

Ontogenesis of the omasum: a comparative analysis of the Merino sheep and Iberian red deer

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Summary. The aim of this study is to describe differences in the ontogenesis of the omasum in sheep (domestic ruminant) and deer (wild ruminant). A total of 50 embryos and fetuses of Merino sheep and 50 Iberian deer were used, from the first stages of prenatal life until birth. For the study, the animals were divided into five experimental groups according to the most relevant histological characteristics. The appearance of the omasum from the primitive gastric tube was earlier in sheep (22% gestation, 33 days) than in deer (25% gestation, 66 days). In both cases it displayed a primitive epithelium of a stratified, cylindrical, non-ciliary type. The appearance of four laminae of different sizes was always earlier in sheep than deer. At around 36% gestation in sheep (53 days) and 36% (97 days) in deer, the omasum consisted of 4 clearly-differentiated layers: mucosa (with epithelial layer and lamina propria), submucosa, tunica muscularis and serosa. The temporal order of appearance of the four order laminae and omasal papillae was always earlier in sheep than deer. The tegumentary mucosa of the omasum was without secretion capability in the first embryonic phases. From 67 days (26% gestation) the neutral mucopolysaccharides appeared in deer and at 46 days (30% gestation) in sheep. In both cases they continued to decrease until birth, this decrease being more pronounced in deer. Finally, the presence of neuroendocrine and glial cells was detected in deer at earlier stages than in sheep.

Key words: Immunohistochemistry, Omasum, Prenatal development, Red deer, Sheep

Introduction

The elevated capacity of ruminants to convert coarse foodstuffs into products of a highly nutritious value has, to a large extent, led to the study of the structure and function of the digestive systems of these animals (Tiwari and Jamdar, 1970; Arias et al., 1978; Molinari and Jorquera, 1988; Stumpff et al., 2009). From a physiological point of view, the omasum plays a fundamental role in the digestion of ruminants, and serves mainly as a dehydration area and as a sieve. As food passes through the omasum it is squeezed and compressed by contractions. The leaves of the omasum absorb a large portion of the volatile fatty acids that were not absorbed through the rumen wall, water and electrolytes, such as potassium and sodium (Redondo et al., 2005). The stomach of sheep was subjected to numerous morphological studies, including immunohistochemical and morphometric analysis (Franco et al., 1992, 1993a,b,c; Regodón et al., 1996). The economic importance of the red deer due to hunting has led to this species being used in histogenetic studies of the gastric compartments in wild animals (Franco et al., 2004a,b; Redondo et al., 2005; Masot et al., 2007). According to their feeding habits, ruminants can be classified as grazers, concentrate selectors and those of intermediate type. There are differences in nutrient acquisition and utilisation between the two species of this study. They have different phylogenetic adaptation to different types of diet (roughage eater vs concentrate selector, Münnich et al., 2008) which has relevance for this study. The objective of this current study was to carry out a comparative analysis of these two species (domestic and wild), fed in a natural way, with the aim of designing a comparative referential base for future studies that consider the possible influence of dietary supplements in histogenetic modifications on the histophysiology of the omasum.

Materials and methods

Animals

Embryos and fetuses of sheep (*Ovis aries*, n=50) and the red deer (*Cervus elaphus*, n=50) from the first prenatal stages until birth were studied. The specimens were divided into 5 groups, according to the most relevant histomorphogenic characteristics. The merino breed ovine embryos and fetuses all came from the Municipal slaughterhouse in Cáceres. To obtain embryos and fetuses at various stages of development, a total of 125 Caesarean sections on the same number of dead females were performed. Deer females were hunted in legal shootings in ten hunting grounds from extensive and non-enclosed estates from the Sierra de San Pedro (north-east of the Province of Cáceres, Spain). The estimates of gestation age were carried out according to the methodology proposed by Evans and Sack (1973).

Sampling and processing

Once the omasum had been separated, small pieces of tissue were dissected from the medial region of the omasum of each animal. The tissue for histological study was fixed in 4% buffered formaldehyde for 24 h, processed by conventional paraffin embedding methods, and sections 5 μ m thick were cut in transverse direction and treated with H-E; Periodic Acid-Schiff (pH 7.2) and PAS-alcian blue (pH 7.2); Masson's Trichrome and Reticuline of Gomori.

Morphometric analysis

Specimens for morphometric analysis were embedded in paraffin, stained with H-E, and viewed through a microscope (Optiphot; Nikon Inc., Tokio, Japan) equipped with a video camera. The image was reflected onto the screen of a semiautomatic image analyzer (Vid IV; Rego and Cia, Madrid, Spain). Variables studied were height of various tissue strata (epithelium, lamina propria and submucosa, tunica muscularis and serosa) and total wall thickness. Eight specimens were selected for each group and 50 measurements were made for each tissue stratum.

