



Differentiation of pancreatic endocrine islets in buffalo fetus

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ABSTRACT

Pancreas of 24 buffalo foetii collected from abattoir and veterinary clinics, GADVASU, Ludhiana were studied. The buffalo foetii were divided into three groups after measuring their CVRL, viz. Group I (CVRL between 0–20 cm), Group II (CVRL above 20 cm and up to 40 cm) and Group III (CVRL above 40 cm) and their approximate age was calculated. The tissues were processed and paraffin sections were cut and stained with different histological stains. In Group I, at 7.5 cm CVRL (62 days), the endocrine cells were scattered either in between the developing acinar cells and primitive tubules or were localized within primitive tubules. The alpha cells were large, round or oval in shape having eosinophilic cytoplasm and large nucleus whereas beta cells were small basophilic cells. At 12.8 cm CVRL (86 days), small groups of cells were present containing purely alpha cells or purely beta cells. Well developed islets of Langerhans with ill-defined capsule were observed at 19 cm CVRL (114 days). At 10.7 cm CVRL (77 days), certain cells which were morphologically different from cells of islets of Langerhans and acinar cells started to assemble and many capillaries were invading these cells. At 12.8 cm CVRL (86 days), these assembled cells formed well developed islets called perilobular islets. These large sized islets were developing at the periphery of lobules. The perilobular islets underwent regressive changes whereas islet of Langerhans increased in number simultaneously.

Keywords: Buffalo, Development, Differentiation, Endocrine pancreas, Foetus

Development of pancreas requires a trophic stimulus, proliferation and differentiation of the mesenchymal tissue (Mc Geady *et al.* 2006). Now-a-days, the biology of the pancreas has been studied intensely, largely driven by the hope of finding better treatments for devastating pancreatic diseases, such as diabetes mellitus, pancreatitis and pancreatic adenocarcinoma. In particular, advancements in stem cell technology have sparked optimism that diabetes could be cured by harvesting stem cells for therapeutic use. This has led to heightened interest in understanding embryonic development of the pancreas, specifically the events involved in cell fate decisions and endocrine cell differentiation (Shih *et al.* 2013). Mammals, birds, reptiles and amphibians have a pancreas with similar histology and mode of development. Recognition of normal development of the pancreas in mammals and other species can help to understand congenital anomalies in humans (Slack 1995). Prenatal development of pancreas had been reported in bovine (Bonner-Weir and Like 1980, Merkwitz *et al.* 2013), rat (Kaung 1994, Inagaki *et al.* 2012), pig (Carlsson *et al.* 2010), sheep (Reddy *et al.* 1988), rabbit (Titlbach and Manakova 2007), but very scanty literature is available in buffaloes (Lucini *et al.* 1999). Keeping in view the paucity in literature, the present research work was planned.

MATERIALS AND METHODS

The present study was conducted on pancreas of 24

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buffalo foetii of different gestational age collected from abattoir and postmortem hall of GADVASU. The CVRL of the foetii was measured with the help of inelastic thread as a curved line along the vertebral column between the most anterior part of frontal bone to the rump at ischiatic tuberosity and approximate age of the foetii was estimated by using the formula given by Soliman (1975) in buffalo. Based on the CVR length, the samples were divided into 3 groups; viz. Group 1 (CVR length between 0–20 cm), Group 2 (CVR length, above 20 cm and up to 40 cm) and Group 3 (CVR length above 40 cm).

The pancreas were dissected out of abdominal cavity and tissues were fixed in 10% Neutral buffered formalin and Bouin's fixative immediately after collection. After the fixation, the tissues were processed for paraffin blocks preparation by acetone benzene schedule (Luna 1968). The blocks were prepared and the sections of 5 - 6 µm were cut with rotary microtome. These paraffin sections were stained with Hematoxylin and Eosin, Masson's trichrome, Gomori's method and Aldehyde Fuchsin.

