

## Goat kids' intestinal absorptive mucosa in period of passive immunity acquisition

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### ABSTRACT

Colostrum intake in newborn goat kids is essential for the acquisition of immunoglobulins (Ig) and influencing development of gastrointestinal mucosa. The present study investigated small intestine structure in the postnatal goat kid fed lyophilized bovine colostrum, an alternative source of antibodies to small ruminants, or goat colostrum using scanning electron microscopy technique. At 0, 7 and 14 h of life 15 male newborns received 5% of body weight of lyophilized bovine colostrum (LBC) and 14 goat colostrum (GC), both with 55 mg/mL of IgG. Samples of duodenum, medium jejunum and ileum were collected at 18, 36 and 96 h of life. Three animals were sampled at birth without colostrum intake (0 h). The enteric tissues were analyzed for villi density (villi/cm<sup>2</sup>) and morphological characteristics. The villi density did not differ between treatment, sampling time and intestinal segments ( $P > 0.05$ ). The morphological characteristics were not different between LBC and GC in all segments. Duodenal villi were fingerlike, thick and short, and with different heights. Duodenal folds could also be verified. Frequent anastomoses in all sampling times were observed in this segment. In the jejunum, fingerlike villi, thin and thick, of different heights were observed in all sampling times as well as leaf-shaped villi. Vacuoles with colostrum were observed in the jejunum of goats sampled at 18 h of life. In ileum, fingerlike villi were observed in all sampling times. At 0 and 96 h of life, thick and low villi were verified while at 18 and 36 h the villi showed different heights and widths. At all sampling times, regularly cell extrusion processes were observed with grouped cells at the apex of the ileum villi and with isolated cells along the villi. In the first 4 days of goat kids' life the small intestine structure was unaffected by different sources of colostrum, goat or lyophilized bovine, and by the replacement of fetal enterocytes, which are able to absorb macromolecules, by adult-type ones.

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### 1. Introduction

In the first hours of life, major changes occur in the intestinal tract, especially in those of ruminants, and the alterations will have important implications in the animals' development

(Fleige et al., 2007; Kelly and Coutts, 2000; Masanetz et al., 2010). Besides the substitution of fetal-type enterocytes, which have the capacity to absorb macromolecules, the small intestinal mucosa undergoes structural changes (Bessi et al., 2002a,b; Boudry et al., 2008; Campbell et al., 1977; Kelly and Coutts, 2000; Skrzypek et al., 2005; Smeaton and Simpson-Morgan, 1985).

Colostrum, a milk secretion responsible for providing antibodies to newborns, also has other components that are associated with the development of the gastrointestinal tract, including the presence of high concentration of insulin-like

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growth factor type I (Georgieva et al., 2003; Hammon and Blum, 2002; Odle et al., 1996). The deprivation of this milk secretion in the first hours of life can result in considerable modifications in the small intestine, such as decrease of intestinal size (Kelly and Coutts, 2000).

In situations with the possibility of Caprine Arthritis Encephalitis (CAE) Virus transmission, provision of colostrum to the newborn goat kid is not recommended, so it is necessary to search for an alternative management of colostrum (Castro et al., 2005; Lima et al., 2009; Logan et al., 1978; Quigley III et al., 2002). Bovine colostrum can provide antibodies to the small ruminants and is also an alternative that is easy to obtain and has high immune quality (Lima et al., 2009; Moretti et al., 2010a,b). Lyophilized colostrum is another alternative management that allows storage for prolonged periods, thus ensuring the biological function and quantity of antibodies (Castro et al., 2005; Quigley III et al., 2002).

Morphological characteristics of goat kid enteric tissue were investigated in newborns fed lyophilized bovine colostrum, as an alternative source of antibodies, and goat colostrum during the period of passive immunity acquisition.

## 2. Materials and methods

The experiment was conducted on the Intensive System of Sheep and Goats Production (ESALQ/USP). In this study, 32 Saanen  $\times$  Boer goat kids were available. The animals were kept, maintained and treated in adherence to accepted standards for humane treatment of animals (authorized by ESALQ/USP ethics committee).

Bovine and goat 1st milking colostrum from two Holsteins cows and 14 Sannen  $\times$  Boer goats were collected before the experiment. The colostrums were homogenized to form a unique pool of bovine colostrum and another of goat colostrum. Thereafter the milking secretions were stored at  $-20^{\circ}\text{C}$ . Samples of each pool were collected for determination of IgG concentration by radial immunodiffusion (Besser et al., 1985; Mancini et al., 1965). The frozen pool of bovine colostrum was conducted to the lyophilization process. The resulting powder was homogenized and stored in a tightly sealed container at  $-20^{\circ}\text{C}$ .

At the time of offering the meals, the pool of goat colostrum was diluted with whole milk until reaching a concentration of 55 mg/mL of IgG. The pool of bovine colostrum powder, however, was resuspended in water until it reached the original chemical composition of the pool taken to the lyophilization process and, subsequently, diluted with whole milk until reaching a concentration of 55 mg/mL of IgG. Samples of final meals were collected for analysis of chemical composition and IGF-I concentration (Table 1).

