Histomorphometrical study of pancreas in Mehraban female sheep

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Summary

In the present study, pancreas glands in Mehraban breed of female sheep were histomorphometrically studied in four different age groups included: fetus, newborn, sexually mature and old ages. The results of histomorphometrical analysis with regard to frequency and percentage of parenchymal and stromal structures such as, exocrine secretory units, secretory ducts, blood vessels, islets of Langerhans and stromal connective tissue showed that minimum and maximum percentages of structures within the whole gland belonged in fetal stage, to blood vessels and stromal connective tissue; newborn stage, to blood vessels and exocrine secretory units; in sexually mature, to islets of Langerhans and exocrine secretory units and in old stage, also to islets of Langerhans and exocrine secretory units, respectively. The diameter changes of islets of Langerhans in different age groups from minimum to maximum were included: fetus, old, sexually mature and newborn stages. In comparison between different age groups, the frequency of exocrine secretory units showed a significant increase from fetal stage to newborn, sexually mature and old stages ($P<0.05$). On the other hand, there was a significant decrease in frequency of secretory ducts from fetal stage to newborn, sexually mature and old stages and there was also a significant decrease in frequency of blood vessels from fetal to newborn stage ($P<0.05$). Significant decrease was also observed in frequency of islets of Langerhans from fetal and newborn stages to sexually mature and old stages. In comparison between groups, there was a significant increase in diameter of islets of Langerhans from fetal to newborn and sexually mature stages and decrease from newborn to sexually mature and old stages. Our study revealed that, there was no significant difference in parenchymal and stromal structures and diameter of islets of Langerhans between different parts of the gland in each age group. However, the above mentioned parameters often showed significant differences between different age groups. These differences were more prominent between fetal stage compared to the other age groups. Therefore, it can be concluded that structural changes of parenchyma and stroma of pancreas gland begin from pre-natal period and will proportionally continue during the period of post-natal development.

Key words: Histomorphometry, Pancreas, Sheep, Mehraban

Introduction

Pancreas is a vital digestive gland which it's exocrine secretions include essential enzymes for digestion along with many electrolytes. It's hormonal secretions are produced by pale staining cell clusters of islets of Langerhans which have various activities such as control of blood sugar concentration (Bloom and Fawcett, 1994). Various histological and morphometrical studies were undertaken on parenchymal structures of pancreas in both human and different domestic animals at both light and electron microscopic level. In human, these studies were consisted of pancreatic development in fetal stage (Conklin, 1962) and cellular diagnosis of islets (Gomori, 1939). Studies of cell populations in fetus and neonatal endocrine tissue and volume density of islets were also reported in human by Malaisse-Lagae *et al*., (1979) and Rahier *et al*., (1981). Extensive studies were done on rat as a laboratory model (Fujii, 1979; Uchiyama and Watanabe, 1984; Ermak and Rothman, 1986; Reaven *et al*., 1987). Cytologic and morphometric studies of pancreas were also reported in post-natal

guinea pig (Assis *et al*., 2003) and rabbit (Thomas, 1937). Pancreatic endocrine portion was also studied in cattle by Galabova and Petkov (1975), in horse by Furuoka *et al*., (1989), in dog by Watanabe *et al*., (1989) and several other mammalian species including cat, buffalo, deer and monkey by Thomas (1937). Mean volume density, mean surface area and average numbers of islets of Lagerhans per unit area were also studied in camel (Tadjalli and Meamary, 1998) and in dog (Redecker *et al*., 1991). Some morphological observations on both pancreatic exocrine and endocrine parenchymal tissues were also reported in chicken and goose using ligth microscope (Nagasao *et al*., 2003; Nurhayat, 2003). In sheep some scattered morphological studies of pancreas are available (Avila and Robinson, 1986; Mukherjes *et al*., 1986 and Mukherjes *et al*., 1988). Recently, histomorphometrical study on both pancreatic exocrine and endocrine tissues in different age groups of Mehraban male sheep was reported by Mansouri *et al*., (2005). However, no histomorphometric evaluation of pancreatic tissues was seen in female sheep. Therefore, present study was undertaken.

