

PRENATAL DEVELOPMENT OF EXOCRINE PANCREAS IN CROSSBRED GOATS: A HISTOLOGICAL STUDY

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ABSTRACT

Back ground: Studies about the normal development of pancreas are beneficial in understanding its normal mode of functioning and thereby help to trace the causes of its dysfunction as in diabetes, pancreatitis or the highly fatal pancreatic cancer. The mechanisms involved in normal prenatal development of exocrine pancreas in goats are poorly understood.

Purpose: The present study was conducted with an aim of contributing some useful information in the field of developmental and pancreatic biology.

Methods and Results: For the present study, the goat embryos from second to fifth month of prenatal life, collected from clinics and farms of Kerala Veterinary and Animal Sciences University and those available in the museum of Department of Veterinary Anatomy, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India were utilized. Samples were fixed in 10% neutral buffered formalin and Bouin's solution and were processed for light microscopic histological studies. Sections of 5-7 μ thickness were cut on a rotary microtome, stained using various routine and special techniques and were examined under Leica DM 2000 LED microscope. During second month of prenatal development, the exocrine pancreas comprised ductules of varying size dispersed within the mesenchyme of both pancreatic primordia. As age advanced and the pancreatic primordia fused, the number and size of ductules increased and developed acini at their end. Later the pancreatic parenchyma was divided into lobules containing groups of acini, blood vessels and intralobular ducts. The present study revealed that the duct system of exocrine pancreas developed before the formation of acini and the number of ducts increased mainly by branching. Acini developed by the third month of development while distinct lobulation could be noticed in the fourth month of development. The arrangement of lobules, duct system and acini in the fifth month of foetal life indicated the development of a well-organized exocrine pancreas in goats in the prenatal life itself.

Potential implications: The results can also be compared to human data for furthering the knowledge of human biology and medicine.

KEY WORDS: Exocrine pancreas, Goats, Histology, Prenatal development.

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INTRODUCTION

The pancreas of mammals is a unique gland due to the presence of both exocrine and endocrine components. The exocrine pancreas constitutes

the larger part of the pancreatic parenchyma and functions as one among the major digestive glands in the body. It is composed of tubuloacinar glands that secrete various digestive enzymes such as amylase, lipase and protease [1]. The secretory units or acini are arranged as groups of sac like structures at the end of ducts. These ducts of varying diameters, originating as the intercalated duct carry the secretory products towards duodenum and drain into it through major or minor duodenal papilla depending upon the species. The lining cells of these ducts are also secretory in nature and produce mucus and bicarbonate rich fluid that helps to neutralize the acidic chyme entering the duodenum [2]. Pancreas develops from dorsal and ventral pancreatic primordia that are outgrowths of endodermal lining at the caudal part of foregut [3]. Cells of the pancreatic primordia multiply and differentiate into ducts and secretory acini. Some cells get detached from the duct system and develop into islets of Langerhans.

Studies about the normal development of pancreas are beneficial in understanding its normal mode of functioning and thereby help to trace the causes of its dysfunction as in diabetes, pancreatitis or the highly fatal pancreatic cancer. It has been reported that any defect in the endocrine part of pancreas as in diabetes adversely affects the function of exocrine part and pathological conditions affecting the exocrine component of pancreas such as chronic pancreatitis, results in impairment in the function of endocrine part [4]. In the pharmaceutical industry, for commercial production of insulin and several digestive enzymes, the pancreas of domestic animals is used. The mechanisms involved in normal prenatal development of exocrine pancreas in goats are poorly understood. Knowledge about these events in goats will add on information to the existing scientific data and thereby contribute to advances in developmental and pancreatic biology and digestive physiology. The results can also be compared to human data for furthering the knowledge of human biology and medicine.

MATERIALS AND METHODS

For the present study, the embryos collected from

clinics and farms of Kerala Veterinary and Animal Sciences University and those available in the museum of Department of Veterinary Anatomy, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India were utilized. Immediately after collection, the embryos were grossly examined and only those without any gross abnormalities were selected. Body weight and crown rump length (CRL) of the embryos were recorded and their age was calculated [5]. The present study was conducted on four groups of embryos/foetuses from second month (31-60 days) to fifth month (121-150 days) of prenatal life. The embryos up to 59 days of age were fixed *in toto* and were serially sectioned to locate the primordia and for studying the early stages of development of the pancreas. From the foetuses of later age groups, the pancreas was harvested by careful dissection.

