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Age Related Histomorphological and Transmission Electron Microscopic Studies of the Pancreatic Islets in Goats

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Abstract: *The study, which was conducted on the pancreatic islets of 30 goats of three different age groups, has not shown the specific pattern of distribution of islets. However, the islets were encountered at the periphery of pancreatic lobes and near to interlobular connective tissue in the vicinity of blood capillaries. It was also confirmed that the abundance of islets were at the tail portion of pancreatic gland. Islets of Langerhans in goats were of mixed types with three varieties of cells viz. alpha, beta and delta. The alpha and delta cells were found located at the periphery while beta cells were placed at the center. Islets cells were distinguished by their cytological and nuclear contents. The connective tissue capsule, which was seems to be the separator between acini and islets became more distinctive with the advancement of age. The size and number of endoplasmic reticulum of alpha cells was on increasing with the increase of age of animal. The elongated nuclei and the evenly distributed lighter chromatin material were recorded in the alpha cells of kid animals. In the young animals, the nuclei of these cells got elongated with densely packed chromatin but in the adult spherical nuclei and condensed chromatin material with clumps was found distributed at the periphery. The secretory granules of alpha cells have shown the disappearance along with the progression of age of animals. The beta cells has shown the polymorphic and uniformly distributed secretory granules. Along with the progression of age, the condensation of chromatin material was occurred in the nuclei of these beta cells. The irregular shaped nuclei with clear cut indentation were the prominent feature of delta cells. Their secretory granules were similar to that of alpha cells.*

Key words: *Histomorphology, Transmission Electron Microscopy, Pancreartic Islets, Goat*

I. Introduction

Pancreas is the distinctive gland which performs exocrine and endocrine functions of the body. Endocrine ductless mass of the pancreas called as islets comprises Alpha, Beta and Delta cells. The secretary activities of these different cells have the undoubted utilization for life. Different cellular constituent of the islet of Langerhans secretes different hormones as to glucagon, insulin and somatostatin. Insulin therapy remains the need of biological system while achieving the satisfactory metabolic control over the blood sugar, which worsens due to the beta cell dysfunction. The hyperglycemia development reportedly discovers that it is directly proportional to the pancreatic beta cell failure and their apoptosis in the mass of pancreatic islets. Hyperglycemia accompanied by decreased rate of carbohydrate utilization and vice versa of lipid and proteins utilization. Glucagon which secrete by the alpha cells has counterchecking action on insulin release while the delta cells secrete somatostatin which is known to be the growth hormone as it controls the rate of nutrient absorption into the blood circulation and also it regulates the activity of alpha and beta cells as the intra islet control device.

Different hormones secreted by different endocrine components of pancreas retain homeostasis pertaining particular with glucose level in the circulation as the source of energy and also helps to absorb the nutrient which works as the resource of blood glucose in the body. This glandular endocrine mass, the pancreatic islet decides the time appropriate release of pancreatic juice for enzymatic digestion of food. Goat is the model of choice in the veterinary research as due to this bio-model owing the physiological, psychological and biomedical similarities with the human being. Since age is the crucial factor concerned with the biological activity therefore it remains very appropriate to choose the comprehensive study of the endocrine portion of pancreas at their morphological, ultra structural and functional level to ascertain the degree of activities with reference to their ageing.

II. Materials And Methods

The present study was carried out on the 30 numbers of fresh pancreatic tissues of healthy local goats which were procured from the local slaughter house of Municipal Corporation. The animals were divided into three groups as to kids, young and adults. Depending upon the age, the kids were grouped into 0 to 7 months of age. The young were included with 7 to 14 months of age while the adults were over and above of 14 months age. Against each group 10 samples were collected. The studies which were confirmed by various workers includes Vijayraghvan and Mariappa (1976) and Meshram et al (2001) that the tail portion of the pancreatic

gland has the abundance of islets. In their concurrence and agreement the sampling in present studies was preferentially restricted over the tail portion as the aim and focus of present study was constrained upon the islets. The collected samples were carried to the laboratory on ice.

The routine Gomori's and Maldonados histological technique was applied as per Singh and Sulochna (1996). The fixation of samples was done in 10% neutral buffered formalin at room temperature. The histomorphological pattern and the cellular details of islets of the endocrine mass of the gland were studied under microscope by using their stained paraffin sections of 5 to 6 µ thickness.

The ultrasructural viz. transmission electron microscopic (TEM) study was carried out as per John and Lonnie (1980). The TEM samples were fixed in 2% glutaraldehyde in 0.05 M phosphate buffer (pH 7.2) for 24 hrs at 4˚ C and post fixed in 2% aqueous Osmium tetroxide in the same buffer for 2 hrs. After the post fixation, samples were dehydrated in a series of graded alcohol, infiltrated and embedded in Spurr's resin. Both, semi thin and ultra thin sections were cut with a glass knife on Leica Ultra cut UCT-GA-D/E-1/00 ultra microtome. Semi thin sections of 200-300 nm thickness were stained with toludine blue and ultra thin sections of 50-70 nm thickness were mounted on grids. Then the sections were stained with saturated aqueous Uranyl acetate and counter stained with 4% lead citrate and observed them at various magnifications under transmission electron microscope model of Hitachi, H-7500.

