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Artigo Original

Structure of the stomach in the canine neonate

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A B S T R A C T

The stomach in the dogs is the key point among the organs of the digestive system that performs secretory and motor functions. Thus, our aim was to determine the morphology of the stomach tissue in the canine neonate. Forty-five canine neonates were obtained from females with dystocic parturitions or from pregnant dogs with problems at the end of the gestational stage, who had problems during parturition and / or cesarean section whose puppies had died. The neonate morphologic analysis was based on data obtained by scanning electron microscopy and common optical microscopy from classical histological techniques. The stomach is divided into four regions in which the abundance of folds is variable in each portion of the organ. The gastric mucosa presents invaginations that form the glandular epithelium and undifferentiated cells. The abundance of folds more and less developed, was observed depending on the region of the stomach. It was also observed at gastric mucosa the invaginations in the tunic to form the glandular epithelium and undifferentiated cells. The data presented in this paper, strongly suggest that the stomach of the canine neonate presents immature morphological characteristics, explaining the reduced functionality of the organ of newborn dogs in relation to the adults.

R E S U M O

O estômago nos cães é o ponto chave entre os órgãos do sistema digestivo, que desempenha funções secretoras e motoras. Assim, nosso objetivo foi determinar a morfologia do tecido estomacal no neonato canino. Quarenta e cinco neonatos caninos foram obtidos de fêmeas com partos distócicos ou de cadelas prenhes com problemas no final da fase gestacional, que tiveram problemas durante o parto e/ou cesariana cujos filhotes haviam morrido. A análise morfológica do neonato foi baseada em dados obtidos por microscopia eletrônica de varredura e microscopia óptica comum a partir de técnicas histológicas clássicas. O estômago é dividido em quatro regiões em que a abundância de dobras é variável em cada porção do órgão. A mucosa gástrica apresenta invaginações que formam o epitélio glandular e células indiferenciadas. A abundância de dobras mais e menos desenvolvidas, foi observada dependendo da região do estômago. Também foram observadas na mucosa gástrica as invaginações na túnica para formar o epitélio glandular e células indiferenciadas. Os dados apresentados neste trabalho, sugerem fortemente que o estômago do neonato canino apresenta características morfológicas imaturas, explicando a reduzida funcionalidade do órgão de cães recém-nascidos em relação aos adultos.

K E Y W O R D S
Canis familiaris, Gastric Mucosa, Newborn, Scanning Electron Microscopy.
P A L A V R A S - C H A V E
Canis familiaris, Mucosa Gástrica, Nascimento, Microscopia eletrônica de varredura

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INTRODUCTION

In dogs and cats, the stomach is the key point among the organs of the digestive system, wich performs secretory and motor functions during the process, as the maintenance of glycemia, once these animals do not have sufficient reserves in the body. One of these periods comprises up to the second week of life, in which we call the neonatal phase (GRUNDY, 2006; HOSKINS, 2001).

Neonatology is the science that studies the neonatal period from the postnatal phase to the development of some resistance characteristics (CAVALCANTE et al., 2015; GRUNDY, 2006; MUNNICH, 2008). In this phase, special care is needed because it has a neurological, immunological and behavioral evolution that is still immature (PETERSON, KUTZLER, 2010). In dogs, this period is characterized by a high mortality rate, approximately 17 to 30% of newborns die until the 15th day of birth (CRESPILHO et al., 2007; MILA, GUERARD & RAYMOND-LETRON, 2021).

One of the first steps to become familiar with animal species is the study of microscope morphology, since it is possible to establish relationships that allow the comprehend of the evolution of that particular species, in addition to supporting applied studies (KERSWELL et al., 2009). Morphological research offers relevant information on the composition and structure of tissues, complementing anatomical, physiological and embryological findings [CONTO et al., 2010, DEREN, 1971; DYCE, WENSING, SACK, 2010; WEINMANN, 1942). Because it allows establishing direct relationships between several diseases and the prenatal period (PETERSON, KUTZLER, 2010). There are few studies about neonate canine digestive tract (CHANDRASOMA, DEMEESTER, 2010). Though, about 30% of puppies die during this period, mainly with pathologies related to neonatal triad (hypoglycemia, hypothermia and dehydration) (MACINTIRE, 2008; MÜNNICH, KÜCHENMEISTER, 2014).

Taking into account that the stomach is a fundamental organ in the development and survival of animals and considering that data related to the microscopic structure of the stomach of newborn canines is scarce, the objective of this study was to analyze the histological and ultrastructural aspects of the stomach of dogs newborns.