After recording the morphological parameters, tissue samples were fixed in various fixatives such as 10% neutral buffered formalin and Bouin's solution. Fixed tissues were processed for histological studies using paraffin embedding method. Sections of 5-7 μ thickness were cut on a rotary microtome and were stained using Ehrlich's Haematoxylin and Eosin (H&E) staining technique [6] and special staining techniques for light microscopical histological studies such as Lison's alcian blue method [7]. Stained sections were examined under Leica DM 2000 LED microscope and digital images were captured and recorded.

RESULTS

In embryos during the second month (31 to 60 days) of development, the parenchyma of both pancreatic primordia contained ductules of varying size dispersed within the loosely arranged mesenchymal connective tissue (Fig. 1). A few ductules showed branching and were lined by cuboidal cells containing dark stained nucleus with high nuclear to cytoplasmic ratio. Ductular lumen was clear and did not show any secretory material. Projecting from the periphery of some ductules, masses of dark stained cells enclosed in a space, indicating the development of endocrine cells were also seen. The loosely arranged mesenchyme contained fine connective tissue fibres and scattered mesenchymal cells.

Fig. 1: Pancreatic primordia containing several ductules in 58 days-old goat embryo. Haematoxylin and Eosin staining. Magnification: 40 x: 1- dorsal pancreatic primordium, 2- ventral pancreatic primordium, 3- developing stomach, 4- developing intestine.

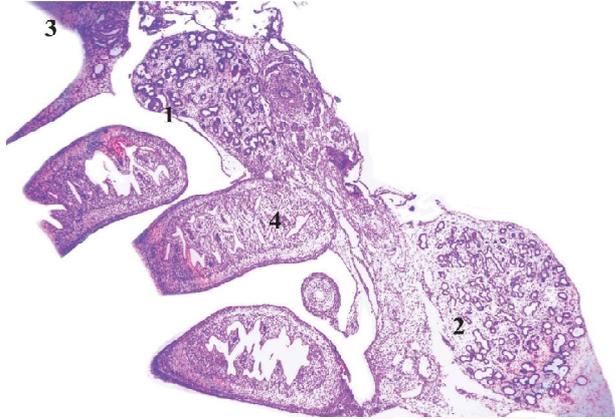
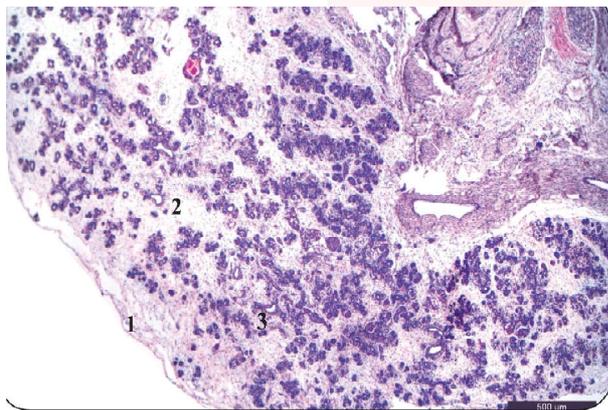


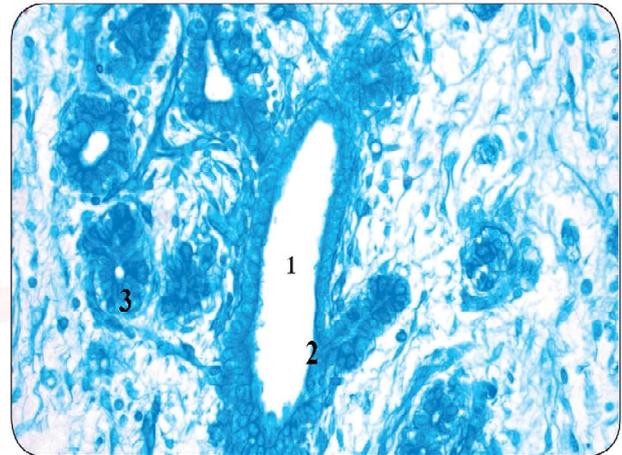
Fig. 2: Section of fused pancreas in 65 days-old goat embryo. Haematoxylin and Eosin staining. Magnification: 40 x: 1- capsule, 2- mesenchyme, 3- ductules.



