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MORPHOLOGICAL STUDY OF THE SMALL INTESTINE OF MOURA PIGS (*Sus scrofa* - Lineaus, 1758) DURING FETAL DEVELOPMENT

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ABSTRACT

The morphology of the small intestine of 39 fetuses and 13 neonates of Brazilian Moura pigs (*Sus scrofa*) was studied. Fetuses were collected on the 30th, 58th and 86th day of fetal life. The entire small intestine was removed and divided into proximal and distal regions (30th day), and into duodenum, proximal jejunum, distal jejunum and ileum on the 58th and 86th days and in neonates. On the 30th day, the small intestine was small and fragile and there was no visible delimitation among the three segments. The length and diameter of the intestine increased significantly ($p<0.001$) from 58 days of gestation to parturition. The length of the small intestine, duodenum, jejunum and ileum increased 2.5, 1.2, 2.6 and 3.0 fold, respectively, whereas the diameter increased 2.7, 2.4, 2.7 and 3.0 fold from 58 days of gestation to parturition. On the 30th day, the immature small intestine consisted of mesenchyme and stratified columnar epithelium. On the 58th day, the mucosa, *muscularis* circular, *muscularis* longitudinal and serosa were observed in the three segments of small intestine and there were no crypts in the distal jejunum and ileum. Goblet cells were common in the duodenum and rare in the jejunum and ileum. Brünner's glands were observed in the submucosa. In 86-day fetuses, the presence of incipient myoblasts indicated that the *muscularis mucosae* was in formation. Crypts were observed in the three segments of the small intestine. In neonates, the *muscularis mucosae* was present and Brünner's glands were more frequent. Peyer's patches were observed in the ileum. These results show that the temporal development of the small intestine of Moura pigs is similar to that of modern breeds. However, macroscopic findings indicate that Moura fetuses have a longer small intestine and heavier body weight at birth than modern breeds.

Key words: fetuses, histology, pig, small intestine, villus

INTRODUCTION

At birth, the gastrointestinal tract must cope with a shift from parenteral nutrition before birth to enteral nutrition after birth. In preparation for this event, circulating and lumenal factors act on the gastrointestinal tract to hasten its development in the weeks before birth and to allow it to support the dramatic changes in nutrition that occur after birth. In the weeks before parturition, the pig intestine grows more rapidly than the body and its relative weight increases 70-80% over the last 3 weeks of gestation [6].

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Morphogenesis is important in the study of development [1]. The development of the gut includes the morphogenesis of intestinal mucosa from a simple tube into a tube lined with villi and the differentiation of immature epithelium into four major epithelial cell types [2]. Organogenesis in the intestine involves the progressive re-organization of the endoderm into a monolayered epithelium that lines basal crypts in which cells proliferate and where villus cytodifferentiation occurs. From this stage onwards, the epithelium is continuously renewed from the crypt stem cells [3].

The anatomic growth of the small intestine during embryogenesis in pigs has been described [4], but little is known about the histogenesis and differentiation of the tissue layers.

Changes in the weight and length of the gastrointestinal tract in fetuses of the white pure-

breed pig (*Sus scrofa*) at 74, 84, 94, 104 and 114 days of gestation show that the absolute length of the small intestine increases 2.4 fold (from 135 cm to 323 cm) during this 40 day period [7].

The porcine small intestine of a cross-breed (Duroc x Hampshire x Yorkshire) developed from a simple tube of stratified epithelium into a tube of simple columnar epithelium containing villi and intervillar regions. The formation of the villi had begun by day 35 in a proximal to distal direction and goblet cells were present in the duodenum at 40 days of gestation. By day 60 crypt formation had begun in a proximal to distal direction and in the duodenum there was some communication with the submucosal glands. The *muscularis mucosae* developed by day 90 [2].

The morphological development and differentiation of the cells and tissue layers of pig jejunum have been studied in White Bulgarian pigs [4]. The small intestine in 30-day-old embryos consisted of mesenchyme and stratified columnar epithelium. The villi had formed by about the 40th day of embryogenesis, and the differentiation of absorptive epithelium, and goblet cells, as well as the formation of the crypts and *muscularis mucosae*, occurred by the end of the second and beginning of the third (between 60th and 90th day) month of the prenatal development [4].

