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Histomorphometric and immunohistochemical study of the goat abomasum during prenatal development

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Summary. This study sought to chart the morphological changes taking place in the goat abomasum during prenatal development, using histomorphometric and immunohistochemical techniques. A total of 140 goat embryos and fetuses, from the first stages of prenatal life until birth. Differentiation of the abomasum as a separate compartment of the primitive gastric tube was observed at 35 days of prenatal life (CRL 3 cm, 23% gestation). Primitive abomasal folds were first observed at 38 days (CRL 4.3 cm, 25% gestation). The muscularis mucosae was visible by 64 days (CRL 13.5 cm, 43% gestation). Transformation of pseudostratified epithelium to simple cylindrical epithelium was also observed at this stage. Differentiation of gastric pits and glands first became apparent at 75 days (CRL 17.5 cm, 50% gestation) and 84 days (CRL 20 cm, 55% gestation), respectively. Neuroendocrine cells were detected by synaptophysin (SYP) at 64 days (CRL 13.5 cm, 43% gestation), while glial cell markers (glial fibrillary acidic protein - GFAP, and vimentin-VIM) were observed at 64 days (CRL 13.5 cm, 43% gestation) and 38 days (CRL 4.3 cm, 25% gestation), respectively. Neuropeptide Y (NPY) and vasoactive intestinal polypeptide (VIP) were detected at 75 days (CRL 17.5 cm, 50% gestation). Gastrin-immunoreactive cells first appeared in the abomasum at 76 days (CRL 18 cm, 50% gestation). In conclusion, prenatal development of the abomasum appears to take place somewhat earlier in goats than in sheep or cattle, but at a similar rate to that reported in wild ruminants such as deer.

Key words: Abomasum, Immunohistochemistry, Capra hircus, Prenatal development

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Introduction

The stomach is considered as the first functional part of the digestive system, in that it is able to digest food and transform it into nutrients which are subsequently absorbed through the intestinal wall (Al-Saffar, 2012). The ruminant stomach is particularly remarkable for its ability to transform low-quality forage into products of great nutritional value (Lombardi, 2005). Differences in ruminant feeding habits led Hofmann (1973) to assign them to three groups: grazers, concentrate selectors and intermediate feeders. The goat is classed as an intermediate feeder, able to make use of marginal pasturelands (Boyazoglu et al., 2005; Rancourt et al., 2006). Browse appears to be an important component in the diet of goats, which are regarded as the best users of poor roughage among ruminants (Gihad et al., 1980).

In order to meet its functional needs, the ruminant stomach has developed four separate compartments, each with its own morphological particularities. The abomasum is the fourth compartment, the glandular portion which is equivalent to the true stomach in monogastric animals (Nickel et al., 1973). The main function of the abomasum is to prepare food for digestion by secreting gastric juices (Age et al., 2007). Like the stomach of other mammals, the abomasum contains gastric pits and glands (Asari et al., 1985). Several endocrine cell groups are to be found in the abomasal mucosa, including gastrin-producing cells. Gastrin is one of the major hormones in the gastrointestinal endocrine system. Among other things, it is essential for the normal growth of the gastrointestinal mucosa, so that any disruption in gastrin production may give rise to digestive disorders (Yu et al., 2011).

Although there has been considerable research into the organization of the forestomach in cattle (Vivo et al., 1990), sheep (Wardrop, 1961; Franco et al., 1989, 1992, 1993a-c; Redondo et al., 1997; Regodón et al., 1996),

and deer (Franco et al., 2004a,b, 2011, 2012; Redondo et al., 2005, 2011; Masot et al., 2007a,b), few studies have addressed the prenatal development of the forestomach in goats (Mcsweeny, 1988; Ramkirshna and Tiwari, 1979; Nwaogu and Ezseaor, 2008; El-Gendy and Derbalah, 2010; Garcia et al., 2012), and fewer still have dealt specifically with the abomasum (Asari et al., 1985; Masot et al., 2007a), the present paper being among the first to do so.

The aims of this study were as follows: (1) to chart the histological development of the goat abomasum during prenatal life; (2) to determine morphometric changes taking place in the various components of the abomasal wall over the same period; (3) to apply immunohistochemical techniques for the detection of neuroendocrine cells using synaptophysin (SYP), of glial cells using glial fibrillary acidic protein (GFAP) and vimentin (VIM), of peptidergic innervation markers neuropeptide Y (NPY) and vasoactive intestinal peptide (VIP), as well as examine the distribution of gastrin cells; and finally (4) to examine the surface of the abomasum using scanning electron microscopy from the early embryonic stages until birth.

Materials and methods

Animals

A total of 140 goat (*Capra hircus*) embryos and fetuses, ranging from the first prenatal stages to birth, were sampled. Specimens were divided into 5 sequential groups, according to major histomorphogenic characteristics: group I (crown-rump length[CRL] 1.5-4.3 cm, age 13-38 days, 1-25% gestation), group II (CRL 4.4-8 cm, 39-52 days, 25-35% gestation), group III (CRL 9-17.5 cm, age 53-75 days, 35-50% gestation), group IV (CRL 18-32 cm, 76-112 days, 50-75% gestation), and group V (CRL 33-47 cm, 113-150 days, 75-100% gestation). Embryos and fetuses were all obtained at municipal slaughterhouse in Caceres (Spain) from pregnant females. These pregnant females were slaughtered by the usual process in the slaughterhouse, where the embryos and fetuses were obtained after opening the abdominal cavity and uterus. These actions were carried out in accordance with the regulation required for the protection of animals at the time of slaughter in slaughterhouses (Spanish Royal Decree 54/1195). Gestational age was estimated following Evans and Sack (1973) and Sivachelvan et al. (1996), as well as in the light of age classifications previously reported for sheep (Franco et al. 1992) and deer (Franco et al. 2004a,b, 2012). Ten specimens were selected for each group for histomorphometric, immunohistochemical and scanning electron microscopic analysis. In group I of these 10 individuals, 2 belonging to the age of 13 days, 3 in 35 days and 5 for 38 days; in group II, 3 fetuses of 39 days, 3 of 46 days and 4 of 50 days were selected; in group III, 2 fetuses of 53 days, 3 of 62 days, 3 of 70 days and 2 of 74 days were chosen; in group IV, 3 fetuses of 76 days, 3 of 84 days, 2 of 95 days and 2 of 112 days were selected; in group V, 2 fetuses of 113 days, 3 of 125 days, 3 of 138 days and 2 fetuses of 150 days were chosen.

