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Pancreas in Sheep Histochemical and Immunohistochemical Analysis

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Abstract

Current research aimed to investigate immunohistochemical and histomchemical study of pancreatic tissue of the adult sheep. Histological sections prepared from the specimens of their pancreas were stained with general and special stains then photographed with Dino-eye piece camera and analyzed with its image software. The endocrine portion reside primarily in the islet's of Langerhans which associated well with extra-blood vessels and exocrine ducts. microscopically, each islet was structured of several cells (alpha and beta) which were different in size, shape and color.

Pancreas is the specific exocrine and endocrine secretion gland. Islet of Langerhans is the endocrine part which has certainly been used for life by its secretary activities. Specific cell islets have specific characteristics and the specific hormone secretes. Such various cells of the Langerhans islet synthesize and secrete different hormones that the biological system needs to achieve adequate metabolic control over blood sugar. Regional distribution, numerical frequency, and presence of glucagon (Alpha-cell)- & glucose-dependent insulinotropic peptide (Beta-cell)-secreting cells in sheep's endocrine and exocrine pancreas were investigated using the immunohistochemistry process. Glucagon immunopositive alpha-cells were found primarily in the central and mantle region of the Langerhans islets, while glucose-dependent insulinotropic peptide immunopositive beta-cells were located in the peripheral, central and pancreatic islet mantles. In addition, some Alpha- and Beta-cells in the exocrine parenchyma and the pancreatic duct epithelium were detected as single cells or clusters of 2 to 3 immunopositive cells. In this study for the first time the presence, regional distribution, and relative frequency of Alpha-&Beta-cells in sheep pancreas were analyzed. It was determined that the immunopositivity and distribution of endocrine cells in the sheep pancreas differs partially from those of other mammalian species such as goat.

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Key words: cell endocrine, immunohistochemistry, islet of Langerhans, sheep pancreas.

Introduction

sheep are small important domesticated ruminants which has served humans earlier and longer than cattle and goat (1, 2). Sheep are wide spread across the world, having adapted to many different climatic conditions and econiches (3). It can be found in all over the world particularly in arid semitropical countries (4).Sheeps pancreas is an important accessory gland and plays a major role in digestion. The pancreas gland is unique glands as consist of exocrine which structured from compound tubuloacinaracini and endocrine parts. The exocrine part is contributed 95% of pancreatic mass.These generate digestive enzymes for carbohydrate metabolism in duodenum and regulatory hormones(5-7) respectively and it comprise frame acinar and duct cells blood vessels with associated connective tissue. Endocrine portion of the pancreas represented by Langerhans Islets contributing 1 to 2 percent of the pancreatic mass and highly vascularized, consisting of several types of endocrine cells that work together to regulate the metabolism of glucose and homeostasis and it synthesize the insulin, glucagon, somatostatine and the pancreatic polypeptide (5).Scientific work on the immunohistochemical analysis of the pancreas in sheep has been minimal. It may also be useful for transplanting pancreatic cells, regenerating stem cells, and tissue engineering in the future.Hence, for a better understanding of immunohistochemical pancreatic in sheep. Therefore the present work has been performed.

Resources and Techniques

Twenty adult clinically healthysheepwere collected from AL-Diwnyiaprovince slaughter house. The pancreas was removed immediately from fresh carcass and washed with normal saline and cleaned from debris and adhered tissues parts were then fixed in10% neutral buffered formalin for 48hrs before paraffin embedding. Tissues were cut into 1 cm thick slices and Routinely treated with a graded sequence of alcohols, dissolved in xylene and soaked with paraffin. Parts of 6μm thickness have been obtained and stained with haematoxyline and eosin for studying the general histology of the pancreas. Some other sections were stained with special stain on the alpha and beta cells demonstrated in the endocrine portion(Langerhans

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islets)Gomori'sTrichrome (6). Parts of the tissue were examined using Olympus light microscope, and photographed using Image Software-fitted Dino-eye piece camera.Immunohistochemical staining (Table.1) Primary Antibody.

Table(1): Antibody of primary and secondary origin.