Moura is a breed of Brazilian pig characterized by its rusticity and its ability to consume a wide range of foods. This breed is a primary source of edible fat or lard. In addition, its efficient conversion of food into weight gain makes it a useful cross with modern breeds. These features make the Moura pig a good alternative for subsistence farming in the warmer regions of Brazil.

A knowledge of how the small intestine develops during fetal life is important for understanding the initial stages of post-natal life. In the case of the Moura, such information could provide a basis for future research into factors that interfere with the patterns of development during gestation. In this study, we examined the morphological development of the small intestine in fetal Moura pigs and compared our findings with reports for other pig breeds.

MATERIAL AND METHODS

Sexually mature gilts of similar age and weight were mated after experiencing two estrus cycles. The day of estrus was designated Day 0. Fetuses were collected by hysterectomy on days 30, 58 and 86 of gestation and neonate were obtained immediately after birth, before the first suckling. Nine individuals of each age were used after weighting. In 30-day fetuses, the small, fragile intestine was removed and divided into proximal and distal regions. In 58- and 86-day fetuses and in neonates, the small intestine was divided into duodenum, jejunum and ileum, as recommended by Schaller [11]. The entire fresh small intestine was dissected and measured (length and diameter), as was each of the three segments. The length (in centimeter) was measured with a simple ruler and the diameter (in millimeters) was measured with a pair of caliper. Both measurements were done in freshly dissected small intestine.

Tissue samples for light microscopy were collected from the middle of each segment in four fetuses and four neonates for each experimental period. The samples were washed with isotonic buffered saline solution to remove debris, then stretched and fixed in Bouin solution (6 h for fetuses and 12 h for piglets) before storing in 70% ethanol. After dehydration, the tissues were embedded in paraffin. For each age, at least 12 sections 5 μm thick were stained with hematoxylin-eosin (HE) and with the periodic acid-Schiff reaction (PAS).

The weights of the pig, and the length and diameter of the small intestine and its segments were expressed as the mean ± standard deviation. The measurements were compared statistically by two non-parametric tests using the computer statistical package SPSS for Windows (standart version, 1989-1999). The Kruskal Wallis test was used to demonstrate the increase in the length and diameter of the small intestine and its segments from the 58th day of fetal life to the time of birth and the Mann Whitney-U test was used to compare the changes in the parameters between the different ages (between the 58th and 86th day and between the 86th and birth).

RESULTS

Macroscopic examination of 30-day fetuses showed a very small, immature and fragile small intestine, with no delimitation among the duodenum, jejunum and ileum (Fig. 2), mainly because the duodenojejunal flexure and ileocecal fold which delimit these segments were not visible at this age. For this reason, no measurements were taken and the small intestine was simply divided into proximal and distal regions.

The weights of 58- and 86-day fetuses and of neonates were 108 ± 17.5 g, 414.9 ± 23.9 g and 1206.7 ± 222.9 g, respectively. The lengths and diameters of the small intestine and its segments are shown in Figure 1.The entire small intestine

increased 3.5 fold in absolute length from the 58th day of fetal development (97.4 cm) to the day of birth (340.8 cm); in the last 28 days of fetal life (from the 86th day, 161.9 cm, to birth), the intestine increased 2.1 fold and this corresponded to the period of greatest growth. Similarly, the duodenum, jejunum and ileum increased 2.2, 3.6 and 4.0 fold between the 58th of gestation and birth, respectively. In the jejunum and ileum, most growth occurred during the last 28 days of fetal life, whereas in the duodenum most growth occurred from the 58th to the 86th day of gestation. The diameter of the duodenum, jejunum and ileum increased 3.4, 3.7, and 4.0 fold, respectively, from the 58th day of fetal life to birth. The increases in the length and diameter of the small intestine and its segments were highly significant ($p<0.001$) from 58 days of gestation to parturition, except for the duodenum where the length was not significantly increased ($p=0.006$) from 86 days to birth.

Histologically, the immature small intestine of 30-day fetuses consisted of stratified columnar epithelium and mesenchyme in the proximal and distal regions. The mesenchymal layer was thicker than the epithelial layer. The *tunica muscularis* was distinguishable by the presence of myoblasts. In cross-section, the lumen of the proximal region of the small intestine had a triangular shape (Fig. 3A). The stratified columnar epithelium extended towards the lumen and mitotic cells were seen at these points (Fig. 3A). In the distal region, the stratified columnar epithelium was thicker than in the proximal region. The lumen of this region was small and elliptical and mitotic figures were distributed throughout this epithelium (Fig. 3B).