Sampling and processing

Once the abomasum had been separated, small pieces of tissue were dissected for analysis. Tissues for histological examination were fixed in 4% buffered formaldehyde for 24 hours, routinely processed and embedded in paraffin. Sections 5μm thick were stained with Hematoxylin-Eosin (H-E), Masson`s Trichrome and Gomori's reticulin.

Morphometric analysis

Small pieces of abomasum were embedded in paraffin, stained with H-E, and viewed through a microscope (NIKON Eclipse 80i) equipped with a digital video camera (NIKON DXMI200F). The computerized image was analyzed using the Nis-Element 2.30 software package. The variables studied were height of various tissue strata (epithelium, lamina propria and submucosa, tunica muscularis and serosa) and total wall thickness. One hundred measurements were made in each tissue layer of each of the selected individuals from each group.

Statistical analysis

The results are shown as mean ± SE. Data was subjected to analysis of variance (ANOVA). Wherever ANOVA revealed significant differences, a post-hoc (Tukey) analysis was carried out to test for significant differences between tissue strata and groups. A value of $P < 0.05$ was considered significant.

Tissue growth models were created using a personal computer and a statistics program (Statistica Six Sigma, 2006). Graphs represent the averages of real growth values together with the adjusted line of regression. The goodness of fit of this adjustment was measured using the rate of determination, r^2. In all cases, embryo body length (crown-rump length, in centimeters) was used as the independent variable; the thickness of each tissue stratum (epithelium, lamina propria + submucosa, tunica muscularis and serosa) served as the dependent variable.

Immunohistochemical analysis

The UltraVision One HRP polymer (polymer conjugated to horseradish peroxidase) was performed on tissue from the abomasum to detect the neuroendocrine cell marker with synaptophysin (SYP), the glial cell marker with glial fibrillary acidic protein (GFAP) and vimentin (VIM), markers of peptidergic innervation as neuropetide Y (NPY) and vasoactive intestinal peptide (VIP) and gastrin. Tissues were deparaffinized, hydrated. Recovery of antigens was performed with citrate and

microwave. Blocking of endogenous peroxidase activity was made with 0.5% hydrogen peroxide for 30 min. Non-specific tissue binding sites were blocked by incubation in 1% normal goat serum for 30 min. Samples were incubated with the following primary antisera: 1:10 mouse monoclonal anti-SYP (Thermo Scientific, MA1-35810); ready to use rabbit polyclonal anti-GFAP (Thermo Scientifc, RB-087- R7), ready to use mouse monoclonal anti-VIM (Thermo Scientific, MS-129-R7), 1:50 rabbit polyclonal anti-NPY (Thermo Scientific, PA1-41576) and 1:50 rabbit polyclonal anti-VIP (AbD serotec, 9535-0204) and gastrin (PA1-36073) for 30 min at room temperature. Sections were finally incubated with polymer conjugated to horseradish peroxidase (Thermo Scientific, UltraVision ONE HRP Polymer, TL-015-PHJ) for 30 minutes at room temperature and without exposure to light. After that, the diaminobenzidine was applied in the tissue (Thermo Scientific, DAB Plus Chromogen TA-001-HCX and DAB Plus Substrate TA-015-HSX) for 5-15 minutes, depending on the desired stain intensity. Finally, the reaction was contrasted with Mayer hematoxylin. The specificity of the staining reaction was determined in control experiments involving either substitution of the primary antibody by PBS or normal goat serum 1:100, or omission of both primary and secondary antibodies. Absorption controls were obtained by incubating sections adjacent to those above with antiserum that contained 25 μg of Ag/ml of diluted antiserum. The antigen used were SPY (33R-6191, Fitzgerald), GFAP protein (30R-AG009 Fitzgerald), VIM (30R-2137, Fitzgerald), NPY (22465, AnaSpec) and VIP (22873, AnaSpec). No staining was found in structures on the sections which served as absorption controls.

Scanning electron microscopy

Small pieces of abomasum were fixed in 2.5% buffered glutaraldehyde for 24 hours, dehydrated through graded ethanol and amyl acetate, and dried in a critical-point dryer. Sections were covered with coating materials including gold and examined and photographed with a Jeol JSM 6300 scanning electron microscope operating at 30Kv at various tilt angles and at a magnification of 10 to 800x.

Results

Gross findings

At 38 days (25% gestation), the abomasum became apparent as an individual compartment, a tubular cavity lined by a smooth whitish mucosa. At 60 days (40% gestation), small surface elevations of the mucosa were observed corresponding to the abomasal folds. As development progressed, folds increased in both height and number. By the final stages of gestation, these folds gave the abomasal surface an irregular appearance similar to that observed in adults, but they were smaller than adult abomasal folds.

Abomasal histomorphogenesis

Group I (CRL 1.5 to 4.3 cm, 13-38 days, 1-25% gestation)

The abomasum became visible as a separate compartment of the primitive gastric tube at 35 days (23% gestation). The abomasal wall comprised three layers: an internal epithelial layer, a middle layer of pluripotential blastemic tissue and an external serosa (Fig. 1a).

The pseudostratified epithelium (365.6±27.5 μm) was composed of columnar cells whose nuclei appeared at different heights.

The middle layer of pluripotential blastemic tissue (492.95±34 μm) consisted of mesenchymal cells embedded in abundant amorphous ground substance.
The external serosa (44.12±16.3 μm) comprised a layer of loose connective tissue lined by a mesothelium composed of flat cells.

At 38 days (25% gestation), primitive abomasal folds were apparent as small elevations of the epithelial layer towards the abomasal lumen (Fig. 1a).

Group II (CRL 4.4 to 8 cm, 39-52 days, 25-35% of gestation)

The cylindrical pseudostratified epithelium (167.95±41 μm) was divided into two well-differentiated zones: a darker basal zone formed by uniformly-arranged nuclei, and a lighter apical cytoplasmic zone.