Materials and Methods

In this study, 12 clinically healthy Iranian Mehraban female sheep were selected in 4 age groups. Fetuses (2.5-3 months) were selected on the basis of crown-rump length. Newborn (1-week-old), sexually mature (6-7 months) and old (more than 3-year-old) were selected on the basis of dental formula. Pancreas glands were removed and washed by normal saline. Glands were cleaned from additional tissues and each was divided into three anatomical parts, body, right and left lobes. These parts were initially primary fixed in 10% buffered formalin. After 24 hrs, each part was trimmed and cut into slices of 1 cm thickness and further fixed in Bouin's solution. Subsequently, serial sections were prepared in 5 micron thickness and were stained with H&E, Gomori's aldehyde fuchsin and Gomori's method for pancreatic islet cells (Luna, 1968). Histomorphometric studies on the serial sections were carried out using light microscope. In the present study, the volume density of stromal and parenchymal structures were determined by using the lattice line graticule on 40 sections from the left lobe, 40 sections from the right lobe and 20 sections from the body of each gland. The tissue selection method of these sections from pancreatic part allowed us to study the whole regions of pancreas. Using ocular micrometry standard method, the diameters of islets of Langerhans were also determined and data were finally analysed by one-way ANOVA, using SPSS software. Duncan's multiple range test was also used to detect significant differences.

Results

The histomorphometric results of stromal and parenchymal structures of pancreas are shown in Table 1 to 3. According to Table 1, the volume density of different structures was varied in different age groups and also between different parts of gland. In fetus, the minimum percentage of structures in all parts of the gland was belonged to the blood vessels and maximum to the stromal connective tissue. Comparison of the percentages of volume densities of different stromal and parenchymal structures in different parts of gland revealed only significant decrease in frequency of islets of Langerhans from right to left lobe. In newborn, the minimum and maximum percentages of structures were found in all parts of the gland, in volume density of blood vessels and exocrine secretory units, respectively. In this age, significant increase in volume density of islets of Langerhans was only noticed from right to left lobe and body of the pancreas. Comparison of the glandular structures in sexually mature animals showed that the highest and lowest percentages of structures in right and left lobes were belonged to the islets of Langerhans and exocrine secretory units, respectively while in glandular body were found to be secretory ducts and exocrine secretory units. No significant difference was seen between the volume densities of structures.

In old animals, the lowest and highest

Table 1: Frequency and percentage of volume density of parenchymal and stromal structures in different parts of pancreas in different age groups. Mean ± SE