The newborn goat kids were separated from their mothers immediately after birth, without maternal colostrum intake. Fifteen animals received 5% of body weight of lyophilized bovine colostrum (LBC group) and fourteen goat colostrum (GC group) as soon as possible after birth and at 7 and 14 h of life. Goat kids were randomly slaughtered at 18, 36 and 96 h for the collection of duodenum, medium jejunum and ileum. Three other animals were sampled immediately after birth without colostrum ingestion constituting an additional group (0 h).

**Table 1**

Chemical composition and IGF-I concentration of lyophilized bovine and goat colostrum fed to newborn goat kids.

	Lyophilized colostrum	bovine colostrum	Goat colostrum
Humidity and volatility, %	81.11 $\pm$ 0.19	79.88 $\pm$ 0.18	
Dry matter, %	18.89 $\pm$ 0.19	20.12 $\pm$ 0.18	
Crude protein, %	9.40 $\pm$ 0.07	9.77 $\pm$ 0.06	
Fat, %	3.97 $\pm$ 0.13	7.76 $\pm$ 0.12	
IGF-I (ng/mL)	158.71 $\pm$ 23.04	356.32 $\pm$ 0.97	

The small intestine segments were opened, the mucosa flushed with saline solution, and the samples were fixed in 4% phosphate buffer paraformaldehyde solution overnight and, thereafter, in cacodylate-buffered Karnovsky's fixative. After post-fixation in OsO<sub>4</sub> for 2 h, intestine sections were washed with cacodylate buffered 0.1 M and dehydrated with acetone (30, 50, 70, 90 and 100%, 10 min each concentration; 15 min at 100%). A critical-point drying apparatus (Balzers CPD-0302) was used to dry all tissue sections.

Processed tissues were fixed with adhesive to aluminum stubs and the sections were coated with 40 nm of gold in Balzers MED-0102 sputter coater. The samples were examined with a scanning electronic microscope Zeiss DSM-940A<sup>3</sup>, at 50 kV. Ten images of each intestinal segment were collected for the determination of villi density (villi/cm<sup>2</sup>) and for morphological analysis.

### 2.1. Statistical analyses

A completely randomized design was used. The statistical analysis was performed using SAS software (SAS Institute Inc., 2008). The villi density in each segment was arranged in a 2  $\times$  3 factorial scheme. Treatment and sampling time were considered to be the main effect. The variable was submitted to analysis of variance (F test at 5% probability) using general linear mixed models (MIXED procedure). The 0 h animals were analyzed as an additional group by orthogonal contrasts using PRC GLM program and compared to the F test at 5% probability. The values are presented as means and standard errors.

The values of villi density were also analyzed independently of the treatment given to animals, lyophilized bovine or goat colostrum, through the PROC MIXED procedure of SAS software in a 4  $\times$  3 factorial scheme, with the main effects sampling time (0, 18, 36 and 96 h) and intestinal segments (duodenum, jejunum and ileum).

## 3. Results

The values of villi density (means  $\pm$  standard errors) are presented in Table 2. There was no effect of treatment, sampling time and interaction between the parameters ( $P > 0.05$ ) in any intestinal segment. The villi density did not differ among the intestinal segments and sampling time ( $P > 0.05$ ).

There were no morphological differences in the duodenal villi in different treatments and sampling times. The villi were fingerlike, thick and short, with different heights (Fig. 1). Duodenal folds could also be verified. The frequent presence of anastomoses of two and three villi was observed in this intestinal

**Table 2**Villi density (villi/cm<sup>2</sup>) in each intestinal segment of goat kids (means  $\pm$  standard errors).

	0 h	18 h	36 h	96 h	General mean	Probability			
						Treatment	Sampling time	Interaction <sup>a</sup>	Additional group <sup>b</sup>
<b>Duodenum</b>									
LBC		8797 $\pm$ 1798	7354 $\pm$ 1854	7531 $\pm$ 1821	7894 $\pm$ 1038	0.59	0.53	0.54	0.56
GC		9256 $\pm$ 1799	10,591 $\pm$ 2010	6395 $\pm$ 2353	8747 $\pm$ 1188				
General mean	6927 $\pm$ 700	9027 $\pm$ 1271	8972 $\pm$ 1372	6963 $\pm$ 1507					
<b>Jejunum</b>									
LBC		6513 $\pm$ 532	7049 $\pm$ 550	8271 $\pm$ 538	7278 $\pm$ 307	0.23	0.55	0.17	0.43
GC		8068 $\pm$ 533	7893 $\pm$ 532	7592 $\pm$ 696	7851 $\pm$ 341				
General mean	7003 $\pm$ 274	7290 $\pm$ 376	7471 $\pm$ 382	7931 $\pm$ 445					
<b>Ileum</b>									
LBC		7124 $\pm$ 747	6393 $\pm$ 772	7145 $\pm$ 756	6887 $\pm$ 432	0.31	0.84	0.84	0.52
GC		7382 $\pm$ 748	7533 $\pm$ 747	7738 $\pm$ 977	7551 $\pm$ 478				
General mean	6567 $\pm$ 627	7253 $\pm$ 528	6963 $\pm$ 536	7442 $\pm$ 625					

LBC – goat kids that received 5% of body weight of lyophilized bovine colostrum at 0, 7 and 14 h of life, GC – goat kids that received 5% of body weight of goat colostrum at 0, 7 and 14 h of life.

