RESEARCH ARTICLE Indian Journal of Animal Research, Volume 59 Issue 11: 1937-1942 (November 2025)

A Histomorphochemical Study of Porcine Pancreas with Emphasis on Cellular Mapping

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10.18805/IJAR.B-5636

ABSTRACT

Background: The pancreas is a dual function gland acting as both an endocrine and exocrine. Production of digestive enzymes like lipase, amylase, trypsin and chymotrypsin is regulated by exocrine part. Hormones like insulin, glucagon and somatostatin, which are vital for regulation of blood sugar, are under regulation and control of endocrine part. The present study was aimed to obtain histological and histochemical observations of porcine pancreas.

Methods: Total six samples (n=6) of pancreas were procured immediately after slaughter and preserved in 10% neutral buffered formalin and Bouin's solution. Tissue processing was done by routine paraffin block preparation by alcohol-benzene schedule.

Result: Pancreas of pig was encapsulated by connective tissue capsule. It was divided into lobes and lobules by connective tissue septa. Major glandular parenchyma was exocrine part while lightly stained endocrine part was only about 1-2% of the total mass. Acini and ducts were the constituents of exocrine pancreas. Acini possessed centroacinar and acinar cells. Acinar cells had three different forms, *viz.* active, resting and exhaustive cells. Pancreatic ducts along with goblet cells were moderately positive for glycogen, weakly sulphated mucins and acidic mucopolysaccharides. Islets of Langerhans had three types of cells *i.e.* alpha, beta and delta. Beta cells outnumbered other two cell types forming major part of pancreatic islets of Langerhans. Islets of Langerhans had profound blood supply. The present work will be a complementary contribution to enhance understanding of porcine pancreatic anatomy and its further implication for the betterment porcine production.

Key words: Acini, Endocrine, Exocrine, Islets and pancreas.

INTRODUCTION

The pancreas is present in the dorsal abdominal region and has a close association with the proximal duodenum (Konig *et al.,* 2004). It is a compound tubuloacinar gland with encapsulation and lobulation. Glandular parenchyma is divided in two parts, *viz.* exocrine and endocrine parts (Eurell and Frappier, 2006). The exocrine pancreas is arranged into acini and the ductal structures that carry the pancreatic enzymes to the intestines. There are distinct lightly stained clusters of endocrine cells known as the islets of Langerhans. The exocrine part of the organ covers majority of the pancreatic mass (more than 90-95%) and the islets of Langerhans are scattered throughout the pancreatic tissue (Longnecker and Thompson, 2023). There are five cell types within the islets of Langerhans, *viz.* α, β, δ, ε and PP cells, producing hormones, *viz.* glucagon, insulin, somatostatin, ghrelin and pancreatic polypeptide, respectively. These hormones regulate glucose uptake, release and serum glucose levels (Eurell and Frappier, 2006).

Pigs serve as a valuable source of nutrients and secondary income source to the rural and sub-urban population (Boro *et al*., 2016). Conditions like pancreatic insufficiency or other related illnesses of pancreas can disrupt metabolic pathways, resulting in reduced growth and performance in animals, which in turn affects production. The most common issues faced include chronic pancreatitis, fibrosis and tumors. Therefore, maintaining the pancreatic health is crucial for successful porcine production (Eric, 2021).

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How to cite this article: Bhadu, N., Poonia, A., Gahlot, P.K. and Parkash, T. (2025). A Histomorphochemical Study of Porcine Pancreas with Emphasis on Cellular Mapping. *Indian Journal of Animal Research*. **59(11):** 1937-1942. doi: 10.18805/IJAR.B-5636.

Submitted: 30-05-2025 **Accepted:** 24-07-2025 **Online:** 23-08-2025

According to Nature Medicine (2022), there is hope that xenotransplantation will help to address the severe lack of human donor organs, as evidenced by the recent ground-breaking clinical findings demonstrating the viability of transplanting transgenic pig organs into humans. Making xenotransplantation a commonplace option for transplant recipients and proving its effectiveness and safety in clinical studies provide the next challenge. The present study was aimed to provide a better histoarchitectural and histo chemical knowledge about the pancreas of domestic pig, helping to have a better understanding of physiological functions, which may be helpful in development of pig as animal model for human related research.