III. Results And Discussion

The pancreatic islets were shown scattered appearance in between the acini. The shapes of the islets were round and stretched. The islets were generally distributed along the periphery of pancreatic lobes, adjacent to interlobular connective tissue in the vicinity of blood capillaries (Fig 1). It was separated from the surroundings exocrine acini by delicate network of connective tissue fibers. The capsule of the islets was well organized and consisted of collagen, elastic and reticular fibers. The capsule surrounding the islets became more distinct with the advancement of age. It was also found with blood vessels and nerves (Fig 2). The findings were in complete corroboration with Banks (1981) in dogs, Ladukar and Bhamburkar (1994) in layers and broilers and the Ganguli and Prasad (1995) in caprines. However, Ali et al (1991) were lacking with such evidences of connective tissue surrounding the isolated islets in ultrathin sections of bovines. The islets in kids were found very thinly distinct by the network of connective tissue fibers from surrounding the acini (fig. 3). This distinction of the network of connective tissue fibers were went on very prominent in young animals (Fig. 4) while it has shown loosely arranged appearance in the adults (Fig. 5). After staining the sections with Gomoris and Maldonado's methods, three types of cells were identified viz. alpha, beta and delta (fig 6 and 7). The maximum diameter of islets was recorded in young group of animals which were followed by adults and kid animals. Their mean values with respect to age groups were 76.59 ± 5.85, 98.61 ± 6.68 and 80.5 ± 5.68 µm respectively for kid, young and adult animals. In respect of the number of islets per lobule was found increasing from kids to adults. Their numbers were 1.75 ± 1.35, 7 ± 2.92 and 7.75 ± 3.25 respectively for the groups of kids, young and adults. Sinusoidal blood capillaries were visible in the vicinity of islets. In the present study mixed type of islets were encountered which were contained mostly with beta and alpha cells and few of the delta cells in all age group of animals. The differences in islets cellular frequencies were directly proportional to the activity of islet and the advancement of age along with quantum of carbohydrate intake.

The alpha cells were smaller than beta and delta. The cell boundaries of alpha cells were not clearly visible. Its cytoplasmic content had taken red stain with centrally located heterochromatic nucleus. These cells were located at periphery of the islets and comparatively less in number than that of beta cells. The maximum numbers of alpha cells were recorded in kid group of animals which were followed by adult and young animals. Their numbers were 12.4 ± 2.87, 6.9 ± 2.40 and 7.1 ± 2.95 respectively for kid, young and adult goats.

Ultra structure of alpha cells in kid was revealed that the nuclei of cells were quite elongated with evenly distributed lighter chromatin material. The cytoplasm was contained with densely packed prominent secretory granules surrounded by distinct hollow vacuoles. They were also contained with few mitochondria, numerous vacuoles and ill defined endoplasmic reticulum (Fig. 8). It was noticed that, the numbers of secretory granules were disappeared gradually with the advancement of age. Contrary, the number and size of endoplasmic reticulum found increasing with the advancement of age. In the young group of animals, the nuclei were elongated with densely packed chromatin. The cytoplasm surrounding the nuclei was contained with large number of long slender mitochondria, vacuoles and comparatively less secretory granules. The rough endoplasmic reticulum and the Golgi complex were also noticed. In the adult group of animals, the nuclei of these cells were acquired the spherical shape and their chromatin material was found in the form of condensed clumps which were distributed at the periphery with central and clear nucleoplasm. The centrosomes, mitochondria, Golgi complex, rough endoplasmic reticulum were found increased and the smooth endoplasmic reticulum was dominated over the rough endoplasmic reticulum with prominent nucleus (fig. 9, 10). It was on the record that, as the age advances there were marked changes at intra nuclear as well as intra cytoplasmic levels. The condensation of chromatin was also evident there as an indication of gearing up the protein secretory

activity. The cytoplasmic organelles were more developed in young animals as to cope up the increasing demand of hormonal surge with the greater intake of feed in young animals. There was marked decrease in the secretory granules in the adults which was a marker of future declination in the functional status of islets. The similar findings were on record which were reported by Bloom and Fawcett (1968) in human beings, Ali et al (1991) in bovines, Maala et al (2004) in Philippine water buffalo and Mythili et al (2005) in wild Indian bonnet monkeys.

The maximum vertical diameter of the nuclei of alpha cells was recorded in kids, which were followed by young and adult animals with their mean values of 6.99 ± 0.02, 6.07 ± 0.02 and 5.68 ± 0.64 mμ respectively for kid, young and adult group of animals. The maximum horizontal diameter of the nucleus of these cells was found in adults, which were followed by kid and young animals. Their mean values were 4.43 ± 0.25, 3.99 ± 0.02 and 4.61 ± 0.38 mμ against kids, young and adults group.

The beta cells were more often found located at the center. They were more in numbers as compared to alpha and delta cells. The cytoplasmic granules of these cells were more in number with light bluish color and central rounded nuclei with heterochromatic nature. The number of beta cells per lobule was higher in young group of animals which were followed by kids and adults. Their mean values in respect of age groups were 8.5 ± 1.95, 11.1 ± 3.25 and 7.8 ± 1.97 respectively against kids, young and adults. It reflects the declination in carbohydrate metabolism in adult goats.