Clonality and its starting point	**"Dilutions**	**Host**	**Primary ''antibodies**
Polyclonal,GIP, Biotechnology Santa Cruz, INC., Santa Cruz, CA, USA.	1-300	goat	**Anti-GIP**
Polyclonal,GLP-1, Santa Cruz Biotechnology, CA, USA.	1-300	goat	**Anti-GLP-1**

Secondary antibody	**Label**	**Dilution**	**Source**
Anti-goat, Donkey IgG	Cy3	1:500	IgG (705-166-147) cyanine-conjugated, Stratech Scientific Limited, Suffolk, UK.
Anti-goat, Donkey IgG	FITC	1:500	Fluorescein-conjugated IgG (705-095-147), Stratech Scientific Limited, Suffolk, UK.

The relative frequency of ' ' IR " cells was located in 1 of 5 groups, -not observed; (unusual), ±; mean values were below 2/1 field); few (+; mean levels were below 5/1 filed); fair (+ +; mean values were below 10/1 filed) and elevated (+ + + +; mean values were up to 20/1 filed); according to their experiential mean numbers as shown in ' ' epifluore ' filed.

Results

Exocrine portion

The present study showed that the pancreas of sheep was surrounded by a very thin membrane constructed of a loose connective tissue from which the septa enters to the pancreatic parenchyma and divide it into lobules, these septa contains blood and lymph vessels, nerves and ductal system and excretory ducts(Fig.1). The exocrine portion displayed of numerous small dark staining acini which were the subunits of the lobules,each acinus composed of cluster or spherical mass of cells, these pyramidal cells characterized by an acidophilic cytoplasm with basally located nuclei

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(Fig 2). Flattened with darkly stained cells represent the myoepithelial cells were observed adjacent periphery to the acini (Fig.2). Some of the acini revealed somewhat clear lumen and others showed centroacinal cells were arranged around their Lumina, these cells considered the beginning ofthe secretory duct(Fig.2). All the surrounding thin stromal connective tissue were stain blue after staining with the special stain Masson's trichrome stain especially around the ductal system and blood vessels (Fig.3).

The ductal system in the sheep pancreas was initiated by the centroacinar cells which observed surrounding the Lumina of exocrine acina, they convey the excretory products to the small ducts; the intercalated duct inside the lobule,These ducts are filled by simple cuboid epithelium, very small. The small intercalated ducts formed the intralobular duct, which was lined by simple cuboid epithelium and runs between the acini in the thin connective tissue (Fig.3 A,B). The intralobular ducts converged into interlobular ducts that were lined by clear low columnar epithelium, the interlobular ducts when wide were collected to form layer ducts when the main excretory duct of the pancreas assisted the connective tissue that revealed the abundance of blood vessels (Fig. 3 C&D).

Endocrine portion

Whereas the endocrine portion represented by the islet of Langerhans which showed different shape and size, they appeared as round, oval or irregular shape with different size (large, medium and small sizes and were present distributedwith in The exocrine component and the interlobular connective tissue also (Fig.4) Most of the small islets were created and embedded between the acini and consist of only one, two or three cells (Fig.4).

when using Gomorie's trichrome stain, the endocrine cells of the islets stained dark blue which representing β cells which appeared large with prominent nuclear and located at the periphery, mantle and center of the Langerhans islets, while cells which coloured purple red represented α (alpha) cells which were smaller than βcells and existed mostly in the periphery of islet's(Fig.5, table 2). The Islets of Langerhans was surrounded by collagen fiber, invading capillaries and nerve fibers were observed(Fig. 5).

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GIP and GLP-1 Immunoactive Cells throughout Sheep's Pancreas

In this analysis, two forms of immunoreactive endocrine cells were found in the sheep's pancreas using antisera to glucose dependent insulintropic peptide and glucagon. There were three distinct layers of the pancreatic islets: middle, mantle, and peripheral areas. Various distributions and frequencies of the immunoactive endocrine cells were recorded (Table 2). Langerhans islets were often spindle-circular or sometimes oval-circular(Fig.4).Glucose-dependent insulintropic peptide immunoreactive cells were present in exocrine portions (Fig.6), glucose-dependent insulintropic peptide immunoreactive cells were observed at a high frequency in the main, peripheral regions of the pancreatic islets, and more rarely in the mantle zones (Table 2, Fig. 6).Immunoactive glucagon cells were mainly confined to mantel zones but also found some positive cells in the central zones (Table 2, Figure 7). It was found however in the peripheral regions (Table 2, Figure 7).