MATERIALS AND METHODS

The experiment was conducted at Department of Veterinary Anatomy, (Session 2023-2025) College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana for session 2023-2025.

Sample collection

The present study was conducted on the pancreas of 6 adult pigs (n=6) of either sex of local non-descript breeds. The samples were collected from local meat shops of Hisar, Haryana, India (29.1492°N, 75.7217°E).

Processing of tissue for light microscopy

The tissues were collected from different segments of pancreas and were fixed in 10% neutral buffered formalin solution for 48 hours for histomorphological and histochemical studies. The tissues were fixed in Bouin's solution for Gomori's and Maldonado's stains for pancreatic islet cells. Tissue processing was done by routine paraffin block preparation by alcohol-benzene schedule (Luna, 1968). The blocks were prepared from paraffin wax (58-60°C) and 5-6 μm thick sections were cut by Leica HistoCore multicut-semi-automated rotary microtome and taken on clean glass slides. The tissues were stained with Hematoxylin and Eosin stain for routine histomorphology, special stains for various connective tissue structures and localization of histochemical moieties (Luna, 1968; Crossman, 1937; Humason, 1972 and Pearse, 1968).

Microscopy

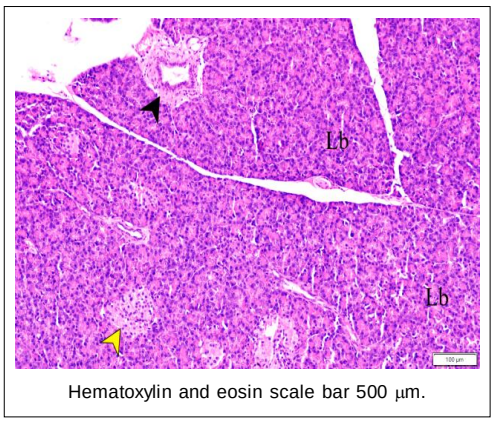
The stained slides were observed under digital Olympus CX33 biological microscope and required photographs were taken with it.

RESULTS AND DISCUSSION

Pancreas of pig was encapsulated by a connective tissue capsule, which divided the parenchyma in lobes and lobules in an irregular manner (Fig 1). These findings were in accordance to the observations of Duhan (1992) in buffalo, Egerbacher and Böck (1997) in humans, Dhoolappa *et al.* (2004) in Indian donkey, Hamodi *et al.* (2013) in common gulls and guinea fowls and Khaleel *et al.* (2020) in goat and Rajathi *et al.* (2023) in guinea pig. The connective tissue septa had collagen (Fig 2) and reticular fibres (Fig 3) as reported earlier by Egerbacher and Böck (1997) in humans and Iniyah *et al.* (2020) in pig. These septae possessed some adipose tissue, rich blood supply, nervous innervations and a few elastic fibres, which were in consonance with the findings of Dhoolappa *et al.* (2004) in Indian donkey and Iniyah *et al.* (2020) in pig. The glandular parenchyma was divided into exocrine and endocrine parts (Fig 1) similar to the findings of Duhan (1992) in buffalo, Iniyah *et al.* (2020) in pig and Khaleel *et al.* (2020) in goat. Exocrine part contributed roughly more than 90% while endocrine part was only 1-2% of the total glandular mass which was consistent to the observations of Pandiri (2014) in rats and mice whereas Khaleel *et al.* (2020) stated that exocrine part was 95% and endocrine part was 1-2% of the total pancreatic mass in goat. However, Hamodi *et al.* (2013) observed 99% exocrine part in pancreas of common gulls and guinea fowls.

