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Observation on the Gross and Histology of the Exocrine and Endocrine Pancreas in Large White Yorkshire Pig

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ABSTRACT

Background: Islet transplantation is a potential treatment option for selected patients with type 1 diabetes. The limited supply of human islet from cadavers and poor islet yield remains a significant barrier to progress in the field. So, the use of porcine islets holds great promise for large scale application for the transplantation of the islet. The primary aim of this study was to describe the gross and morphological studies of the porcine pancreas to rule out its similarity to human pancreas.

Methods: Pancreas was collected from eight apparently healthy Large White Yorkshire pig (6-7 months of age). The gross and morphological studies were analyzed by using different staining techniques.

Result: The pancreas appeared as pale pink triangular lobulated gland located along the dorsal aspect of the abdominal cavity behind the stomach having exocrine and endocrine part. Histologically, two types of acinar cell were identified *i.e.* active and resting acinar cells. The islets were richly vascularized and were separated from the exocrine acini by a thin layer of collagen, elastic and reticular fibers which were seen extending into the interior of the islets. Mainly three types of cells were observed in islets *i.e.* alpha (A), beta (B) and delta (D) cells. Epithelial duct and secretory cells showed strong positive reaction for both Periodic acid Schiff (PAS) and Alcian Blue (pH-2.5) whereas islets of Langerhans showed moderate activity for both.

Key words: Endocrine, Exocrine, Gross morphometry, Histology, Large white yorkshire, Pancreas.

INTRODUCTION

Pigs are the highly adaptable and rapidly growing species that serves as an important source of their livelihood for small and marginal farmers (Lind *et al.,* 2007). The pancreas is considered as an accessory digestive gland which has two regions, an exocrine portion where digestive enzymes are synthesized and the other is an endocrine portion where regulatory hormones are produced and released into blood vessels (Roth and Tuggle, 2015). The endocrine portion consisted of isolated groups of pale staining cells that are called islets of Langerhans (Aughey and Frye, 2001). Pigs served as a useful animal model representing human because of their significant anatomical and physiological similarity between the species (Chen *et al.,* 2016). For these reasons, pig seems to be a potential source for pancreatic islet transplantation. Pigs being bred worldwide for meat production and therefore ethical concerns may only be a minor problem (Krickhahn *et al.,* 2002). Not much scientific information pertaining to the pancreas in pig is available. Hence, the present work was undertaken to gain the comprehensive knowledge on the gross and histological observation of pancreas and to understand the distribution patterns of endocrine cells within the islet of Langerhans.

MATERIALS AND METHODS

The experiment was conducted in Department of Veterinary Anatomy, Madras Veterinary College, in the year 2020-2021.The pancreas for the study has been collected from eight apparently healthy pigs (6-7 months of age) which were slaughtered at Department of Livestock Products Technology (Meat science), Madras Veterinary College, Chennai.

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Gross morphological studies

Immediately after slaughter, the abdominal cavity is exposed to study the location of the organs. The pancreases were separated, washed with normal saline and the macroanatomical features such as colour, shape, weight, length, width and thickness were observed.

Histological and histochemical studies

The tissue pieces from different lobes were collected and fixed in 10% neutral buffered formalin. Tissue pieces were then routinely processed for paraffin sections. Sections of

5-6 µm thickness were obtained by microtome. The sections were subjected to the following stains:

a) Hematoxylin and Eosin stain for general tissue reaction and cytoarchitectural studies (Luna, 1968).
b) Masson's Trichrome method for collagen fibers (Luna, 1968).
c) Weigert's method for elastic fibres (Luna, 1968).
d) Gomori's silver stain for reticular fibers (Gomori, 1937).
e) Maldonado's methods for pancreatic islet cells (Luna, 1968).
f) Periodic acid- Schiff (PAS) stain for neutral mucins (Singh and Sulochana, 1978)
g) Alcian blue (pH 2.5) for weakly acidic sulfated mucins (Luna, 1968).

RESULTS AND DISCUSSION

Gross morphological studies

The pancreas of Large White Yorkshire pig was a lobulated gland with irregular margins and was located along the dorsal aspect of the abdominal cavity behind the stomach left to the median plane. This was similar to the observation made by Iniyah *et al.* (2019). The pancreas was pale pink in colour in fresh state and it is covered by a large amount of fat similar to the reports of Iniyah *et al.* (2019) in pig, in Indian donkey it was greyish pink to pale brown (Dhoolappa *et al.,* 2004) and in camel greyish pink in colour (Ali and Masaad, 2007). It appeared as triangular shape organ, consisted of three lobes *viz.*, splenic, duodenal and connecting lobe. The triangular shape and lobulations was similarly observed in pig by Iniyah *et al.* (2019), in horse, Indian donkey and sheep (Dhoolappa *et al.,* 2004) and shape of caudally opened 'V' in dog (Tsuchitani *et al.,* 2016). Between the splenic and connecting lobes is a "bridge" of pancreatic tissue serving as an anatomical connection between the two lobes. The pancreas of Large White Yorkshire pig has only one pancreatic duct that opened from the duodenal lobe similar to the report of Nickel *et al.* (1973) in pig (Fig 1).