Discussion

Sheep pancreatic islets were circular, oval or irregular in shape, occurring irregularly in the exocrine portion as well as in the interlobular connective tissue. Such results concord with (7) findings in cattle and buffalo (who reported that Ox islets were spherical and smaller but buffalo islets were irregular and larger), (9)in domestic animals and (8) in buffalo. Similar finding were observed in sheep and goat by (10) whereas different from that observed in philippin carabaa in which the islets observed more focussed in the right lobe then other part of the pancreas (11)

Some of the small islets contained only one, two, or three cells that were in agreement with (12) that reported sheep unicellular islets.

Alpha cells were located peripherally, while beta cells were reported toward the middle of islets, similar findings were made in dog and man (8), and in buffalo (13). However, alpha cells present towards the middle of islets and beta cells towards the periphery were observed at (14) in equine and (15) in Indian donkey. This variability can refer to differences between species.Both the two main endocrine cell types were expressed in the sheep's pancreas via the endocrine pancreas. All the two main endocrine cell types were distributed through the endocrine pancreas in the sheep's pancreas. All these endocrine cells appeared predominantly spherical to the shapes of spindles. The insulinotropic glucose-dependent peptide was extracted in the B-cells of

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the pancreatic islets and controls the serum glucose levels as in (16) above.In mammals, the hamster (17), opossum (18) and various laboratory animals (19) reported the regional distribution and relative frequency of pancreatic glucose-dependent insulinotropic peptide immunoreactive cells. We were surrounded by the immunoreactive glucagon cells. Nevertheless, Redy et al (20) stated that these immunoreactive cells are found in most islets where they occur peripherally as cell groups and several marsupial species within the pancreatic islets.Glucagon is synthesized in the pancreatic A cells and also contributes to blood glucose concentration control (21). Morphologically identical cells also exist in a dog's digestive tract (19).Glucagon immunoreactive cells were primarily restricted to mantle zones in the present research, but some of these cells were also present in the central zones of the sheep pancreas. Such results were similar to those found on mammalian pancreatic islets (21,17,18,22,19). Cell clusters consisting of glucagon and glucose-dependent insulinotropic peptide immunoreactive cells located in the connective tissue regions of pancreatic duct portions are usually found in higher mammals (19) also agree with (17) reported the comparative histology of buffalo and ox pancreas, the endocrine portion was reduced in quantity and distributed throughout the acini. Islets formed as a network of cellular cords, and sinusoidal capillaries filled the meshes. And an agreement with (23) defined islets, a pancreatic endocrine portion with cellular aggregations that interposed irregularly between the acini or on the ducts.

TABLE 2. — Numbers of glucose dependent insulinotropic peptide and glucagon -reactive cells in the pancreas of the sheep.

Antibody	Pancreatic Islet			
	Mantle	Center	Peripheral	Exocrine regions
GIP-positive cells	+++	++	++++	++
GLP-1 containing cells	+++	++++	++	+

Relative frequency :
+ = rare, ++ = small number, +++ = moderate amount, ++++ = high number.