Statistical analysis

The results are shown as the mean \pm SE. The data were analysed using Analysis of Variance. In cases where ANOVA was significant, a post-hoc (Tukey) analysis was carried out in order to study the significant differences among the distinct tissue strata in the two species and among the distinct groups. A value of $P \leq 0.05$ was considered significant.

Immunocytochemical analysis

ExtrAvidin Peroxidase Staining (EAS) was

performed on deparaffinized tissue from the dorsal and ventral sac of the rumen to detect the neuroendocrine cell markers [non neuron enolase (NNE)] and glial cells markers [glial fibrillary acidic protein (GFAP) and vimentin (VIM)]. Tissue was deparaffinized, hydrated and treated sequentially with 15% hydrogen peroxide for 30 min in order to block endogenous peroxidase activity. 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: 1:200 monoclonal anti-human NNE (Sigma/Aldrich Química, Madrid, Spain, no. S5768); 1:400 monoclonal anti-human GFAP (Sigma/Aldrich Química, Madrid, Spain, no. G-3893) and 1:20 monoclonal anti-human VIM (Sigma/Aldrich Química, Madrid, Spain, no. V-5255) for 3 h at 20°C. Biotinylated goat anti-mouse IgG (1:200 dilution) (Sigma/Aldrich Química, Madrid, Spain no. B7151) was then added to the sections for 30 min. Sections were finally incubated with diluted (1:50) ExtrAvidin-Horseradish Peroxidase (Sigma/Aldrich Química, Madrid, Spain no. E2886) for 1 h. After diaminobenzidine reaction, nuclear counterstaining with Mayer hematoxylin was applied. The specificity of the staining reaction was determined in control experiments. These comprised prior absorption of the primary antibody, substitution of the primary antibody by PBS or normal mouse serum 1:100, or omission of both primary and secondary antibodies.

Results

Omasal Histomorphogenesis

Group I: sheep (0.4-2.6 cm CRL, 23-33 days: 1-22% gestation); deer (1.4-3.6 cm CRL, 30-60 days: 1-25% gestation).

At the first stage of development, in both species, the gastric outline appeared as a fusiform tube that comprises one unique cavity, and whose wall was formed by two well-differentiated layers: an internal or epithelial layer and another external or pluripotential blastemic tissue. The epithelium was of a non-ciliary, stratified, cylindrical type. Its cells displayed a round nucleus localized in the middle and apical portions of the epithelial layer leaving a band of clear cytoplasm peripherally. The pluripotential blastemic tissue was subepithelial and of a mesenchymatous nature, forming the largest part of the organic wall. It was made up basically of a blastema rich in stellate cells, grouped in various irregularly-arranged layers and immersed in an abundant ground substance.

In sheep at 33 days (22% gestation) the outline of the omasal compartment appeared, of an individualised form and separated from the rest of the compartments (Fig. 1A,B). The individualisation was observed in red deer in the final phases of this first phase, at 60 days-25% gestation (Fig. 1A,B). In both species, the omasal wall, smooth until now, displayed an undulating surface

