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Histological Study on the Prenatal Development of the Pancreas in Camel (*Camelus dromedarius*) with some Emphasis on β- Cells Immunohistochemically

Estudio Histológico del Desarrollo Prenatal del Páncreas en Camellos (*Camelus dromedarius*), con Énfasis en la Inmunohistoquímica de las Células β

Abdelhay M. Ali[1] & Abdelrahman M.A. Elseory[1]

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SUMMARY: This study investigated the histogenesis of the pancreas, with a focus on β-cells differentiation and distribution in the dromedary camel fetus. Twenty-seven fetuses at different stages of gestation were used to examine their pancreas using both histological and immunohistochemical techniques. The findings indicated that during the first trimester, the pancreatic tissue of the fetus featured tubular formations of epithelial cells with individual endocrine cells. By the end of this phase, acinar structures, ducts, and more endocrine cells appeared. In this period, β-cells appeared individually or in small clusters. As gestation progressed into the second trimester, the pancreas developed more acinar units, irregular lobes, and lobules. A distinct capsule also appeared. Towards the end of this stage, fat cells, ganglia, blood vessels, nerve fibers, interlobular ducts, and small pancreatic islets (islets of Langerhans) were observed. β-cells proliferated in the tubules, ducts, and smaller pancreatic islets. In the third trimester, a well-developed capsule and septa were observed. The pancreatic parenchyma showed numerous acini in densely packed lobes. Pancreatic islets were tiny and expanded, with fewer individual endocrine cells among the parenchyma. The β-cells are grouped into many small and a few larger pancreatic islets. In conclusion, the embryo's pancreatic structure in dromedary camels evolves from tubular formations in early gestation to compact lobes and lobules with more pancreatic islets before birth.

KEY WORDS: Dromedary camel; Fetus; Immunohistochemistry; Histology; Pancreas.

INTRODUCTION

Much research has focused on the biology of the pancreas in an effort to develop better therapies for disorders such as pancreatitis, diabetes mellitus, and pancreatic cancer. The pancreas is composed of exocrine cells, which release digestive enzymes into the digestive tract, and endocrine cells, which secrete hormones into the bloodstream. Developmentally, the organ arises from two separate buds (dorsal and ventral) that originate from the distal foregut endoderm and later fuse. The dorsal bud appears first and gives rise to most of the pancreatic tissue. Structurally, the exocrine component consists of acinar cells and ducts, while the endocrine component is organized into single endocrine cells and clusters known as the pancreatic islets (islets of Langerhans) (Wessells & Cohen, 1967; Slack, 1995; St-Onge & Wagner, 2006).

The endocrine pancreas comprises four principal cell types: β-cells, which secrete insulin; a-cells, which produce glucagon; d-cells, which secrete somatostatin; and PP-cells, which produce pancreatic polypeptide. These hormones play crucial roles in carbohydrate and lipid metabolism, particularly in maintaining blood glucose homeostasis. In addition, the pancreas produces several peptide hormones, including somatostatin and pancreatic polypeptide, which further regulate digestive and metabolic processes (Bonner-Weir & Orci, 1982; Reddy & Elliott, 1985).

The development of the fetal pancreas has been studied in several animal species using morphological and immunohistological approaches (Conklin, 1962; Spooner *et al*., 1970; Laitio *et al*., 1974; Clark & Grant, 1983; D'Agostino

[1] Department of Anatomy, College of Veterinary Medicine, King Faisal University, Al-Ahsa, Saudi Arabia.
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et al.,1985; Field, & Frazier 1985; Slack, 1995; Böck *et al*., 1997; Bouwens *et al*., 1997; Gupta *et al*., 2002; Piper *et al*., 2004; Carlsson *et al*., 2010; Merkwitz *et al*., 2011; Krivova *et al*., 2012; Jennings *et al*., 2015; Tiwari *et al*., 2015; Gupta *et al*., 2020; Singh *et al*., 2021; Deka, 2025). Numerous investigations have also described the adult pancreas of the dromedary camel (Adeghate, 1997; Al-Ajlan & Bailey, 2000; Baragob *et al*., 2011; Elamin *et al*., 2014; Hafez *et al*., 2015; Althnaian *et al*., 2019; Al Khodair & Elseory, 2025), with some studies addressing its endocrine component (Hafez *et al*., 2015; Zghair, 2016). However, research on the prenatal development of the camel pancreas remains scarce (Mohammed *et al*., 2019; Zolain & Babiker, 2023), likely due to the limited availability of fetal material.

Therefore, the present study was undertaken to provide a histological investigation of the prenatal development of the pancreas in dromedary camel fetuses, with a particular emphasis on the distribution and differentiation of b-cells using immunohistochemical techniques.

MATERIAL AND METHOD

Animals and sampling

Specimens of the pancreas were collected from 27 healthy dromedary camel fetuses of both sexes and various ages. The fetal curved-crown-rump lengths (CVR) ranged from 8 to 115 cm, which was estimated to be from 87 to 390 days of gestation. All specimens were obtained from Omran slaughterhouse, Al-Asha, Saudi Arabia.

The fetuses were divided into three groups according to age: first trimester (0–130 days), second trimester (131–260 days), and third trimester (261–423.5 days). The age of each fetus was determined using the equation GA = (CVRL + 23.99)/0.366, where GA (gestational age) and CVRL (crown-vertebral-rump length) are both expressed in centimeters (cm) (Elwishy *et al*., 1981).

All procedures were approved by the King Faisal University Research Ethics Approval Committee (KFU-2025-ETHIC 3574).