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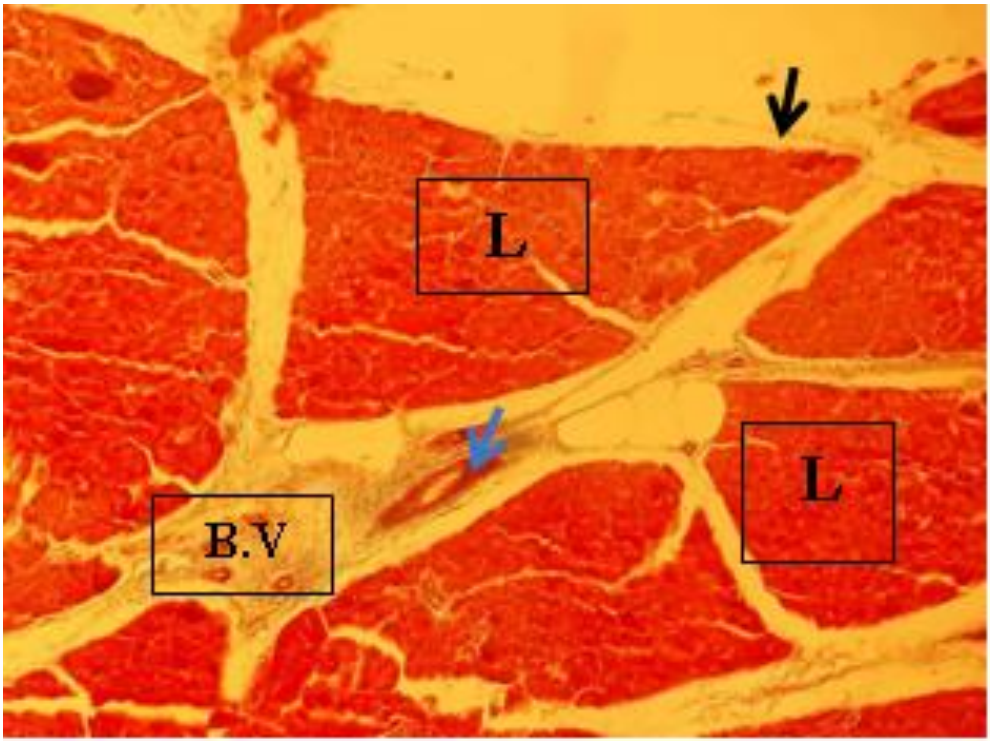

Fig.1:Histological section of sheep pancreas showed pancreatic lobule(L), mesentery(black arrow), duct(blue arrow) and blood vessels (B.V.) , (Masson's Trichrom).X100.

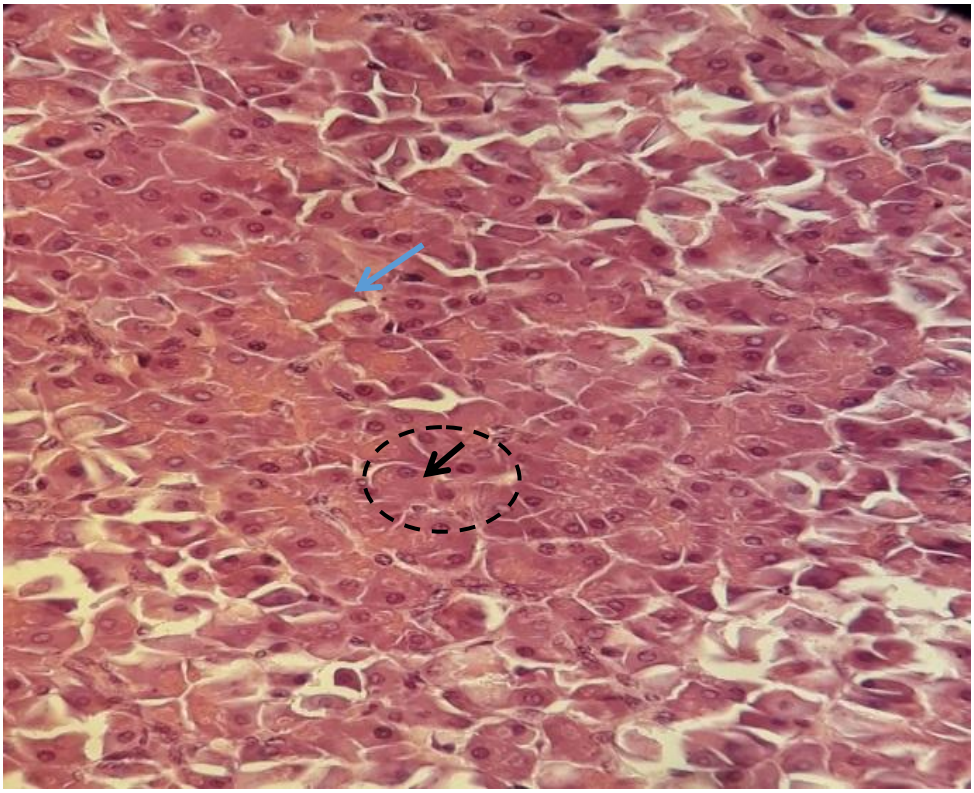

Fig.2:Histological section of sheep pancreatic exocrine showed centroacinur cell (black arrow), myoepithelial cell (blue arrow). H&E X100.