For electron microscopy, fresh tissues were washed and fixed in Karnovsky's fixative. The secondary fixation was done for 2 h in 2% Osmium tetroxide. The tissues were dehydrated, cleared, infiltrated, embedded and polymerized. The ultrathin sections (70–90 nm) were cut and stained with uranyl acetate (15 min) followed by lead citrate (10 min) (Bozolla and Russell 1999). Finally, the grids with sections were examined under Transmission Electron Microscope

for ultrastructural details of pancreatic cells and required photographs were taken.

RESULTS AND DISCUSSION

During foetal life, two distinct types of islets were developing - Islets of Langerhans and perilobular giant islets.

Islets of Langerhans: In Group 1, at 7.5 cm CVRL (62 days), the endocrine cells were scattered either in between the developing acinar cells and primitive tubules or were localized within primitive tubules (Fig. 1A). So it was believed that these cells originated from these primitive tubular/ductular cells along with the acinar cells. The common origin of exocrine and endocrine cells from the primitive tubular cells. However, Bencosme (1955) found that mature islets developed from differentiated acini and not from the tubules. The formation of endocrine cells from the primitive tubule has been described as stage of budding islets by Robb (1960) in human foetal pancreas. At this

stage, the alpha cells were more in number as compared to beta cells. Earlier Park and Bendayan (1993) observed that the glucagon producing alpha cells started to appear in the endocrine pancreas on the 12th day of pregnancy and the beta cells appeared on the 14 day in foetal rat pancreas. Reddy and Elliott (1985) reported that in mammals for most of gestation, the glucagon containing alpha cells predominate and were the first cells to be identified clearly. Reddy *et al.* (1988) reported the presence of alpha cells at 40–45 days and beta cells at 80–100 days of gestation in foetal sheep pancreas. These alpha cells were large, round or oval in shape having eosinophilic cytoplasm and large nucleus whereas beta cells were small basophilic cells. Our observations agrees with the previous observations of Bonner-weir and Like (1980) in bovine pancreas who reported the presence of insulin and glucagon immunopositive cells and Lucini *et al.* (1998) in buffalo foetal pancreas who reported the presence of alpha cells and beta cells at 60 days of gestation. Whereas Carlsson *et*