Ultra structurally, the nucleus of beta cells in kids shown the circular nuclei. The chromatin material was distributed at the periphery and in the center. The cytoplasm had less number of endoplasmic reticulum with scanty ribosomes. These cells were contained the numerous polymorphic secretory granules with the uniform distribution (Fig. 11). The number of mitochondria were very few so also the Golgi apparatus with abundant vacuoles (Fig. 12 and 13). Along with the advancement of age the condensation of chromatin material was started and the endoplasmic reticulum was also obtained well developed. While attaining the adult age, the endoplasmic reticulum and the mitochondria turned on to the abundance however very few secretory granules were noticed with reduced vacuoles. The chromatin material was found as clumps which were distributed at periphery and center (fig. 14 and 15). The findings in present studies were in the line with that of Dorsche (1979) in rats, Bloom and Fawcet (1968) in humans, Mikami et al (1985) in quails, Tahir et al (1982) in rats, Maala et al (2004) in Philippine water buffalo and Mythili et al (2005) in Wild Indian bonnet monkeys. However, the observations revealed by Ali et al (1991) in isolated bovines islets were not in agreement with the present observations.

The maximum vertical diameter of the nuclei of beta cells was recorded in kid group of animals which were followed by adults and young age groups. Their mean values were 5.99 ± 0.01, 5.23 ± 0.09 and 5.60 ± 0.05 mμ respectively for kid, young and adult animals. The maximum horizontal diameter of the nuclei of these cells was recorded in young animals. It was followed by adults and kids. Their mean values against kids, young and adults were 4.31 ± 0.19, 4.75 ± 0.25 and 4.62 ± 0.45 mμ respectively.

The delta cells were found scanty in numbers as compared to the beta and alpha cells. These cells were located at the periphery of islets, particularly between the alpha cells and external connective tissue reticulum limiting the islets. These delta cells were seen irregular in shape with oval to elongated nuclei with grayish cytoplasmic granules. Their maximum numbers were noticed in adults followed by kids and young animals. Mean values against the kids, young and adults were 0.9 ± 0.90, 0.8 ± 1.01 and 1.3 ± 1.15 respectively.

The ultra structure of the delta cells in the kid group of animals was shown in irregular shapes. The nuclei of them were also irregular with clear cut indentation. The chromatin material was noticed with uniform distribution. The clumps of chromatin material were seen adhered to the nuclear membrane and also suspended in the center. The secondary granules were small, rounded and electron dense. The secretory bodies were similar to that of alpha cells. Less number of mitochondria and poorly developed endoplasmic reticulum was also observed. Secretory granules were increasing with the advancement of age (Fig. 16 and 17).

The decrease in the secretory granules in the alpha and beta cells was indicative of decreased secretion of glucagon and insulin hormone. However, this study has established that the numbers of secretory granules were increased in delta cells as the age advances. The logic was quite acceptable that the stomatostatin hormone which secreted by delta cells has critical stimulating action on the islets. Such type of stimulation is essential when the cellular activity of alpha and beta cells goes on decline. The findings in the present studies were in accordance with those of Mikami et al (1985) in quails, Salakij and Watanabe (1996) in chickens and Maala et al (2004) in Philippine water buffalo. The maximum vertical diameter of the nuclei of delta cells was recorded in kids which was followed by adult and young animals. Their mean values were 6.56 ± 0.85, 5.15 ± 0.03 and 5.28 ± 0.01 mμ respectively against kid, young and adult animals. The maximum horizontal diameter of the nuclei of delta cells was found in kids which was followed by young and adult animals. Their mean values were 3. 58 ± 0.10, 3.57 ± 0.36 and 3.57 ± 0.02 mμ respectively for the kid, young and adult animals.

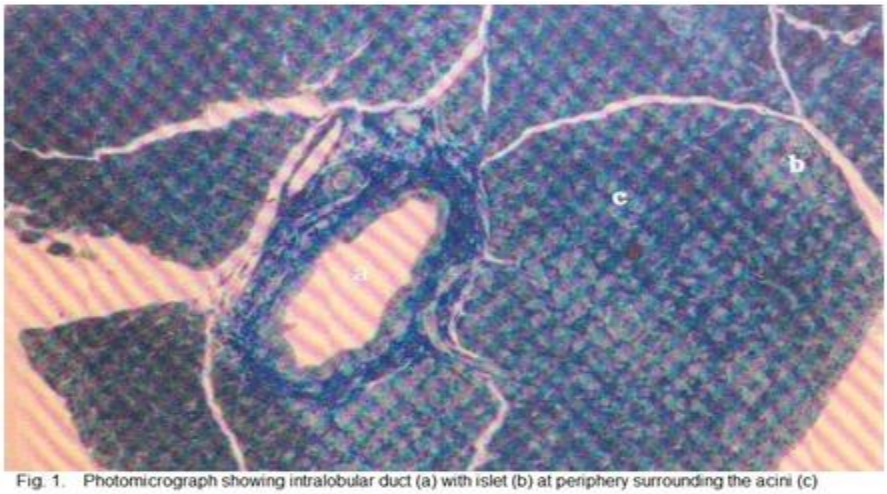

Fig. 1. Photomicrograph showing intralobular duct (a) with islet (b) at periphery surrounding the acini (c)