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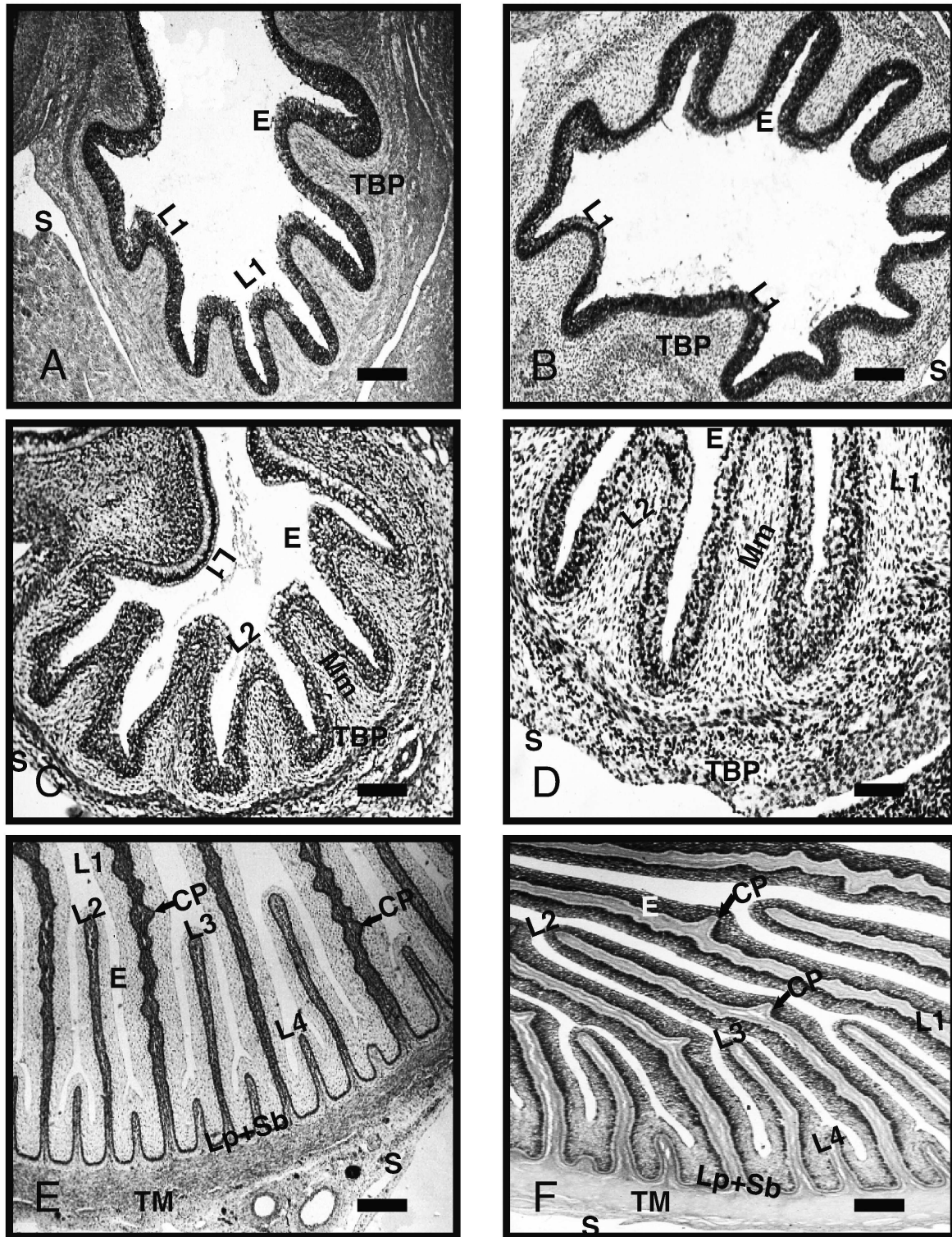


Fig. 1. Omasal histomorphogenesis in sheep (23-79 days, 1-53% gestation) and red deer (30-135 days, 1-50% gestation). **A and B.** Photomicrograph of a transverse section of the omasal wall of sheep (**A**, 33 days, 22% gestation) and red deer (**B**, 60 days, 25% gestation). Three layers are visible: epithelium (E), pluripotential blastemic tissue (PBT) and serosa (S). In the epithelial layer the primary smaller laminae can be seen (L1). H-E. **C and D.** Photomicrograph of a transverse section of the reticular wall of sheep (**C**, 52 days, 35% gestation) and red deer (**D**, 90 days, 35% gestation). Myoblastic fibres of the tunica muscularis infiltrating into the smaller laminae and constituting the muscularis mucosae (Mm) were observed. The epithelium is stratified in two zones: a basal zone or stratum germinativum and another apical zone or stratum granulosum. Serosa is visible (s). The secondary smaller laminae (L2) can be observed in the spaces between the primary smaller laminae (L1). H-E. **E and F.** Photomicrograph of a transverse section of the reticular wall of sheep (**E**, 79 days, 53% gestation) and red deer (**F**, 135 days, 50% gestation). Four layers can be detected: epithelium (E), lamina propria-submucosa (Lp+Sb), tunica muscularis (TM) and serosa. Presence of the primary (L1), secondary (L2), tertiary (L3) and quaternary smaller laminae (L4). Presence of cornu papillae (Pc) in the smaller laminae is visible. H-E. Bars: A-C, E, F, 30 μ m; D, 25 μ m.

and appeared as three well defined layers. These undulations were interpreted as rudimentary or first-order laminae, all similar in height (Fig. 1A,B). The internal epithelial layer, with no histochemical reaction to the mucopolysaccharides, was thicker in sheep ($66\pm 6\ \mu\text{m}$) than in deer ($46\pm 5\ \mu\text{m}$). The middle layer, or pluripotential blastemic tissue, was thicker in sheep ($223\pm 10\ \mu\text{m}$) than in deer ($183\pm 10\ \mu\text{m}$) and displayed in its medial zone the appearance of fusiform myoblastic cells. It was more defined in sheep, constituting the ontogenetical base for the future tunica muscularis. The external layer or serosa displayed a similar thickness in both species ($77\pm 6\ \mu\text{m}$ in sheep and $71\pm 5\ \mu\text{m}$ in deer) and was formed by a mesothelium of flat cells and a lax connective tissue of cellular support.