<sup>a</sup> Interaction between treatment and sampling time.

<sup>b</sup> Effect of additional group (group sampled at birth without colostrum intake) by orthogonal contrast, F test ( $P < 0.05$ ).

segment (Fig. 2). Villi with dome shape were found in one animal sampled at 0 h (Fig. 3). Mucin exocytosis by goblet cells could also be detected.

Columnar enterocytes could be observed in the absorptive epithelium of goat kids (Fig. 4).

The jejunum, like the duodenum segment, did not show differences in morphology of the villi in the different treatments and sampling times. Fingerlike villi both thin and thick and of different heights were observed (Fig. 5). Leaf-shaped villi could also be verified. The structure of the absorptive mucosa was higher than the other intestinal segments. Anastomoses were not observed in this intestinal segment. The brush border of enterocytes was well developed, with microvilli that delimit the border of the cells, and goblet cells were evident along the villi. Vacuoles of absorption were verified in the jejunum of goat kids sampled at 18 h of life (Figs. 6 and 7). Fig. 8 suggests the presence of cells, probably in the process of cell death, with holes in their membranes and without microvilli. After cell loss, a region of extrusion can be observed.

In the ileum, treatments did not affect morphological characteristics of the villi. Fingerlike villi were observed in

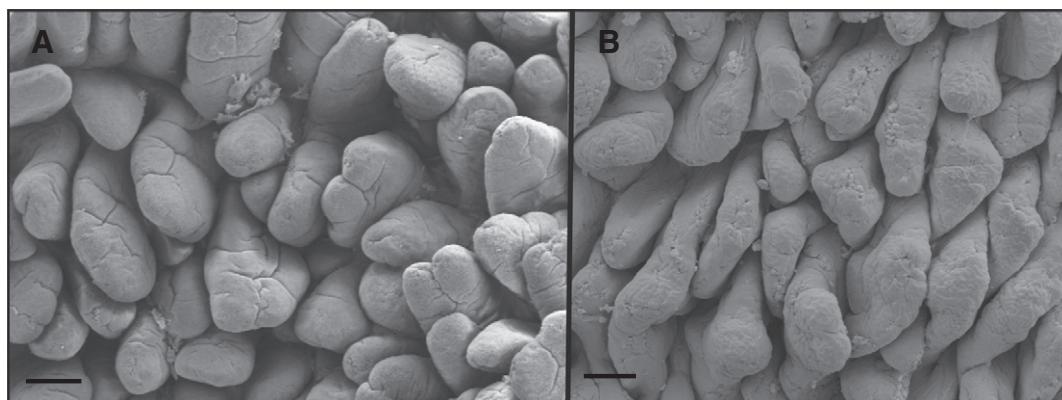
all sampling times. At 0 and 96 h of life, thick and low villi were verified while at 18 and 36 h, the villi showed different heights and widths (Fig. 9). Anastomoses were observed in this intestinal segment, but with less frequency compared to the duodenum. The frequent presence of grooves was also found in some animals, which led to a rocky shape to villi (Fig. 10).

In the ileum, frequent cell extrusion processes, at all sampling times, were observed at the apex and along the villi (Fig. 11). When the cell loss was observed at the apex of the villi, it was often with a group of cells, while along the villi, the death of isolated cells was observed.

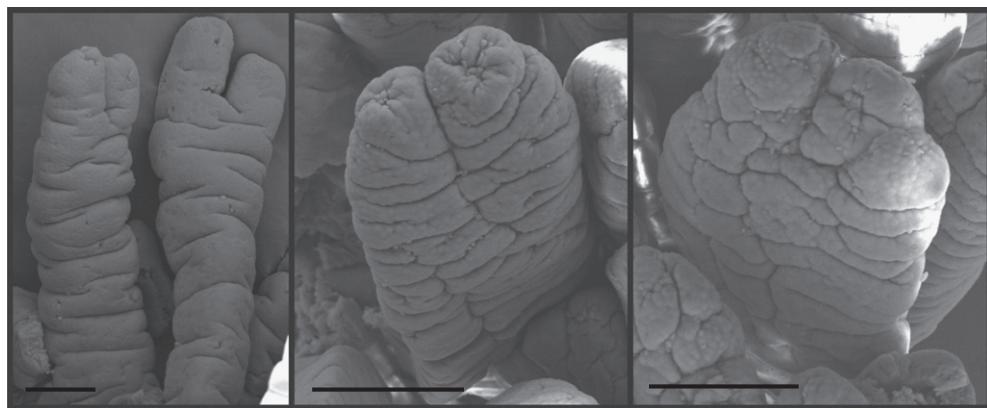
Projections of Peyer's patches, lymph nodes present in the ileum segment, were observed among the villi of all animals (Fig. 12).