By the 58th day of fetal development, the small intestine consisted of a mucosal layer (epithelium and lamina propria) organized into villi and crypts, *muscularis* circular, *muscularis* longitudinal and serosa layers (Fig. 4A). It was possible to differentiate the lamina propria and submucosa by the number of cells present: the lamina propria had more cells than the submucosa (Fig. 4A). In the simple columnar epithelium of the villi, the nuclei were located in the apical cell cytoplasm instead of the basal cytoplasm (Fig. 4B). Crypts were observed principally in the duodenum and only rarely in the jejunum and ileum (Fig. 4A-D). Crypts in formation had more than one cell layer. Goblet

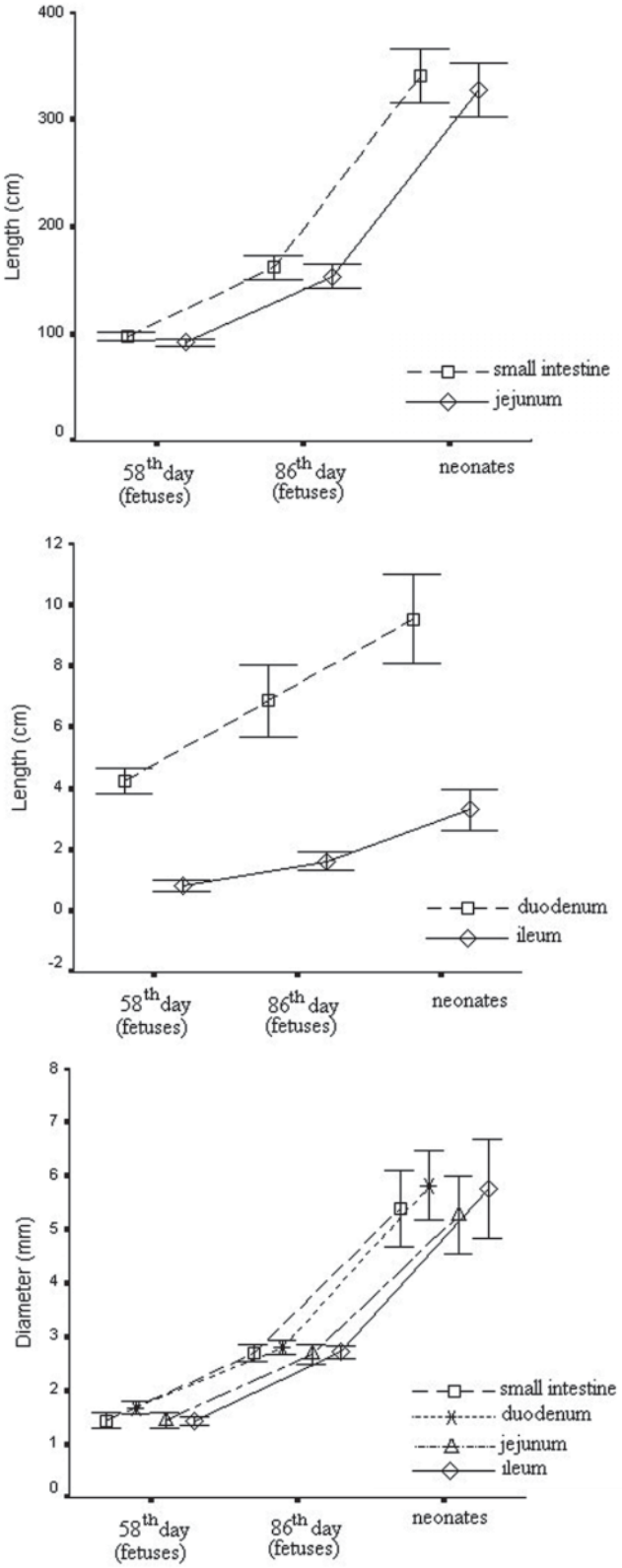

Figure 1. Length (cm) and diameter (mm) of the small intestine and its segments (duodenum, jejunum and ileum) in 58-day and 86-day-old fetuses and in neonate Moura pigs. The points are the mean ± standard deviation (SD) of 9 pigs for each time interval.