The pluripotential blastemic tissue layer (334.99±8 μm) was separated from the epithelium by a clearly-defined basal membrane. At 50 days (33% gestation), the appearance of fibroblasts and collagen fibers embedded in amorphous ground substance led to the differentiation of this blastemic tissue layer into lamina propria and submucosa (Fig. 1b).

By this stage, too, the tunica muscularis was distinguishable from pluripotential blastemic tissue, comprising 3-4 layers of myoblasts deriving from mesenchymal cells (Fig. 1b).

The serosa was formed by a subserosa of loose connective tissue underlying a mesothelial layer of flat cells.

At 52 days (35% gestation), abomasal folds were clearly apparent as elevations of the epithelium, involving both the lamina propria and the submucosa. Folds were larger and more numerous than at the previous stage.

Group III (CRL 9 to 17.5 cm, 53-75 days, 35-50% of gestation)

At 53 days (35% gestation), the abomasal wall was formed by four distinct tissue layers: epithelium, lamina

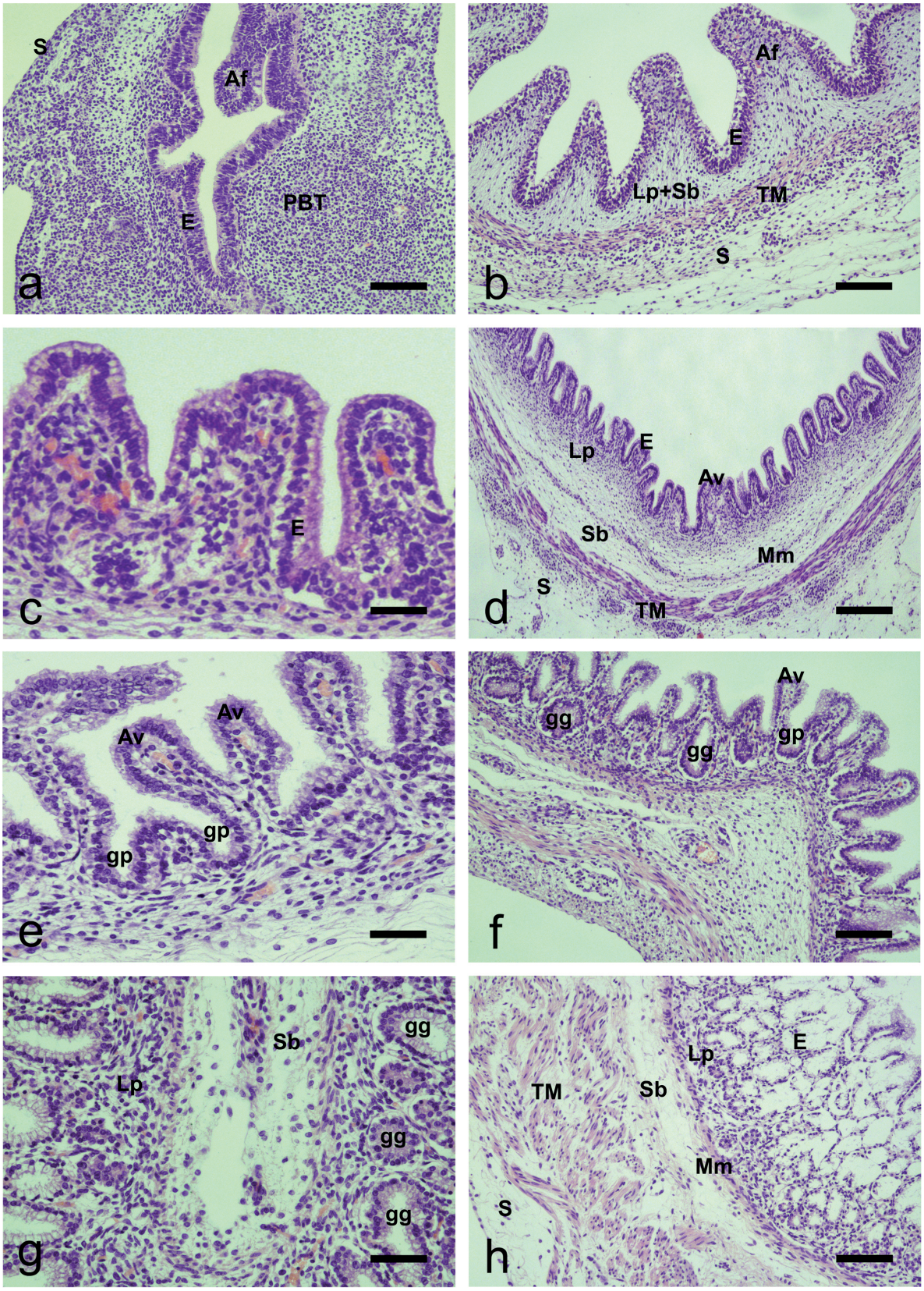

Fig. 1. Abomasal histomorphogenesis from 35 days (23% gestation) to 150 days (100% gestation). **a.** Photomicrograph of section of the differentiated abomasum (35 days, 23% gestation). The wall consisted of three layers: epithelium (E), pluripotential blastemic tissue (PBT) and serosa (S). Primitive abomasal folds are visible (Af). H-E. **b.** Photomicrograph of section of abomasal wall at 52 days (35% gestation). The wall is formed by epithelium (E), lamina propria + submucosa (Lp+Sb), tunica muscularis (TM) and serosa (S). Abomasal folds (Af) are larger. H-E. **c.** Photomicrograph of section of abomasal wall at 64 days (43% gestation). Simple columnar epithelium visible (E). H-E. **d.** Photomicrograph of section of abomasal wall at 64 days (43% gestation). The wall is formed by epithelium (E), tunica muscularis (TM), serosa (S) and lamina propria (Lp) and submucosa (Sb) separated by muscularis mucosae (Mm). Abomasal villi (Av) visible on the surface. H-E. **e.** Photomicrograph of section of the abomasal wall at 75 days (50% gestation). Gastric pits (gp) visible in the spaces between abomasal villi (Av). H-E. **f.** Photomicrograph of section of abomasal wall at 87 days (57% gestation). Outlines of gastric glands (gg) visible in the bottom of gastric pits (gp) and numerous abomasal villi (Av). H-E. **g.** Photomicrograph of section of abomasal wall at 113 days (75% gestation). Gastric glands (gg) now fully developed in lamina propria (Lp) and submucosa (Sb). H-E. **h.** Photomicrograph of section of abomasal wall (150 days, 100% gestation). All wall layers visible: epithelium (E), lamina propria (Lp), submucosa (Sb), muscularis mucosae (Mm), tunica muscularis (TM) and serosa (S). H-E. Scale bars: a, b, 25 μm; c, e, g, 20 μm; d, f, h, 30 μm

propria + submucosa, tunica muscularis and serosa.