Histological methods

For the histological study, samples of the pancreas were collected from 27 camel fetuses. They were cut into slices for proper fixation, then fixed in 10 % buffered formalin. The tissues were processed routinely and embedded in paraffin wax, as described by Bancroft & Stevens (1990). A rotary microtome was used to cut sections about five µm thick. Haematoxylin and eosin (H&E) and trichrome stains were used for general histology. The slides were visualized and photos captured with a light microscope (Leica DM6000B, Germany) connected to a digital camera (Leica DFC420, Germany).

Immunohistochemical methods

For the immunohistochemistry, pancreatic tissue samples were collected and fixed overnight in 4 % paraformaldehyde. Then, they were processed by dehydration, clearing, infiltration, and embedding in paraffin. Sections 5 µm thick were cut using a microtome (Leica, Germany), mounted on SuperFrostedTM Plus charged slides, and then deparaffinized with xylene. The hydration of the sections followed the procedure, which involved dipping them for 30 seconds in alcohol with varying concentrations (100 %, 95 %, 80 %, 70 %). Using the avidin-biotin-peroxidase complex technique as described by (Adeghate *et al*., 2001), the slides were washed twice for 5 minutes each in Tris-buffered saline (1X TBS) plus 0.025 % Triton X-100 with gentle agitation for antigen retrieval. The slides were then incubated in 10 % normal serum with 1 % bovine serum albumin (BSA) in 1X TBS for two hours at room temperature to prevent nonspecific binding.

The slides were then wiped with tissue paper. Polyclonal guinea pig anti-insulin (Dako, A0564) and a mouse- and rabbit-specific HRP/DAB (ABC) detection IHC kit (Abcam, ab64264) were used to detect insulin immunoreactivity. The slides were counterstained by immersing them in Mayer's haematoxylin and washing them under tap water. After that, the sections were dehydrated by dipping them in graded alcohols (70 %, 95 %, 100 %, and 100 %), cleared with xylene, and then mounted. The negative control experiment was conducted by omitting the primary antibody from the protocol. Finally, the staining was observed by a light microscope, and photomicrographs were taken.

RESULTS

The histological observations

First trimester. The fetal pancreas at 87 days of gestation (CVRL 8cm) showed that the pancreas parenchyma was formed of branching tubular epithelial structures. These epithelial structures were surrounded by loose mesenchymal stroma and embedded in a highly vascularized matrix of mesenchymal tissue (Fig. 1A). The tubules were composed of cuboidal epithelial cells with spherical to oval-shaped nuclei. Individual endocrine cells were observed among the tubular cells (Fig. 1B).

At 98 days of gestation (CVRL 12 cm), the pancreatic parenchyma showed increased branching of the tubular epithelium (Fig. 1C).