#### 4. Discussion

Colostrum is an important source of hormones and bioactive factors that have activities associated with the maturation and development of the gastrointestinal tract (Blum and Hammon, 2000; Morise et al., 2008; Odle et al., 1996; Pauletti et al.,



**Fig. 1.** (A) Fingerlike villi, thick and short, with different heights in the duodenum of goat kids; (B) Duodenal folds in the duodenum of goat kids; Bar = 100  $\mu$ m.

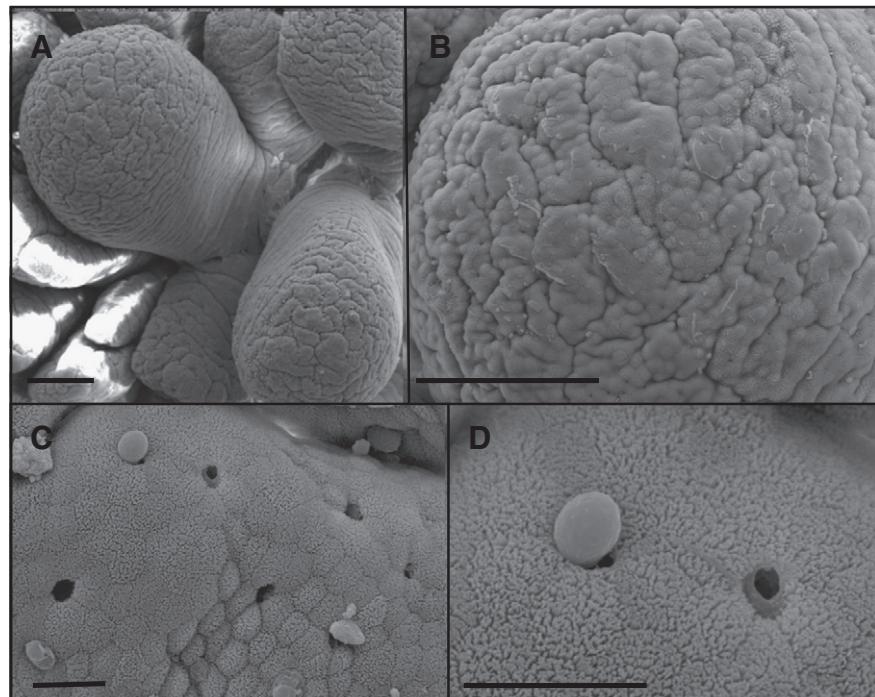


**Fig. 2.** Anastomoses between duodenal villi of goat kids; Bar = 100  $\mu$ m.

2007). Among the hormones and bioactive factors present in this lacteal secretion, the insulin-like growth factor type I (IGF-I) is one of the most investigated (Blum and Baumrucker, 2002; Buhler et al., 1998; Georgieva et al., 2003; Kelly and Coutts, 2000; Odle et al., 1996; Playford et al., 2000; Zhang et al., 1997). According to Kindlein (2006), the amount of IGF-I provided may influence villi density. The authors observed that the ingestion of this bioactive factor less than 500  $\mu$ g determined 25% of decrease in the ileal villi density of calves. In the present work, the amount of IGF-I ingested by the animals that received lyophilized bovine and goat colostrum was very low,

approximately 91 and 197  $\mu$ g, respectively, which may be responsible for the absence of differences in the villi density in different treatments, sampling times and intestinal segments. It is known, however, that the absorptive capacity depends not only on villi density. The height of the villi and the microvilli also has a significant influence (Macari, 1995, 1999). Therefore, the morphology of intestinal villi is also an important feature to be investigated in the intestinal epithelium.

In this study, there were no morphological differences between treatments in all segments studied. In the duodenum,



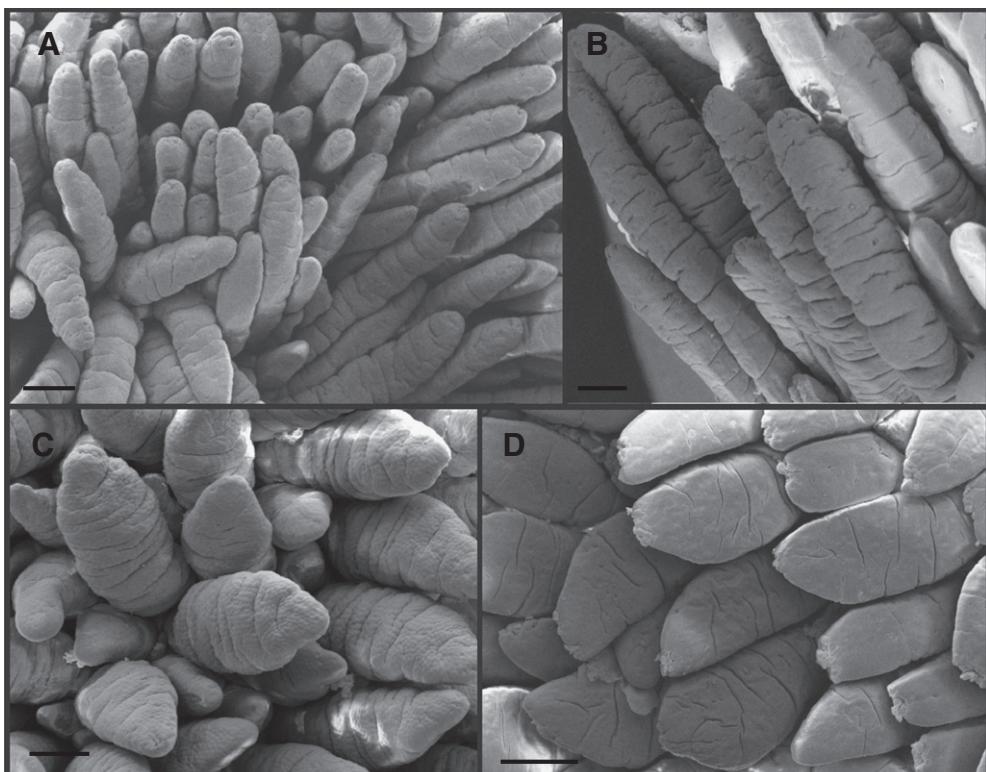
**Fig. 3.** (A) and (B) Villi with dome morphology found in one animal at 0 h of life; Bar = 40  $\mu$ m; (C) and (D) Mucin exocytosis by goblet cells in the duodenal villi; Bar = 10  $\mu$ m.