Group II: sheep (4-8 cm CRL, 39-52 days: 26-35% gestation); deer (4.5-7.2 cm CRL, 67-90 days, 26-35% gestation)

At 42 days (28%) in sheep and at 90 days (35%) in deer, epithelial swellings in the interlamina space gave rise to the appearance of second-order laminae and were formed from pluripotential blastemic tissue, which initiated a division of lamina propria and submucosa (Fig. 1C,D). In both species the stratified epithelial layer undergoes a sharp increase in thickness, more pronounced in sheep ($168\pm 10\ \mu\text{m}$) than in deer ($119\pm 11\ \mu\text{m}$), at identical percentages of gestation. This epithelial layer was bizonal with a dark-staining basal zone rich in germinativum cells and another more external apical zone of globular cells of clear cytoplasm (Fig. 1C,D). For the first time we detected neutral mucopolysaccharides in the basal zone of the epithelium at 67 days (26% gestation) in deer and at 46 days (30% gestation) in sheep. The pluripotential blastemic tissue, thicker in sheep ($307\pm 15\ \mu\text{m}$) than in deer ($206\pm 13\ \mu\text{m}$), appeared separated from the epithelium by a clearly defined basal layer (Fig. 1C,D). It was highly vascularised and in its depth a thin tunica muscularis, comprising two layers of supposed myoblasts, circularly and longitudinally arranged, began to appear. This tunica muscularis exhibited a series of undulations coinciding with the implantation base of each smaller laminae. The appearance of the second-order laminae coincided with the appearance of a thin layer of smooth muscle fibers between the submucosa and the lamina propria of the first-order laminae. These fibers, which formed the muscularis mucosae (Fig. 1C,D), originated in the inner bundle or the tunica muscularis. The serosa, of a similar thickness in both species ($45\pm 5\ \mu\text{m}$), was situated immediately below the previous layer and was formed by an epithelium of flat cells and a highly cellular tissue or subserosa.

Group III: sheep (8.5-19 cm CRL, 53-79 days: 36-53% gestation); deer (8-19 cm CRL, 97-135 days, 36-50% gestation).

The wall was formed by 4 layers: mucosa (with

epithelial layer and lamina propria), submucosa, tunica muscularis and serosa (Fig. 1E,F). The stratified epithelial layer continued growing in thickness, more pronounced in sheep ($243\pm 16\ \mu\text{m}$) than in deer ($219\pm 30\ \mu\text{m}$) due to the increase in size as well as number of cells. It was made up of a basal zone, or stratum germinativum, formed by three or four layers of oval cells and of basophilic cytoplasm, together with an apical layer consisting of polyhedral cells, with a small nucleus and clear-coloured cytoplasm, arranged in a mosaic pattern and referred to as the stratum granulosum. In both cases, the presence of neutral mucopolysaccharides in this stratum with interlamina spaces was positive, although not for acidic mucopolysaccharides, mucins or mucoid compounds. The appearance of these two strata was always earlier in deer (97 days, 36% gestation) than in sheep (72 days, 48% gestation). The lamina propria, adjacent to the epithelial layer, was formed by morphologically stellate cells and a small quantity of ground substance. The submucosa, close to the tunica muscularis, displayed less cellular richness and a greater quantity of ground substance than the previous group. Both layers together were of a similar thickness in both species ($101\pm 9\ \mu\text{m}$ in sheep and $99\pm 8\ \mu\text{m}$ in deer). The tunica muscularis displayed a greater thickness than in the previous group in sheep ($121\pm 19\ \mu\text{m}$) as well as in deer ($79\pm 8\ \mu\text{m}$), projected into the center of the laminae. The serosa, ($52\pm 7\ \mu\text{m}$ in sheep and $32\pm 7\ \mu\text{m}$ in deer) showed a mesothelium held in a subserosa rich in ground substance, reticuline fibres, and with an intense vascularisation and innervations. At 53 days (36%) in sheep and at 97 (36%) in deer, first- and second-order laminae had grown considerably, and the initial stages of third-order laminae were visible between them. Development of the fourth-order laminae also took place at 59 days (40%) in sheep and at 122 (45%) in deer (Fig. 1E,F), appearing at this moment some lateral connective tissue evaginations of the stratum basale of first-order laminae towards the epithelial surface. These were the primitive corneum papillae and were visible in second-order laminae by 79 days (53%) in sheep and at 135 (50%) in deer (Fig. 1E,F).