By third month (61 to 90 days), the pancreatic primordia fused to form a single mass which was enveloped by a distinct connective tissue capsule. The mesenchyme was composed of loosely arranged connective tissue fibres; numerous stellate mesenchymal cells and large number of ductules (Fig. 2). Small ductules were more in number compared to larger ones and were lined by simple cuboidal epithelial cells with well developed dark stained nucleus and high nuclear to cytoplasmic ratio. These ductules showed extensive branching. Adjacent to ductules, solid clusters of cells prior to the development of tubular lumen were also noticed. These cells were pyramidal in shape and were arranged in circular fashion with the apex of cells coming close together at the centre. Commencement of lumen formation occurred at the centre of cell clumps to form new ductules. Large ductules showed branching to form new ones

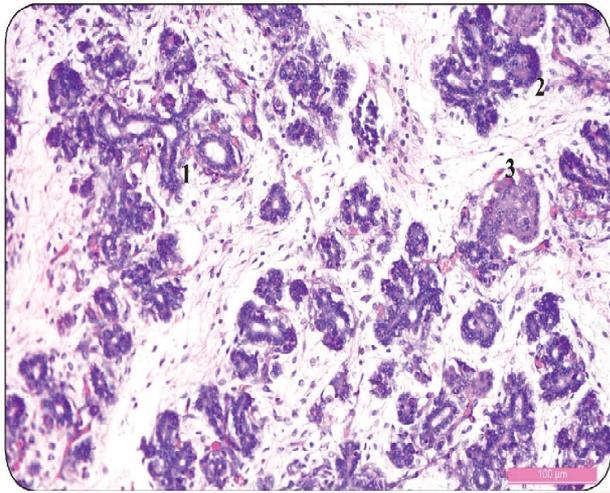
and budding to form new islets (Fig. 3). Lining cells of these larger ductules were lightly stained and taller with clear intercellular borders, whereas the cell buds contained darkly stained cells with large vesicular nucleus.

Fig. 3: Section of pancreas showing branching of large ductules in 65 days-old goat embryo. Lison's alcian blue staining. Magnification: 400 x: 1- lumen of large ductule, 2- branching from large ductule, 3- small ductule.



In 70 days-old embryos, the pancreas was covered by a well developed capsule containing connective tissue fibres and blood vessels. Within the parenchyma, the number of ductules, cell clusters and developing islets increased and they started to group together. Capsule and the mesenchyme contained connective tissue cells and fibres including a fine reticulum. As the number of ductules and islets increased, the amount of mesenchyme got reduced. Ductules showed extensive branching and elongation. Branches of ductules showed the formation of acini at their ends (Fig. 4). Developing acini were seen as small sac like enlargements at the end of ductular branches. The cells lining these developing acini were pyramidal with large, spherical, heterochromatic basal nuclei and eosinophilic cytoplasm. From 71 days, the lobulation of the gland became evident with grouping of the ductules, acini and islets. Within each group, ductules showed branching as well as elongation. Ductular cells were cuboidal with large spherical or oval heterochromatic nuclei and scanty cytoplasm. The developing acinar cells were cuboidal or pyramidal with large spherical and heterochromatic basally located nuclei. The apical cytoplasm was more eosinophilic.

Fig. 4: Section of pancreas showing branching of ductules and development of acini in 70 days-old goat embryo. Haematoxylin and Eosin staining. Magnification: 200 x: 1- branching of ductule, 2- developing acini, 3- developing islet.



By the beginning of the fourth month (91 to 120 days) of development, division of the pancreatic parenchyma into clear lobules of varying size was observed (Fig. 5). Within the lobules, groups of acini and islets of varying size were present. Cross sections of ducts of varying size lined by cuboidal cells and large blood vessels were also seen in the connective tissue between acini and in the interlobular septa. Acini contained cells with basally placed, dark stained nuclei and eosinophilic cytoplasm indicating the presence of zymogen granules. Corresponding to the increase in number of acini, ductules and islets, amount of mesenchyme was reduced considerably. Within the lobules, pancreas from 98 days old embryos showed large ducts of varying diameter. These ducts were lined by simple columnar epithelium with microvilli and their lumen showed some secretory material (Fig. 6). Small intralobular ducts with free cells similar to centroacinar cells in the lumen were observed. After 105 days, the capsule became thick and each lobule contained several secretory acini. Acini were mostly tubular and were lined by cuboidal cells with spherical, dark, basally located nuclei. Among acini, ducts of varying size, arterioles, venules, nerve bundles and isolated neurons were also observed. Small intralobular ducts were lined by thick simple squamous cells or cuboidal cells having lightly stained spherical or oval nuclei. Some of these ducts showed isolated cells within

the lumen. Larger intralobular ducts were lined by darkly stained cuboidal cells with vesicular, spherical or oval, basal nuclei having distinct nucleoli. Large interlobular ducts were lined by columnar epithelial cells with large spherical or basal nuclei. Luminal border presented numerous microvilli.