MATERIALS AND METHODS

This study was conducted at the Federal University of Piaui (UFPI), in the Health Science Center, Morphology Department in collaboration with Federal University of Rio de Janeiro (UFRJ) in the Histology Laboratory of the biomedical sciences institute. Forty-five neonatal dogs (*Canis familiaris*), mixed breed, were obtained from private veterinary clinics in Teresina-Piauí and at the Veterinary Hospital of UFPI. The animals were obtained from females with dystocia or pregnant, at the end of gestational stage, who had problems during delivery and/or cesarean section whose puppies died. This research is approved by the institution's Animal Research Ethics Committee

These animals were sent to the Research Laboratory in Morphological Sciences (UFPI) to be washed with saline solution, then dissected and the stomach removed. Stomach fragments 0.5 cm thick were obtained from the four portions of the organ (cardia, fundus, body and pylorus) and immediately immersed in a 10% formaldehyde solution buffered (pH around 7.0) and fixed for 48 hours. After fixation, the fragments were prepared by routine histological process, sectioned and stained with hematoxylin and eosin. The slides obtained were analyzed using a light microscope (Leica Mycrosistems DM 400, Germany) and photomicrography using a digital photomicrography system (Nikon® Eclipse E200, Japan).

Three canine neonates were perfused with 4% paraformaldehyde, 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) through the carotid arteries. After perfusion, 0.3 cm thick fragments were obtained from the four stomach regions and fixed in Karnovsky's Fixative Solution (1% paraformaldehyde + 3% glutaraldehyde in 0.07 M cacodylate buffer pH 7.2) and sent to the Histology Laboratory of the Institute of Biomedical Sciences (UFRJ) for processing and analysis in scanning electron microscopy. After 24 hours in the Karnovsky's fixative, these samples were post-fixed in 1% osmium tetroxide and subsequently dehydrated in increasing concentrations of acetone. Then, the samples were completely dried at a critical point in the chamber with carbon dioxide. The material was assembled in pieces of metal, sputtering coated with gold and observed in a scanning electron microscope (Topcon SM-300, Japan).

RESULTS

The neonate canine stomach is a dilated saccular tube that begins in the caudal portion of the esophagus and ends in the cranial part of the small intestine. It is located in four abdominal regions: xiphoid, left hypochondriac, left umbilical and lateral, with predominance in the left umbilical and lateral regions. The convex surface faces the caudoventral region and to the left (Figure 1). It is divided into four regions: the cardia, the fundus, the body and the pylorus. The fundus and body regions have identical microscopic structure and, therefore, only three regions are considered histologically.

Optical microscopy revealed that the canine neonate's stomach wall has all the layers of a typical tubular organ of the gastrointestinal tract (TGI): mucosa, submucosa, muscular and adventitia (Figure 1a). The mucous tunic is formed by a glandular epithelium. The layer exibed a large number of gastric folds varies according to the stomach region, that open into gastric pits, covered by simple columnar epithelium.

The cardiac region shows the mucosa with protruding folds (Figure 2a). The folds were less prominent in the body and fundic region, and in the transitional portion between the two regions there is a sudden reduction in folds (figure 2b). The pyloric region presents more developed folds that overlap the opening of the glands (Figure 2d). The grooves have continuity with the gastric glands. These are clustered in the lamina propria whose thickness varies according to the region of the stomach. The cardiac region has short, bent, branched, simple and tubular glands that release their products into relatively shallow pits. In the fundic region and in the body, the glands are tubular, larger and straight. The amount of connective tissue is reduced as the glands are closely grouped (Figure 1d). The histological

organization of the pyloric glands is similar to that of the cardiac region, but the gastric invaginations are deeper than in the other regions.