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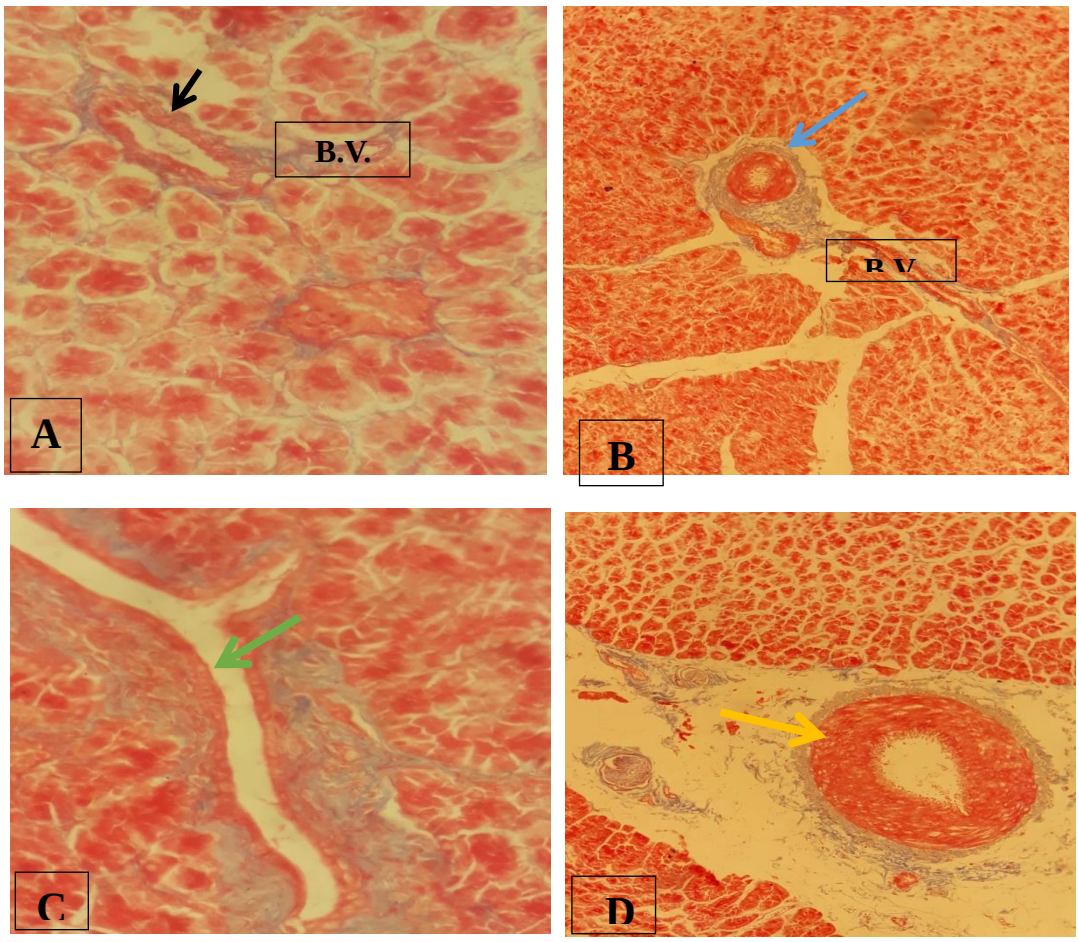

Fig.3:Histochemical section of sheep pancreatic exocrine showed : A: intercalated duct (black arrow); B: Intralobular duct (blue arrow); C: Interlobular duct(green arrow) and D: Main pancreatic duct (yellow arrow), blood vessles(B.V.). Masson'Trichrom, A&C: X400, B: X100 and D:X200.