Transformation of the primary pseudostratified epithelium into simple cylindrical epithelium (85.17±5.6 μm) was observed at 64 days (43% gestation). Nuclei were basally located, while the apical area was composed of cell cytoplasm (Fig. 1c).

The lamina propria and submucosa (619.79± 32 μm) were composed of fibroblast-rich connective tissue and sparse ground substance. At 64 days (43% gestation), the muscularis mucosae was visible in the form of 2-3 layers of smooth muscle fibers arising from the inner bundle of the tunica muscularis (Fig. 1d), which separated the lamina propria from the submucosa.

The tunica muscularis (249.49±29.7 μm) was formed by two bundles of smooth muscle fibers, a circular internal bundle and a longitudinal external bundle.

The serosa (85.62±12.81 μm) was composed of loose connective tissue underlying a layer of flat mesothelial cells.

By this stage of development, abomasal folds were taller and wider. At 64 days (43% gestation), a series of irregularities began to display on the surface of abomasal folds, together with the formation of abomasal peak areas. These irregularities or depressions corresponded with abomasal villi. By 75 days (50% gestation), the distance between peak areas was increased due to the formation of gastric pits in the intervening furrows (Fig. 1e).

Group IV (CRL 18 to 32 cm, 76-112 days, 50-75% gestation)

The abomasal surface was lined by a simple cylindrical epithelium (66.71±4 μm). Gastric pits were clearly visible in the widening spaces between gastric

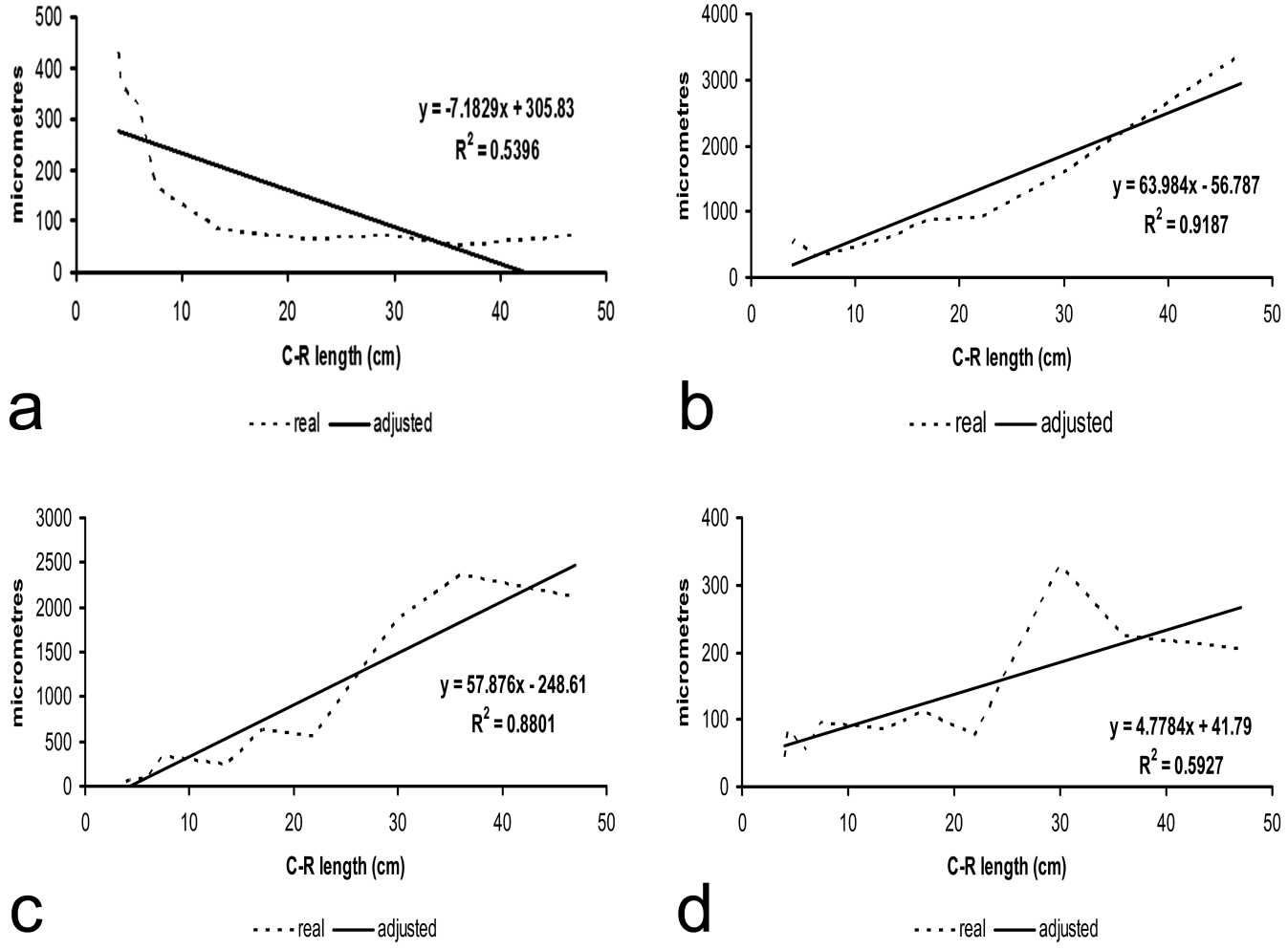

Fig 2. Mathematical growth models for abomasal tissue layers. **a.** Mathematical model of abomasal growth (epithelium). **b.** Mathematical model of abomasal growth (lamina propria and submucosa). **c.** Mathematical model of abomasal growth (tunica muscularis). **d.** Mathematical model of abomasal growth (serosa).

peak areas. At 87 days, rudimentary gastric glands were visible in the form of clumped cells at the bottom of gastric pits, but without differentiation of the various types glandular (Fig. 1f). The muscularis mucosae comprised 2-3 layers of smooth muscle fibers.