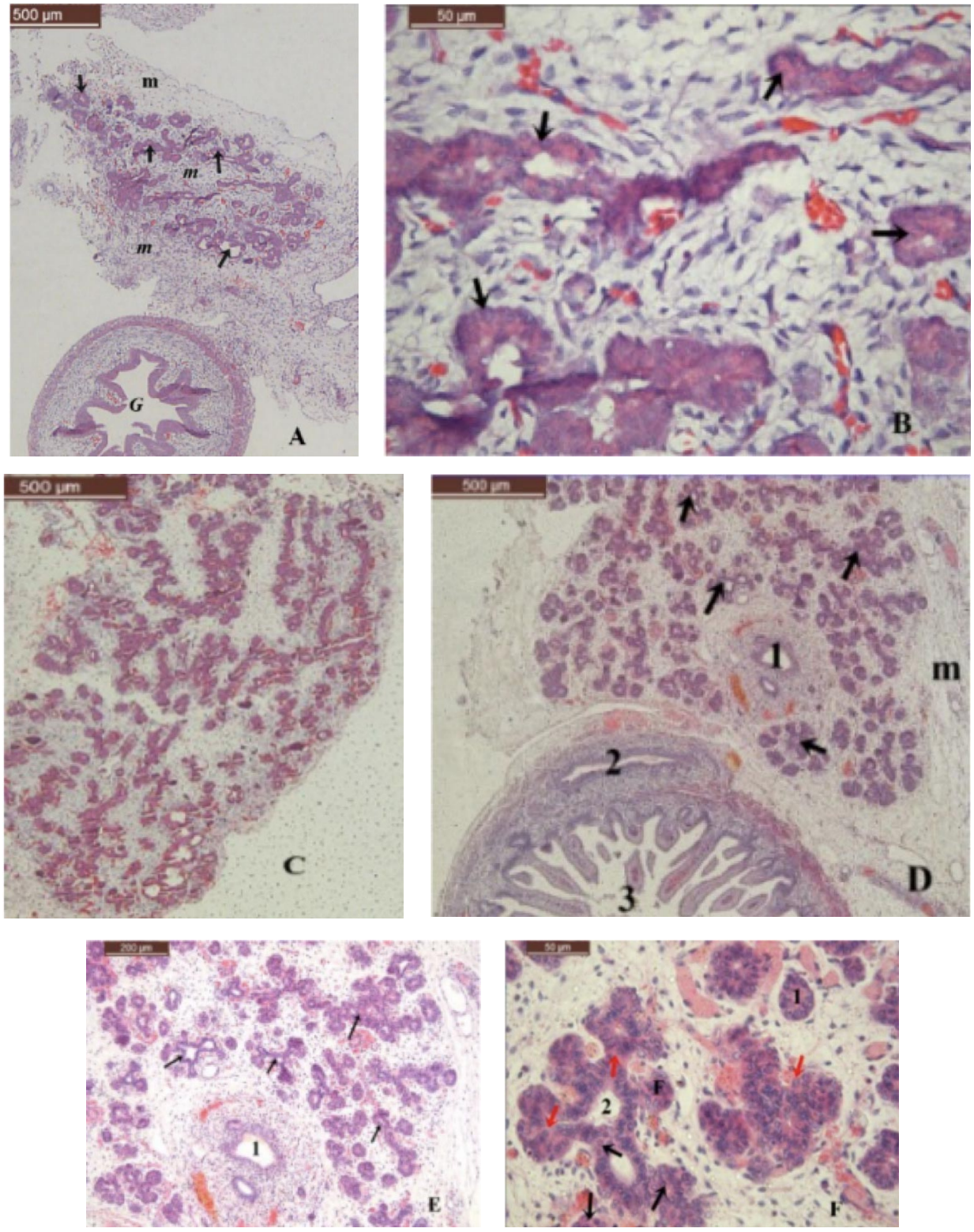


Fig. 1. Photomicrograph of sections of the fetal pancreas (first trimester). A, at 80 d. (CVRL 8 cm), showing branching tubular epithelium (arrows), mesenchyme (m), and the gut (G). B, individual endocrine cells within the tubular cells (arrows). C, at 98 d. (CVRL 12 cm), the tubular epithelium increased in branching. D & E, at 125d. (CVRL 22 cm), showing the budding of masses of cells in the form of tubulo-acinar complexes (arrows), mesenchyme (m), 1, sizeable pancreatic duct; 2, hepatopancreatic ampulla. F, small clusters of endocrine cells (black arrows), single endocrine cells (red arrows), 1, acinus; 2, tubule-acinar complex. H&E stain.

With advanced age of gestation (125 days, CVRL 22cm), the pancreatic parenchyma showed budding of masses of cells from the tubules in the form of the tubulo-acinar complex. The buds were composed of stratified epithelium. Some of these groups of cells detached from the tubules as small acini units with a narrow lumen or as some solid masses of cells (Figs. 1D, E, F). A sizeable pancreatic duct was near the gut and lined by a simple columnar

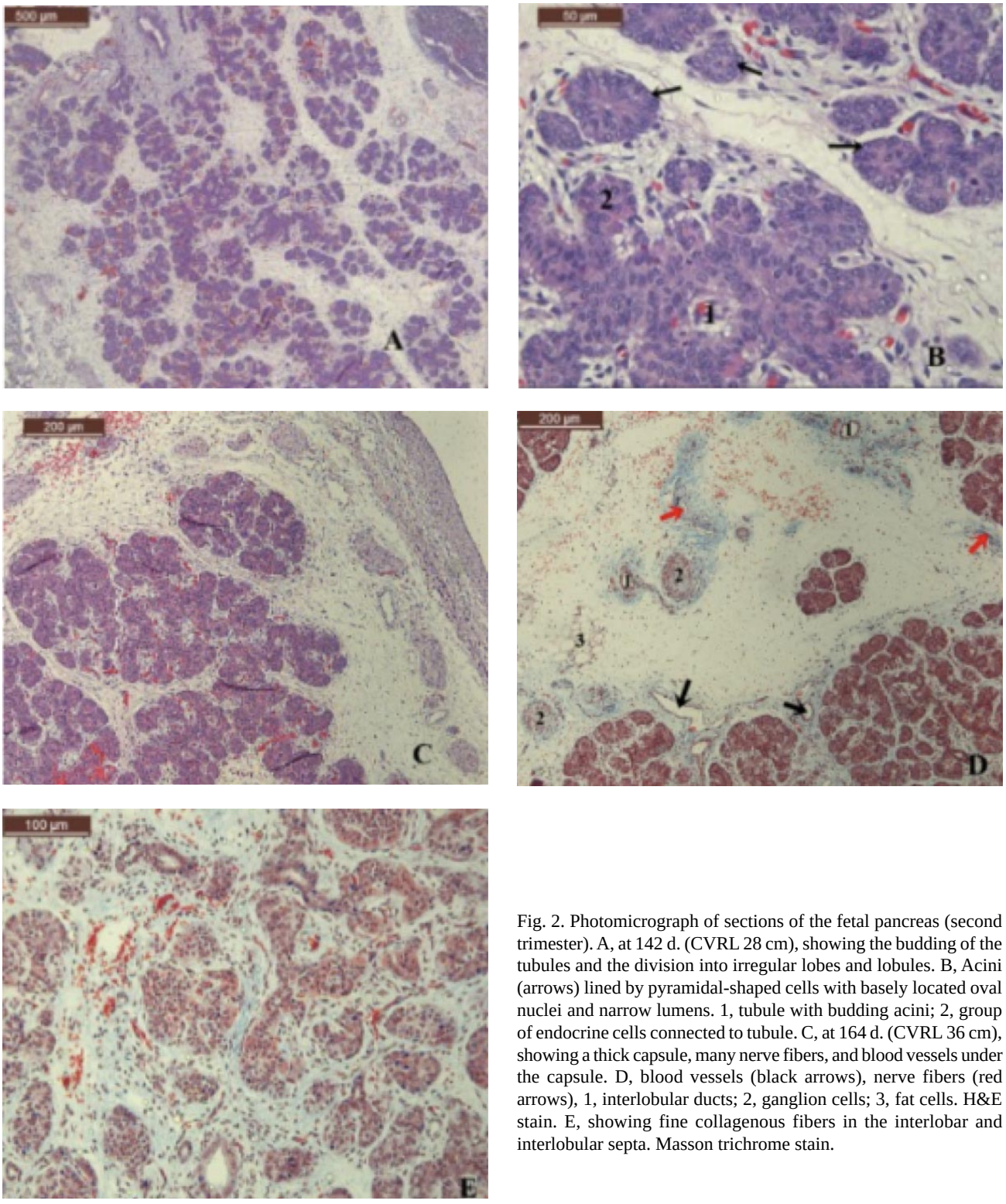
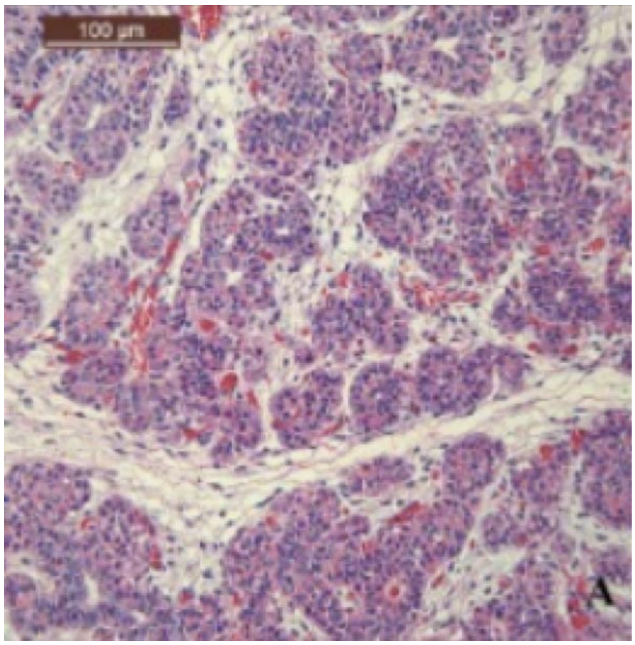