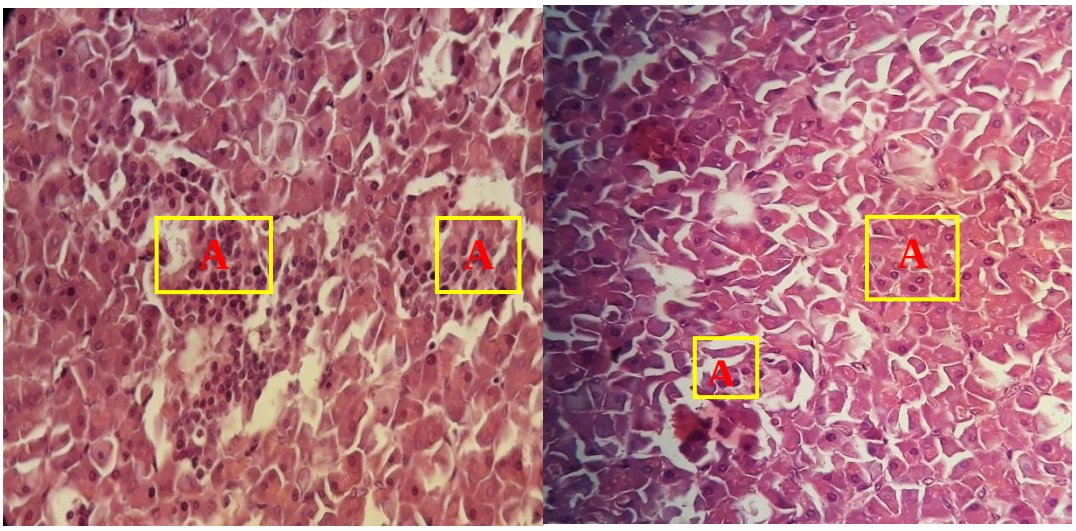

Fig.4:Histological sections of sheeppancreas showed, islet ofLangerhans(A),(H&E)X100.

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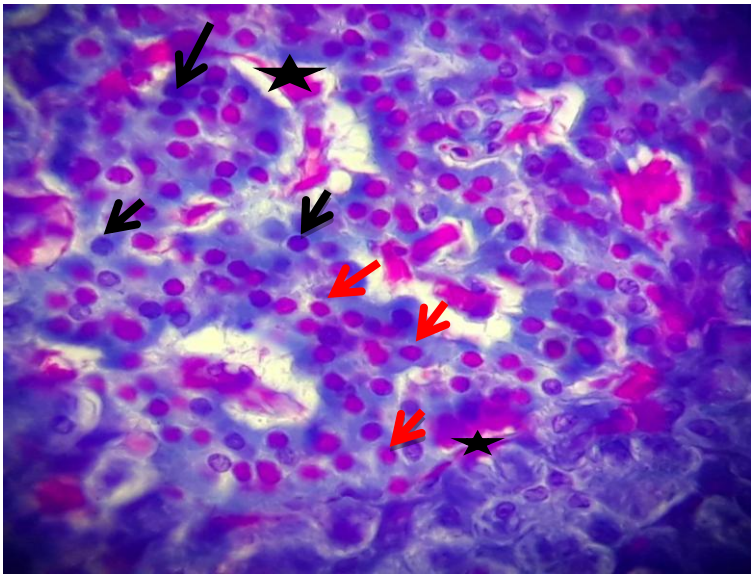

Fig.5: pancreas photomicrograph in sheep that reveals alpha (red arrow) and beta cells (black arrow), Capillaris (black star).Gomori's Trichrom stain × 400.

