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Histomorphogenesis of exocrine part of pancreas in prenatal goat (*Capra hircus*)

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Abstract
The stained foetal serial sections of early prenatal stage showed that the primordium of foetal goat pancreas was present in close vicinity of developing duodenum. At 69 day old goat foetus the pancreatic parenchyma was formed by tubules of different length and diameter, tubular buds, blood capillaries and islets of Langerhans. The beginning of the process of acini formation in the foetal goat pancreas was first observed from 76 days of gestation. Cells of several buds became loosely arranged and their peripheral cells began to arrange in the form of either a complete or a part of circle to form a central lumen. From 115 days onwards the cytoplasm of few acinar cells around the nucleus had a little bluish tinge and centroacinar cells were seen in few acini. From 132 days to full term foetus the pancreas was chiefly formed by developed acini, however at few places some immature acini were also found.

Keywords: Pancreas, histogenesis, exocrine part, prenatal goat

Introduction
The pancreas is an organ containing two distinct population of cells, the exocrine cells that secrete enzyme into the digestive tract and endocrine cells that secrets hormones into the bloodstream (J.M.W. Slack, 1995) [10]. The exocrine parts made up of acini or alveoli. Each acinus has a single layer of acinar cells with a lumen in the centre. The acinar cells contain zymogen granules, which possess digestive enzyme i.e. pancreatic juice. These enzymes have greatest role in digestion of proteins, lipids and carbohydrate. So alteration and impairment in the structure of foetal exocrine parts cause profound effects on performance of goat. Publication devoted to the histogenesis of these part of foetal goat pancreas is scanty. Therefore, present study was undertaken to see the origin and development of these parts of pancreas during different stage of gestation.

Material and Methods
The present study was conducted on the pancreas of 24 goat foeti of either sex ranging from 42 days to 147 days of gestation. In current research, ethical approval is exempted from the university. The samples were taken from the aborted foeti which has collected from the clinics of college of Veterinary Science & Animal Husbandry, Mathura and local Veterinary hospital of Mathura, Uttar Pradesh, India. The approximate age of foeti were estimated by using the formula derived by Singh *et al.* (1979) [9]. in goat. The foeti were divided into early prenatal (0 to 50 days gestation), mid prenatal (51 to 100 days gestation) and late prenatal (101 to 147 days gestation) periods. As the separation of the pancreas from the adjacent organ was not possible before 50 days gestation, therefore from 42 and 44 days old foeti a mass of the tissue having group of the organ was collected and was fixed in neutral buffered formalin. Tissues were processed by standard protocol of tissue processing and five to six micron thick paraffin sections were obtained. The sections were stained with haematoxylin and eosin for general histoarchitecture, Wilder's reticular stain for reticular fibres, Verhoeff's method for elastic fibres and Masson's trichome stain for collagen fibres (Luna, 1968) [7]. The micrometric observations were conducted with the help of computerized Motic Image Pluse 2.0 software. For each micrometric parameter 10 readings were taken at different places in the same slide.

Results
Early Prenatal Stage
The stained serial sections of 42 days old foetus showed that the primordium of foetal goat

pancreas was present in close vicinity of developing duodenum (Fig.1). The reticular, collagen and elastic fibers were absent in the goat pancreas at early prenatal period.

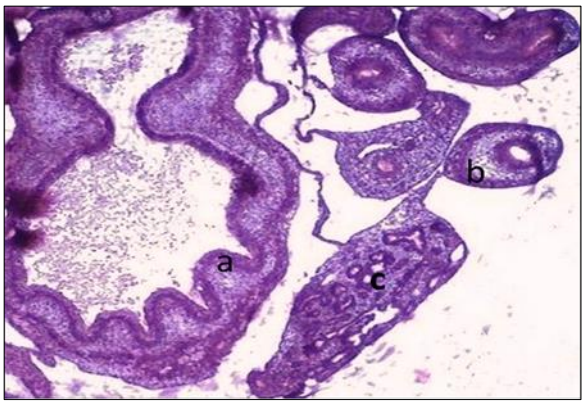

Fig 1: Histological serial section of 42 days old goat foetal pancreas showing abomasum (a), developing duodenum (b) and primordial of pancreas (c). H&E×100