Toiludine blue, 100X

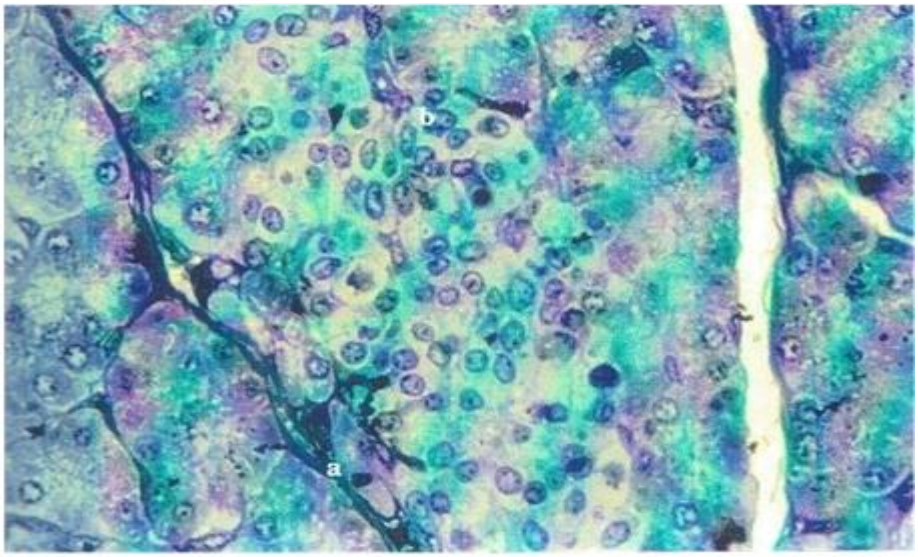

Fig. 2. Photomicrograph showing an islet with surrounding capsule (a) and blood vessels (b)

Toiludine Blue, 400X

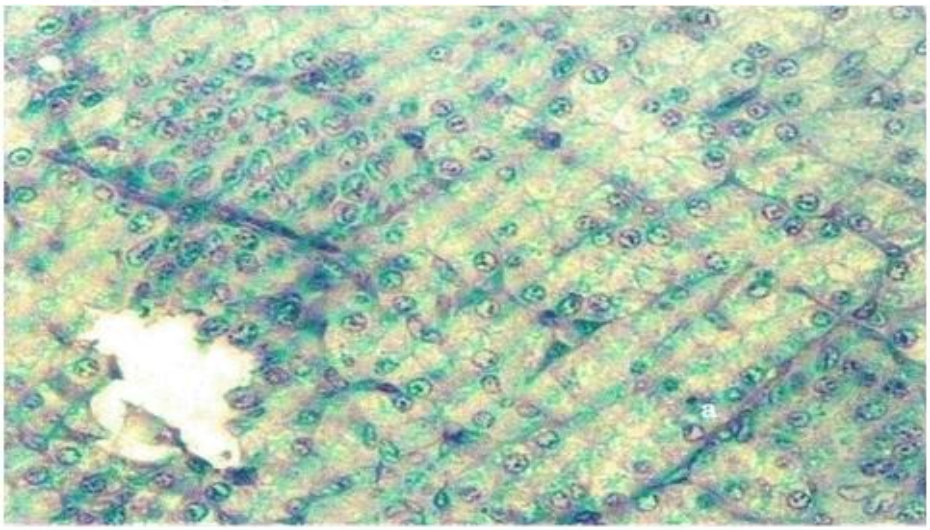

Fig. 3. Photomicrograph showing an islet of kid goat surrounding connective tissue (a)

Toiludine Blue, 400X

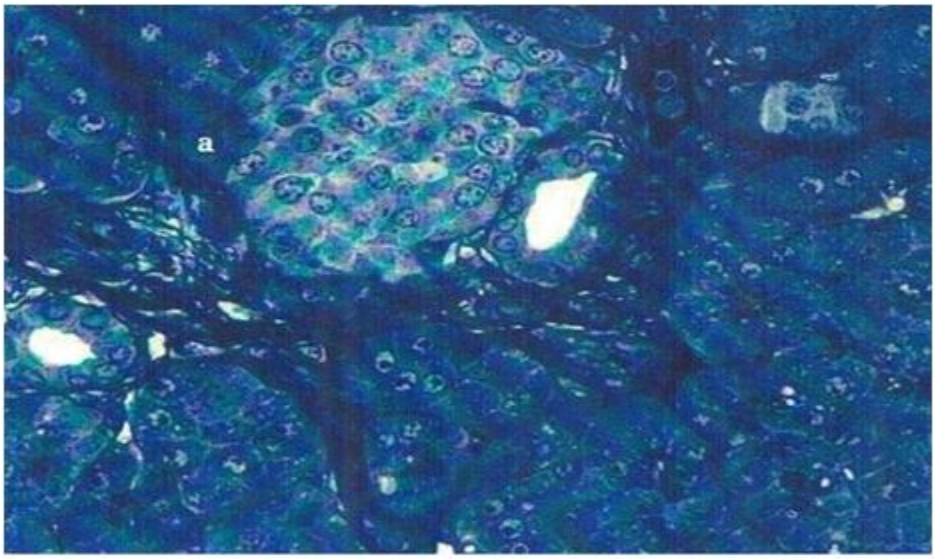

Fig. 4. Photomicrograph showing an islet of young goat surrounding network of connective tissue (a)
Toiludine Blue, 400X