Figure 2. Photomicrograph of the small intestine of a Moura fetus on the 30th day of gestation. Arrowhead - proximal small intestine, arrow- distal small intestine. Bar = 2.5 cm.

cells (PAS-positive) were observed in the duodenum and only rarely in the jejunum and ileum (data not shown). In the submucosa of the duodenum, incipient Brünner's glands were seen as a cluster of acini below some crypts (Fig. 4B). The cells of these glands were of the mucous type.

On the 86th of fetal life, all of the layers of the small intestine were present, except for the *muscularis mucosae* (Fig. 5). The lamina propria and submucosa can be differentiated by the number of cells (fewer in the submucosa), and by the presence of incipient myoblasts indicating the formation of the *muscularis mucosae*. Crypts were observed in the three segments of the small intestine. The nuclei of villus epithelial cells continued in an apical position (Fig. 5). Goblet cells were now present in all segments of the small intestine. The appearance of Brünner's glands was similar to that observed in fetuses on the 56th day of life.

The *muscularis mucosae* layer was present at birth (Fig. 6). The nuclei of villus epithelial cells were now in a basal position. Brünner's glands present were now more frequent in the duodenum and appeared as a tubuloacinar gland (Fig. 6). The lamina propria and submucosa of the ileum contained aggregates of lymph nodules (Peyer's patches).

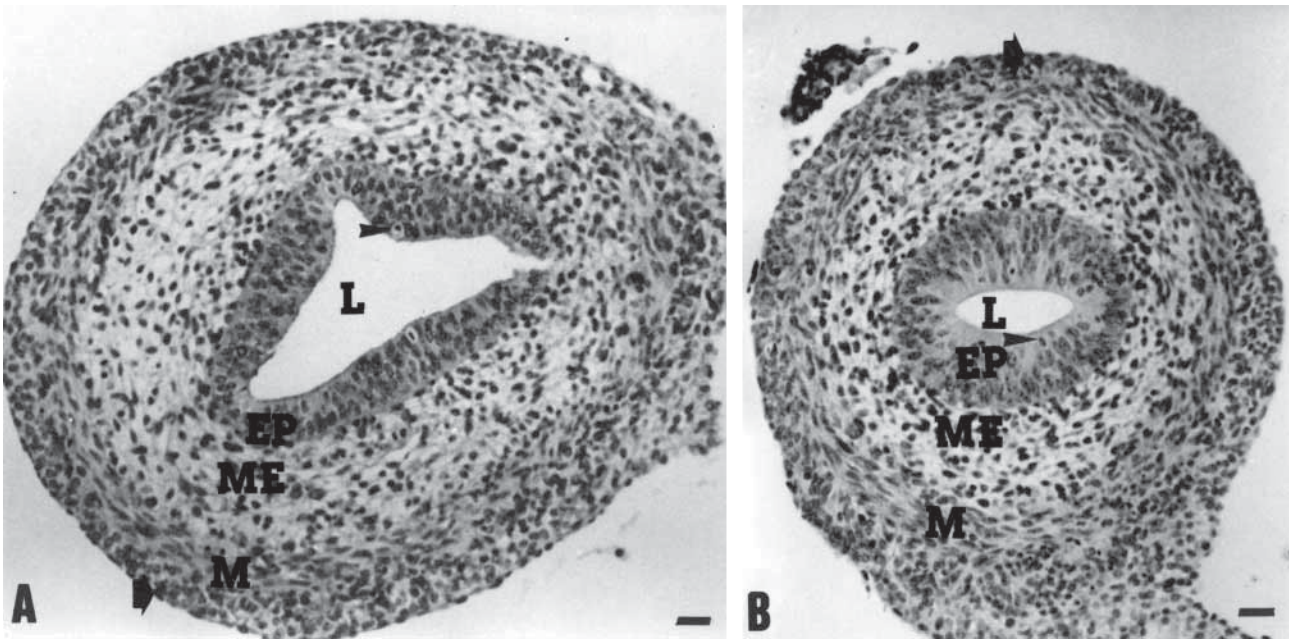

Figure 3. Photomicrographs of the proximal **(A)** and distal regions **(B)** of the small intestine of a 30-day-old Moura fetus. (EP) epithelium, (L) lumen, (M) myoblasts, (ME) mesenchyma. Arrow- mesothelial cells, Arrowhead - mitotic figure. HE. Bar = 25 µm.