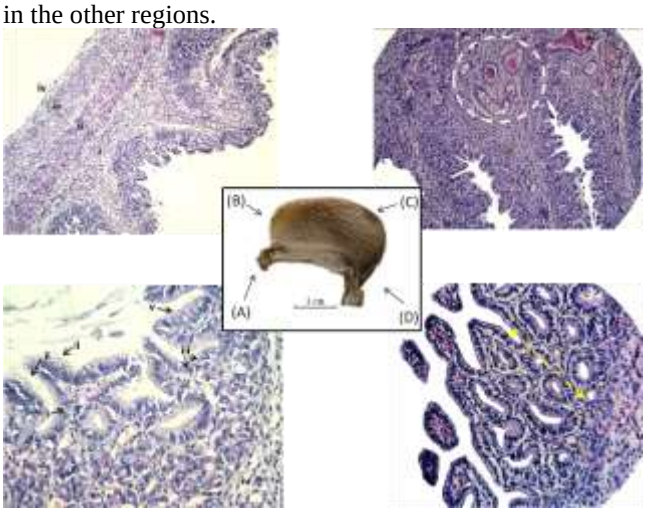

Figure 1. Sections of four transverse areas of the canine stomach of neonates with their histology. (A) The cardia region indicating: (i) suggestive stem cells in the isthmus region of the gland, (ii) surface region with suggestive proliferative activity, (iii) suggestive cells with embryonic characteristics, (iv) submucosal region, (v) suggestive stem cells mucosal surface. 400x. (B) Background region: (E) simple columnar epithelium, (Sb) submucosa, (M) Three-layered muscular tunic: (i) oblique layer of muscle fibers, (ii) circular layer of muscle fibers, (iii) muscular layer with longitudinal fibers, (S) serous layer.100x. (C) Body region with intense vascularization, (F) Gastric pit. 200x. (D) Pyloric region demarcated by the yellow arrow indicating: (G) gastric glands, (L) lamina propria. Hematoxylin-eosin (HE).

These glands are responsible for producing mucus that adheres to the epithelial surface (Figure 2). The presence of this mucous secretion is observed over the whole organ, but mostly in the region of the body (Figure 2c). In addition to the superficial mucous cells, suggestive the cells with proliferative activity and embryonic characteristics were observed in the epithelium of the cardiac region (Figure 1a). Suggestive stem cells are also found in the region of the isthmus of the gland. Among the lamina propria, the presence of the compact stratum was not observed.Under the lamina propria there is a thin layer of muscle, the mucosal muscle, formed by bundles of longitudinal, oblique, and circular fibers that invade the lamina propria and advance to the glands.

The submucosal tunic is formed by a loose connective tissue. In this layer there is the presence of several suggestive mesenchymal cells and poorly organized collagen fibers, as well as blood vessels and nerves. Stomach regions have blood supply formed until birth. However, there was variation in relation to the region. The body region is the most vascularized (Figure 1c), while the pyloric region had the smallest blood supply. The muscular tunic is formed by three layers of muscles with different fiber arrangements: oblique, circular and longitudinal (Figure 1b). Between these layers is the myenteric plexus. Externally lining the organ is the serous layer formed by simple squamous tissue.

DISCUSSION

The stomach in dogs is not fully mature at birth. Although the well-defined structure of the stomach layers, as well as its cells, is observed, some cell structures suggest that they are still undifferentiated. Full development of the organs that make up the gastrointestinal tract (TGI) is necessary to cope with changes in diet (SANGILD, FOWDEN, TRAHAIR, 2000). In preparation for this event, TGI needs to grow and mature rapidly in the weeks before birth, but its development continues in the postnatal period to expand its physiological capacities (BIRCHENOUCH et al., 2017; BUDDINGTON et al., 2003; CONTO et al., 2010; DANIEL, WANG, 1999; DEREN, 1971; EMANUILOV, MASLUIKOV, NOZDRACHE, 2018; PAULSEN, BUDDINGTON, BUDDINGTON, 2003; SANGILD, FOOWDEN, TRAHAIR, 2000).

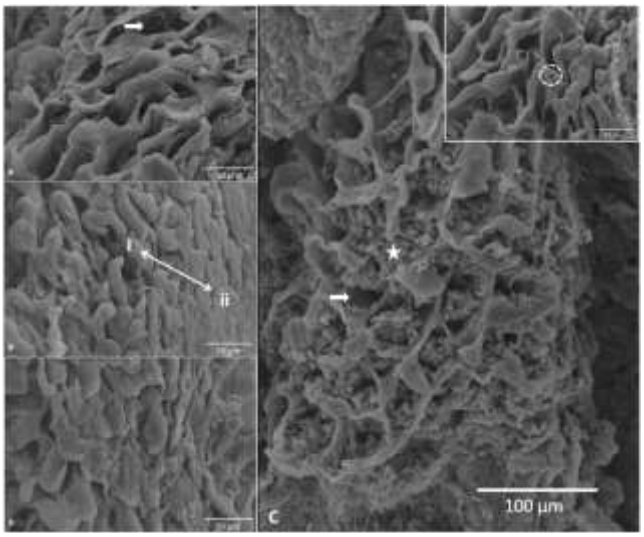

Figure 02. The four stomach regions of canine newborns indicating the appearance of these mesoscopes analyzed by Scanning Electron Microscopy (SEM). (A) The region of the cardia, with obvious folds. Note the opening of the gastric glands (arrow). (B) Background region, where the transition from one area with thicker folds more evident (i) to another with less prominent folds (ii) was observed. (C) Body region with plenty of mucus (star). The opening of the gastric glands is also observed in this region (arrow). In particular, the highlighted structure represents the orifice of the gland mucosa (circle). (D) pyloric region, with more developed folds that overlap the opening of the glands. (A, B, D) and detail 500x, (C) 150x.