Group IV: sheep (20-3.5 cm CRL, 81-112 days: 54-75% gestation); deer (21 to 33 cm C-R, 142-191 days, 51-75% gestation).

In both species, the epithelial layer underwent a sharp increase in thickness, this being more pronounced in sheep ($406\pm 28\ \mu\text{m}$) than in deer ($390\pm 28\ \mu\text{m}$). This thickening was due to greater stratification, distinguished by four strata (Fig. 2A-D). The stratum germinativum, situated in the basal zone displayed intense staining of the basophils. The stratum granulosum was formed by many polyhedral vesiculiform cells, of a clear cytoplasm and nuclei polarised towards one of the membranes. The appearance of a transition zone external to stratum granulosum represented the morphological expression of

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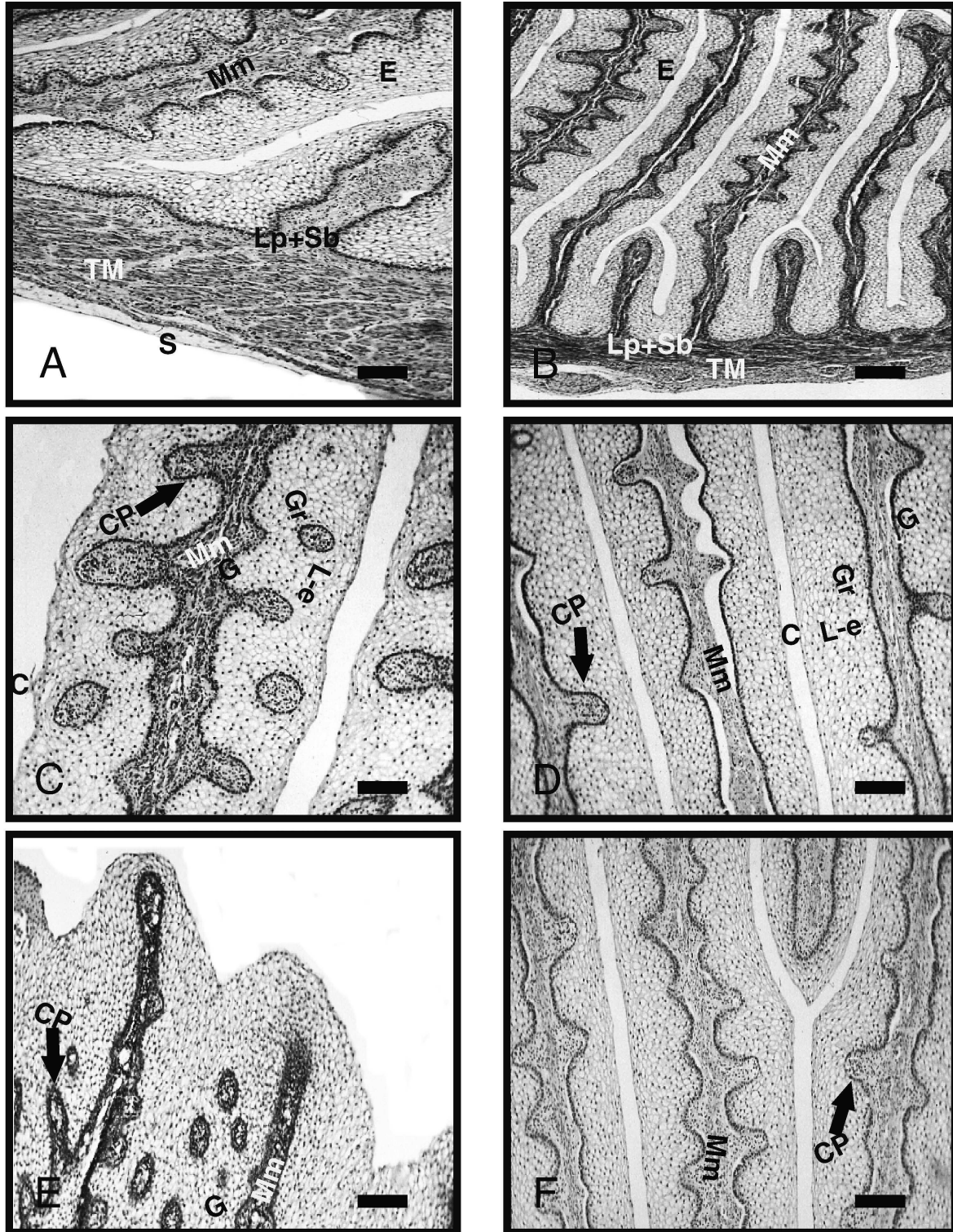