Mid Prenatal Period

At 69 day old goat foetus the pancreatic parenchyma was formed by tubules of different length and diameter, tubular buds, blood capillaries and islets of Langerhans (Fig.2). The beginning of the process of acini formation in the foetal goat pancreas was first observed at 76 days of gestation where cells of peripheral part of several buds became loosely arranged and peripheral cells of some buds were arranged to form either a complete or part of a circle. In many buds some cells were circularly arranged whereas others were arranged in the form of clusters (Fig.3).

The differentiation of buds into acini became relatively more pronounced from 91 days gestation. In these stages at several places the peripheral bud cells formed a continuous circular lining and the cells located in the centre developed empty spaces between them. At some places the developing acini had a large lumen which was surrounded by a layer of spherical bud cells having spherical vesicular nuclei. At some other places the developing acini were lined by two layers of the cells. Some of these cells were spheroid or cuboidal in shape whereas the other cells were relatively larger and appeared as pyramidal in shape. The transformation of buds cells into acinar cells was often seen at these stages where the spheroid buds cells gradually became larger and attained pyramidal shape (Fig-4).

At some places, particularly at 100 days of gestation, more than one developing acini were in continuation to each other having a common lumen. In addition to the developing acini some developed acini were also found which was lined by pyramidal cells having bright eosinophilic supranuclear part and spheroid or spherical bright basophilic nuclei. The lumen of the developed acini was relatively narrower than many developing acini. The centroacinar cells were absent in the foetal goat pancreas at mid prenatal period (Fig-5).

At 69 days the reticular fibers were in almost complete thin layer around the developing pancreatic mass and formed partial or complete circularly dispersed sparse network around the tubules, buds and developing islets of Langerhans. The network of fine reticular fibers was also observed between the parenchymal patches. The collagen and elastic fibers were absent at this stage. By 76 days, the thin reticular fibers encircled the individual tubule and bud and also bound the groups of tubules and buds into small compartments (Fig. 6).

From 91 to 100 days gestation the tubules and developing acini were contained chiefly fibroblasts and fine to coarse reticular fibers. Dense layers of fibroblasts along with relatively coarser reticular fibers were observed in the capsule at these stages (Fig.7). The distinct collagen fibers were absent in all the stages of mid prenatal period. The elastic fibers were absent in the foetal pancreas of goat

The developed acini of mid prenatal period had an average diameter of 20.22±2.43 µm with the lumen having average diameter 6.98±0.52 µm. The average height of the acinar cells were 6.62±0.42 µm with nuclear size of 2.93±0.31 µm.

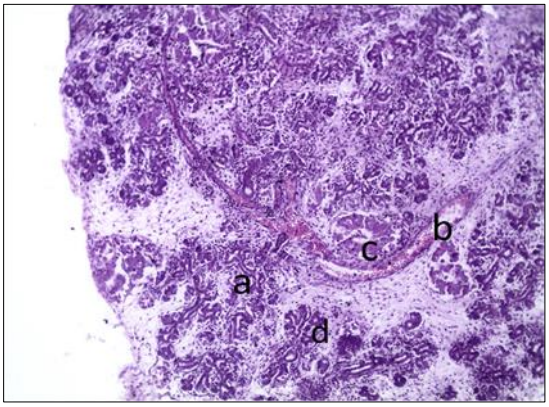

Fig 2: Histological section of 69 days old goat foetal pancreas showing tubules (a), blood capillaries (b), developing islets of Langerhans (c) and tubular buds (d). H&E×100