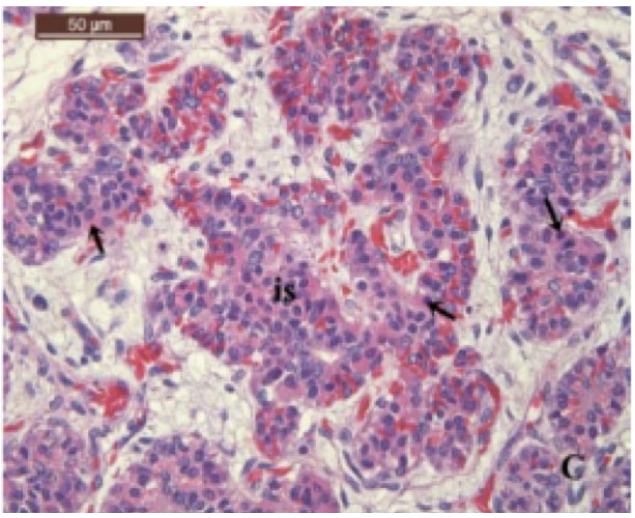
Fig. 2. Photomicrograph of sections of the fetal pancreas (second trimester). A, at 142 d. (CVRL 28 cm), showing the budding of the tubules and the division into irregular lobes and lobules. B, Acini (arrows) lined by pyramidal-shaped cells with basely located oval nuclei and narrow lumens. 1, tubule with budding acini; 2, group of endocrine cells connected to tubule. C, at 164 d. (CVRL 36 cm), showing a thick capsule, many nerve fibers, and blood vessels under the capsule. D, blood vessels (black arrows), nerve fibers (red arrows), 1, interlobular ducts; 2, ganglion cells; 3, fat cells. H&E stain. E, showing fine collagenous fibers in the interlobar and interlobular septa. Masson trichrome stain.

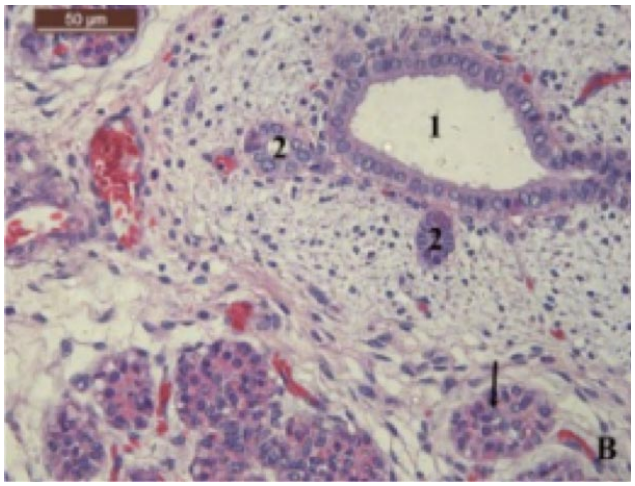
epithelium, the main pancreatic duct. A thick layer of connective tissue supported this duct. Smaller other ducts in its connective tissue were lined by cuboidal to low simple columnar epithelium. In addition, another duct was observed in contact with the gut and was lined by simple columnar epithelium, which may be the hepatopancreatic ampulla (Figs. 1D, E). At this stage, the pancreatic islets were not visible. However, many individual endocrine cells were observed, especially in the wall of the tubules or as small clusters in their contact (Fig. 1F).