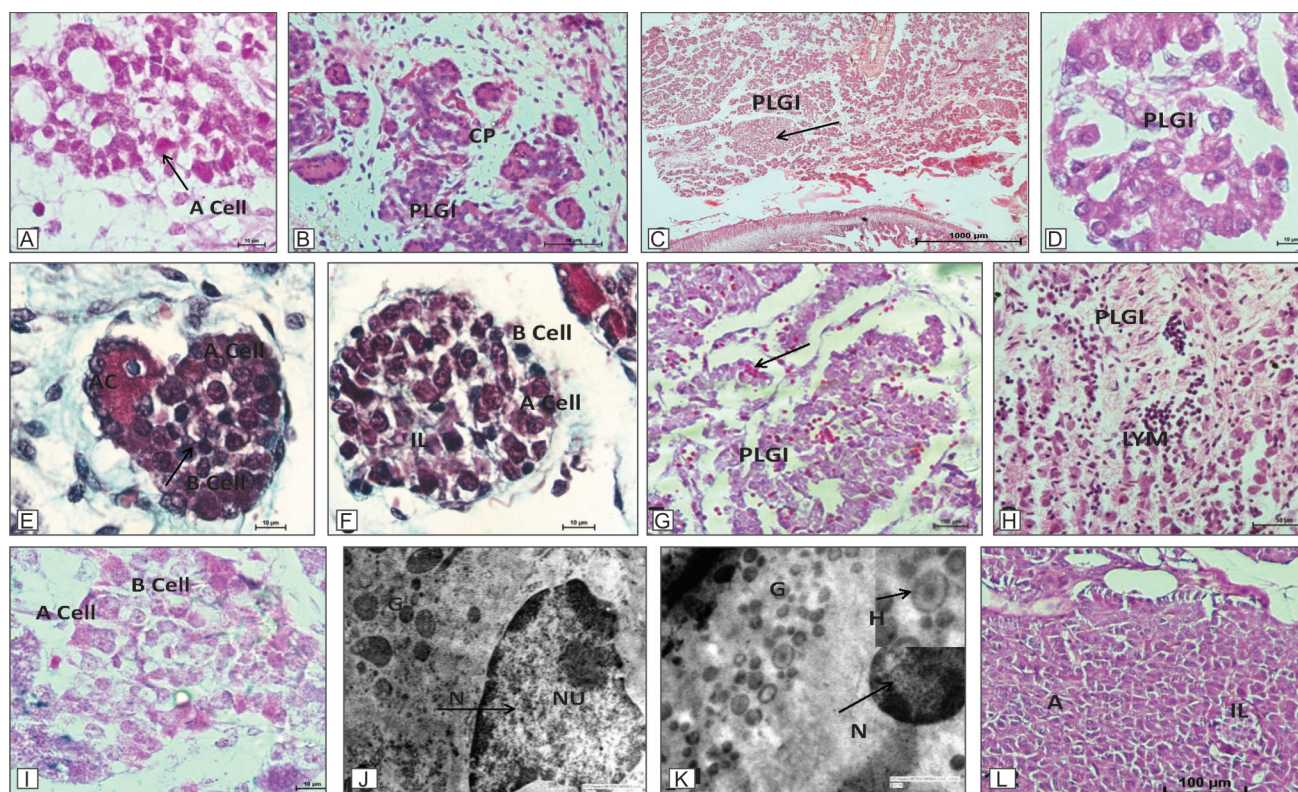


Fig. 1. Photomicrographs of paraffin sections of foetal pancreas at— A. 7.5 cm CVRL (62 days) showing alpha cells (A cell). Gomori's method $\times 1000$; B. 10.7 cm CVRL (77 days) showing formation of perilobular giant islets (PLGI) with invasion of capillaries (CP). Hematoxylin and Eosin $\times 400$; C. 12.8 cm CVRL (86 days) showing formation of lobes and lobules and fully formed perilobular giant islets (PLGI). Hematoxylin and Eosin $\times 20$; D. 19 cm CVRL (114 days) showing perilobular giant islet (PLGI). Hematoxylin and Eosin $\times 1000$; E. 19 cm CVRL (114 days) showing close association of acinar cells (AC), alpha cells (A cell) and beta cells (B cell) indicative of common origin from progenitor cells. Masson's Trichome $\times 1000$; F. 19 cm CVRL (114 days) showing intralobular islet or islet of langerhans (IL) containing alpha cells (A cell) and beta cells (B cell) with ill-defined connective tissue capsule. Masson's Trichome $\times 1000$; G. 20 cm CVRL (119 days) showing perilobular giant islet cells (PLGI) and extravasation of RBCs (arrow). Gomori's method $\times 400$; H. 22.6 cm CVRL (125 days) showing presence of lymphocytes (LYM) in regressing perilobular giant islet cells (PLGI). Hematoxylin and Eosin $\times 400$; I. 32 cm CVRL (146 days) showing less number of alpha cells (A cell) and more number of beta cells (B cell). Gomori's method $\times 100$; J. Electron micrograph at 32 cm CVRL (146 days) showing irregularly shaped nucleus (N), granules (G) and a prominent nucleolus (NU) in alpha cell. $\times 1700$; K. Electron micrograph at 32 cm CVRL (146 days) showing round nucleus (N), granules (G) having the halo (H) in beta cell. $\times 1700$; and L. 100.5 cm CVRL (300 days) showing islet of Langerhans (IL) and acini (A). Hematoxylin and Eosin $\times 100$.