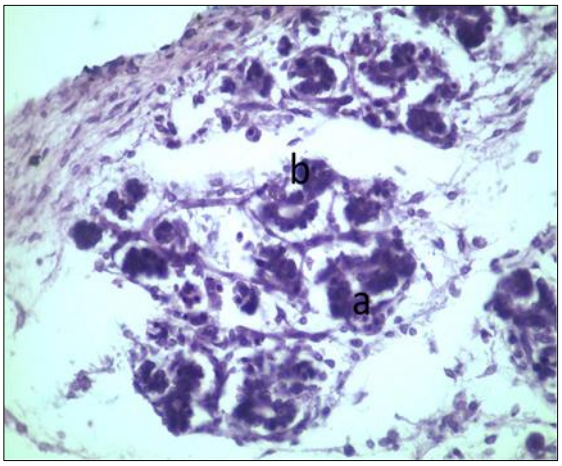

Fig 3: Histological section of 76 days old goat foetal pancreas showing loosely arranged cells (a) and tubular bud (b). H&E×400

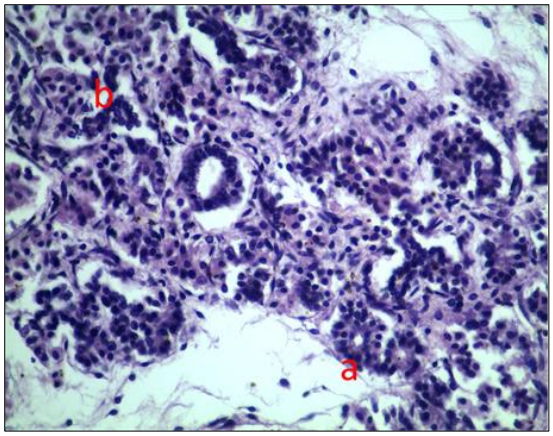

Fig 4: Histological section of 91 days old goat foetal pancreas showing developing acini (a) and tubular bud cells (b) H&E×400

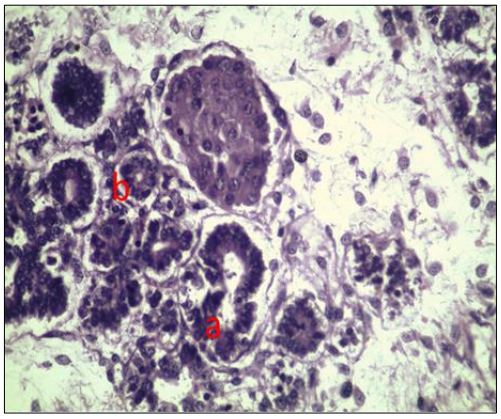

Fig 5: Histological section of 100 days old goat foetal pancreas showing developing acini with common lumen (a) and almost developed acini (b). H&E×400

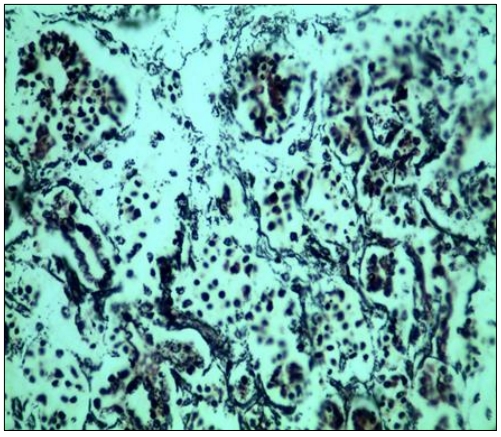

Fig 6: Histological section of 76 days old goat foetal pancreas showing thin reticular fibers around developing acini and tubules. Wilder`s reticular stain×400

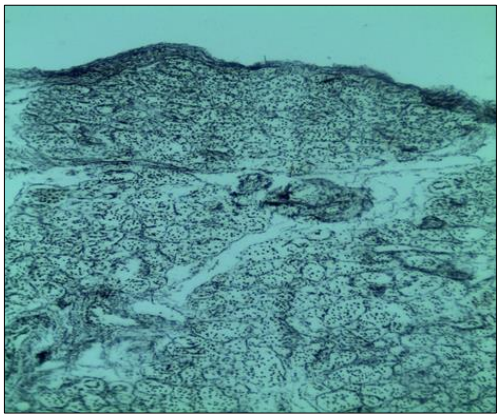

Fig 7: Histological section of 100 days old goat foetal pancreas showing coarse reticular fibers in capsule and around the acini. Wilder`s reticular stain×100

Late prenatal period

The histological appearance of the developing and developed acini in the pancreas of goat foetus of 101 to 112 days gestation was almost similar as it was found at 100 days stage. However, at 112 days some developing acini were lined by low columnar cells having nuclei either in the central or basal part of the cells.