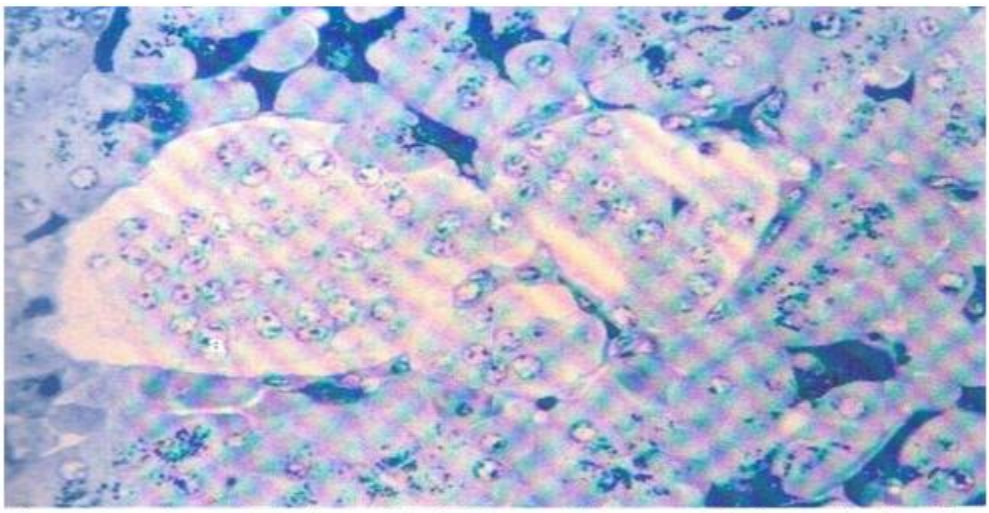

Fig. 5. Photomicrograph showing am islet of adult goat with loose network of connective tissue (a)
Toiludine Blue, 630X

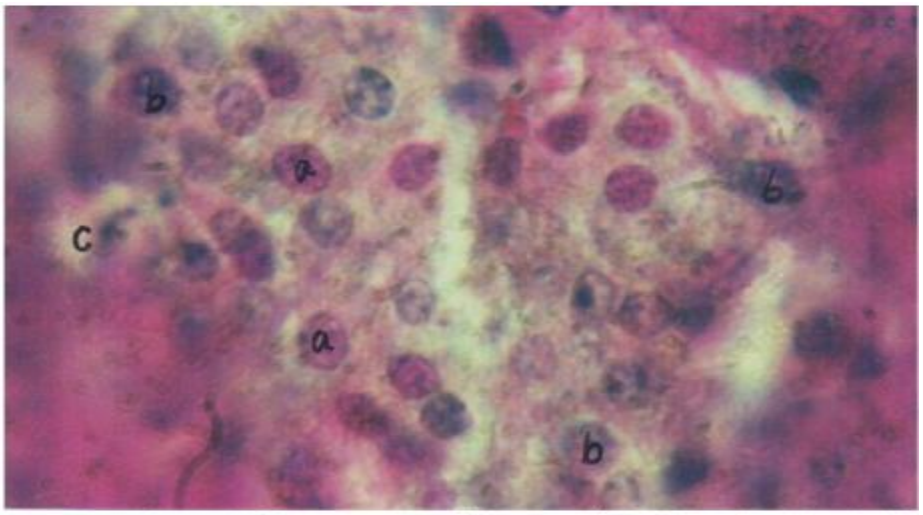

Fig. 6. Photomicrograph of caprine pancreas of adult group of animals showing alpha cells (a), beta cells (b) and delta cells (d)
Gomori's 1000X

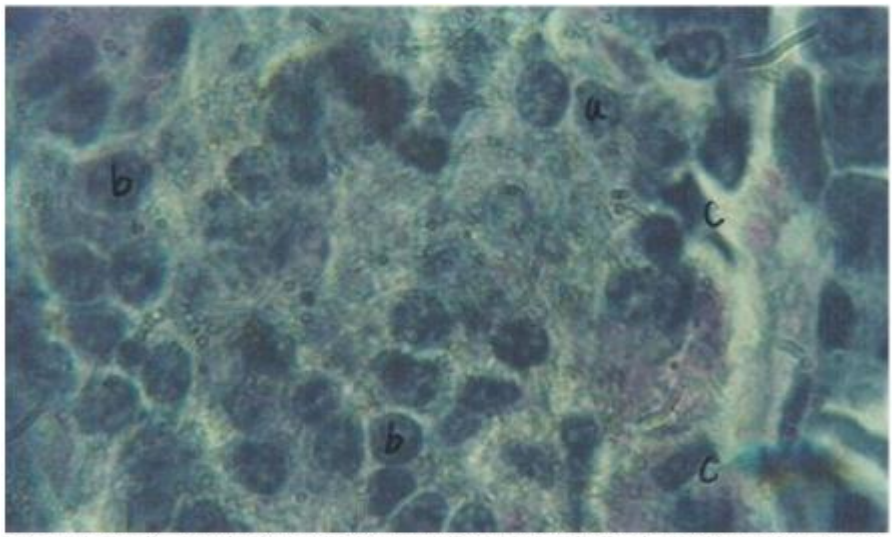

Fig. 7. Photomicrograph of caprine pancreas of kid group of animals showing alpha cells (a), beta cells (b) and delta cells (c)