Understanding the morphological findings allows interpretations of alterations in the functioning of the TGI (ARCISZEWSKI, BARABASZ, CALKA, 2008; CAVALCANTE et al., 2016; DEREN, 1971; DOOM et al., 2016; SANDERSON et al., 2017). Through changes in cellular and tissue attitudes it is possible to prepare the best biological and nutritional management that meets each phase of the neonatal period (NEAL-KLUEVER et al., 2019; SINGH et al., 2017). Delayed embryo implantation or premature delivery can lead to the late development of certain organs, such as the stomach, affecting their physiological parameters (DANIEL, WANG, 1999).

Çetin and Eşrefoğlu (2014) observed the prenatal and postnatal development of Wistar rats and identified that localization changes that occur during organogenesis promote changes that reflect morphological features such as stomach wall thickening and cell differentiation. Among the layers that form a stomach wall, the mucous and muscular tunics are those that undergo significant changes in thickness during the gestational period until birth (WALTHALL et al., 2005). In pig, the gastric mucosa is well developed before birth with prominent gastric folds and pits, as well as those reported in this research (GEORGIEVA, GEROV, 1975).

Dietary variety and hormonal factors are involved in the growth and physiology of the gastric mucosa (JAIN, SAMULESON, 2006). The interaction between epithelial and mesenchymal cells induces hormone production and growth factors that regulate epithelial cell proliferation and differentiation (ÇETIN, ESSEFOGLU, 2014; KARAN, 1998). The canine neonate's glandular mucous membrane has suggestive cells in embryonic activity that allow maturation and thickening of the epithelial layer after birth (ÇETIN, ESSEFOGLU, 2014; WALTHALL et al., 2005). The expression of retinoblastoma protein (PRb) in canine stomach mucosa is directly related to the cellular increase and renewal of epithelial regulation of gastric mucosa of canine neonates, confirming proliferative action at birth (CAVALCANTE et al., 2016; CONTO et al., 2010).

The cardia region, suggestive cell proliferation was observed on the surface, revealing stages of fetal development. The cells found in the cardia are typical of the epithelium: cells of the superficial mucosa, with protective function and lining of the stomach surface, as well as stem cells (ROS, PAWLINA, 2011). Stem cells are arranged in the isthmus of the stomach gland and when differentiated or in the process of differentiation, dependently migrate to the function that the gastric unit will perform (JAIN, SAMULESON, 2006).

With changes in diet, the release of stomach products through the mucosal cells begins (SINGH et al., 2017). Gastrin is one of the hormones responsible for the maturation of cells related to acid secretion (JAIN, SAMULESON, 2006). Buddington et al. (2003) reported that in the stomach of dogs the onset of pepsin secretion occurs approximately 21 days after birth (WALTHALL et al., 2005). With breastfeeding, renin is produced in large quantities in the neonate stomach, promoting the release of hydrochloric acid and the beginning of milk digestion TRENTO, OTA, 2015). At this time, bacterial colonization also begins, mainly by lactobacilli from birth canal, however in rabbits the stomach environment remains sterile during the lactation period (BAJAREK et al., 2019; BUDDINGTON, 2003; WATHALL et al., 2005).

Proper control of the digestive tract depends on the functioning and integrity of the nervous system (ARAUJO, SILVA, MENDES, 2012). The antagonistic relationship between sympathetic and parasympathetic innervations is responsible for this command. Postnatal changes include establishing connections between the sympathetic ganglia and the stomach, increasing the number of sympathetic neurons until the tenth day of life and thus remaining unchanged throughout life (EMANUILOV eta l., 2019; EMANUILOV, MASLUIKOV, NOZDRACHE, 2018).

CONCLUSION

In summary, the canine neonate's stomach is anatomically divided into four regions: cardia, fundus, body and pylorus. The canine neonate's stomach has four well-developed stomach layers, but with the presence of suggestive undifferentiated cells at birth, which may explain the reduced functionality of the organ during this phase. However, after birth, these tunics mature to differentiate and specialize their cell types. The glandular epithelium of the stomach is formed by mucosecretory columnar cells. The arrangement of the gastric folds varies with the region of the stomach. In this way, the canine newborn's stomach is formed until birth. In view of this, the morphological understanding of the canine neonate's stomach favors the development of research in neonatology, nutrition and endocrinology.

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