In 115 days old goat foetus the occurrence of the developed acini was relatively more than the developing acini. At these stage the lining cells of several acini were either pyramidal or low columnar in shape whereas the several developing acini were lined by polyhedral and cuboidal cells. The cytoplasm of lining cells of developing acini was bright eosinophilic and granular. The nuclei of these cells were rounded and oval in shape and were bright basophilic. Some developed acini contained Centro acinar cells in central part of their lumen (Fig-8).

In 118 days onwards goat fetuses the occurrence of the developed acini was relatively more than the previous stages of late prenatal period. The lining cells of the developed acini had eosinophilic granular cytoplasm. Lumen of several acini was narrower and ill developed whereas several other acini had distinct lumen. The lining cells of the developed acini had eosinophilic granular cytoplasm. Lumen of several acini was narrower and ill developed whereas several other acini had distinct lumen (fig.9).

In late prenatal period, the reticular fibers gradually became coarser in around the acini with the increase of the foetal age from 103 to 132 days gestation. Distinct collagen fibers were not found up to 115 days of gestation. At 118 days gestation the of foetal goat pancreas few fine collagen fibers were present around the acini (Fig.10). Elastic fibres were not found in any stages of developing pancreas.

In late prenatal period the average diameter of acini along with the average diameter of its lumen, height of acinar cells and size of the nuclei was relatively less in late prenatal period than mid prenatal period. In late prenatal period the values of these parameters were 19.36±2.17 µm, 6.46±0.71 µm, 6.44±0.57 µm and 2.86±0.26 µm respectively**.**

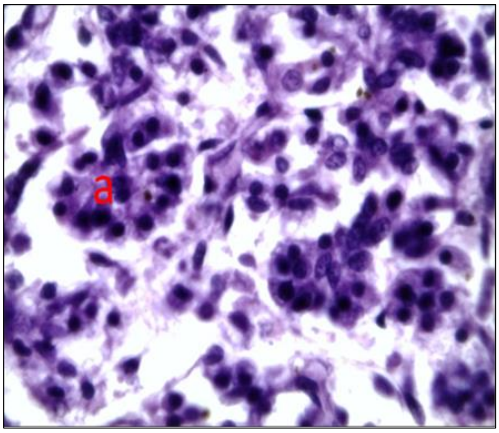

Fig 8: Histological section of 115 days old goat foetal pancreas showing developed acini with centroacinar cells in centre (b). H&E×1000

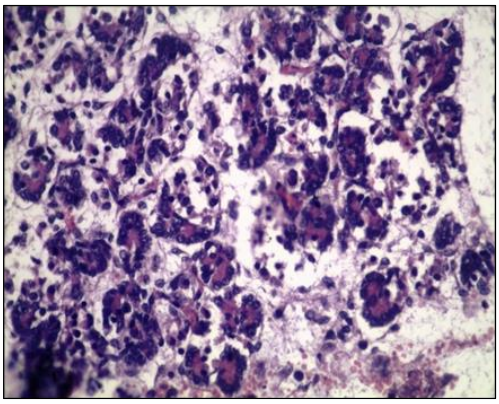

Fig 9: Histological section of 118 days old goat foetal pancreas showing the developed acini having bright basophilic nuclei and darker eosinophilic supranuclear cytoplasm. H&E×400

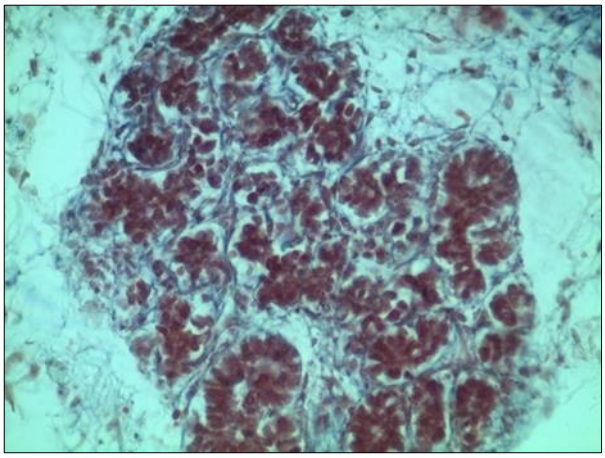

Fig 10: Histological section of 118 days old goat foetal pancreas showing thin collagen fibers around acini. Masson`s trichome stain×400