Maldonado's 1000X

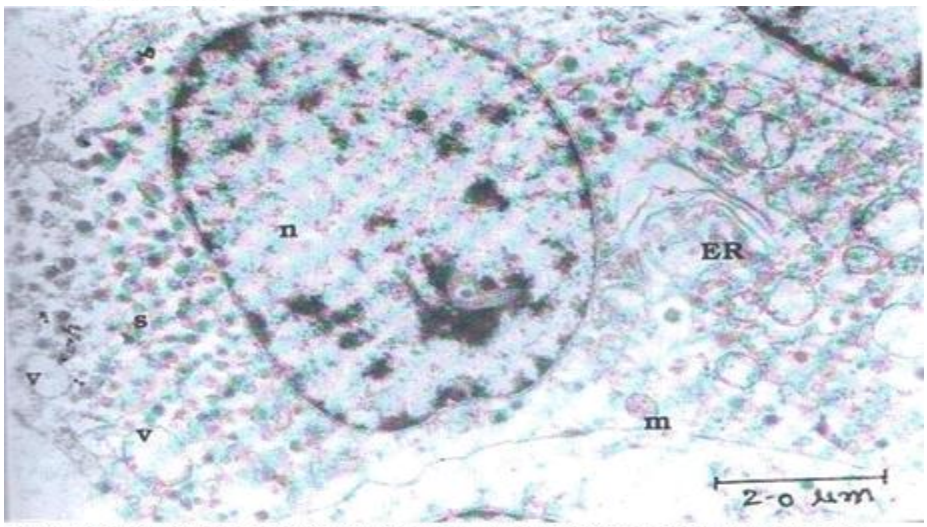

Fig. 8. Transmission electron photomicrograph of alpha cells of kid group showing nucleus (n), mitochondria (m), endoplasmic reticulum (ER), secretory granules (s) and vacuoles (v)

5KX

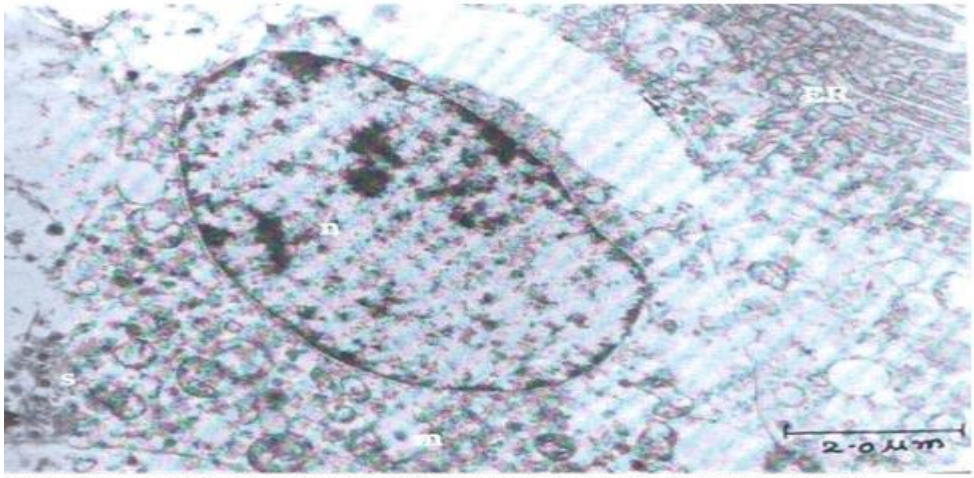

Fig. 9. Transmission electron micrograph of alpha cell og young group of goat showing nucleus (n), mitochondria (m), secretory granules (s) and endoplasmic reticulum (ER)

5KX

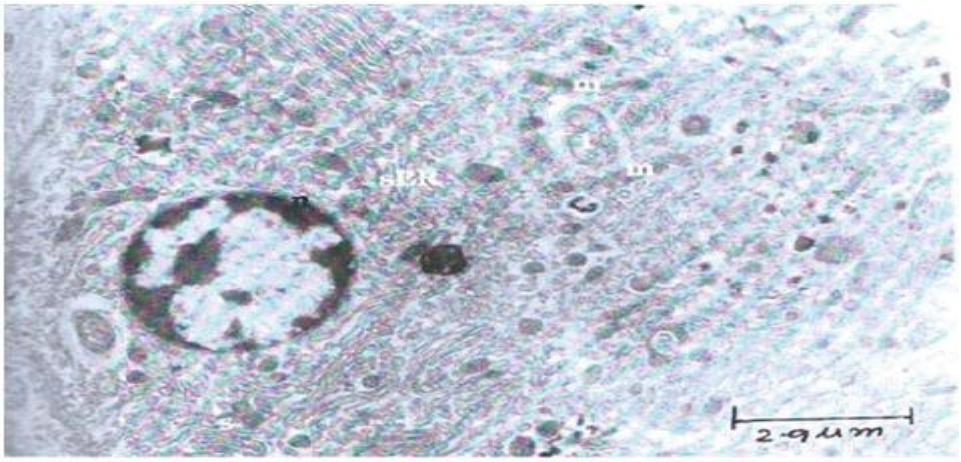

Fig. 10. Transmission electron photomicrograph of alpha cell of adult group showing nucleus (n), mitochondria (m), secretory granules (s), rough endoplasmic reticulum (rER), Golgi apptatus (g), lysosomes (l) and smooth endoplasmic reticulum (sER)
3.5KX