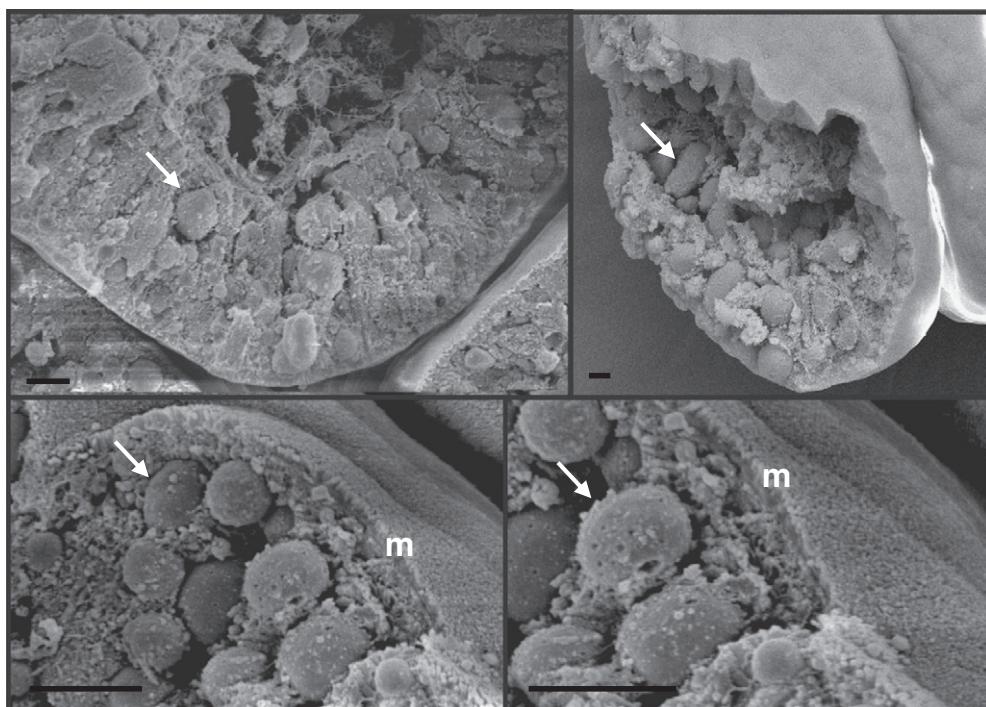
**Fig. 4.** Columnar enterocytes in the absorptive epithelium of goat kids' small intestine; Bar = 40  $\mu$ m.

the intestinal epithelium did not change villi shape in the first hours of goat kids' life. [Bessi et al. \(2002b\)](#) and [Kindlein et al. \(2008\)](#), in turn, observed morphological differences between the duodenum villi of newborn calves and animals 24-hours old and 3-days old, respectively. The presence of intestinal anastomoses in this segment is in agreement with results found by [Bessi et al. \(2002b\)](#) and [Kindlein et al. \(2008\)](#) in calves and by [Skrzypek et al. \(2005\)](#) in pigs. However, the frequency of these structures in the goat kids was higher than that found in cattle and pigs, which may have determined the presence of duodenal folds.