The average weight of the pancreas in the adult Large White Yorkshire pig was 90.91±0.32 g. However, the average weight of donkey pancreas was 171 g (Ali and Masaad, 2007) and 95 g in Indian donkey (Dhoolappa *et al.,* 2004). The changes in the weight of pancreas may be due to variation in age and species difference. Among the three lobes, it was observed that the splenic lobe is the longest and widest, the duodenal lobe was long but thinnest and the connecting lobe was the shortest and thickest (Table 1). This was similar to the observation made by Iniyah *et al.* (2019) in pig.

Histological and histochemical studies

The pancreas of Large White Yorkshire pig was an encapsulated, lobulated compound tubuloacinar gland consisted of both exocrine and endocrine parts. It is covered by a connective tissue capsule made up of collagen fibres predominantly, but reticular and elastic fibres were sparse in the capsule. These observations were similar to report of Dhoolappa *et al.* (2004) in the Indian donkey, Garg *et al.* (2007) in porcupine, Kalita *et al.* (2019) in Zovawk pig and Rajathi *et al.* (2023) in guinea pig.

The septa from the connective tissue capsule extended into the parenchyma and divided into complete and incomplete lobules (Fig 2A). Each lobule consisted of secretory units and intralobular ducts in exocrine part and islets of Langerhans in the endocrine part. The septa contained collagen fibers in the interlobular locations especially around the ducts and blood vessels (Fig 2B). Furthermore, fine networks of collagen fibres were noted between the acini and islets of Langerhans. These observations were in accordance with the findings of Dhoolappa *et al.* (2004) in the Indian donkey, Morini *et al.* (2006) in rat and Kalita *et al.* (2019) in Zovawk pig. The secretory units were tubuloacinar with prominent acinar portion. These were similar to the report of Kalita *et al.* (2019) in Zovawk pig: Tubuloacinar but showed dominance of the tubular portion Stinson and Calhoun (1981) in ruminants. However, the secretory units of the donkey and that of the horse are tubulo-alveolar (Sisson and Grossman, 1964). The acinar cells were pyramidal in shape, have

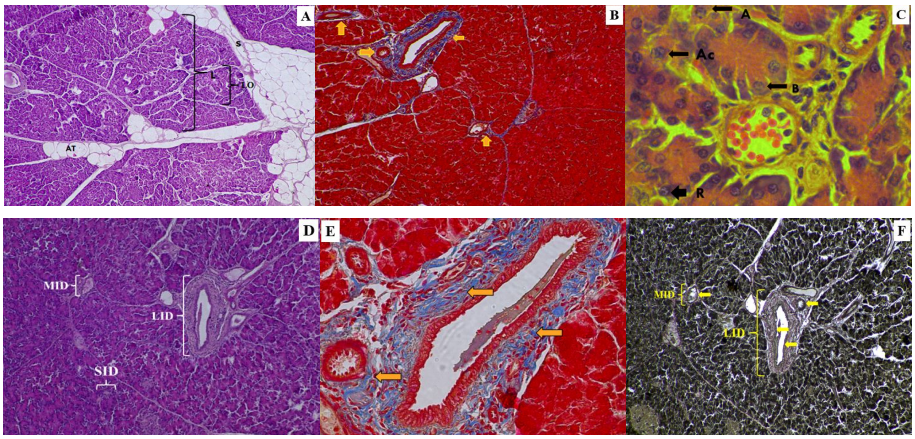
Fig 1: Pancreas of large white Yorkshire pig showing the different lobes.

Table 1: Biometrical observations of various parameters of the pancreas of Large White Yorkshire pig.

Parameters	Splenic lobe (Mean ± SE)	Duodenal lobe (Mean ± SE)	Connecting lobe (Mean ± SE)
Length (cm)	16.65±0.30	10.34±0.30	7.28±0.25
Width (cm)	3.38±0.24	2.16±0.08	3.22±0.07
Thickness (cm)	1.15±0.11	0.98±0.10	1.21±0.07