al. (2010) observed the alpha cells at 26th dpc and insulin stained cells at 27 dpc in bovine fetal pancreas and in pig foetal pancreas, they detected both alpha and beta cells at 19 dpc. The appearance of these endocrine cells in different species at different time occurs due to difference in length of gestation period (Reddy *et al.* 1988). Ultrastructurally, the alpha cells showed large number of empty vesicles and irregular shaped nucleus. Empty vesicles at this stage indicate that the cells were degranulated. Although the endocrine cells were scattered in the mesenchyme yet no definite islets were formed at this stage and even at 9.6 cm CVRL (72 days) though the cells started to assemble. At 12.8 cm CVRL (86 days), it was observed that small groups of cells were present containing purely alpha cells or purely beta cells (Fig. 1C). Well-developed islets of Langerhans with ill-defined capsule were observed at 19 cm CVRL (114 days) (Fig. 1E). These islets were having more number of alpha cells than beta cells (Fig. 1F). These islets of Langerhans has been named as secondary islets by Liu and Potter (1962) in human foetal pancreas, small islets by Bonner-weir and Like (1980) and intralobular islets by Merkwitz *et al.* (2013) in bovine foetal pancreas. Many capillaries started to invade the endocrine cells of these islets. This stage was described as Centre capillary clusters at 10–16 weeks of gestation by Robb (1960) in human fetal pancreas. Distribution of alpha and beta cells in these islets was variable. Lucini *et al.* (1998) reported that this variability in the organization of islet endocrine cells may reflect differences in the interactions between the cells of the islets. Zabel *et al.* (1995) observed in pig fetal pancreas that all four endocrine cell types could be detected at day 20 of gestation.

In Group 2, at 28.3 cm CVRL (137 days), capillaries were very prominent in these islets along with nerves and ganglia. In some islets, alpha cells were grouped at one side and beta cells were at the other side. This stage has been described as stage of bipolar islets by Robb (1960) in human foetus at 16 week of age. These islets were still budding off from primitive tubules. At 32 cm CVRL (146 days), the alpha and beta cells had increased in number as well as in size (Fig. 1I). Kaung (1994) stated that the accelerated growth of these islet cell populations was accomplished by a high cell proliferative activity at 20–22 days of gestation and a large influx of undifferentiated epithelial cells into the specific islet cell population during this period. Ultrastructurally, the granules of alpha and beta cells were well established and densely arranged within the cytoplasm at this stage. The alpha cells were relatively more in number than the beta cells at this stage also. Granules of alpha cells were electron dense and did not have any space between core and limiting membrane (Fig. 1J) while granules of beta cells had space between a dense core and limiting membrane known as halo (Fig. 1K) as reported by Bonner-weir and Like (1980) in cattle fetal pancreas. Long slender mitochondria and scanty rER cisternae were interspersed among numerous beta granules. Nucleus of alpha cell was irregular in shape while that of beta cell was

round in shape. The granules were of variable size and density as earlier reported by Titlbach and Manakova (2007) in rabbit foetal pancreas. The shape of cell granules varied from round, spherical or tear drop as reported earlier in bovine fetal pancreas by Bonner-weir and Like (1980). Fowden (1995) reported that foetal alpha and beta cells have important roles in foetal growth and development. Insulin secretion from the beta cells is essential for glucose uptake by insulin sensitive tissues of the foetus. Ablation of the foetal beta cells leads to hyperglycemia, reduced glucose utilization and ultimately, growth retardation of the foetus. The fetal beta cells therefore acts as sensors of nutrient sufficiency and via insulin secretion, match the rates of glucose utilization and growth by the foetus to its rate of glucose supply. Whereas, foetal alpha cells appeared to act as sensors of nutrient insufficiency particularly of oxygen. Glucagon raises foetal glucose levels has effects on the fetal cardiovascular system at high concentrations. These changes may help to maintain a glucose supply to essential fetal tissues such as brain during adverse intra-uterine conditions. The nerves and ganglia in fetal pancreas during gestation are involved in regulating islet cell response to stressful stimuli (Fowden and Hill 2001).