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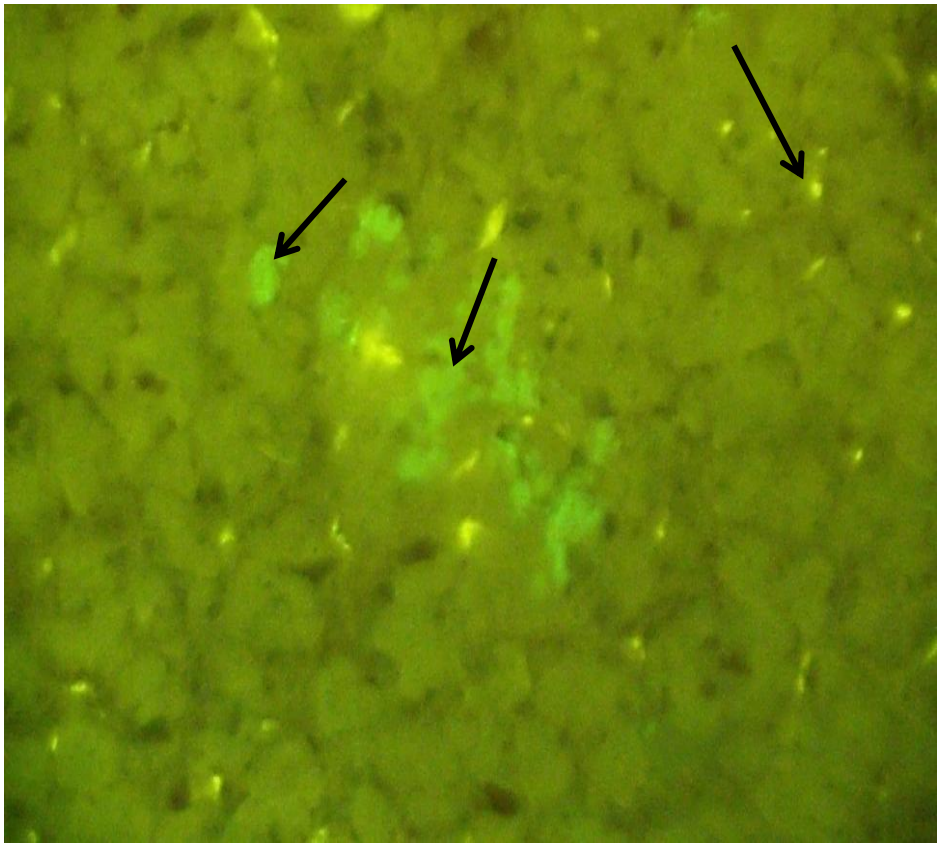

Fig.6:ImunofluercentImage of the pancreas of the sheep, using immunostaining of Glucose dependent insulinotropic peptide-producing cells (GIP) ,Beta cells were distributed throughout the islet in the center, mantle, the periphery and exocrine region(black arrows). X400

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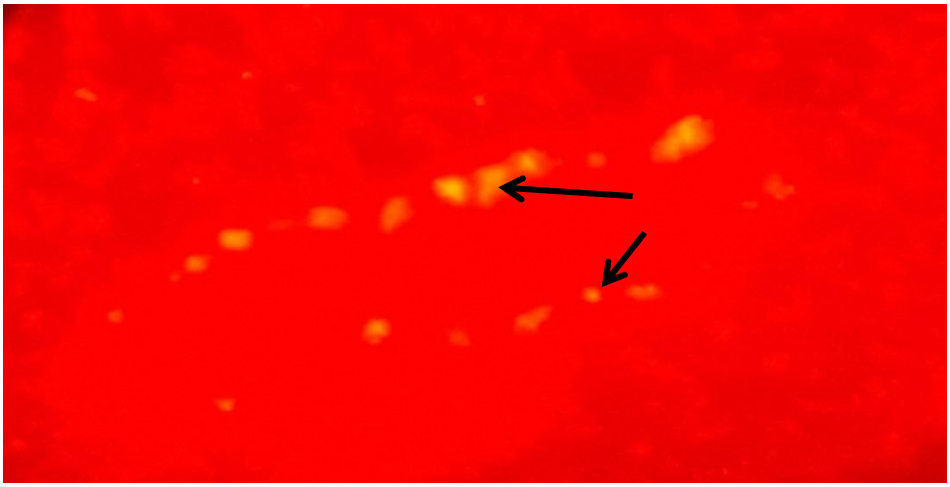

Fig.7:Imunofluercent image of pancreatic islets of sheep, using immunostaining of Glucagon -producing cells (GLP-1) ,Alpha cells were distributed throughout the islet in the center &mantle(black arrows).X400

Reference:

1-Yalcin, B. C. (1986). Sheep and goats in Turkey. Anim. Prod. Health. FAO. 60

2- Haenlein, G. F. (1992). Digestion extension goat handbook. ttp://www. inform.umd.edu/EdRes /Topic /AgrEnv/ndd/goat.digestion.htm

3- Al-Baggal, H. A.R., Al-Dahash, S.Y. A. and Alwan, A.F. (1993). Macroscopic study of the female genital system in Iraqi goats. Small. Rum. Res. 9: 341-346.

4-Noseir, M.B., Kassem, M. Mand EL-Amrawy (1987). Ovario-hysterectomy in different stages qf gestation, Assiut.Vet.Med.J.18(36):172-175.