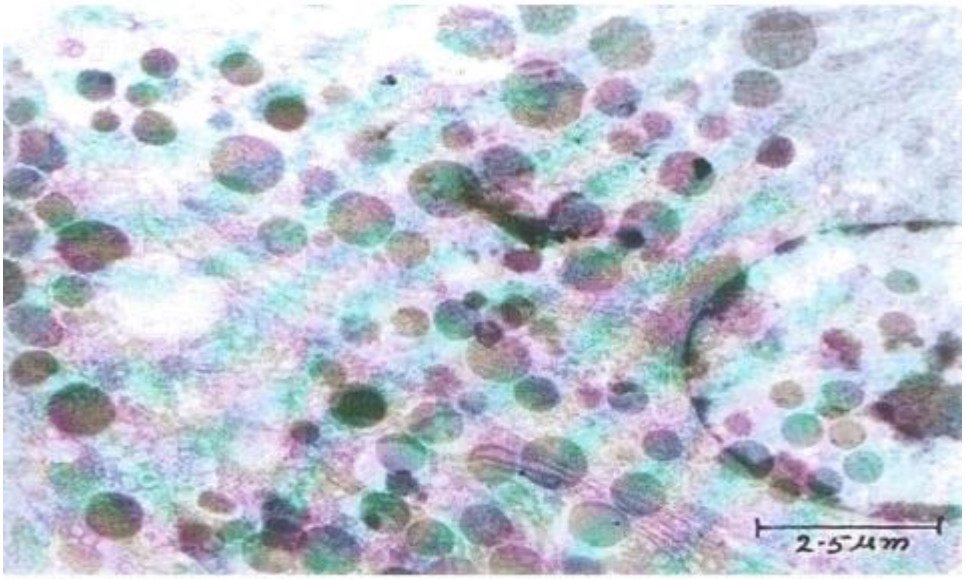

Fig. 11. Transmission electron photomicrograph showing polymorphic secretory granules.
4KX

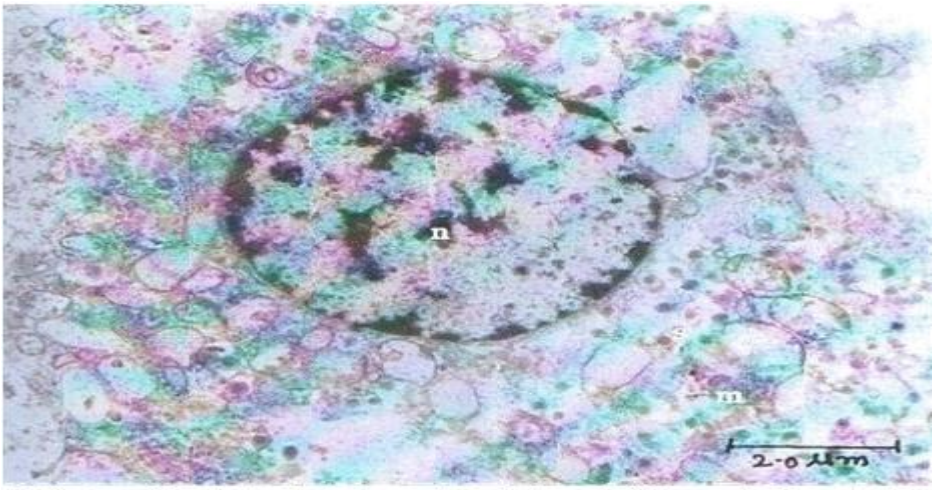

Fig. 12. Transmission electron photomicrograph showing beta cell of kid group showing nucleus (n), nitochondria (m) and secretory granules (s)
5KX

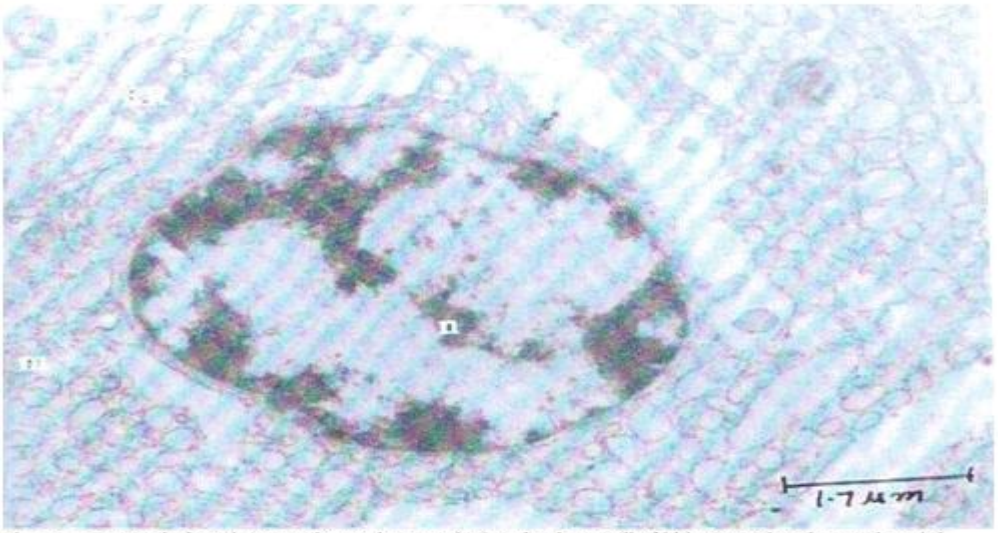

Fig. 13. Transmission electron photomicrograph showing beta cell of kid group showing nucleus (n), mitochondria (m), endoplasmic reticulum (ER) and vaculoes (v)