Fig. 2. Omasal histomorphogenesis in sheep (81-150 days, 54-100% gestation) and red deer (142-235 days, 51-100% gestation). **A and B.** Photomicrograph of a transverse section of the omasal wall of sheep (**A**, 112 days, 75% gestation) and red deer (**B**, 191 days, 75% gestation). It was formed by 4 layers: mucosa, with epithelial layer (E), lamina propria and submucosa (Lp+Sb) and muscular mucosae (Mm); tunica muscularis (TM); serosa (S). **C and D.** Photomicrograph of a transverse section of the omasal wall of sheep (**C**, 112 days, 75% gestation) and red deer (**D**, 191 days, 75% gestation). Primary lamina with stratified epithelium with stratum germinativum (G), stratum granulosum (Gr), stratum lucidum (L-e) and stratum corneum (C). Corneum papillae (CP) and muscularis mucosae can also be seen. **E and F.** Photomicrograph of a transverse section of the omasal wall of sheep (**E**, 150 days, 100% gestation) and red deer (**F**, 235 days, 100% gestation). Inner fascicle of the tunica muscularis filling the width of the smaller laminae and forming the muscularis mucosae (Mm). Abundant presence of corneum papillae (CP) can also be observed. H-E. Bars: A, 25 μ m; B, 30 μ m; C-F, 20 μ m.

the stratum lucidum. Finally, the stratum corneum was formed by a simple layer of extended cells, morphologically flat and in contact with the lumen. This completed stratification began to be formed first in deer, at 142 days of prenatal life (51% gestation) and later in sheep (83 days, 55% gestation). Corneum papillae (Fig. 2C,D) were abundant in the first-order laminae as well as in the second-order, but were not found in lower order laminae. The neutral mucopolysaccharides began to be detected in the lucidum-spinosum stratum at the same time that they decreased in the granulosa stratum, for both species. The lamina propria-submucosa was thinner than in the previous group ($81\pm 6\ \mu\text{m}$ in sheep and $85\pm 7\ \mu\text{m}$ in deer) and highly vascularised. In this phase, we also witnessed a sharp development in thickness of the tunica muscularis, especially of the internal fasciculus that formed the greater part of the thickness of the laminae, making up the muscularis mucosae (Fig. 2C,D). Finally, the thinner serosa ($21\pm 4\ \mu\text{m}$ in sheep and $26\pm 4\ \mu\text{m}$ in deer) did not display differential characteristics with respect to the previous stage.

Group V: sheep (32-40 cm CRL, 113-150 days: 76-100% gestation); deer (36 to 40 cm C-R, 205-235 days, 76-100% gestation).

The histogenetic structure of the omasal wall did not change substantially with respect to the previous stage. The epithelium was keratinized squamous stratified, corresponding to a tegumentary-type. The corneum papillae (Fig. 2E,F) had increased in primary and secondary laminae and appeared in the two remaining generations: in sheep at 113 days (76% gestation) in the tertiary laminae and at 216 days (80%) in quaternary laminae; in deer at 200 days (85%) and 223 days (95%), respectively. In the interior of the corneum papillae, reticulin and collagen fibres were seen, as well as smooth muscular fibres coming from the muscularis mucosae (Fig. 2E,F). In the lamina propria, submucosa, tunica muscularis and serosa, we found no significant differences with respect to the previous stage for either species.

Morphometric observations

Table 1 shows the tissue layer thickness in the omasum of sheep and red deer during prenatal development. A factorial ANOVA indicated that the mean growth value of group I epithelium was significantly lower than in groups II-V in sheep ($P=0.003$) and in red deer ($P=0.004$). By contrast, the mean growth value of group I serosa was significantly higher than in groups II-V in sheep ($P=0.003$) and in red deer ($P=0.002$) and the same for wall in sheep ($P=0.003$) and in red deer ($P=0.002$). As indicated by main factor analysis in factorial ANOVA, the lamina propria and submucosa of group III was significantly different from these layers of groups IV and V in sheep ($P=0.002$) and in red deer ($P=0.002$), and the tunica muscularis of

groups III was also significantly from this tunica in groups IV and V in sheep ($P=0.002$) and in red deer ($P=0.002$). When comparing the thickness of each layer in two species, a factorial ANOVA indicated that the mean growth value of epithelium of sheep was significantly higher than red deer in all groups ($P=0.004$) and the same for wall ($P=0.003$). The mean growth value of tunica muscularis of red deer was significantly higher than sheep in groups IV and V ($P=0.004$). There was no significant difference in the case of the serosa.