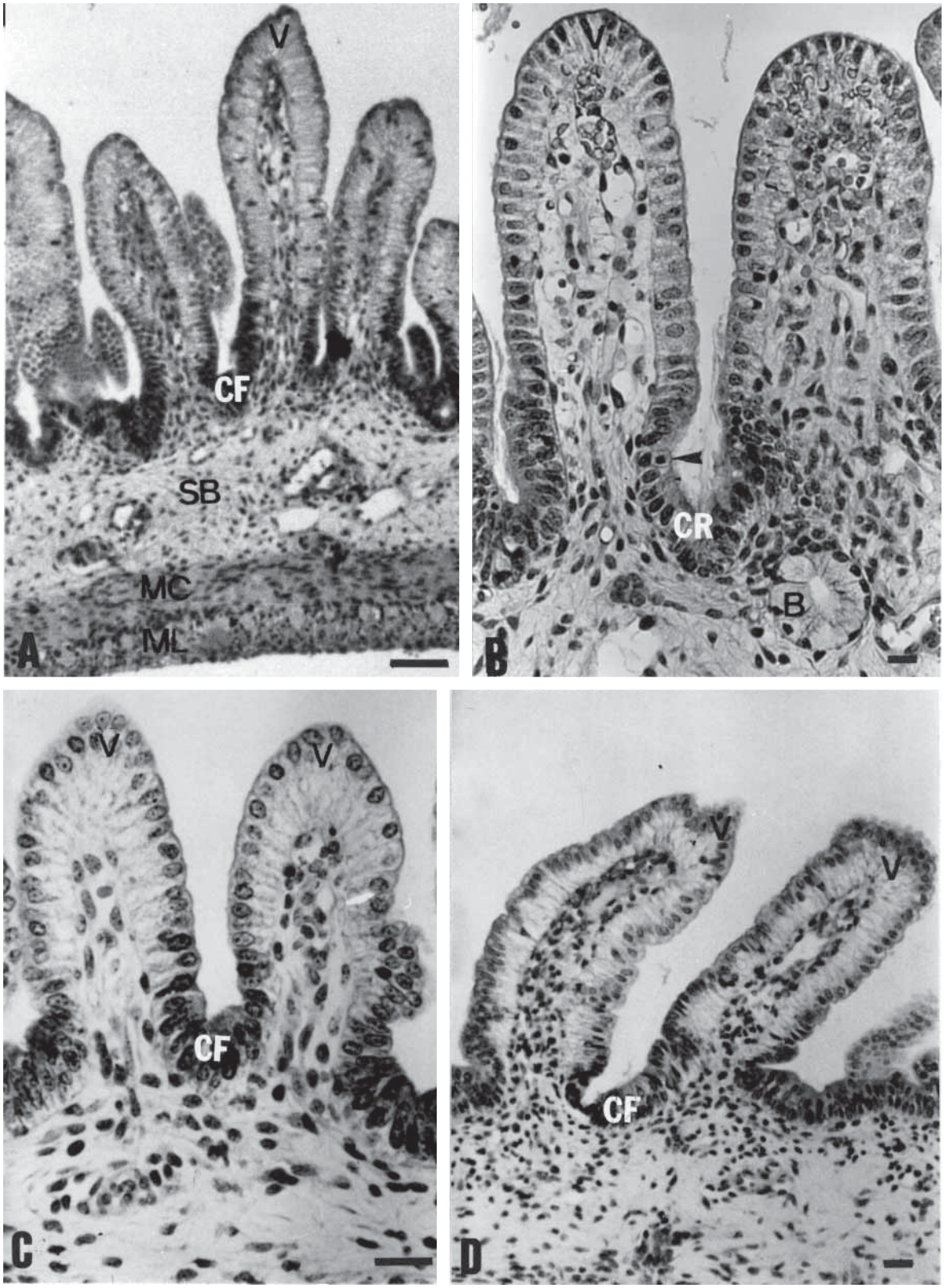

Figure 4. Photomicrographs of the proximal jejunum **(A)**, duodenum **(B)**, distal jejunum **(C)** and ileum **(D)** of a 58-day-old Moura fetus. Note the villi with apically positioned nuclei. (B) Brünner's gland, (SB) submucosa, (CF) incipient crypts, (CR) crypts, (MC) *muscularis* circular, (ML) *muscularis* longitudinal, (V) villi. Arrowhead - mitotic figure. HE. Bars: A = 50 µm, B = 12.5 µm, C and D = 25 µm.