6KX

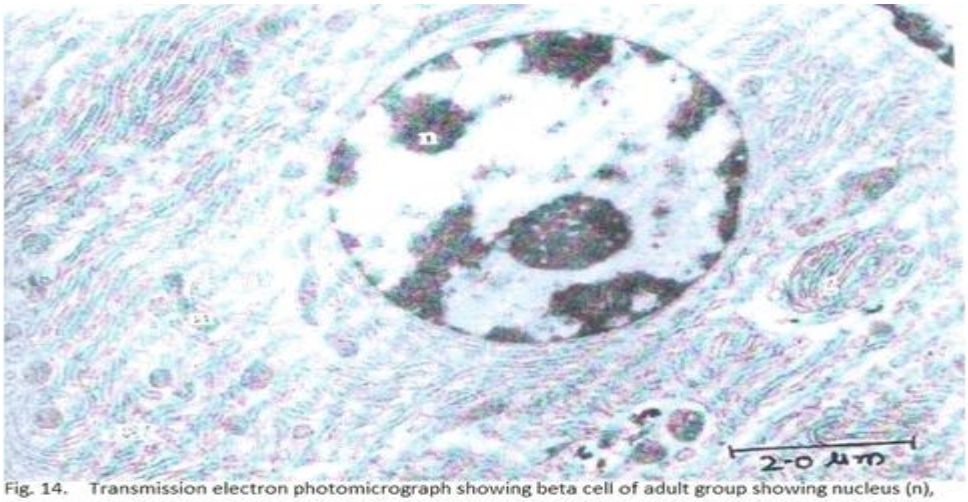

Fig. 14. Transmission electron photomicrograph showing beta cell of adult group showing nucleus (n), mitochondria (m), rough endoplasmic reticulum (rER) and Golgi appratus (g)

5KX

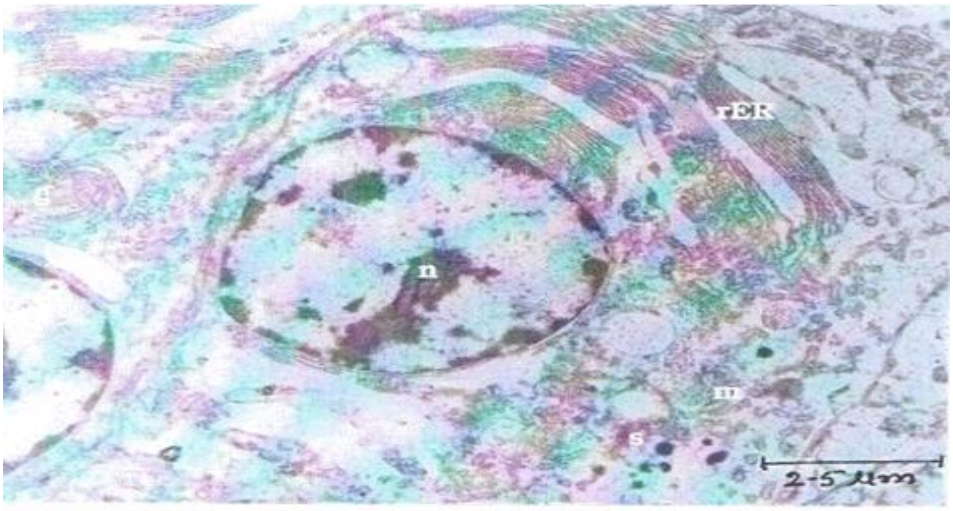

Fig. 15. Transmission elecron photomicrograph showing beta cell of adult group, showing nucleus (n), mitochondria (m), rough endoplasmic reticulum (rER), secretory granules (s) and Golgi appratus (g)

5KX

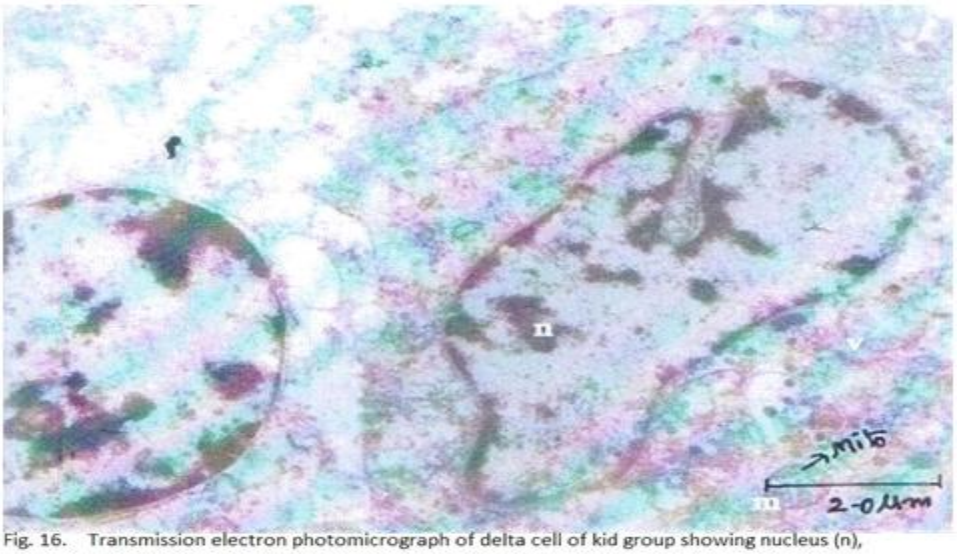

Fig. 16. Transmission electron photomicrograph of delta cell of kid group showing nucleus (n), mitochondria (m), vesicles (v) and cytoplasmic granules (g)
5KX

Fig. 17. Transmission electron photomicrograph of delta cell of adult group showing nucleus (n), cytoplasmic granules (c) and mitochondria (m)
5KX

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