In Group 3, at 48 cm CVRL (182 days), in some islets, alpha cells were present in the core and beta cells were present at periphery while some islets were of mixed type and in some islets, beta cells formed the core of islet while alpha cells were present at periphery. This stage has been described earlier by Robb (1960) as stage of mantle islets in human fetal pancreas at 20 weeks of age. The relative number of beta cells was more as compared to other groups. With progression of foetal age, these islets were scattered in the parenchyma of pancreas and more number of islets were formed at 70 cm CVRL (232 days) and later on at 100.5 cm CVRL, these islets contained more number of beta cells than alpha cells (Fig. 1L). Lucini *et al.* (1998) reported that beta cell comprised the highest percentage at 8 month old buffalo fetuses. Carlsson *et al.* (2010) reported that around 155 dpc, insulin stained cells were markedly more abundant than glucagon stained cells in bovine foetal pancreas and at 60 dpc in pig foetal pancreas.

Micrometrical study revealed that these islets were having diameters ranging from 48.72 μ m to 73.43 μ m in Group 1 with mean diameter of $58 \pm 3.45 \mu$ m; 72.91 μ m to 106.22 μ m in Group 2 with mean of $89.6 \pm 11.81 \mu$ m and 76.42 μ m to 150.32 μ m in Group 3 with mean of $82.50 \pm 7.57 \mu$ m. While Merkwitz *et al.* (2013) reported the intralobular islets during foetal life of bovine with diameter not exceeding above 100 μ m even in older foetuses. The alpha cell were having mean diameter of $10.78 \pm 0.41 \mu$ m in Group 1, $11.21 \pm 0.48 \mu$ m in Group 2 and $11.80 \pm 0.74 \mu$ m in Group 3. The beta cell were having mean diameter of $8.96 \pm 0.25 \mu$ m in Group 1, $9.31 \pm 0.41 \mu$ m in Group 2 and $9.51 \pm 0.29 \mu$ m in Group 3. Earlier, Prashar (1995) also reported the mean diameter of alpha and beta cells as $10.80 \pm 0.18 \mu$ m and $8.93 \pm 0.32 \mu$ m respectively in neonatal buffalo pancreas. Statistically, the diameter of alpha and beta cells did not differ significantly

between Group 1, Group 2 and Group 3 ($P < 0.05$). Also the diameters of islets of Langerhans differed significantly between three groups ($P < 0.05$).

Perilobular giant islets: At 10.7 cm CVRL (77 days), it was observed that certain cells which were morphologically different from cells of islets of Langerhans and acinar cells started to assemble and many capillaries were invading these cells (Fig. 1B). Further at 12.8 cm CVRL (86 days), these assembled cells formed well developed islets. These large sized islets were developing at the periphery of lobules and thus had been named as perilobular giant islet by Merkwitz *et al.* (2013) in bovine fetal pancreas at 11 cm CRL. They reported that these perilobular giant islets most probably originated from endodermal epithelium and at very ends of primitive ductulo acinar complexes contiguous with connective tissue and fibrous septae at later stages. These islets were very large in size and composed of single or double rowed anastomosing polymorphic cell bands which were of rosette like or gyriform appearance. At 19 cm CVRL (114 days), these cells were either cylindrical or cuboidal in shape and their cytoplasm was eosinophilic and their nuclei were predominately centrally or apically located (Fig. 1D). Earlier similar type of islets had also been reported in bovine foetal pancreas and were named as large islets by Bonner-weir and Like (1980) at 9th foetal week and primary islets by Liu and Potter (1962) at 8th week in human foetal pancreas. These special type of cells were also observed by Laguesse in 1895 (Titlbach and Manakova 2007) who called these cells as cellulles troubles and named these as Islets of Laguesse in foetal sheep pancreas. At 20 cm CVRL (119 days), perilobular giant islets were more developed and most of the cells revealed to be like beta cells. The spaces between the cell bands of the islet were infiltrated by delicate capillary network (Fig. 1G).