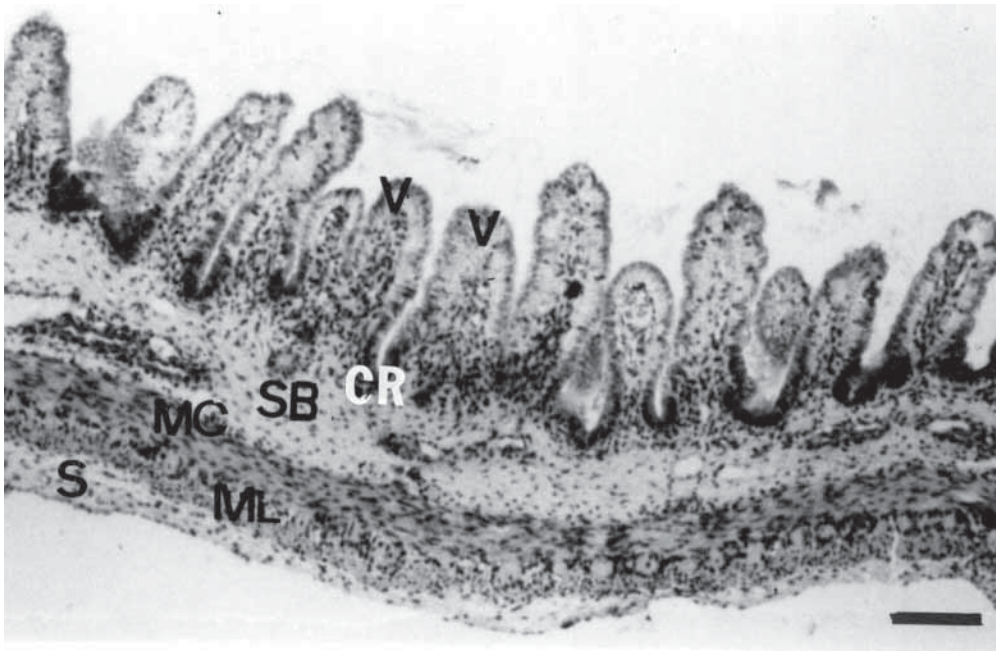

Figure 5. Photomicrograph of the duodenum of an 86-day-old Moura fetus. Note the apical position of the nuclei in the villi. (CR) crypts, (MC) *muscularis* circular, (ML) *muscularis* longitudinal, (S) serosa, (SB) submucosa, (V) villi. HE. Bar = 100 μm.

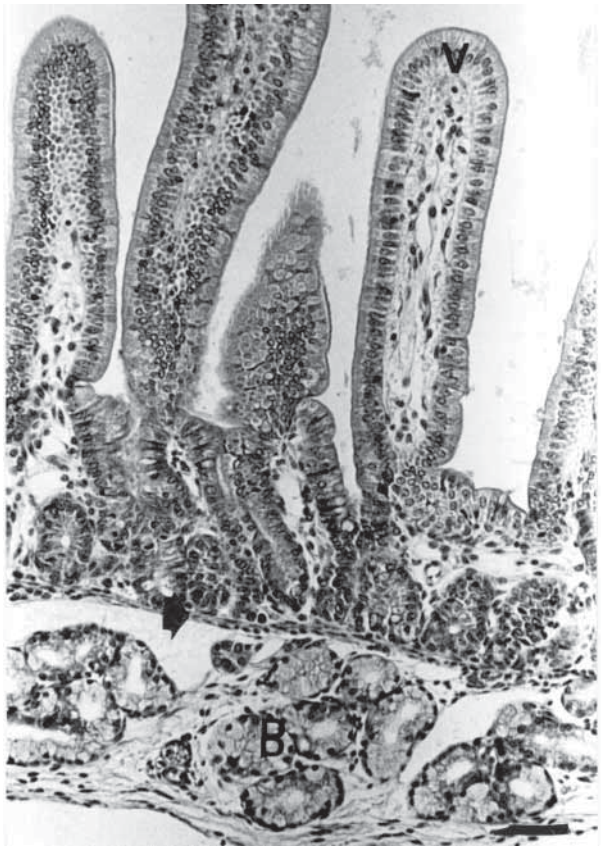

Figure 6. Photomicrograph of the duodenum of a neonatal Moura pig. Note the basal position of the nuclei in the villi. (B) Brünner's glands, (CR) crypts, (V) villi. Arrow- *muscular mucosae*. HE. Bar = 20 μm.