Second trimester. At the age of 142 days (CVRL 28cm), the tubules increased in branching and budding, especially at the periphery of the gland. The branching tubules showed more budded acini at their peripheral end; the acinar secretory units had narrow lumens. More acini were detached from the tubules at this age. Extensive loose mesenchymal tissue septa projecting from the capsule divided the tubules and the acini into irregular lobes and lobules. In addition, there are many blood capillaries (Fig. 2A). Simple to stratified epithelium lined the tubules. The acinar cells were pyramidal with basally located oval nuclei and narrow lumens (Fig. 2B). Endocrine cells were also observed individually, especially in the epithelium of the tubules or as small groups near or connected to the tubules (Fig. 2B).

At the age of 164 days (CVRL 36 cm), the capsule became thicker, and the connective tissue septa divided the







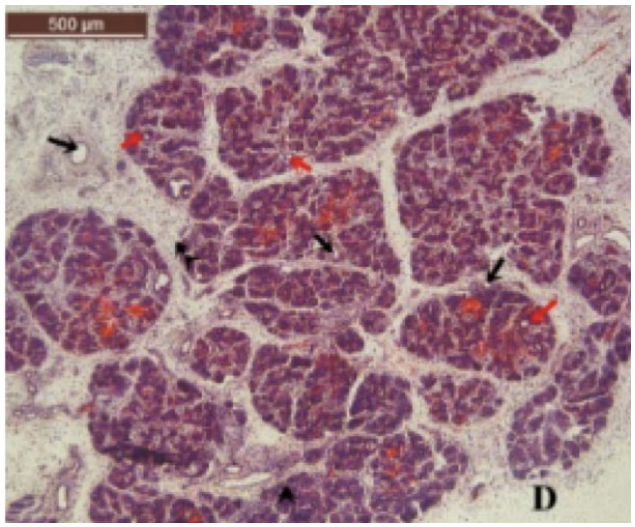


Fig. 3. Photomicrograph of sections of the fetal pancreas (second trimester). A, at 180 d. (CVRL 42 cm), showing many small acinar secretory units with central narrow lumens and fewer tubulo-acinar structures. B, showing 1, large pancreatic duct; 2, small ducts; centroacinar cells (arrow). C, showing a small pancreatic islet (is), single endocrine cells (arrows). D, at 245 d. (CVRL 66 cm), showing interlobular ducts (black arrows); intralobular ducts (red arrows). H&E stain.

parenchyma into more regular lobes and lobules of variable sizes. The acini increased in number, and a few interlobular ducts, as well as many nerve fibers and blood vessels, were observed, particularly under the capsule (Fig. 2C). Additionally, fine collagenous fibers, nerve fibers, and blood vessels were detected in the interlobar and interlobular septa, and more extensively around the interlobular ducts. Moreover, ganglion and fat cells were also observed between the interlobular septa (Figs. 2D, E). The endocrine cells were still present as individual cells or in small groups associated with tubules and ducts. However, some small pancreatic islets were detected.

At 180 days (CVRL 42 cm), as in the previous age, the pancreas was covered by a well-developed capsule containing connective tissue fibers and blood vessels. The connective tissue septa were rich in blood vessels and divided the parenchyma into larger lobes and lobules. The lobules contained more acinar secretory units, composed of pyramidal epithelial cells with a central narrow lumen (Fig. 3A). The acinar cells have spherical or oval basally located nuclei and centroacinar cells. The intralobular ducts were lined by low cuboidal cells, and the interlobular ducts were lined by cuboidal cells. The large pancreatic duct was lined by simple columnar epithelium. The branches in its vicinity were lined by cuboidal epithelium (Fig. 3B). Individual cells or small groups and some pancreatic islets represented the endocrine portion (Fig. 3C).

At the age of 245 days (CVRL 66 cm), the exocrine acinar portion became more evident. As lobules of various sizes increased, they were separated by connective tissue septa extending between the secretory units. Additionally, intralobular, interlobular, and blood vessels increased (Fig. 3D).

Third trimester. Between 297 and 389 days (CVRL 85–115 cm), the exocrine portion of the pancreas developed a thick connective capsule from which robust septa extended, dividing the organ into large, variably sized lobes. Each lobe contained compact lobules of acini (Fig. 4A), while the connective tissue septa were rich in blood vessels and adipose tissue. The duct system included both intralobular and interlobular ducts. During this phase, the number of Pancreatic islets increased, with fewer individual endocrine cells being scattered among the pancreatic parenchyma. Most islets were small, with a few large ones present (Fig. 4B).