In Group II, at 22.6 cm CVRL (125 days), some of these perilobular islets showed regressive changes in them which included shrunken cellular bands, broadened interepithelial spaces, lymphocytic infiltration and small amount of collagen fibres (Fig. 1H). Such types of regressive changes had been reported in lambs by Titlbach *et al.* (1985) and in bovines by Merkwitz *et al.* (2013). Liu and Potter (1962) and Homo-Delarche and Drexhage (2004) have described the lymphocyte infiltration concomitant with islet degeneration in human foetal pancreas during last three months of intrauterine life. Jansen *et al.* (1993) showed that foetal and neonatal human pancreas harbour large focal leukocyte infiltrates consisting of mainly T- cells which were also present in the connective tissue septa and in the capsule. At 28.3 cm CVRL (137 days), large number of ganglia and nerve fibers were observed in close proximity of these perilobular giant islets. The cells of perilobular giant islets were much more developed than the earlier ages. At 48 cm CVRL (182 days), perilobular giant islets were of variable sizes. At 54 cm CVRL (195 days), some of the perilobular giant islets were undergoing regression and still other were developing and were innervated by nerve-fibers and ganglia. Such type of association has

been described as neuro-insular complex by Merkwitz *et al.* (2013) in bovine fetal pancreas. The regression of these islets was even evident at 70 cm CVRL (232 days). At 100.5 cm CVRL (300 days), very few perilobular giant islets were observed. Involution of the perilobular giant islets seems to be a physiological rather than a pathological process and may be necessary to prevent neonatal hyper insulin anemic hypoglycaemia (Titlbach *et al.* 1985). Bonner-Weir and Like (1980) reported that the early appearance of perilobular giant islets in the pancreas of bovine foetus and disappearance of these islets at around 1st year suggests that these islets are physiologically important only in foetus and neonates. Since the present study was on the pancreas of buffalo during foetal life, so it can't be concluded whether the perilobular giant islets persists after birth or not.

Micrometrical observations on perilobular giant islets revealed the diameter ranging from 134.17 μ m to 436.62 μ m in Group I, 186.33 μ m to 500.24 μ m in Group II and 213.13 μ m to 1133.46 μ m in Group III. The values observed in present study corroborates well with the earlier values reported by Merkwitz *et al.* (2013) who found the perilobular giant islets with diameter ranging from a few 100 μ m in younger foetuses and to over 1 mm in older foetuses.

It seems implicit that distinct mechanisms and regulatory factors must be involved in the development of two populations of islets from one and the same tubulo-acinar tree in cattle and other ruminants (Bonner-Weir and Like 1980, Lucini *et al.* 1998). The perilobular giant islets differentiated earlier than the Islets of langerhans. But they were few, larger in size, located at periphery of lobules and were in close proximity to ganglia and nerves. Whereas Islets of Langerhans were more in number, smaller in size, embedded within the exocrine tissue and contained alpha and beta cells. Bonner-Weir and Like (1980) reported that the prominence of small islets in the adult suggest that they are physiologically significant throughout the life of cow and probably represented the adult endocrine pancreas while the prominence and growth of the large islets during foetal life and their apparent subsequent cessation of growth after birth suggest that they are physiologically important in foetus and neonate but may be a development vestige in the functioning adult ruminant. Presence of two types of islets permit perinatal changes in digestion/carbohydrate metabolism in ruminants (Bonner-Weir and Like 1980, Titlbach *et al.* 1985). The relationship of the two populations of islets to the exocrine tissue and in addition, to neuronal structures of the autonomic and sensory nervous system (Myojin *et al.* 2000) suggest the presence of effective insulo-acinar communication of the intra-lobular small islets and intense insulo-neuronal communication of the perilobular giant islets.

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