5- Balasundaram, K., (2018).Histomorphology of pancreas in Goats. Journal of pharmacognosy and Phytochemistry. 1711-1713.

6- Luna L. G. (1968).Manual of histological staining methods of armed forces institute of pathology. 3rd edn. The blakistan Division Mc-Graw Hill Book Company, New Yark.

7- Malik MR, Prem Prakash (1972) Comparative histology of pancreas of buffalo and ox, A note. Ind J Ani Sc 42(9): 681-682.

8-Monroe CW, Spector B (1963) Tannic acid iron haematoxylene, Alcian blue and basic fuchsin for staining islets, reticular fibres of the pancreas. Stain Technol 38: 187-192.

9- Banks WJ (1993). Applied veterinary histology. (3rd edn), Mosby- Year Book Inc, USA, pp. 371-373.

10- Mahesh, Gadre KM, Ashok P., Girish HM., Sharanagouda and Annapurana Awati (2017).Comparative Histomorphology of Endocrine Pancreas in Deccani Sheep and Bidri Goat .MOJ Anat & Physiol 3(1): 00079.

11- BALTAZAR E.T., KITAMURA N., HONDO E., YAMADA J., MAALA C.P., SIMBORIO L.T.(1998). Immunohistochemical study of en- docrine cells in the gastrointestinal tract of the Philippine carabao (*Bubalus bubalis*). Anat. Histol. Embryol., 27(6): 407-411.

12- Mukherjee G, Singh LP, Barnwal AK, Sharan A (1988) Endocrine pancreas of sheep. Ind J Ani Sc 58(1): 91-93.

13- Ladukar ON, Pandit RV (1992) Identification of cells of islets of Langerhan's of buffalo Pancreas by Bielschowsky's Silver lmpregnation Method. Cherion 21(2): 56-59.

14- Helmstaedter,V., Feurle, G. E. and Frossmann, W. G. (1976). Insulin, glucagon and somatostatin- immunoreactive endocrine cells in the equine pancreas. Cell and Tissue Research. 172: 447-454.

15- Dhoolappa, M., Pawar Ashok, Ramakarishna, V and Gadre, K. M. (2004). Gross and histomorphology of pancreas in donkey. Indian Journal of Animal Science. 74(9): 926-929.

16- Kocamis H, Karadag Sari E, Nazli M, Gulmez N, Aslan S, Deprem T.(2009). Immunohistochemical distribution of insulin-, glucagon-, and somatostatin-containing cells in the pancreas of the rat (Wistar albino). Kafkas Univ Vet Fak Derg; 15: 611–614.

17- Cabrera O, Berman M, Kenyon NS, Ricordi C, Berggren P, Caicedo A. (2006). The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. P Natl Acad Sci USA; 103: 2334–2339.

18- Elayat AA, El-Naggar MM, Tahir M.(1995).An immunocytochemical and morphometric study of the rat pancreatic islets. J Anat; 186: 629–637.

19- WIECZOREK G., POSPISCHIL A., PERENTES E.A.(1998). Comparativeimmunohisto-chemical study of pancreatic islets in laboratory animals(rats, dogs, minipigs, nonhuman primates). Exp. Toxicol. Pathol., 50(3): 151-172.

20- REEDY S.N., BIBBY N.J., FISHER S.L., ELLIOTT R.B.(1986). Immunolocalization of insulin, glucagon, pancreatic polypeptide and somatostatin in the pancreatic islets of the possum, Trichosorus vulpecula. Gen. Comp. Endocrinol., 64(1): 157-162.

21-HSU W.H., CRUMP M.H.,(1989).The endocrine pancreas. In: Mcdonald, L.E. and Pineda MH (ed), Veterinary endocrinology and reproduction,pp. Lea & Febiger, Philadelphia. 186-201.

22- SASAKI M., ARAI T., USUI T., OKI Y.(1991). Immunohistochemical,ultrastructural, and hormonal studies on the endocrine pancreas of voles (Microtus arvalis) with monosodium aspartate-induced diabetes. Vet. Pathol., 28(6): 497-505.

23- Copenhaver, W. M. and R. P. Duglus (1975). Bailey's Textbook of Histology.16th Edn. The Wilhams and Wilkins Co. : 466-473.