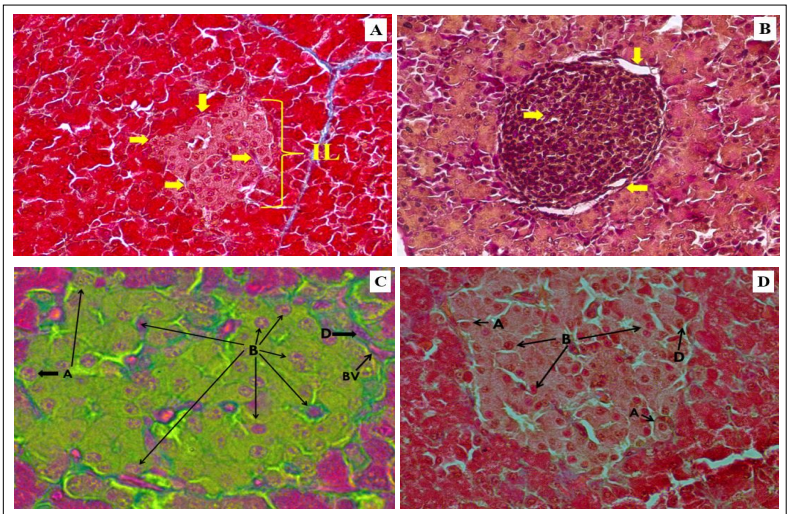
spherical or oval nuclei resting upon a basal lamina and were surrounded by delicate reticular fibers. Similar observation was reported by Kalita *et al.* (2019) in Zovawk pig. Two types of acinar cells were identified on the basis of shape, location of the nucleus and its chromatin content *i.e.* active acinar cell and resting acinar cells (Fig 2C). The nucleus of the active acinar cells were spherical in shape, located at the center and are euchromatic whereas the nucleus of resting acinar cells were also spherical in shape, heterochromatic and placed very close to the base of the cell. The amount of zymogen granules was more as compared to active acinar cells. Similar observation was report by Kalita *et al.* (2019) in Zovawk pig. In addition to the above, there is presence of exhausted cells, in which the nucleus was spherical and located almost in the center of the cell in human (Bloom and Fawcett, 1968), in camel and donkey (Mukherjee *et al.,* 1986).

The duct system of the pancreas consisted of large interlobular, medium intralobluar and small intercalated



(A) S-Septa, L-Lobes, LO-Lobules and AT- Adipose tissue, H and E X50; (B) arrow showing distribution of Collagen fibres in Ducts and Blood, Masson's Trichome X100; (C) acinar cell showed two distinct zones A-Apical zone and B-Basal zone, two secretory acinar cells Ac- Active cells and R-Resting cells, H and E X1000; (D) showing LID- Large interlobular duct, MID-Medium intralobular duct and SID-Small intercalated duct, H and E X100; (E) arrow showing distribution of collagen fibres in large interlobular ducts, Masson's Trichome X400 and (F) arrow showing distribution of reticular fibres in LID-large interlobular duct and MID-Medium intralobular duct, Gomori's X400.

Fig 2: Photomicrograph of pancreas.



(A) arrow showing distribution of collagen fibres between acini and IL-Islets of Langerhans, Masson's Trichome X400; (B) arrow showing distribution of elastic fibres in islets of Langerhans, Weigert's stain X400; (C) showing A-A cells, B-B cells, D-Delta cells and BV-Blood vessel, Maldonado's Method X1000 and (D) showing A-A cells, B-B cells and D-Delta cells, Masson's Trichome X400.

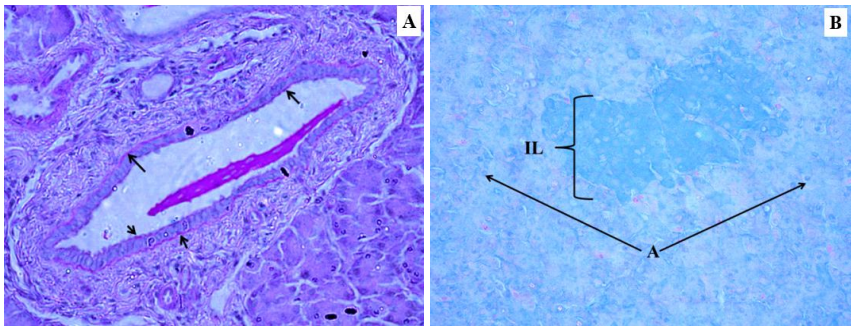
Fig 3: Photomicrograph of endocrine pancreas.

ducts (Fig 2D). The mucosa of the small intercalated ducts was lined by flattened cuboidal type of epithelium. The cells from the ductal epithelium extended into the lumen of the acinus as centroacinar cells of pancreas. These cells can be clearly demarcated from the acinar cells, as these cells were smaller than the acinar cells and had few granules in the cytoplasm. This is similar to the observation reported in buffalo (Davinder and Gupta, 1999) and in Zovawk pig (Kalita *et al.,* 2019). The large intralobular duct was lined by single layered cuboidal epithelium. These ducts consisted of distinct basal lamina and were surrounded by collagen, reticular and few elastic fibres around the ducts (Fig 2E and 2F). The intralobular ducts were present within the lobules. This is similar to human pancreas (Bloom and Fawcett, 1968) and in Zovawk pig (Kalita *et al.,* 2019). However, the intralobular duct is lined by columnar cells and not cuboidal cells in sheep (Gemmel and Heath, 1973). The interlobular ducts were found between the lobules in the connective tissue septa. The interlobular duct was lined by a single layered cuboidal type of epithelium and is supported by numerous collagen and reticular fibres. These observations were in accordance with the findings in human pancreas (Bloom and Fawcett, 1968) and in Zovawk pig (Kalita *et al.,* 2019). This duct showed distinct smooth muscle layer in its middle and surrounded by numerous connective tissue fibres as in buffalo pancreas (Davinder and Gupta, 1999).