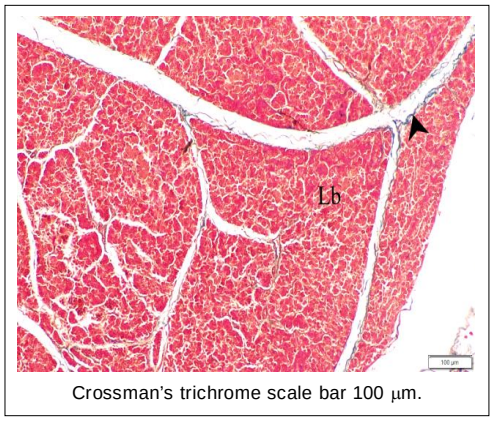
Histology of exocrine part

Exocrine part of pancreas was consisted of two components *i.e.* the secretory portion known as acini (Fig 1 and 4) and the duct system (Fig 1). The pancreatic acini were of variable shape and size, surrounded by a fine network of collagen (Fig 2) and reticular fibres (Fig 3), which was in agreement to the observations of Dhoolappa *et al.* (2004) in Indian donkey and Khaleel *et al.* (2020) in goat. Spindle shaped, elongated and basophilic cells considered as myoepithelial cells (Fig 4) were observed on the peripheral part of acini similar to the observations of Iniyah *et al.* (2020) in pig and Khaleel *et al.* (2020) in goat. Acinar cells were having typical cellular detail *i.e.* the basal region possessed the nucleus and the cytoplasm was basophilic in nature whereas the apical region was eosinophilic in nature containing zymogen



Hematoxylin and eosin scale bar 500 μm.

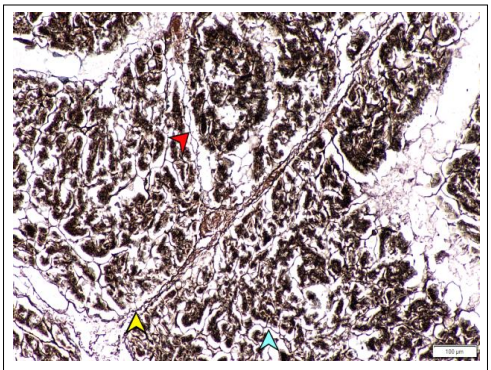
Fig 1: Photomicrograph of pancreas showing islets of Langerhans (yellow arrowhead), ducts (black arrowhead) and acini present in lobules (Lb).



Crossman's trichrome scale bar 100 μm.

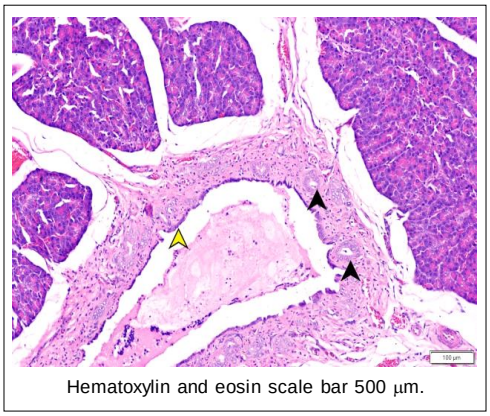
Fig 2: Photomicrograph of pancreas showing blue colored collagen fibres (black arrowhead) in connective tissue septae dividing into lobules (Lb).

granules, which was in concurrence with the findings of Duhan (1992) in buffaloes, Hamodi *et al.* (2013) in common gulls and guinea fowls and Iniyah *et al.* (2020) pig. On the basis of difference in morphological character, size and location of nucleus and density of zymogen granules, acinar cells were classified into three types (Fig 4) *i.e.* active cells, resting cells and exhausted cells. Each type of acinar cell indicated a different physiological state. Similar observations were made by Duhan (1992) in buffaloes. The active cells had euchromatic type of nucleus which was at the basal part of the cell. The apical portion of active cells contained abundance of pink coloured zymogen granules. The resting cells had heterochromatic nucleus, which was located towards the basal portion. The heterochromatic nucleus suggested the reduced activity of the resting cells. The zymogen granules were densely packed in resting cells. The exhaustive cells were elongated in shape and possessed spherical to oval, highly euchromatic nucleus, which was located at middle part of the cell. Euchromatic nucleus suggested high cellular activity. Centroacinar cells (Fig 4) were found at the centre of acini as reported earlier by Egerbacher and Böck (1997) in humans and Iniyah *et al.* (2020) in pig. On contrary, centroacinar cells were not observed in common gulls and guinea fowls by Hamodi *et al.* (2013). Intercalated ducts observed to originate from central portion of an acinus as reported earlier by Rajathi *et al.* (2023) in guinea pig. The intercalated duct had flat type of epithelium, which drained its secretion into intralobulated duct. The intralobulated ducts had low cuboidal type of epithelium. The intralobulated ducts joined the small interlobular ducts, which further joined the major interlobular ducts, that had simple columnar type of epithelium (Fig 5). It was in conformity with the observations made by Egerbacher and Böck (1997) in humans and Dhoolappa *et al.* (2004) in Indian donkey. However, Hamodi *et al.* (2013) stated that ductal epithelium was simple squamous to cuboidal type in common gulls and guinea fowls. These ducts contained collagen fibres along with a few reticular and scanty elastic fibres in their walls, as reported



