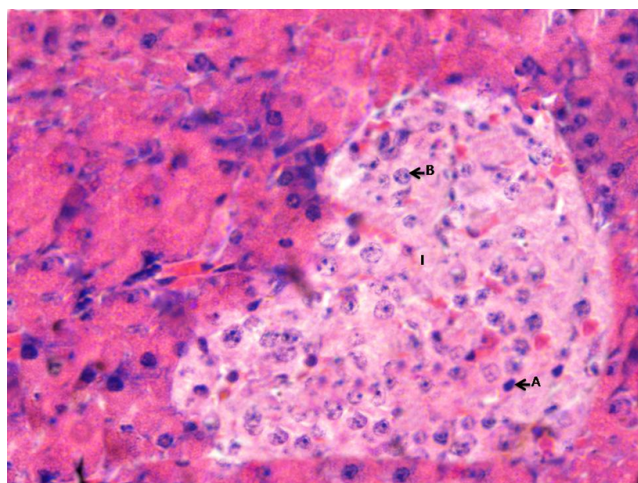


Fig. 3: Endocrine portion (I) of pancreas showing A= A cells, B= B cells, C= Centro acinar cell. H&E 100X

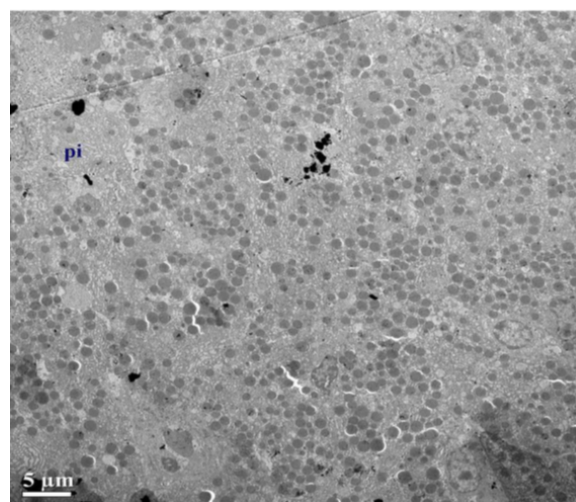


Fig. 5: Demonstration of Islets of Langerhans and exocrine (pi) portion of pancreas. TEM 160X

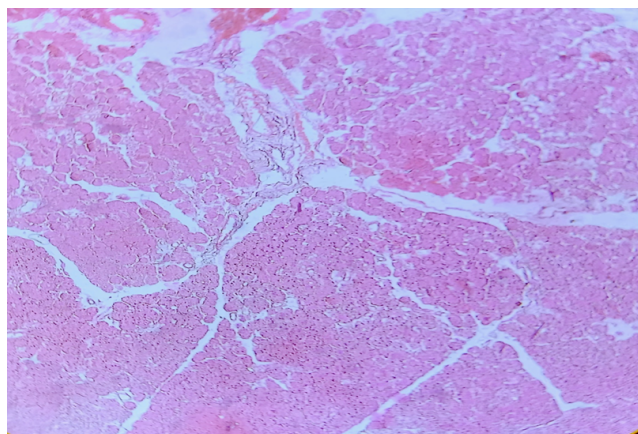


Fig. 4: Demonstration of interlobular duct system. H&E 10X

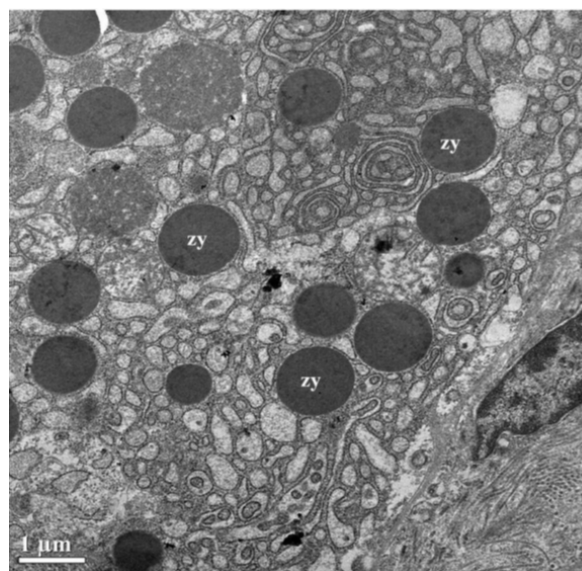


Fig. 6: Demonstration of more evident individual zymogen granules (zy) in the acinar cells at high magnification. TEM, 720X