Groups	Parts	Exocrine secretory units	Secretory ducts	Blood vessels	Islets of Langerhans	Connective tissue	Empty space
	Right	11.71±2.18(%34)^{abc}	3.48±0.38(%11.77)^{abc}	1.83±0.47(%6.19)	2.7±0.27(%9.13)^{abci}	14.94±0.58(%43)^{abc}	0.02±0.001(%0.02)
Fetus	Body	12.39±0.51(%38.77)^{def}	2.89±0.39(%9.04)	1.66±0.33(%5.19)	2.19±0.18(%6.85)	12.82±0.59(%40.12)^{def}	0.02±0.003(%0.03)
	Left	11.58±0.49(%36)^{ghi}	4.46±0.9(%13.86)^{def}	1.33±0.57(%4.13)^{abc}	1.66±0.12(%5.16)^{di}	13.13±0.50(%40.82)^{ghi}	0.023±0.003(%0.03)
	Right	19.32±0.34(%68.82)^{a}	1.79±0.22(%6.12)^{a}	1.00±0.00(%3.56)	1.89±0.11(%6.73)^{ajk}	4.14±0.38(%14.74)^{a}	0.02±0.005(%0.03)
Newborn	Body	19.19±0.4(%62.85)^{d}	3.22±0.1(%10.54)	0.87±0.12(%2.84)	2.55±0.19(%8.35)^{j}	4.70±0.64(%15.39)^{e}	0.023±0.003(%0.03)
	Left	18.94±0.34(%65.31)^{g}	2.57±0.28(%8.86)^{d}	0.66±0.33(%2.27)^{a}	2.52±0.14(%8.68)^{defk}	4.31±0.24(%14.86)^{g}	0.016±0.003(%0.02)
	Right	18.85±0.22(63.46)^{b}	2.30±0.22(%7.74)^{b}	1.66±0.18(%5.58)	1.60±0.07(%5.38)^{b}	5.29±0.66(%17.81)^{b}	0.023±0.003(%0.03)
Sexually mature	Body	18.62±0.33(%61.02)^{e}	1.46±0.14(%4.78)	2.54±1.00(%8.32)	1.92±0.12(%6.29)	5.97±1.00(%19.56)^{e}	0.026±0.003(%0.03)
	Left	19.02±0.27(%66.8)^{h}	1.84±0.14(%6.46)^{e}	1.63±0.32(%5.72)^{b}	1.60±0.07(%5.61)^{e}	4.38±0.27(%15.38)^{h}	0.023±0.003(%0.03)
	Right	19.94±0.25(%68.05)^{cm}	1.47±0.16(%5.01)^{c}	2.25±0.69(%7.67)	1.52±0.12(%5.11)^{cn}	4.14±0.26(%14.12)^{cmn}	0.026±0.008(%0.04)
Old	Body	17.16±0.39(%57.28)^{fmn}	2.41±0.98(%8.04)	2.25±0.52(%7.51)	2.10±0.22(%7.01)^{no}	6.03±0.38(%20.14)^{fm}	0.016±0.003(%0.02)
	Left	20.13±0.93(%64.29)^{in}	2.1±0.31(%6.7)^{f}	2.37±0.48(%7.56)^{c}	1.5±0.11(%4.79)^{fo}	5.21±0.32(%16.64)^{in}	0.01±0.003(%0.02)

Similar superscripts in vertical columns show significant differences (P<0.05)

Table 2: Comparison between frequency and percentages of volume densities of parenchymal and stromal structures of pancreas in different age groups. Mean ± SE

Groups	Exocrine secretory units	Secretory ducts	Blood vessels	Islets of Langerhans	Connective tissue
Fetus	11.84±0.63(%35.61)^{abc}	3.72±0.37(%11.19)^{def}	1.66±0.89(%4.99)^{g}	2.26±0.13(%6.79)^{hi}	13.76±0.33(%41.39)^{lmn}
Newborn	19.15±0.21(%65.85)^{a}	2.43±0.29(%8.45)^{d}	0.88±0.07(%3.02)^{g}	2.29±0.08(%7.87)^{jk}	4.33±0.21(%14.86)^{l}
Sexually mature	18.85±0.15(%63.16)^{b}	1.99±0.18(%6.76)^{e}	1.86±0.27(%6.28)	1.74±0.06(%5.87)^{ik}	5.17±0.39(%17.46)^{m}
Old	19.37±0.36(%64.07)^{c}	1.90±0.21(%6.28)^{f}	2.29±0.37(%7.57)	1.69±0.09(%5.59)^{hj}	4.98±0.18(%16.47)^{n}

Similar superscripts show significant differences (P<0.05)

Table 3: Comparison between diameters (µm) of islets of Langerhans in different parts of pancreas in different age groups. Mean ± SE

Groups	Right	Body	Left	Total
Fetus	43.95±2.85^{ab}	45.68±3.09	40.96±5.04^{ef}	43.59±2.09^{gh}
Newborn	53.19±2.00^{ac}	51.27±2.64	53.53±2.64^{e}	52.75±1.35^{gi}
Sexually mature	52.15±1.97^{bd}	50.66±2.66	51.27±1.75^{f}	51.56±1.20^{hj}
Old	47.02±2.81^{cd}	43.77±2.87	46.91±3.6	46.20±1.81^{ij}

Similar superscripts show significant differences (P<0.05)

percentages of structures in right lobe were seen in secretory ducts and exocrine secretory units, respectively whereas in left lobe and body were noticed in islets of Langerhans and exocrine secretory units. Comparison of the percentages of glandular structures revealed significant increase in the exocrine secretory unites from body to left and right lobes, decrease in islets of Langerhans from body to left and right lobes and increase in stromal connective tissue from right to left lobe and body.