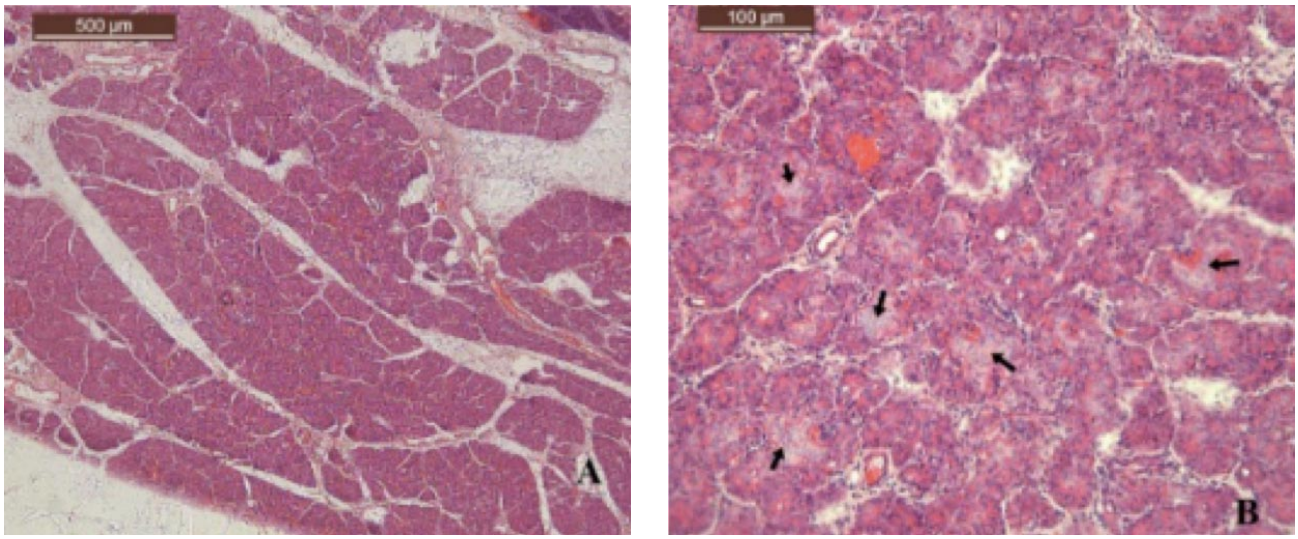


Fig. 4. Photomicrograph of sections of the fetal pancreas (third trimester). A, at 333d. (CVRL 98 cm), showing the pancreatic large lobe and lobules consisting of compact acini. B, at 379 d. (CVRL 115 cm) showing an increased number of the pancreaticislets (arrows). H&E stain.

Immunohistochemical observations

During the first trimester (125 days, CVRL 22 cm), immunoreactive insulin cells (β-cells) first appeared as single cells in the wall of the tubules, or as small groups in contact (Fig. 5A), marking the initial stage of their development.

In the second trimester at 142 days (CVRL 28 cm), β-cells appeared as single cells or small clusters in the tubule walls and in the duct walls in the septa (Fig. 5B). By 164 days of gestation (CVRL 36 cm), the number of b-cells increased. They appeared as single cells, clusters, and small pancreatic islets (Fig. 5C). The pancreatic islets became more numerous toward the end of this phase (Fig. 5D).

During the third trimester (297-389 days, CVRL 85-115 cm), immunoreactive insulin cells (β-cells) were grouped in many small and a few large pancreatic islets; in contrast, fewer β-cells appeared individually scattered in the parenchyma or ducts of the pancreas (Fig. 5E).