The lamina propria and submucosa (913.71±47.6 μm) were separated into two distinct layers by the muscularis mucosae. Although both layers comprised connective tissue, the lamina propria was more cellular than the submucosa.

The tunica muscularis (560.03±52 μm) was composed of two bundles of smooth muscle fibers: an external longitudinally-arranged bundle and an internal circular bundle.

The serosa (198±25.31 μm) displayed no histological differences with respect to earlier stages of development.

Group V (CRL 33 to 47 cm, 113-150 days, 75-100% of gestation)

At 113 days (75% gestation), the abomasal wall was formed by the following layers: mucosa, submucosa, tunica muscularis and serosa.

The abomasal mucosa was lined by a simple cylindrical epithelium (70.77±7.5 μm) composed of surface mucous cells. Gastric pits, evident in the form of small depressions over the whole mucosal surface, contained gastric glands, visible as fully-organized cell clusters arranged around a central lumen (Fig. 1g). At 113 days, different abomasal regions were differentiated with corresponding glandular types:

- cardial glandular region was located at the junction omasum-abomasum and cardial glands were observed closest to the gastric pits
- fundic region and abomasal body with oxyntic glands situated more deeply in the gastric pits.
- pyloric glandular region located in the distal part of abomasums, where the pyloric glands were observed.

The lamina propria (265.23±90.2 μm), composed of dense connective tissue, occupied the space between gastric glands. The muscularis mucosae was formed by several layers of smooth muscle fibers, separating the mucosa from the submucosa.

The submucosa (769.94±22.4 μm), underlying the muscularis mucosae, was composed of highly-vascularized connective tissue.

The tunica muscularis (2370±38.22 μm) comprised two well-developed bundles of smooth muscle fibers, with occasional interspersed nerve plexuses.

The serosa (206.86±9.12 μm) was formed by loose connective tissue, blood vessels and nerve fibers, with an overlying layer of flat mesothelial cells.

At 150 days (100% gestation), the abomasal wall consisted of four fully-formed layers, and was similar in composition to that of the adult abomasum (Fig. 1h).

Table 1. Morphometrical findings of the tissue layer thickness in the abomasum of goat during prenatal development (μm).

	Group I	Group II	Group III	Group IV	Group V
E	397.75±5	246.6±8	80.8±13[a]	68.77±21[a]	63±33[a]
Lp + Sb	pbt*	pbt	751.27±14	1261.41±24[b]	2811.71±37[b]
TM	Pbt	pbt	441.14±22	1217.06±27[b]	2242.81±41[b]
S	63.65±6	74.21±12	99.09±13	203.71±8	215.78±7

Group I (1.5-4.3 cm CRL, 26-38 days: 1-25% gestation); Group II (4.4-8 cm CRL, 39-52 days: 25-35% gestation); Group III (9-17.5 cm CRL, 53-75 days: 25-35% gestation); Group IV (18-32 cm CRL, 76-112 days: 50-75% gestation); Group V (33-47 cm CRL, 113-150 days: 75-100% gestation). E: Epithelium; Lp + Sb: Lamina propria and submucosa; TM: Tunica muscularis; S: Serosa; pbt: pluripotential blastic tissue. * The pluripotential blastic tissue, which will later give rise to the lamina propria-submucosa and tunica muscularis; were not statistically compared owing to the fact that one structure will give rise to various others. [a]P<0.005 vs Group I y II; [b]P<0.005 vs Group III.

Morphometric observations

Changes in the thickness of abomasal wall tissue layers during prenatal development are shown in Table 1.

The thickness of the epithelial layer was significantly higher in groups I and II compared to groups III to V (F= 15.01; P=0.008), but no significant differences were found between group I and II, or between group III, IV and V. The lamina propria and submucosa showed a thickness significantly higher in group IV and V than group III (F=10.86; P=0.016), but there were no significant differences between group IV and V. The thickness of tunica muscularis was significant differences between group III with group IV and V (F=8.15; P=0.02, but without significant differences between group IV and V. The mean value of the serosa of group I, II and III was significant different from group IV and V (F=1.57; P= 0.003), but no significant difference were found between group I, II and III, or between group IV and V.

The epithelial growth rate declined from the early embryonic stages until 64 days (43% gestation; Fig. 2a). The growth rate of the lamina propria and submucosa also declined from differentiation until 64 days (43% gestation). Thereafter, both layers displayed faster growth (Fig. 2b). The tunica muscularis grew progressively from differentiation until birth (Fig. 2c), as did the serosa, though the growth-rate was less marked (Fig. 2d).

Immunohistochemical observations

The results of immunohistochemical staining of the abomasum for SPY, GFAP, VIM, NPY, VIP and gastrin during prenatal development are shown in Table 2.

Neuroendocrine cells were first detected by SYP staining at 64 days (43% gestation), in the lamina propria, submucosa, tunica muscularis, serosa and myenteric plexuses (Fig. 3a,b).

VIM-immunoreactive glial cells were observed at 38 days (25% gestation), in pluripotential blastemic tissue and serosa (Fig. 3c). By 64 days (43% gestation), they

were also detected in myenteric plexuses, and scattered throughout the lamina propria, submucosa, tunica muscularis and serosa. Glial cells immunoreactive to GFAP were first observed at 64 days (43% gestation), in the lamina propria, submucosa, serosa, tunica muscularis and myenteric plexuses (Fig. 3d).

The peptidergic innervation markers NPY and VIP were first detected at 75 days (50% gestation), in the lamina propria, submucosa, tunica muscularis, serosa and myenteric plexuses (Fig. 3e, f).

Gastrin-immunoreactive cells were first detected at 76 days (50% gestation) in some areas of the epithelium (Fig. 3g), appearing at a later stage in pyloric glands (Fig. 3h).

Scanning electron microscopy observations

At 38 days (25% gestation) the mucosal surface of the abomasum appeared irregular (Fig. 4a). By 50 days (33% gestation), primitive abomasal folds were visible as small elevations of the mucosal surface (Fig. 4b).

At 64 days (43% gestation), abomasal folds were larger, and were observed over the whole surface (Fig. 4c); thereafter, folds grew in both size and number throughout development (Fig. 4d).