The islets of Langerhans were embedded within the exocrine portion of the pancreas. It appeared as pale stained rounded or oval or irregular areas between the darkly stained acini. They were separated from the acini by a thin layer of reticular and elastic fibers which were seen extending into the interior of the islets; but no distinct capsule encircling the islets was observed (Fig 3A and 3B). This is similar to observation in Zovawk pig (Kalita *et al.,* 2019). The islets were richly vascularized and the blood capillaries were enlarged forming cyst-like structure. The cells of islets were arranged in irregular branching and anastomosing cords separated by blood capillaries with delicate collagen fibres. Similar arrangement of islets cells were noticed in pancreas of domestic pig (McGeddy *et al.,* 2006), albino rat (Fattah, 2008) and in Zovawk pig (Kalita *et al.,* 2019). Three different types of islets were observed *i.e.* small, medium and large islets. The large islets were usually ovoid in shape and are found closer to the larger blood vessels and major ducts. The number of endocrine cells were more in large islets followed by medium and small. This is similar to observation in albino rat (Rayaz, 2013). However, four different types of islets are noticed in pancreas of rat *viz.*, large sized (150мм and above), medium sized (75-150мм), small (50-75мм) and very small islets (Hughes, 1946).

Mainly three types of cells were found in the islets *i.e.* alpha (A), beta (B) and delta (D) cells. The alpha and delta cells occupied the peripheral region of the islet, whereas beta cells present mostly in the interior and only a few delta cells were found at intermediate portion (Fig 3C and 3D). This is similar to the finding in Zovawk pig (Kalita *et al.,* 2019) and in dog (Kumar *et al.,* 2024). The alpha cells were characterized by a pale cytoplasm, dense round granules and a pale nucleus. These cells appeared round to ovoid in shape, slightly larger than the beta cells and make up about 15 % of the total islet cell population as observation in camel (Sultan, 1999) and in albino rat (Rayaz, 2013). The beta cells were the predominant cell type. These cells were spherical in shape and constituted about 80 % of the total islet cell population. These cells had dark cytoplasm containing dense, round granules and a dark nucleus. The cells have round or oval-shaped nuclei. Finding is similar to observation made in Zovawk pig (Kalita *et al.,* 2019). However, the beta cells are polyhedral in shape in islets of rat, as described by Patent and Alfert (1967). The delta cells were long, flattened or irregularly shaped with cytoplasmic processes. These cells were characterized by the presence of small granules and make up about 3 % of the total islet cell population as reported by Elayat *et al.* (1995) in rat pancreas.

For histochemical studies, islets of Langerhans showed moderate activity for both Periodic acid Schiff (PAS) and Alcian Blue (pH-2.5) whereas the acini and the epithelial



(A) arrow showing the presence of PAS activity in the duct epithelium, PAS stain x 400 and (B) arrow showing presence of Alcian blue activity in A-acinus and IL-Islets of Langerhans, Alcian blue X400.

Fig 4: Photomicrograph of pancreas.

cells of the pancreatic ducts shows strong positive reaction for both Periodic acid Schiff (PAS) and Alcian Blue (pH-2.5) (Fig 4A and 4B). This is similar to observation in albino rat (Rayaz, 2013). Moderate reaction for Alcian blue were observed in larger excretory ducts, but no affinity for Alcian blue in smaller pancreatic ducts of Japanese quail (Sivakumar, 1997; Das *et al.,* 2001).

CONCLUSION

As detailed in this study, the pancreas of Large White Yorkshire appeared as pale pink triangular gland, shows two portions *i.e.* exocrine and endocrine portions. Focusing on the pancreatic islets there is a great variability in islets profile depending on number, size and collagen content. This may exert greater influence on islets yield. Therefore, better understanding of histomorphological analysis is needed prior to islet isolation. Porcine pancreatic islets may be regarded as a major challenge to biotechnology as pig serves as a useful animal model representing humans because of the comparable organ size and many physiological and biochemical similarities between the species. The result of the present study may be used as research baseline for the comparative studies in other animals.

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Conflict of interest

The authors declare the existence of no conflict of interest.

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