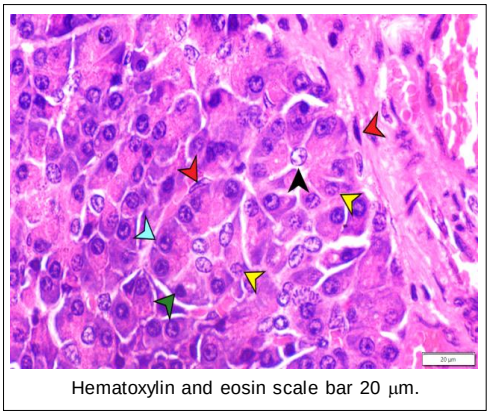
Gomori's method for reticular fibres Scale bar 100 μm.

Fig 3: Photomicrograph of pancreas showing reticular fibres in connective tissue septae (yellow arrowhead), in between islet of Langerhans, in exocrine part (red arrowhead) and around the acini (blue arrowhead).



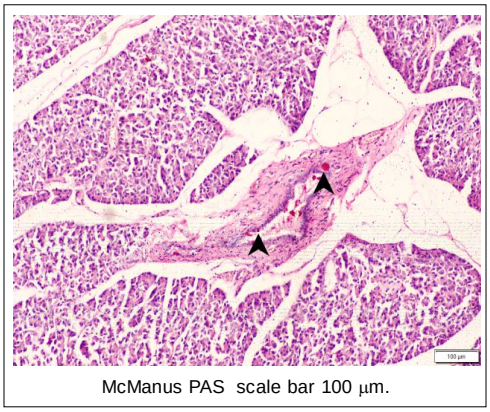
Hematoxylin and eosin scale bar 500 μm.

Fig 5: Photomicrograph of pancreas showing major pancreatic duct lined by columnar epithelium (yellow arrowhead) and smaller ducts opening in it (black arrowhead).



Hematoxylin and eosin scale bar 20 μm.

Fig 4: Photomicrograph of pancreatic acini showing active cells, centroacinar cell, exhaustive cells and resting cells (blue, black, yellow, red and green arrowhead respectively)



McManus PAS scale bar 100 μm.

Fig 6: Photomicrograph of pancreas showing PAS positive reaction in the goblet cells (black arrowhead) in interlobular duct.

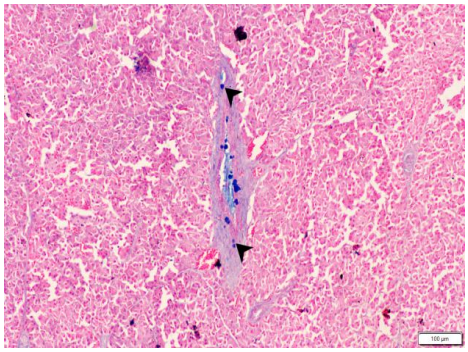
earlier by Dhoolappa *et al.* (2004) in Indian donkey and Singh *et al.* (2024) in pig.