By 113 days (75% gestation), incipient peak areas and gastric pits were evident as small irregularities on the surface of abomasal folds (Fig. 4e).

In the final stage of prenatal development, abomasal folds were abundant, and gastric pits were clearly visible as furrow-like depressions on the fold surface, which also displayed mucous residue (Fig. 4f).

Discussion

Differentiation of the goat abomasum took place at 35 days (23% gestation), as also observed by Molinari and Jorquera (1988). Similar findings are reported by Franco et al. (1993b) in sheep and Masot et al. (2007a) in red deer. By contrast, differentiation occurs slightly earlier, at 30 days (11% gestation) in cattle (Asari et al., 1985; Vivo et al., 1990). Differentiation has been reported in goats at 28 days (19% gestation) by Mutoh and Wakuri (1988).

The pseudostratified epithelium observed here at 38 days (25% gestation) has also been reported at a similar stage in sheep (Wardrop, 1961; Fath El-Bab et al., 1983). Transition from pseudostratified to simple cylindrical epithelium took place at 64 days (43% gestation), a finding also noted in goats by Lee et al. (1994), and in sheep by Del Rio Ortega (1973) and Franco et al. (1993b). However, this transition was observed in goats at an earlier stage (10% gestation) by Mutoh and Wakuri (1988). In cattle, a similar transformation has been reported at 32% (Asari et al., 1985) and at 27% gestation (Vivo et al., 1990), while in red deer it has been observed by Masot et al. (2007a,b) at 97 days (35% gestation). In a study by Fath El-Bab et al. (1983), epithelial transformation was not recorded in sheep until 63% gestation.

At 38 days (25% gestation), primitive abomasal folds were visible as small undulations in the abomasal wall; similar findings are reported in sheep by Del Rio Ortega (1973) and Franco et al. (1993b), and in red deer by Masot et al. (2007a). However, Molinari and Jorquera (1988) and Fath El-Bab et al. (1983) report abomasal folds at a later stage in goats and sheep (49% and 40% gestation, respectively), while in cattle it occurs earlier, at 14% gestation (Vivo et al., 1990).

At 64 days (43% gestation), gastric peak areas were observed on the surface of abomasal folds, a finding also reported at a similar stage in goats (Molinari and Jorquera, 1988) and in sheep (Del Rio Ortega, 1973; Fath El-Bab et al., 1983). By contrast, gastric peak areas have been noted at an earlier stage of 35% gestation both in sheep (Franco et al., 1993b) and in red deer (Masot et al., 2007a).

At 75 days (50% gestation), gastric pits were formed in the space between gastric peak areas, while gastric glands were observed at 84 days (55% gestation) and the differentiation of cardial, oxyntic and pyloric glands took place at 113 days (75% gestation). In red deer the gastric glands were reported at similar ages by Masot et al., 2007a. The present results, however, differ from

Table 2. Immunohistochemical findings in the abomasum of goat during prenatal development.

	Group I				Group II				Group III				Group IV				Group V			
	E	Lp+Sb	TM	S	E	Lp+Sb	TM	S	E	Lp+Sb	TM	S	E	Lp+Sb	TM	S	E	Lp+Sb	TM	S
SYP	-	-	-	-	-	-	-	-	-	++	++	+	-	++	++	+	-	+++	+++	+
GFAP	-	-	-	-	-	-	-	-	-	+	+	+	-	++	++	+	-	++	++	+
VIM	-	+	+	+	-	+	+	+	-	´+	+	+	-	++	++	++	-	+++	+++	+++
VIP	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+	-	++	+	+
NPY	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+	-	++	+	+
GAS	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	++	-	-	-

Group I (1.5-4.3 cm CRL, 26-38 days: 1-25% gestation); Group II (4.4-8 cm CRL, 39-52 days: 25-35% gestation), Group III (9-17.5 cm CRL, 53-75 days: 25-35% gestation), Group IV (18-32 cm CRL, 76-112 days: 50-75% gestation); Group V (33-47 cm CRL, 113-150 days: 75-100% gestation). E: Epithelium, Lp + Sb: Lamina propia and submucosa, TM: Tunica muscularis, S: Serosa. SYP: synaptophysin, GFAP: glial fibrillary acid protein, VIM: vimentine, VIP: vasoactive intestinal peptide, NPY: neuropeptide Y, GAS: gastrin. -, non immunoreactivity; +, low immunoreactivity; ++, moderate immunoreactivity; +++, high immunoreactivity.