Fig. 5: Section of pancreas showing division of parenchyma into clear lobules in 91 days-old goat embryo. Haematoxylin and Eosin staining. Magnification: 40 x: 1- lobule, 2- interlobular connective tissue septum, 3- developing islets.

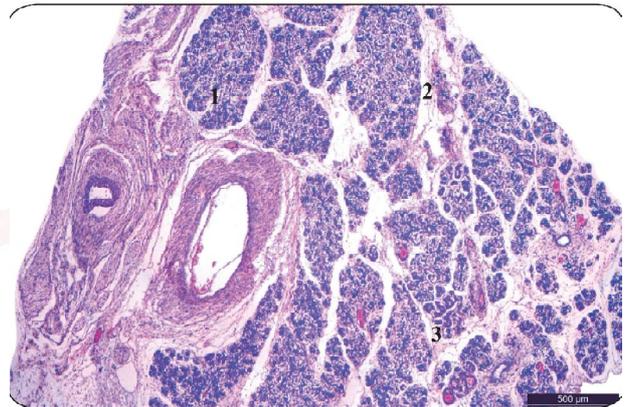
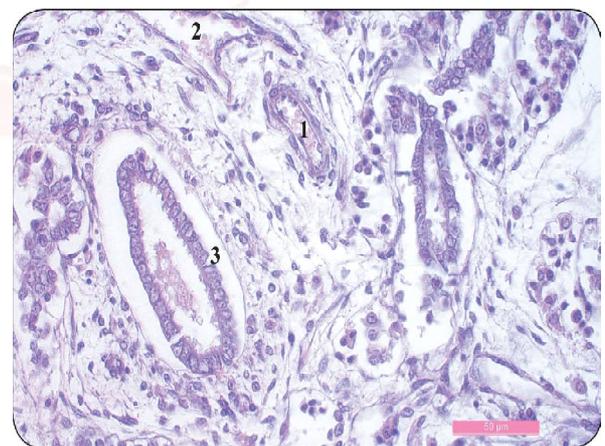


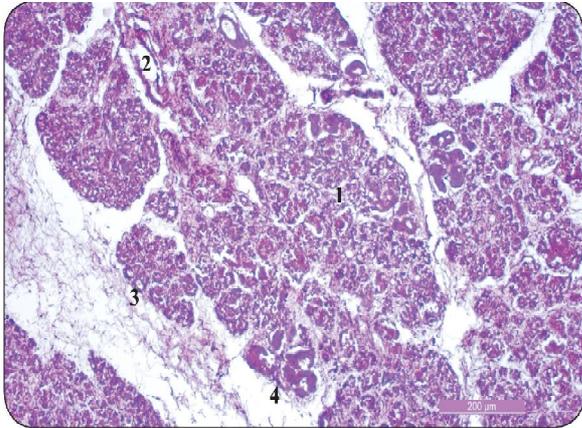
Fig. 6: Section of pancreas showing large duct with secretion and microvilli in 98 days-old goat embryo. Haematoxylin and Eosin staining. Magnification: 400 x: 1- artery, 2- vein, 3- large duct with secretion.



In the fifth month (121 to 150 Days) of prenatal life, most of the pancreatic lobules showed a tree like arrangement around a central trunk that contained blood vessels and intralobular ducts with cuboidal epithelial lining (Fig. 7). The interlobular septa made of loosely arranged collagen and reticular fibres contained blood vessels, ducts, nerve bundles and ganglia. Within the lobules, groups of acini and ducts of varying size were further separated by thick intra lobular septa. Acini were supported by connective tissue frame work composed of

collagen and reticular fibres.

Fig. 7: Section of pancreas showing well developed lobules in 148 days-old goat embryo. Haematoxylin and Eosin staining. Magnification: 100x: 1- lobule containing acini, 2- large intralobular duct, 3- interlobular septum, 4- large islets with sinus.



DISCUSSION

An understanding about the steps involved in the normal development of exocrine pancreas is important in the field of developmental biology and pancreatic cancer biology. In this study, the parenchyma of both pancreatic primordia in embryos during second month of development contained ductules of varying size dispersed within the mesenchymal connective tissue and some ductules showed branching. According to earlier reports, the pancreas in 40 to 45 days-old sheep foetuses, contained numerous ductules [8]. The loosely arranged mesenchyme contained fine connective tissue fibres and scattered mesenchymal cells similar to the findings in foetal goats at 42 days of gestation [9].