Discussion

The beginning of the process of acini formation in the foetal goat pancreas was first observed at 76 days of gestation where cells of several tubule buds were loosely arranged and peripheral cells of some buds began to arrange in the form of either a complete or a part of circle. At this stage many buds contained circularly disposed cells along with the cells arranged in the form of clusters. According to Lucini *et al.* (1998) [6] in buffalo and Arey (1954) [1], Liu and Potter (1962) [5] and Latio *et al.* (1974) [4] in the pancreas of man, the acini formation was observed in 3rd month of gestation. In the present study the acini were formed from the buds which were originated from the tubule. Vincent (1924) [12] and Arey (1954) [1] in man mentioned the formation of acini from tubules and primitive side buds respectively. Contrary to the finding of Vincent (1924) [12] in man the transition of acini into islets and vice versa was not found in the present study. This was in the agreements with the findings of Conklin (1962) [3], Clark and Grant (1983) [2] and Van Assche *et al.* (1984) [11] in man who stated that the tubular cells developed either into the islets or acini and both were developmentally and functionally independent of each other. In some developing acini of the pancreas of goat foetus particularly at 91 days gestation, the peripheral cells formed either in the complete circle or a part of it and the central part had either ill developed lumen or contained loosely arranged group of 2 to 4 cells. These findings may be correlated with the observation of Laitio *et al.* (1974) [4] who stated that in human pancreas at 12 to 14 week gestation the differentiated exocrine cells were rare and randomly distributed in the peripheral region of early outgrowth along with single cell in epithelial lining of tubules. In the present study the differentiation of buds into acini was relatively more pronounced from 91 to 100 days gestation where several developing acini had a continuous peripheral circular lining and the cells located in the centre developed empty spaces between them. At these stages in some developing acini a central large lumen was surrounded by a layer of spherical bud cells having spherical vesicular nuclei whereas, some other developing acini were lined by two layers of the cells. In some acini cells were spheroid or cuboidal in shape whereas the other cells were relatively larger and appeared as pyramidal in shape. At some places, particularly at 100 days of gestation, more than one developing acini were in continuation to each other having a common lumen. These findings may indicate the various stages in the process of transformation of buds in to acini. Liu and Potter (1962) [5] in human foetus reported that after appearing in third month gestation the acini matured rapidly. At 100 days gestation of goat foetal pancreas, some acini were completely lined by pyramidal cells having bright eosinophilic supra nuclear part and large spheroid or spherical bright basophilic vesicular nuclei. According to Conklin (1962) [3] in human foetal pancreas from 12.5 to 14.5 weeks of gestation the nuclei of the acinar cells were large, vesicular and contained prominent nuclei. In the present study showed the lumen of the developed acini was relatively narrower than developing acini. These findings showed that in goat foetal pancreas the differentiation of acini from buds gradually became more pronounced in the late stages of mid prenatal period and the developed acini began to appear at 100 days of gestation.

Latio *et al.* (1974) [4] found that the maturation of acini in human foetal pancreas took place up to 5th month of gestation. The present study indicated that in initial stages the process of differentiation of acini from buds the irregular empty spaces were developed between the bud cells and then the cells were arranged either in single or more than one peripheral layer around a central lumen. Subsequently the bud cells formed a single peripheral layer of cuboidal cells around a large lumen. In the last stage of differentiation of acini the cuboidal lining cells became elongated to attained columnar and pyramidal shape. Conklin (1962) [3] reported that in man at 12.5 to 14.5 weeks gestation the pancreatic acinar cells enlarged, became pyramidal in shape and grouped around a central lumen. The developed acini of mid prenatal period had an average diameter of 20.22±2.43 µm with the range of 15.35 to 23.18 µm and lumen having average diameter 6.98±0.52 µm with the range of 2.83 to 9.24 µm. The average height of the acinar cells was 6.62±0.42 µm with the range of 6.26 to 6.97 µm and nuclear size of 2.93±0.31 µm with the range of 2.71 to 3.17 µm.