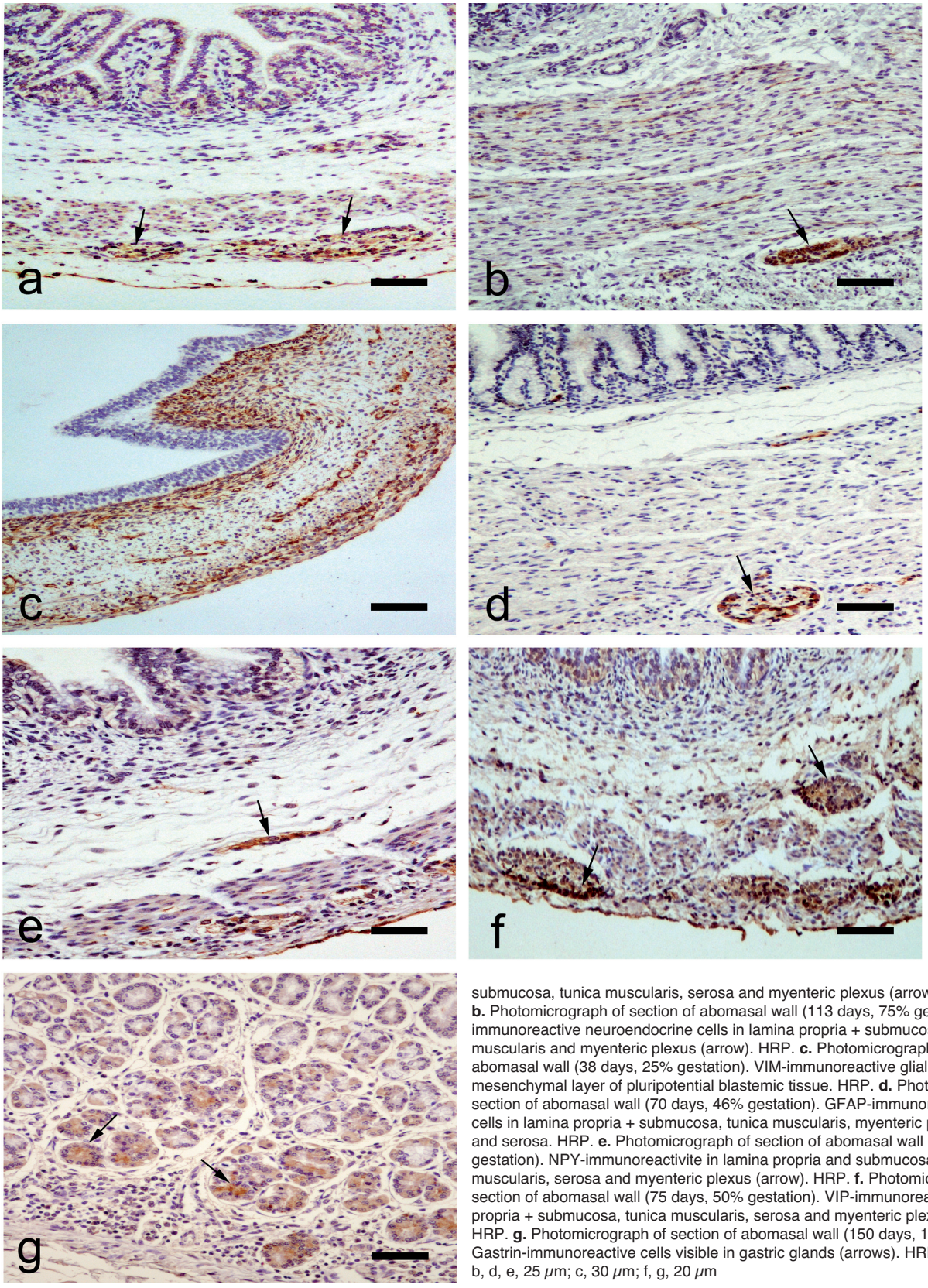

Fig. 3. Immunohistochemical findings in goat abomasum. **a.** Photomicrograph of section of abomasal wall (53 days, 35% gestation). SPY-immunoreactive neuroendocrine cells in lamina propria + submucosa, tunica muscularis, serosa and myenteric plexus (arrows). HRP. **b.** Photomicrograph of section of abomasal wall (113 days, 75% gestation). SYP-immunoreactive neuroendocrine cells in lamina propria + submucosa, tunica muscularis and myenteric plexus (arrow). HRP. **c.** Photomicrograph of section of abomasal wall (38 days, 25% gestation). VIM-immunoreactive glial cells in mesenchymal layer of pluripotential blastemic tissue. HRP. **d.** Photomicrograph of section of abomasal wall (70 days, 46% gestation). GFAP-immunoreactive glial cells in lamina propria + submucosa, tunica muscularis, myenteric plexus (arrow) and serosa. HRP. **e.** Photomicrograph of section of abomasal wall (75 days, 50% gestation). NPY-immunoreactivite in lamina propria and submucosa, tunica muscularis, serosa and myenteric plexus (arrow). HRP. **f.** Photomicrograph of section of abomasal wall (75 days, 50% gestation). VIP-immunoreactivite lamina propria + submucosa, tunica muscularis, serosa and myenteric plexus (arrows). HRP. **g.** Photomicrograph of section of abomasal wall (150 days, 100% gestation). Gastrin-immunoreactive cells visible in gastric glands (arrows). HRP. Scale bars: a, b, d, e, 25 μm; c, 30 μm; f, g, 20 μm