DISCUSSION

Macroscopic analyses of the small intestine in porcine fetuses have been reported only for 30 days of gestation or more [2,7], since before this period, the organ is too small and fragile, and shows little differentiation of the various regions, as also observed here.

The length of the small intestine of fetuses varies during development in each one of the breeds studied. In 30-day fetuses of a cross-breed (Duroc x Hampshire x Yorkshire), Dekaney *et al.* [2] found a very small organ that could not be divided into proximal and distal regions as done here. In 58-day Moura fetuses, the small intestine was 97.4 cm long and could be subdivided into duodenum, jejunum and ileum. In 84-day fetuses, the small intestine was longer (194 ± 20.5 cm) in white pure-breed pigs [4] compared to Moura pigs (161.9 ± 14.74 cm). Although major growth occurs from the 84th day of gestation to birth in Moura fetuses as well as in white pure-breeds [4], at birth the small intestine in Moura fetuses is longer (340.8 ± 9.89 cm) than in white pure-breeds (323 ± 56.1 cm), indicating a greater growth and body weight gain in this period. At day 84, Moura fetuses are lighter than white pure-breeds (414.9 ± 23.94 g versus 506 ± 94.3 g) whereas at birth they are heavier (1206.7 ± 236.4 g versus 1012 ± 345.3 g). There are no

data in the literature on the length and diameter of the duodenum, jejunum and ileum. Consequently, no comparisons can be made, except that the diameter increases steadily in the different segments of the small intestine.

The development of the small intestine of the Moura pig occurs from the proximal to the distal end. This finding agrees with those described by Georgieva and Gerov [4] and Dekaney *et al.* [2], although these authors did not report a triangular-shaped lumen in the proximal region as observed here. Such a lumen has been described only in the small intestine of chick embryos [1], in which the morphogenesis occur in three phases, circular, elliptic and triangular [1]. In the current work, a triangular-shaped lumen was observed in the proximal region whereas an elliptical-shape lumen was observed in the distal small intestine of fetuses on day 30. This finding confirms that small intestine develops faster in a proximal to distal direction, and that this begins early in the gestational period.

Villi were observed on the 58th day in Moura fetus, as also reported for the Duroc x Hampshire x Yorkshire cross-breed [2]. The nuclei of villus epithelial cells have an apical position from the 56th to 86th days, but a basal position at birth. This variable pattern of nuclear position was also verified in cross-breed pigs [2] in which electron microscopy revealed short microvilli by the 60th day of fetal life. These authors related the irregular nuclear position to the presence of vacuolated cells. In Moura pigs, there was no relationship with vacuolated cells. The presence of goblet cells and Brünner's glands seen in the 58th day in Moura fetuses confirmed the results of Dekaney *et al.* [2]. The presence of crypts in the duodenum on the 58th day and in all regions by the 86th day of gestational life agrees with Dekaney *et al.* [2] and confirms that crypt formation occurs in a proximal to distal direction. Georgieva and Gerov [4] reported that crypt formation begins between the 60th and 90th day of fetal life. However these authors studied the jejunum and the late presence of crypts in this region was also observed in Moura fetuses.

According to Dekaney *et al.* [2], the formation of the *muscularis mucosae* begins on the 90th day in white pure-breed fetuses. In 86-day-old Moura fetuses, the presence of incipient myoblasts and the presence of this layer in neonates confirmed that the formation of this layer occurred during the last 28 days of intra uterine development.

The presence of Paneth cells in the porcine small intestine has been disputed in the literature. In this study, Paneth cells were not detected by light microscopy, thus confirming the data of Dekaney *et al.* [2], who reported an absence of Paneth cells based on light or electron microscopy. On the other hand, Georgieva and Gerov [4] described the presence of Paneth cells in the blind bottoms of crypts in porcine small intestine in the periods shortly after birth.

This is the first report to evaluate the morphological development of the fetal porcine small intestine in a Brazilian breed raised in warm regions. Although the development of the small intestine in Moura pigs proceeds in a temporal pattern similar to that of modern breeds, macroscopic findings show that Moura fetuses have a longer small intestine and heavier body weight at birth than modern breeds. This may indicate a morphological advantage of rustics pig compared to modern breeds.

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