Histochemistry of exocrine part

Acini exhibited PAS negative reaction for glycogen while ducts along with goblet cells displayed moderate reaction showing the presence of glycogen (Fig 6), On contrary, Conklin (1962) reported strong PAS activity in pancreatic acini of humans while Singh and Gupta (1999) in buffalo reported mild PAS positive reaction in exocrine part of the pancreas. Negative alcianophilic reaction was observed in the acini showing absence of weakly sulphated mucopolysaccharide. Intense alcianophilic reaction was observed in the duct along with goblet cells (Fig 7), which was consistent to the findings of Singh and Gupta (1999) in buffalo. Earlier, Dhoolappa *et al.* (2004) reported a strong positive reaction while Hamodi *et al.* (2013) observed a mild positive reaction for both PAS and Alcian blue in larger ducts of exocrine part in Indian donkey and in common gulls and guinea fowls, respectively. Iniyah *et al.* (2020) observed PAS positive goblet cells along with some other areas in larger ducts of exocrine pancreas of large white Yorkshire pigs. Positive PAS-AB reaction demonstrated presence of acidic mucopolysaccharide in the lumen of duct and in goblet cells along with some areas for neutral mucopolysaccharide (Fig 8). No reactions were observed for the presence of mucin. Acini displayed the presence for cysteine protein (>4%), while Hamodi *et al.* (2013) reported moderate to weak reaction for proteins in common gulls and guinea fowls. Mast cells were observed randomly in whole exocrine part except for the ducts. No keratin or prekeratin was observed in whole pancreatic parenchyma.

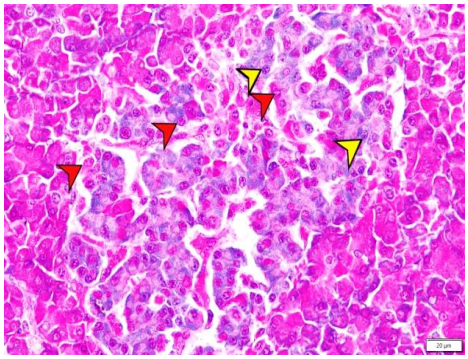
Histology of endocrine part

It comprised the islet of Langerhans, scattered throughout the exocrine part in various shapes and sizes. The reticular fibres were predominately observed surrounding the islets. These islets had a profound blood supply exhibited by presence of numerous capillaries as reported earlier by Duhan (1992) in buffalo and Singh *et al.* (2024) in pig. These pancreatic islets comprised three major type of cell population *i.e.* alpha (α), beta (β) and delta (δ) cells, in accordance to the observations of Duhan (1992) in buffalo and Jagapathi *et al.* (2012) in the cat. However, Hamodi *et al.*



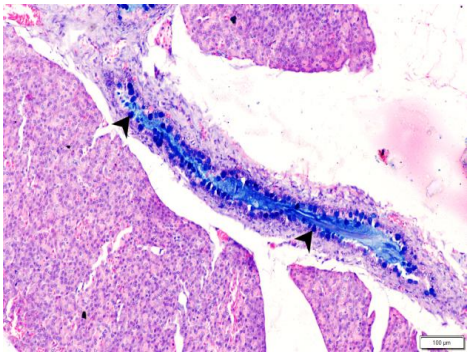
Alcian blue scale bar 100 μm.

Fig 7: Photomicrograph of pancreas showing alcianophilic reaction in goblet cells (black arrowhead) in interlobular duct.



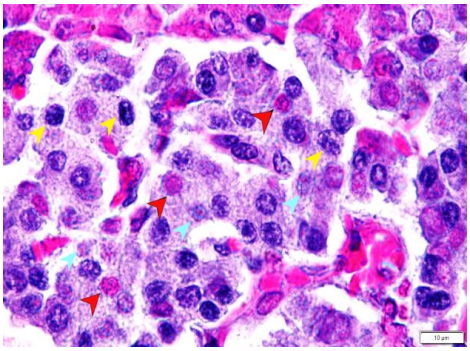
Gomori's for pancreatic islets Scale bar 20 μm.

Fig 9: Photomicrograph of alpha dominant islet of Langerhans showing alpha cell (red arrowhead) and beta cells (blue arrowhead).



PAS-AB scale bar 100 μm.

Fig 8: Photomicrograph of pancreas showing presence of acidic mucopolysaccharides in goblet cells (black arrowhead) of interlobular duct.



Maldonado's scale bar 20 μm.