Immunohistochemical observations

Immunohistochemical findings in the omasum of the five groups of sheep and red deer studied are summarized in Table 2. The presence of neuroendocrine cells (NNE-positive cells) in the omasal mucosa was not detected until 97 days (36% gestation) in deer and 81 days (54% gestation) in sheep (Table 2, Fig. 3A,B). In both species, these cells were located in the lamina propria and submucosa, close to the epithelium and in the thickness of the intermuscular connective tissue and in the tunica muscularis (Table 2, Fig. 3A,B). The presence of GFAP-positive cells occurred at around 142

Table 1. Morphometrical findings of the tissue layer thickness in the omasum of sheep and red deer during prenatal development (μm).

	Group I	Group II	Group III	Group IV	Group V
Epithelium					
sheep	66±6	186±10 ^a	243±16 ^a	406±28 ^a	572±35 ^a
red deer	46±5 ^c	119±11 ^{ac}	219±30 ^{ac}	390±28 ^{ac}	525±30 ^{ac}
Lp + sb					
sheep	pbt	pbt	101±9	81±6 ^b	83±10 ^b
red deer	pbt	pbt	99±8	85±7 ^b	88±8 ^b
Tm					
sheep	223±10*	307±15*	121±19	166±12 ^b	172±11 ^b
red deer	183±10*	206±13*	79±8	188±12 ^{bc}	190±11 ^{bc}
Serosa					
sheep	77±6	46±5 ^a	52±7 ^a	21±4 ^a	22±4 ^a
red deer	71±5	45±5 ^a	32±7 ^a	26±4 ^a	24±6 ^a
Wall					
sheep	366±10	539±14 ^a	517±18 ^a	674±29 ^a	849±38 ^a
red deer	300±15 ^c	370±15 ^{ac}	429±24 ^{ac}	689±41 ^{ac}	827±31 ^{ac}

Group I: sheep (0,4-2,6 cm CRL, 23-33 days: 1-22% gestation), red deer (1,4-3,6cm CRL, 30-60 days:1-25% gestation); Group II: sheep (4-8 cm CRL, 39-52 days: 26-35% gestation), red deer (4,5-7,2 cm CRL, 67-90 days, 26-35% gestation); Group III: sheep (8,5-19 cm CRL, 53-79 days: 36-53% gestation), red deer (8-19 cm CRL, 97-135 days, 36-50% gestation); Group IV: sheep (20-31,5 cm CRL, 81-112 days: 54-75% gestation), red deer (21 to 33 cm C-R, 142-191 days, 51-75% gestation); Group V: sheep (32-40 cm CRL, 113-150 days: 76-100% gestation), red deer (36 to 40 cm C-R, 205-235 days, 76-100% gestation). Lp+sb: Lamina propria and submucosa. Tm: tunica muscularis. pbt: pluripotential blastoc tissue. *The pluripotential blastoc 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; ^b $P<0.005$ vs Group III, ^c $P<0.005$ vs sheep.