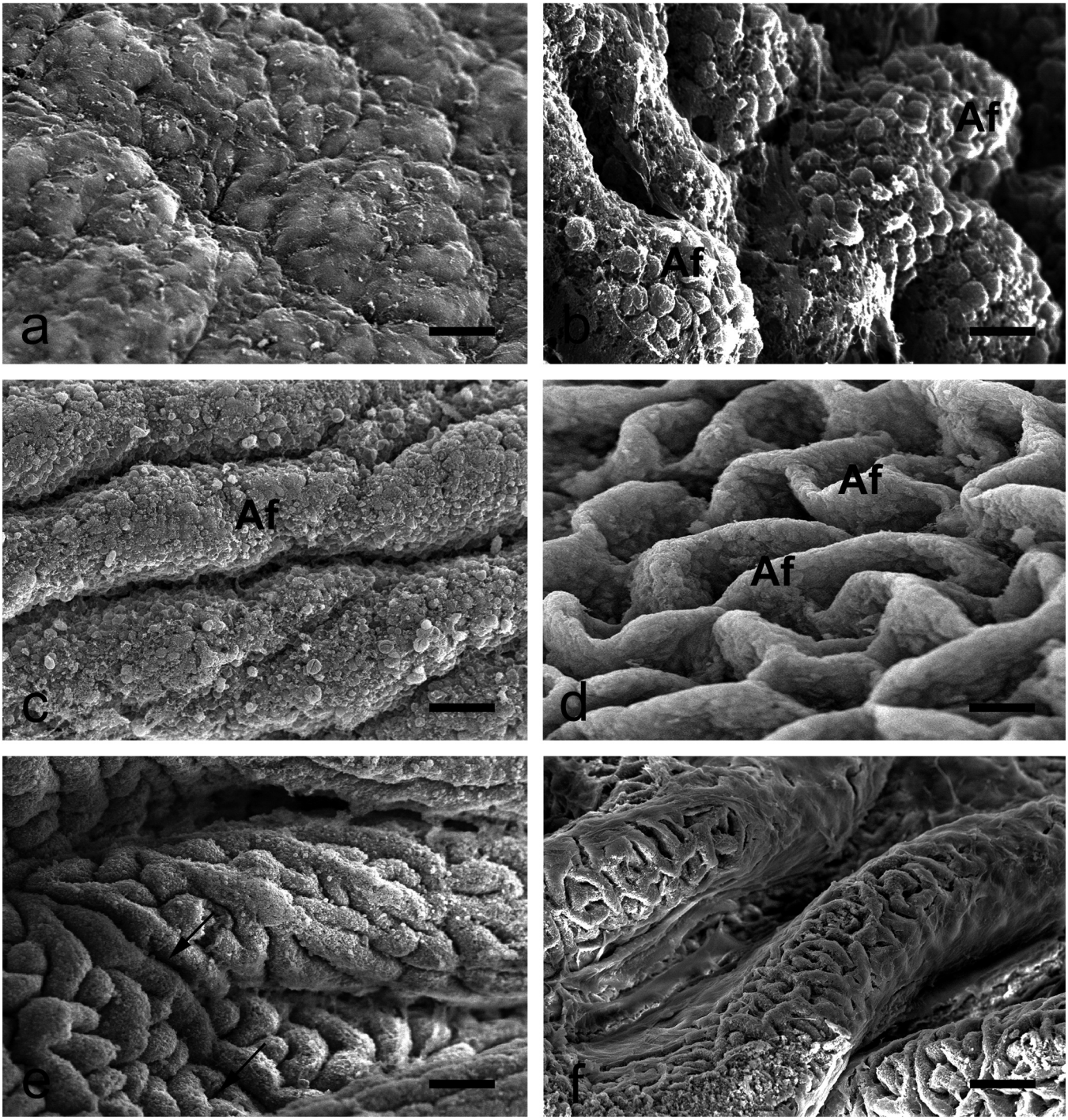

Fig. 4. Scanning electron microscopy of the goat abomasum (38 to 150 days, 25-100% gestation). **a.** Photomicrograph of section of abomasal wall (38 days, 25% gestation). Abomasal mucosa visible as irregular surface. SEM. **b.** Photomicrograph of section of abomasal wall (52 days, 35% gestation). Primitive abomasal folds (Af) visible as small elevations of the mucosal surface (Af). SEM. **c.** Photomicrograph of section of abomasal wall (64 days, 43% gestation). Abomasal folds have increased in size (Af). SEM. **d.** Photomicrograph of section of abomasal wall (75 days, 50% gestation). Abomasal folds (Af) larger and more numerous. SEM. **e.** Photomicrograph of section of abomasal wall (113 days, 75% gestation). Gastric pits (arrows) visible as small depressions on the surface of folds. SEM. **f.** Photomicrograph of section of abomasal wall (150 days, 100% gestation). Abomasal mucosa showing fully-developed folds and mucous residue. SEM. Scale bars: a, b, 8 μm; c-e, 6 μm; f, 4 μm.

those of Molinari and Jorquera (1988), who observed gastric pits at 46% gestation and gastric glands at 69% gestation, also in goats. In sheep, they have been reported both at earlier stages (Del Rio Ortega, 1973; Franco et al.. 1993b) and at a later stage of development (Fath El-Bab et al., 1983).

Differentiation of the lamina propria and the submucosa from pluripotential blastemic tissue took place at 50 days (33% gestation), a finding also reported for the goat rumen (Garcia et al. 2012). Both layers were composed of fibroblasts and collagen fibers, as also indicated by Age et al. (2007, 2008) in cattle and sheep, respectively. The muscularis mucosae appeared at 64 days, separating the lamina propria from the submucosa. This layer has been observed at an earlier stage in both sheep (33% gestation; Franco et al., 1993b) and red deer (35% gestation; Masot et al., 2007a).

Differentiation of the tunica muscularis from pluripotential blastemic tissue also took place at 50 days (33% gestation), as noted in red deer by Masot et al. (2007a). The two muscle-fiber bundles constituting the tunica muscularis were visible at 64 days (43% gestation), a finding also reported in the goat rumen (Garcia et al., 2012).

The serosa was composed of loose connective tissue underlying a layer of flat mesothelial cells, as also noted for the goat abomasum by Al-Saffar (2012).

SPY-immunoreactive neuroendocrine cells were detected at 64 days (43% gestation) in the lamina propria and submucosa, tunica muscularis, serosa and myenteric plexuses, a finding also reported by Masot et al. (2007a). VIM-immunoreactive glial cells were observed at 38 days, and GFAP-immunoreactive glial cells at 64 days, confirming that VIM is an earlier marker, as noted by Franco et al. (1997)) in a study of prenatal pineal-gland development in sheep. Glial cells were observed at earlier stages in red deer (Masot et al., 2007a).

Neuropeptide Y-immunoreactive and VIP-immunoreactive were detected at 75 days (50% gestation) in the lamina propria, submucosa, tunica muscularis, myenteric plexuses and serosa, as also reported in red deer by Masot et al. (2007a). These neuropeptides have also been detected in the postnatal sheep abomasum (Groenewald, 1994).

Gastrin-immunoreactive cells were first detected at 76 days (50% gestation) in the pyloric region, as reported for other ruminant species: they have been detected in sheep at 77 days (50% gestation; Franco et al., 1993d), in red deer at 142 days (50% gestation; Masot et al., 2007a) and in fallow deer between the 11th and 17th weeks of prenatal development (Dall´Aglio et al., 1999).

Under the scanning electron microscope, the abomasal mucosa appeared as an irregular surface, with small elevations which would subsequently give rise to the abomasal folds. As development progressed, small furrow-like depressions observed on the surface of these folds developed into gastric pits. Similar findings have been reported in the human stomach by Otani et al. (1993), who observed rudimentary gastric pits in the form of depressions or furrows between 5 and 8 weeks of fetal life.

In summary, the present observations and comparison with findings reported for other ruminants suggest that the prenatal development of the abomasum as a functional structure with secretional capacity takes place somewhat earlier in goats than in sheep or cattle, but at a similar stage to that observed in red deer. The similar abomasal growth-rate in goats and deer may reflect similarities in maternal feeding patterns due to there is growing appreciation of the influence of diet on the ontogeny of the gastrointestinal (Newburg and Walker, 2007), which explains the differences in the stomach prenatal development of the different species. Goat and deer have a maternal diet similar (Garcia Gonzalez and Cuartas, 1992) based on grazing and particularly on more marginal pasturelands. These reasons may explain the coincidence in time of prenatal development abomasal between goat and deer. By contract, the abomasum of sheep and cattle has a later prenatal development that goat. This difference may be due to sheep and cattle digest low quality forage (Hofmann, 1989) and receive feed supplements in adulthood, so they do not need an early development of abomasum to adapt to the environment.

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