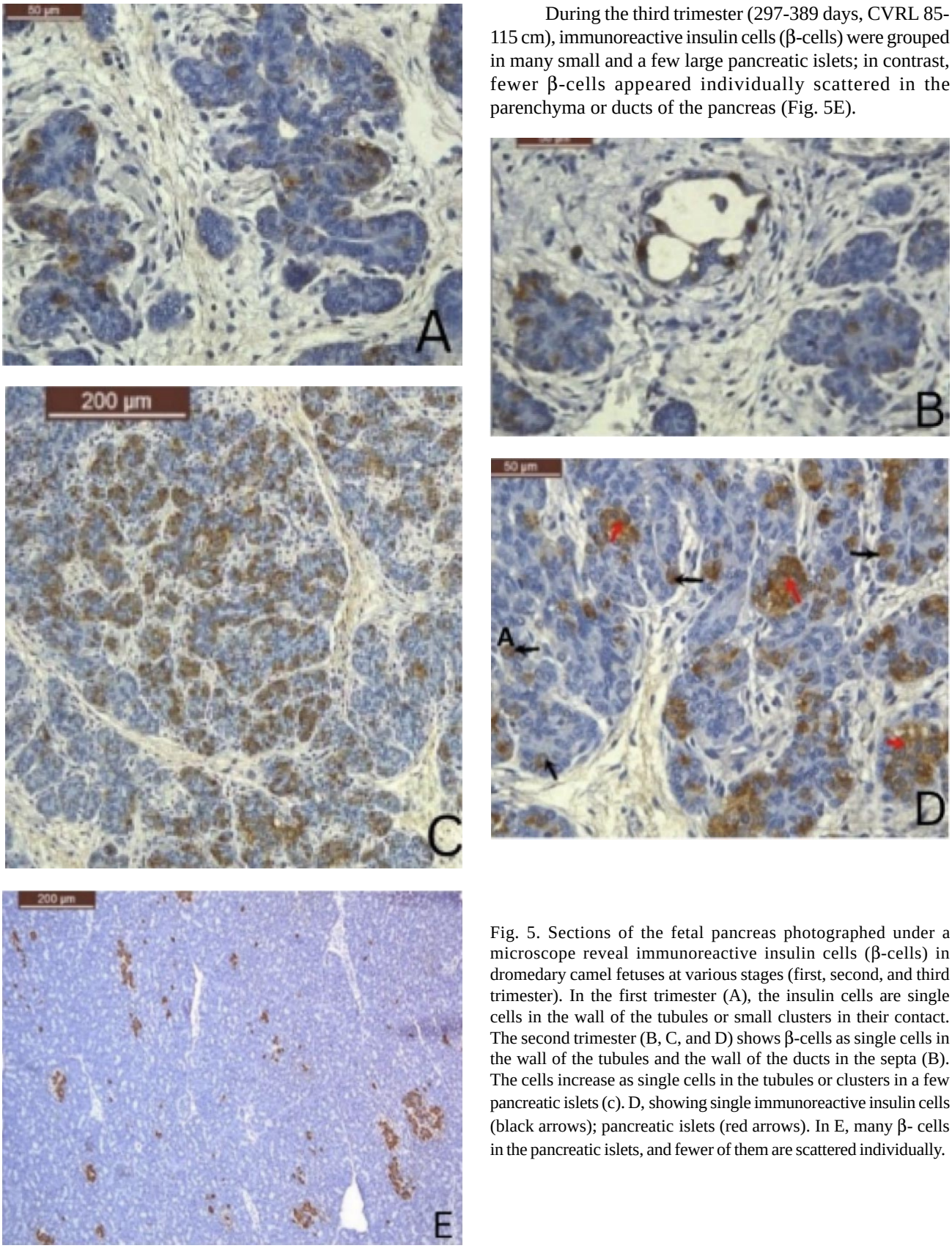


Fig. 5. Sections of the fetal pancreas photographed under a microscope reveal immunoreactive insulin cells (β-cells) in dromedary camel fetuses at various stages (first, second, and third trimester). In the first trimester (A), the insulin cells are single cells in the wall of the tubules or small clusters in their contact. The second trimester (B, C, and D) shows β-cells as single cells in the wall of the tubules and the wall of the ducts in the septa (B). The cells increase as single cells in the tubules or clusters in a few pancreatic islets (c). D, showing single immunoreactive insulin cells (black arrows); pancreatic islets (red arrows). In E, many β- cells in the pancreatic islets, and fewer of them are scattered individually.

DISCUSSION

The present study showed the histological development of the pancreas in the dromedary camel fetuses. In the first trimester, at 87 days of gestation, the pancreas consists of tubular epithelial structures embedded in and surrounded by mesenchymal connective tissue. Furthermore, it was revealed that the endocrine cells were distinct cells embedded in the tubular epithelium. Similar results were observed in human fetuses at 52 days (Krivova *et al*., 2012), in goats at 60 days (Sreeranjini *et al*., 2015; Sreeranjini & Ashok, 2016), in buffalo at 65 days (Singh & Sethi 2012; Gupta *et al*., 2020), and in rats at 15 days (40) (Hisaoka *et al*., 1992), of gestation. Previously, it has been reported that pancreas organogenesis requires trophic signals from the embryonic mesenchyme (Slack, 1995; Landsman *et al*., 2011). At 98 to 125 days, the pancreatic parenchyma exhibited buds of masses of cells from the tubules, in the form of tubular-acinar complexes, small acini, or solid cell clusters. There was also a sizeable pancreatic duct in the pancreas near the gut lined by simple columnar epithelium, which is the main pancreatic duct. A thick connective tissue layer supported this duct, and smaller other ducts were present within its connective tissue, lined by cuboidal to low simple columnar epithelium. The individual endocrine cells within the wall of the tubules increased in number, and some endocrine cells formed clusters in close contact with the tubules. The pancreatic islets were not observed. Similar to this, other studies conducted on human fetuses (Robb, 1961), bovine fetuses (Merkwitz *et al*., 2011), and buffalo fetuses (Gupta *et al*., 2020) at 52–62 days of age reported that endocrine cells were dispersed among the growing acinar cells or tubular walls. In contrast, Mohammed *et al*. (2019), reported that only the acini, connective tissue, and blood vessels were visible in the first trimester of the camel fetal pancreas in their morphometric study.

In the present study, tubule branching and budding increased significantly at 142 days in the second trimester, and there were more acinar secretory units than in the first trimester. Additionally, irregular lobes and lobules were seen in the pancreatic parenchyma. A similar division was observed in the fetal pancreas of other mammals, such as buffalo, at 125 days (Singh & Sethi, 2011), while humans showed it at 84 days (Mclean, 1979). As in the first trimester, endocrine cells were still observed as single cells in the tubular epithelium or as small clusters. Nevertheless, the pancreatic islets were still not observed. Comparable results were noted in the camel fetal pancreas in the same trimester (Zolain & Babiker, 2023) and in humans at 11–12 weeks of gestation by Krivova *et al*. (2012). In the present study, at 164 days, the capsule became thicker, and a few interlobular ducts, as well as numerous nerve fibers and blood vessels, were observed, especially under the capsule. Fine collagenous fibers, nerve fibers, and blood vessels were detected in the interlober and interlobular septa and more extensively around the interlobular ducts. Moreover, ganglion and fat cells were also observed between the interlobular septa. Equivalent findings were noted in the goat pancreas, where blood vessels, ducts, nerve bundles, and ganglia are seen in the interlobular septa (Sreeranjini & Ashok, 2016).

Meanwhile, Krivova *et al*. (2012), documented that endocrine cells and neurons are closely integrated in the developing human pancreas. They added that appropriate islet morphogenesis requires the existence of neurons and nerve fibers. Furthermore, this study demonstrated that endocrine cells remain in single or tiny clusters. Nonetheless, a few tiny pancreatic islets were found. These results are consistent with those previously reported by Krivova *et al*. (2012), who noted that the endocrine cells in the human developing pancreas appeared spatially as single cells or in small clusters in early fetuses (8–11 weeks), bipolar islets (after 15–16 weeks), and adult-typical islets (after 25–27 weeks).