By third month of development, fused pancreatic mass with well developed capsule was noticed. The loosely arranged mesenchyme presented large number of ductules of varying size. Small ductules were numerous compared to larger ones and showed extensive branching. Adjacent to them, solid clusters of cells prior to the development of tubular lumen were also noticed. The presence of large number of small ductules and solid clusters indicated an increase in the mass of the exocrine component. The branching of large ductules and presence of cell clusters adjacent to ductules indicated that an increase in number of ductules occurred both by budding of new cell masses in the

mesenchyme as well as by branching of large ductules. The pancreas of human embryos at nine weeks of age also showed a similar structure [10].

The pancreas of 60 days-old buffalo foetuses contained cell clusters and ductules, both surrounded by an abundant mesenchymal mass [11]. However, the pancreatic primordium in mice between 9.5 and 12.5 days embryonic age developed multiple micro lumens which coalesced later [2]. It was also found that in mice, duct morphogenesis involved initial epithelial stratification and formation of multiple small lumens [12]. These further remodelled through changes in shape and position of cells, fused and formed a ramifying duct system with single lumen. In 70 days-old embryos, the pancreas was covered by a well developed capsule containing connective tissue fibres and blood vessels. Within the parenchyma, the number of ductules, cell clusters and developing islets was more. Capsule and the mesenchyme contained connective tissue cells and fibres including a fine reticulum.

As the number of ductules and islets increased, the amount of mesenchyme got reduced. Extensive branching and elongation of ductules indicated rapid increase in the mass of exocrine tissue. Branches of ductules showed the formation of small sac like acini at their ends. The cells lining these developing acini were pyramidal with large, spherical, heterochromatic basal nuclei and eosinophilic cytoplasm. In the pancreas of rat foetuses, secretory acini with zymogen granules developed after the development of ductules [12]. In mouse embryos at 13.5 days, acinar cells developed from the extending tips of several finger-like protrusions in the mesenchyme and increased in number by duplication [2]. In the pancreas of human embryos the acini started to appear by 14 to 16 weeks and by 20 weeks, well defined acini with significant number of zymogen granules were noticed [9]. Grouping of the ductules and islets within the parenchyma from 71 days of development was indicative of the initiation of lobule formation within the pancreas.

By 91 days of development, division of the pancreatic parenchyma into clear lobules of varying size was observed. Within each lobule,

ductules showed branching as well as elongation. Ductular cells were cuboidal with large spherical or oval heterochromatic nuclei and scanty cytoplasm. The developing acinar cells were cuboidal or pyramidal with large spherical and heterochromatic basally located nuclei. The apical cytoplasm was eosinophilic. Similar observations have been reported in buffalo foetuses by third month [11]. Contrary to the present study, distinct lobulation of the pancreas was recorded at 125 days in buffalo foetuses [13] and at 14 to 16 weeks of gestational age in human foetuses [9, 14].

From fourth month of development onwards, the pancreas showed a distinct capsule composed of loose connective tissue and the parenchyma was organized into a large number of lobules of varying size. Large ducts of varying diameter were also seen within the lobules and their lumen showed some secretory material. Presence of such a ductular arrangement and the presence of secretory material in large ducts could be a sign of the beginning of secretory activity of the exocrine pancreas.

In the embryos of fifth month of gestational age, the capsule became thicker and was made of collagen and reticular fibres. This gland showed distinct lobulation with a large number of lobules of varying size. Most of the lobules showed a tree like arrangement around a central trunk that contained blood vessels and intralobular ducts lined by cuboidal epithelium. Such an arrangement is indicative of the establishment of a well-developed exocrine system within the pancreas. Moreover, at this age, the amount of secretory acini increased to a considerable extent compared to the initial stages of development and a systematic arrangement of ducts was also noticed in this age group. In correspondence with the advancement of foetal age, there was a progressive increase in the proportion of exocrine portion of the pancreas and a progressive decrease in the amount of mesenchyme and interlobular connective tissue. These findings support the observations in the pancreas of human embryos that the interstitial tissue became progressively less conspicuous with age [9]. In buffalo foetuses maximum growth of exocrine pancreas occurred during late gestation [13].

CONCLUSION

The present study revealed that the duct system of exocrine pancreas developed before the formation of acini as early as second month of foetal life. Formation of acini could be noticed in the third month of development while distinct lobulation as seen in postnatal animals could be noticed in the fourth month of development. The arrangement of lobules, duct system and acini within the pancreas in the fifth month of foetal life indicated the formation of a well organized exocrine system in goats in the prenatal life itself.

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Conflicts of Interests: None

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