Although among the stages of late prenatal period there was a gradual increase in the occurrence of developed acini in the exocrine part of foetal goat pancreas from 101 to full term foeti but the average diameter of acini along with the average diameter of its lumen, height of acinar cells and size of the nuclei was relatively less in late prenatal period than mid prenatal period.

The histological appearance of the developing and developed acini in the pancreas of goat foetus of 101 to 112 days gestation was almost similar as it was found at 100 days stage. However, at 112 days some developing acini were lined by low columnar cells having nuclei either in the central or basal part of the cells. In 115 days old goat foetus the occurrence of the developed acini was relatively more than the developing acini and the lining cells of several acini were pyramidal in shape. From this stage the cytoplasm of few acinar cells around the nucleus had a little bluish tinge. Conklin (1962) [3] in the foetal pancreas of man reported that during 14.5 to 17 weeks gestation the acinar epithelia demonstrated the greatest change and the entire cytoplasm of these cells became intensely basophilic but gradually the basophilia became most prominent in the basal areas of cell. However, in the present study the supra nuclear part of most acinar cells up to full term foetus had bright eosinophilic cytoplasm. At this stage the developing acini were generally lined either by low columnar or by polyhedral and cuboidal cells. The Centro acinar cells were first found in few acini at 115 days stage and at this stage interlobular ducts were lined by a single layer of

cuboidal cells and had wider lumen. Conklin (1962) [3] in human foetal pancreas reported the presence of Centro acinar cells at 12.5 to 14.5 week gestation and stated that in a few of the acini intercalated duct cells became partially encircled by acinar cells which gave rise to Centro acinar cells.

From 118 days gestation the exocrine part of the pancreas in goat foetus was highly developed and had numerous developed and developing acini along with the intra lobular and inter lobular ducts. Moreover, at this stage the occurrence of the developed acini was relatively more than the previous stages of late prenatal period. These findings may be correlated with the observation of Lucini *et al*. (1998) [6] in buffalo who observed that during the late foetal and postnatal period the exocrine portion was noticeably increased in mass. Moreover, Singh and Sethi (2012) [8] also reported that maximum growth of exocrine pancreas in buffalo foetus in late gestation. At this stage several pancreatic acini in goat foetus had a well-developed lumen but in many other acini the lumen was relatively ill developed. The central part of some acini contained Centro acinar cells. The lining cells of the acini had eosinophilic granular cytoplasm. Conklin (1962) [3] found zymogen granules in most of the acini of human foetal pancreas of 17 to 22-week stage. Moreover, according to Latio *et al*. (1974) [4] in human foetal pancreas scattered zymogen granules could be observed in some acini by the end of 4th month but significant number of granules were not found until the 5th foetal month.

From 132 to full term foetus the pancreas was chiefly formed by developed acini, however at few places some immature acini were also found. The lumen of many acini showed the presence of Centro acinar cells. The histilogical details of the pancreas in goat foeti at late stages almost resembled with the adult domestic animals. However, according to Conklin (1962) [3] and Latio *et al*. (1974) [4] reported that in human foetal pancreas the acinar cells arrangement resembled to the adult pancreas at 12.5 to 14.5 weeks and in 14 to 16 weeks of gestation, respectively.

Conclusion

In present investigation it seems that, the acini formation in pancreas of prenatal goat was first observed at 76 days of gestation, where cells of several buds became loosely arranged and their peripheral cells began to arrange in the form of either a complete or a part of circle to form a central lumen. From 115 days onwards the cytoplasm of few acinar cells around the nucleus had a little bluish tinge. From 132 to full term foetus the pancreas was chiefly formed by developed acini. Therefore, it considered as from 132 days onwards the exocrine part of pancreas may be ready to work.

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