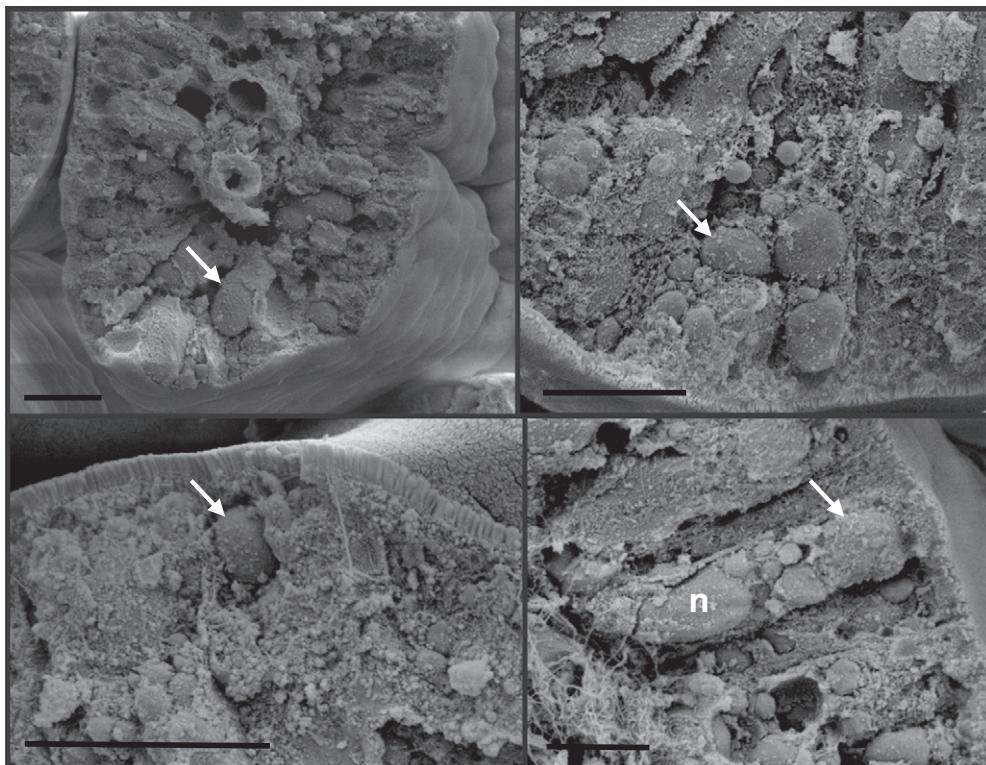
In the jejunum, the villi were high and finger-like in accordance with the literature ([Bessi et al., 2002a,b](#); [Kindlein et al., 2008](#); [Poole et al., 2003](#)). Villi in leaf form, such as those observed in this study, were also detected by [Kindlein et al. \(2008\)](#) in 3-day-old calves. The tall villi present in the jejunum, as seen in the goat kids, are related to an increase in surface area and consequently with the highest condition of absorption in this portion of the small intestine ([Poole et al., 2003](#)). In ruminants, this segment has significant importance to the newborn animals, since the absorption of immunoglobulins in the first hours of life occurs primarily in cells of jejunum and



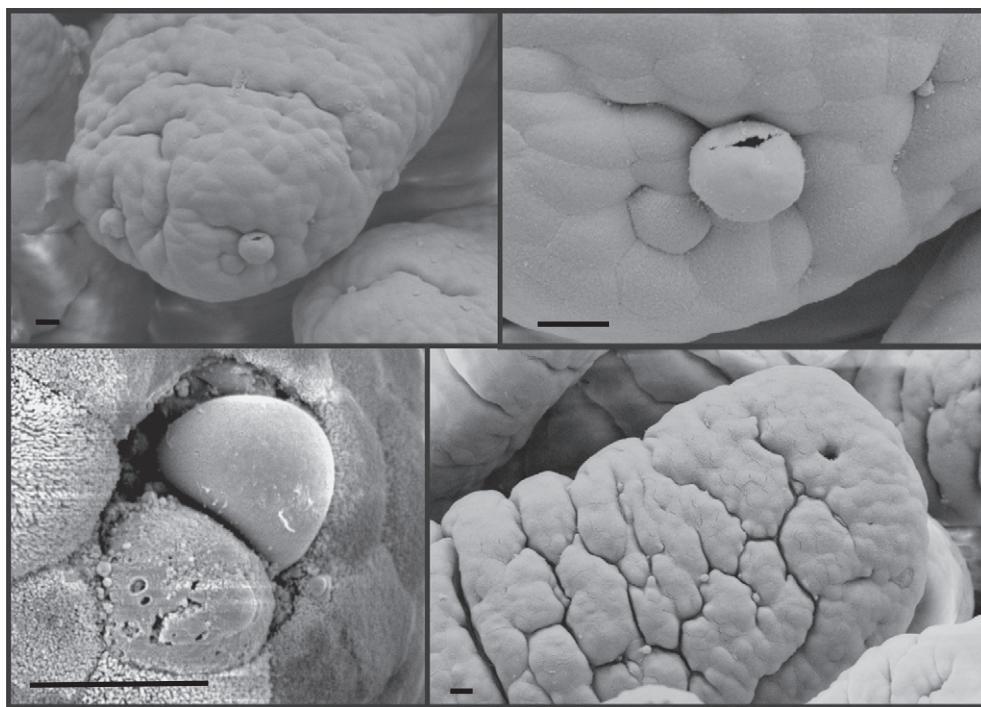
**Fig. 5.** (A), (B) and (C) Fingerlike villi thin and thick and with different heights observed in the jejunum of goat kids; (D) Leaf shaped villi observed in the jejunum of goat kids; Bar = 100  $\mu$ m.



**Fig. 6.** Jejunum of goat kids sampled at 18 h of life showing vacuoles of colostrum absorption; Arrow: vacuoles of colostrum absorption; m: microvilli; Bar = 5  $\mu$ m.