nucleoli (Figure 7). The cisterns of the granular endoplasmic reticulum occupy the cytoplasm in linear arrays from the base to the apex of the cells. A few pale vesicles are seen occasionally at the base of the cell. Granules of moderate density appear in the supranuclear region of the cell to be replaced by very dense granules toward the apex (Figure 7). The centroacinar cells are distinguished by irregular nuclei and cytoplasm that is pale in comparison with that of the acinar cells. The cytoplasm has only a moderate supply of ribosomes. The centroacinar cells have relatively few apical microvilli (Figure 8). A rounded pancreatic islet made up of a collection of large pale cells is located within a lobule of acini, in which the cells appear smaller and darker (Figure 5). This is similar to the findings of Bloom and Fawcett (1986) in the bat and dog.

4. Conclusion

In conclusion, it can be said that an authentic and till date only base line data about the gross and ultra structural features of pancreas were established in this study. Prominent features were, two types of prominent cells were found in the pancreatic islets, i.e. A cells or alpha cells and B cells or beta cells. B cells were more numerous than the A cells in the islets of Langerhans which is characterized by the spherical nucleus and located almost all over the islets. Whereas A cells were characterized by the oval nucleus and distributed mainly in the center of the islets. Numerous zymogen granules were present in the acinar cells of zovawk

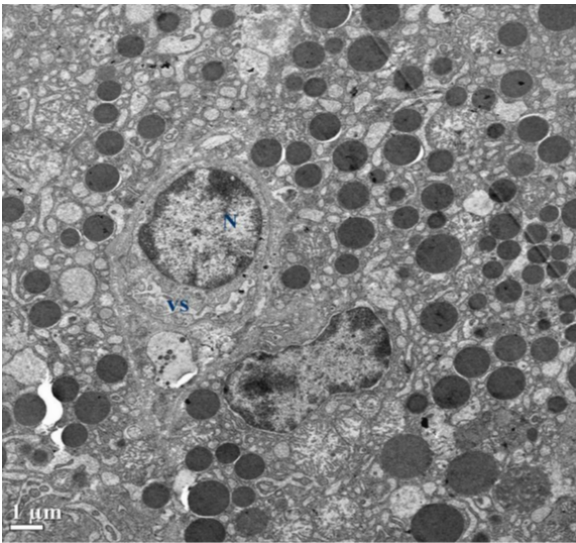


Fig. 7: Demonstration of individual acinar cells showing its tiny and round nucleus (N) with very large nucleoli and a pale vesicles (vs). TEM 9200X (Pancreas)

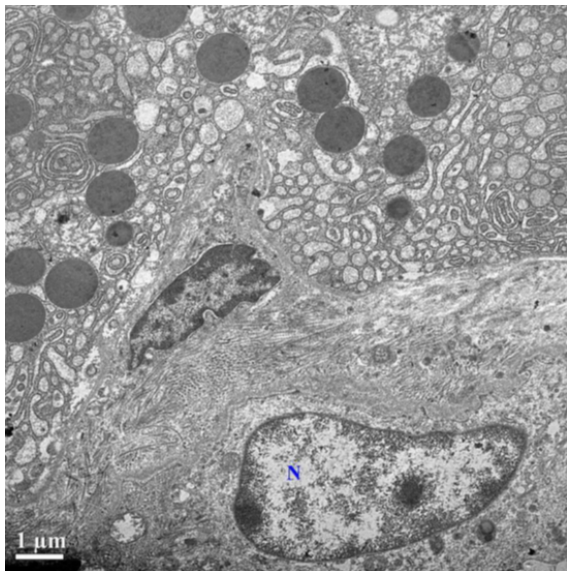


Fig. 8: Demonstration of centroacinar cell, showing its irregular nuclei (N) and cytoplasm. TEM, 11250X (Pancreas)

pancreas which are thought to be the reason for the higher enzymatic properties in the zovawk pancreas.

5. Source of Funding

None.


6. Conflict of Interest

The authors declare no conflict of interest.

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