Comparison of the percentages of volume densities of different stromal and parenchymal structures of pancreas between all age groups showed that there was significant increase in volume density of exocrine secretory units in all parts of the gland from fetus towards old animals, decrease in secretory ducts in right and left lobes of fetus compared to the same lobes of other ages. Significant decrease in blood vessels between fetus and newborn and increase between fetus and sexually mature and old animals were observed in left lobe. Significant decrease was noticed in volume densities of islets of Langerhans in right lobe between the fetus and other age groups and in left lobe between newborn and other age groups. Significant decrease was also noticed in volume densities of stromal connective tissue in all parts of the gland between fetus and other ages.

According to Table 2, within the whole gland the highest and lowest percentages of glandular structures were belonged in fetus to blood vessel and stromal connective tissue, in newborn to blood vessel and exocrine secretory units and in sexually mature and old age to islets of Langerhans and exocrine secretory units, respectively. Comparison of structures between all age groups revealed significant increase in frequency of exocrine secretory units and decrease in secretory ducts, respectively in fetus compare to the other age groups. Significant decrease in frequency of blood vessels was only noticed between fetus and newborn. Significant decrease was also seen in frequency of islets of Langerhans between fetus, sexually mature and old ages and again between newborn, sexually mature and old animals. As shown in Table 3, in both right and left lobes of pancreas the diameters of islets of Langerhans were significantly increased from fetal period towards newborn and sexually mature but only in right lobe a significant decrease was present between newborn, sexually mature and old ages. Diameter changes of islets of Langerhans from minimum to maximum within the whole gland were respectively seen in fetal, old, sexually mature and newborn stages.

Discussion

On the basis of present study, significant difference was noticed in the volume density of exocrine secretory units between fetal and post-natal stages. These parenchymal changes indicated highly digestive enzymes requirement in animals of advanced ages with regard to increasing food consumption. According to this phenomenon, the pancreatic exocrine tissues developed in pre-natal and reached it's maximum growth in maturation. On the other hand, significant decrease was seen in stromal connective tissue from fetal stage towards the post-natal periods. This decrease may be due to developing of other parenchymal structures, particularly the exocrine secretory units. Similarly, significant increase in volume density of exocrine acini and decrease in stromal connective tissue was noticed from fetal stage towards the post-natal ages in Mehraban male sheep (Mansouri *et al*., 2005). Decrease in stromal connective tissue from 28% in neonate to 25% in adult human pancreas was also reported by Rahier *et al*., (1981). The volume density of blood vessels was increased from peri-natal periods towards the older ages showing highly vascularized pancreas in old animals. Increased blood supply was also reported in male sheep (Mansouri *et al*., 2005). Due to an increased parenchymal exocrine tissues, the blood supply of organ has to be equally increased. In the present study, the volume density of endocrine tissue was varied in different regions. Age related reduction in volume density of endocrine tissue was noticed between neonate and older animals although there was some increase in neonatal period. Rahier *et al*., (1981) in a study on human pancreas also reported age

related reduction from 15% to 5-7% to 2-4% in neonate, infant and adult, respectively. Decrease in volume density of the endocrine tissue (0.93%) was also noticed in adult camel by Tadjalli and Meamary (1998). Age related reduction in volume density of endocrine tissue was also noticed between neonate and old ages of male sheep although there was some increase from fetal towards the neonate (Mansouri *et al*., 2005). The diameter ranges of islets of Langerhans were found to be varied in human and domestic animals (Getty, 1975; Bloom and Fawcett, 1994). These diameter ranges were recorded 100-200 µm in human (Bloom and Fawcett, 1994), 50-370 µm in dog (Redecker *et al*., 1991) and 25-200 µm in small islets and 100-1600 µm in large islets of bovine, respectively (Bonner and Like, 1990). In our study, the diameter ranges of islets of Langerhans were obtained from 25.74 to 72.62 µm and in whole gland the minimum was belonged to fetus and maximum to newborn animals.

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