**Fig. 7.** Enterocytes with vacuoles of colostrum absorption in goat kids sampled at 18 h of life; Arrow: vacuoles of colostrum absorption; n: nucleus of the enterocytes; Bar = 10  $\mu$ m.

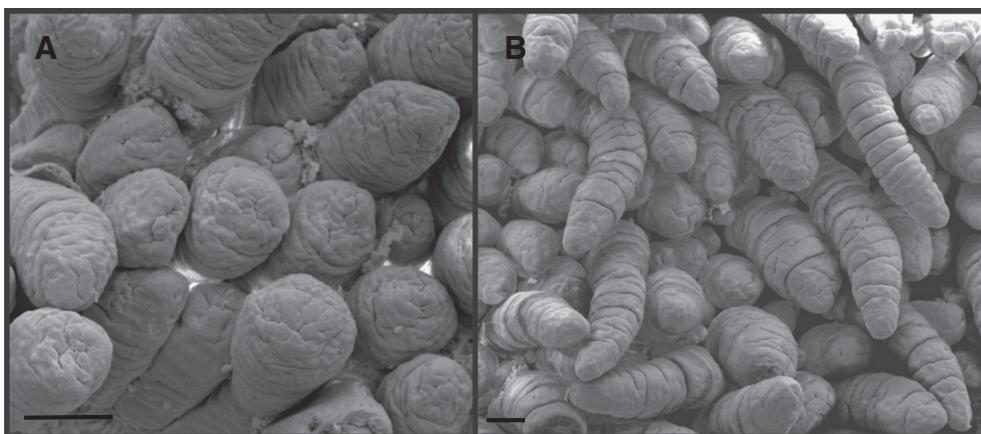


**Fig. 8.** Process of cell death and extrusion in the jejunum villi of goat kids; Bar = 10  $\mu$ m.

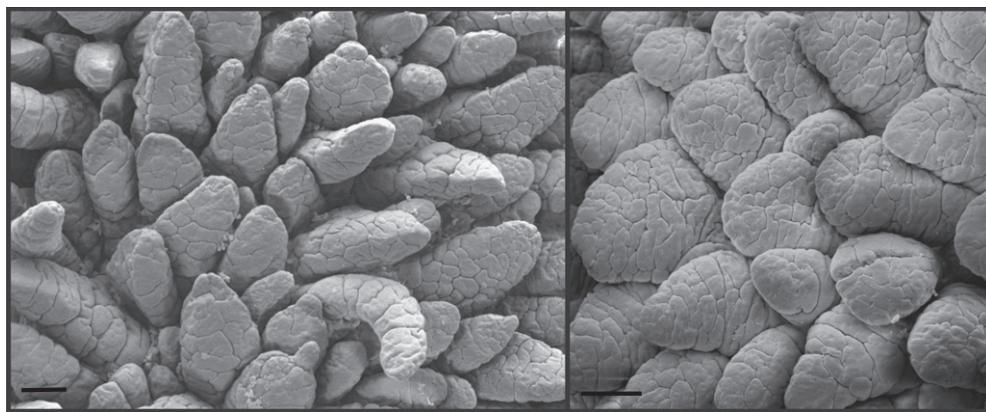
ileum, while duodenum cells have negligible contribution in this process (Trahair and Robinson, 1989). In this study, vacuoles of absorption containing colostrum were verified in jejunum enterocytes of goat kids sampled at 18 h of life, a period of intense activity of macromolecule internalization. These vacuoles are absorbed by pinocytosis and can appear in the bloodstream of the newborn within one to three hours after its ingestion (Castro et al., 2009; Lima et al., 2009; Moretti et al., 2010b; Pauletti et al., 2007). However, after 24 to 36 h, the membranes of enterocytes are altered and lose their ability to absorb macromolecules, resulting in intestinal closure (Bessi et al., 2002a;

Campbell et al., 1977; Smeaton and Simpson-Morgan, 1985). Probably because of the decrease of macromolecule internalization, vacuoles of absorption could not be observed by scanning electron microscopy at 36 and 96 h of life.

In this study, there were changes in the morphology of the ileal epithelium of goat kids at 18 and 36 h of life and, moreover, this was the segment that showed a higher frequency of cells undergoing cell death. These characteristics indicate that this region of the small intestine is greatly affected by colostrum ingestion. It is known that as epithelial cells are heading to the top of the villi, they undergo apoptosis and



**Fig. 9.** (A) Thick and low villi observed at 0 and 96 h of goat kids' life; (B) Different heights and widths of ileal villi observed at 18 and 36 h of goat kids' life; Bar = 100  $\mu$ m.