Fig 10: Photomicrograph of higher magnification of islets of Langerhans in pancreas showing alpha (red arrowhead), beta (yellow arrowhead) and delta (blue arrowhead) cells.

(2013) observed only alpha and beta cells in common gulls and guinea fowls. Gomori's stain (Fig 9) for pancreatic islets imparted purple colour to nucleus of alpha cells while, beta cell nucleus was stained dark blue with granulations in it.

Maldonado's stain enabled to identify all the three types of cells (Fig 10). Alpha cells had round shaped, purple coloured nucleus. Majority of the alpha cell population was located at the periphery of the islet but some cells were also found towards the centre of islet of Langerhans, which was in consonance to the observation of Kim *et al.* (2009) in dog and pig. Whereas, Kim *et al.* (2009) in reported a different arrangement of alpha and beta cells in rodents and monkeys than that of dog and pig. Whereas, Malik and Prakash (1972) and Jagapathi *et al.* (2012) reported that alpha cells were located at the periphery of the islets in buffalo and ox and cats, respectively. Contrarily, Khaleel *et al.* (2020) reported that alpha cells were located only at center of the islets in goat. Beta cells had dark blue coloured nucleus with some granular texture. The beta cells outnumbered other two cells types and were located mainly at the central part of the islet while some cells were also noticed at the periphery as reported earlier by Mukherjee *et al.* (1988) and Jagapathi *et al.* (2012) in sheep and cat respectively. Whereas Khaleel *et al.* (2020) reported that the beta cells were only found at periphery of islets in goat. Delta cells had oval to elliptical nucleus with light blue colour having purplish tinge. Delta cells were least in number amongst all three types of cells and were mostly observed in association with alpha cells which was in concurrence with the findings of Elayat *et al.* (1995) in rat. Whereas, Jagapathi *et al.* (2012) reported that delta cells were found randomly in islets of cats while Khaleel *et al.* (2020) observed the delta cells at periphery of islets in goat. Majority of the islets were beta cell-dominating but some alpha cell-dominating islets were also observed. Similar findings were reported by Hamodi *et al.* (2013) in common gulls and guinea fowls and Tsuchitani *et al.* (2016) in experimental animals.

Histochemistry of endocrine part

No activity was noticed in the islets of Langerhans with PAS and PAS-AB stains indicating absence of glycogen, acidic and neutral mucopolysaccharide, respectively. However, Duhan (1992) in buffalo reported presence of glycogen in alpha cells while Hamodi *et al.* (2013) observed PAS and alcianophilic positive reactions in islets. Weakly sulphated acidic mucopolysaccharide, strongly acidic mucopolysaccharide and mucins were not observed within the pancreatic islets depicted by negative reaction for alcian blue, colloidal iron and Mayer's mucicarmine stains, respectively. Islets showed weak to mild reaction for presence of proteins, which was in accordance to Hamodi *et al.* (2013) who observed that islet cells highlighted weak to moderate reaction for bromophenol blue. Mast cells were not observed in the islets.

CONCULSION

The exocrine pancreas primarily has secretory acini and a branched ductal system, which work together to produce and transport digestive enzymes. Active, resting and exhaustive acinar cells highlights different physiological states. The duct system originates from centroacinar cells and proceeds as intercalated, intralobular and interlobular ducts, which progressively gets taller epithelial cells which facilitate the effective transport of pancreatic secretions. Ducts and goblet cells highlighted presence of glycogen and acidic and neutral mucopolysaccharides but were not observed in acini. The islets of Langerhans were scattered randomly in the exocrine part. These islets consisted of three types of cells *i.e.* alpha, beta and delta cells. Beta cells were most numerous in the present study, whereas delta cells were least in number. The pancreas has a significant role in digestion and assimilation food, which is highlighted by the structural arrangement and histochemical characteristics of the gland.

ACKNOWLEDGEMENT

The authors are grateful to the Department of Veterinary Anatomy, COVS for providing research facility and Department of Veterinary Parasitology for providing microphotography facility, COVS, LUVAS, Hisar (125 004), Haryana, India.

Conflict of interest

The authors declare that there is no conflict of interest.

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