The pancreatic capsule was fully formed around 180–245 days at the end of the second trimester, and the size and quantity of lobes and lobules grew. In the meantime, the number of pancreatic islets and acini increased. Our findings were validated by Zolain & Babiker (2023) and Mohammed *et al*. (2019), who noted a rise in acini and the first detection of the ducts and pancreatic islets in camel fetuses during this trimester. The pancreatic parenchyma of goats between the ages of 91 and 120 days of development also displayed distinct lobules, acini, and islets of different sizes (Sreeranjini & Ashok, 2016).

According to this study, the camel embryo's pancreas at 297–389 days in the third trimester had a thick capsule from which the septa separated into lobes of different sizes. Each lobe contained numerous compact lobules and numerous acini. The number of individual endocrine cells dispersed throughout the pancreatic parenchyma decreased, whereas the number of Pancreatic islets rose. There were a few large islets and many little ones. This form aligned with the results of Zolain & Babiker (2023) and Mohammed *et al*. (2019), on the fetal pancreas of camels and Gupta *et al*. (2020) in buffalo.

In the present study, during the first trimester, immunoreactive insulin cells were found as single cells in the wall of the tubules or in small groups in contact. These results have also been reported in the fetal pancreas of bovines (Bonner-Weir & Like, 1980) and buffaloes (Lucini

et al., 1998) at 60 days of gestation, as well as in human fetuses at 12 weeks (Gupta *et al*., 2018). Additionally, it was noted that the human embryonic pancreas's singular insulin-positive endocrine cells were the most abundant during the first trimester (Clark & Grant, 1983; Piper *et al*., 2004).

In the current study, β-cells increased in the second trimester of the developing pancreas, appearing as single cells in the wall of the tubules or small clusters in contact with the wall of the ducts in the interlobular septa. In addition, they are found in small pancreatic islets. Whereas, in the third trimester, the immunoreactive insulin cells became mainly aggregated in an increased number of small and a few large pancreatic islets. However, fewer β-cells were found scattered individually in the parenchyma or the ducts of the pancreas. D'Agostino *et al*. (1985), reported that the immunoreactivity of insulin in the fetal pancreas of bovines increased progressively during gestation, reaching up to 7-fold in the third trimester, higher than that in the adult. Moreover, Reddy and Elliott (1985) stated that most endocrine cells in the bovine fetus were insulin and glucagon cells from day 100 of gestation to term. Similarly, the predominance of insulin and glucagon endocrine cells was observed by Reddy *et al*. (1988), in the ovine fetal pancreas. Gupta *et al*. (2018), stated that the endocrine cells were initially a small number of cells at 12 weeks, then as groups of cells from 19 weeks, and as islets at 30-40 weeks of gestation.

CONCLUSION

In conclusion, the pancreas of the dromedary camel contains tubular structures with endocrine cells throughout the first trimester. By the second trimester, acinar units, lobes, and lobules begin to form, along with a thickening capsule and an increase in endocrine cells. The exocrine portion of the pancreas grows in size at the end of the second trimester. During the third trimester, the pancreas features a thick capsule from which substantial septa partition the organ into large lobes of varying sizes. Each lobe consists of dense lobules, and there is a greater presence of pancreatic islets and insulin-producing cells. Therefore, the fetal pancreas displays significant developmental features during the later stages of pregnancy. β-cells commence insulin production earlier, and the synthesis of this hormone escalates throughout pregnancy.

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ALI, A. M. & ELSEORY, A. M. A. Estudio histológico del desarrollo prenatal del páncreas en camellos (*Camelus dromedarius*), con especial énfasis en la inmunohistoquímica de las células β. *Int. J. Morphol., 44(1)*:211-220, 2026.

RESUMEN: Este estudio investigó la histogénesis del páncreas, centrándose en la diferenciación y distribución de las células β en fetos de camellos dromedarios. Se utilizaron veintisiete fetos en diferentes etapas de la gestación para examinar su páncreas mediante técnicas histológicas e inmunohistoquímicas. Los hallazgos indicaron que, durante el primer trimestre, el tejido pancreático del feto presentaba formaciones tubulares de células epiteliales con células endocrinas individuales. Al final de esta fase, aparecieron estructuras acinares, conductos y más células endocrinas. En este período, las células β aparecieron individualmente o en pequeños grupos. A medida que la gestación avanzaba hacia el segundo trimestre, el páncreas desarrolló más unidades acinares, lóbulos irregulares y lobulillos. También apareció una cápsula diferenciada. Hacia el final de esta etapa, se observaron células grasas, ganglios, vasos sanguíneos, fibras nerviosas, conductos interlobulillares y pequeños islotes pancreáticos. Las células β proliferaron en los túbulos, conductos e islotes pancreáticos más pequeños. En el tercer trimestre, se observaron una cápsula y septos bien desarrollados. El parénquima pancreático mostró numerosos acinos en lóbulos densamente agrupados. Los islotes pancreáticos eran diminutos y expandidos, con menos células endocrinas individuales en el parénquima. Las células β se agrupan en muchos islotes pancreáticos pequeños y algunos más grandes. En conclusión, la estructura pancreática del embrión en los camellos dromedarios evoluciona desde formaciones tubulares al inicio de la gestación hasta lóbulos y lobulillos compactos con más islotes pancreáticos antes del nacimiento.

PALABRAS CLAVE: Camello dromedario; Feto; Inmunohistoquímica; Histología; Páncreas.

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Corresponding author:
Abdelrahman M. A. Elseory
Department of Anatomy
College of Veterinary Medicine
King Faisal University
Al-Ahsa 31982

SAUDI ARABIA

E-mail: amamohamed@kfu.edu.sa