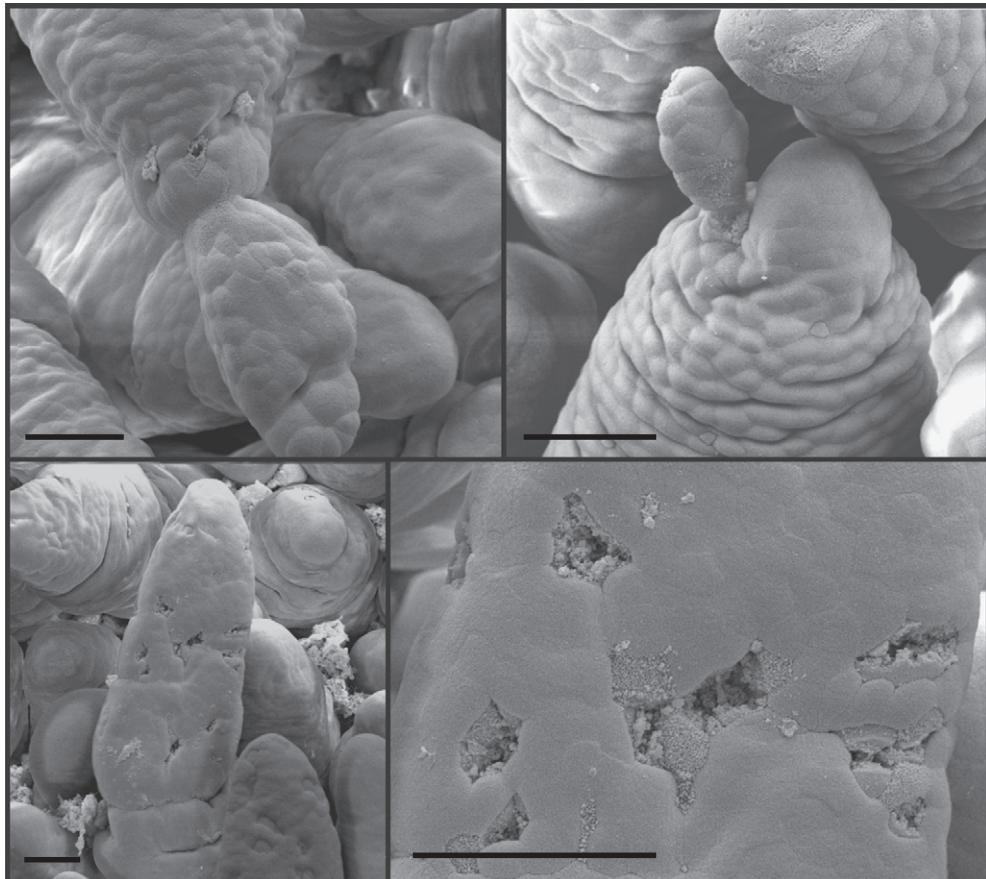
**Fig. 10.** Grooves in the ileal segment of goat kids leading to a rocky aspect of the villi; Bar = 100  $\mu$ m.

extrusion into the intestinal lumen. However, this study shows that cell loss may also occur along the villi, indicating that other components that arrive in this segment can be harmful to intestinal cells and, consequently, stimulates early death.

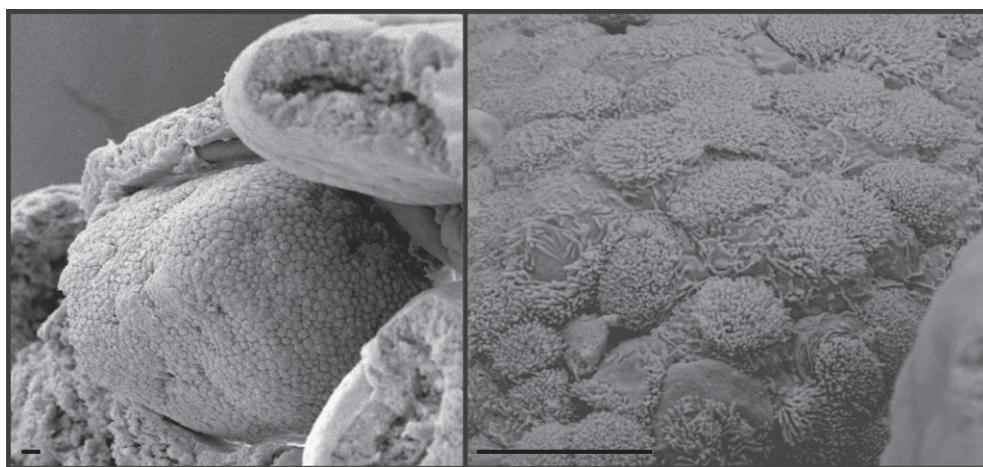
The absence of morphological differences in the villi of goat kids suckled lyophilized bovine or goat colostrum indicates

that the source of heterologous antibodies does not cause injury on the development of the intestinal epithelium of these small ruminants. Thus, the present work ensures this source of Ig as a possible substitute for goat colostrum.

In the first 4 days of goat kids' life the small intestine structure was unaffected by different sources of colostrum, goat or lyophilized bovine, and by the replacement of fetal enterocytes,



**Fig. 11.** Cellular extrusion process occurring at the apex of the villi with a group of cells and empty spaces corresponding to isolated dead cells; Bar = 40  $\mu$ m.



**Fig. 12.** Projections of Peyer's patches observed among the ileal villi of goat kids; Bar = 10  $\mu$ m.

which are able to absorb macromolecules, by adult-type ones.

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