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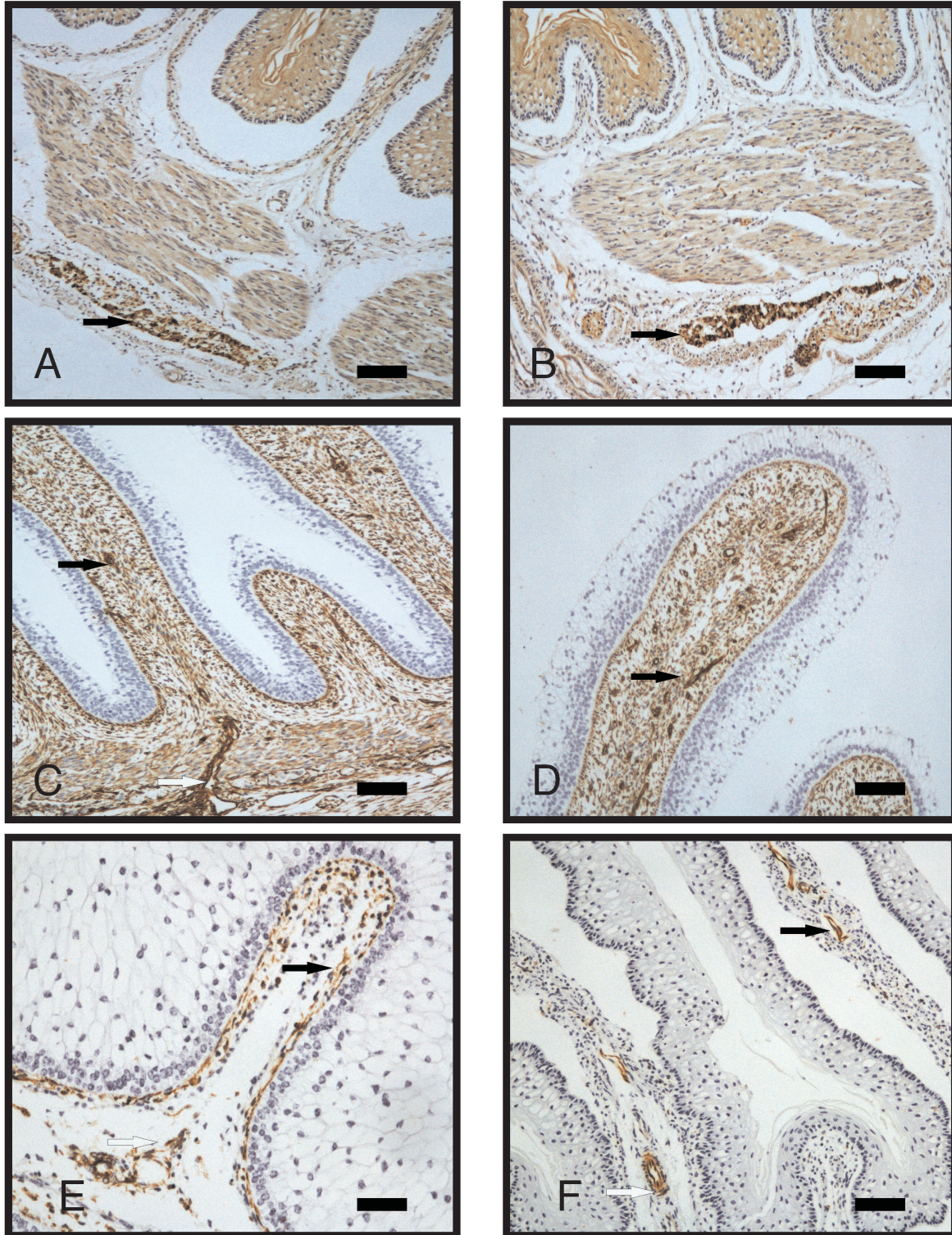


Fig. 3. Omasal immunohistochemical findings in sheep (23-150 days, 1-100% gestation) and red deer (30-235 days, 1-100% gestation). **A and B.** Photomicrograph of a transverse section of the omasal wall of sheep (**A**, 81 days, 54% gestation) and red deer (**B**, 135 days, 50% gestation). Presence of non neuron enolase positive cells in myenteric plexus (arrows). EAS. **C and D.** Photomicrograph of a transverse section of the omasal wall of sheep (**C**, 112 days, 75% gestation) and red deer (**D**, 191 days, 75% gestation). Abundant presence of GFAP positive cells in the lamina propria-submucosa (white arrow), and in the tunica muscularis. (black arrow). EAS. **E and F.** Photomicrograph of a transverse section of the reticular wall of sheep (**E**, 97 days, 36% gestation) and red deer (**F**, 67 days, 26% gestation). Presence of VIM positive cells in the interior of the omasal laminae (black arrow) and in the inter-vascular connective tissue of lamina propria-submucosa (white arrow). EAS. Bars: A, B, 30 μ m; C, D, 25 μ m; E, F, 20 μ m.

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Table 2. Immunohistochemical findings in the omasum of sheep and red deer during prenatal development.

	Group I				Group II				Group III				Group IV				Group V			
	E	LP-S	TM	S	E	LP-S	TM	S	E	LP-S	TM	S	E	LP-S	TM	S	E	LP-S	TM	S
NNE Sheep	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	++	++	-
NNE Reed Deer	-	-	-	-	-	-	-	-	-	+	+	-	-	++	++	-	-	++	++	-
GFAP Sheep	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	--	++	++	++
GFAP Reed Deer	-	-	-	-	-	-	-	-	-	-	-	-	-	++	++	++	--	++	++	++
VIM Sheep	-	-	-	-	-	-	-	-	-	+	+	+	-	++	++	++	-	+++	+++	+++
VIM Reed Deer	-	-	-	-	-	+	+	+	-	++	++	++	-	++	++	++	-	+++	+++	+++

Group I: sheep (0,4-2,6 cm CRL, 23-33 days: 1-22% gestation), red deer (1,4-3,6 cm CRL, 30-60 days: 1-25% gestation); Group II: sheep (4-8 cm CRL, 39-52 days: 26-35% gestation), red deer (4,5-7,2 cm CRL, 67-90 days, 26-35% gestation); Group III: sheep (8,5-19 cm CRL, 53-79 days: 36-53% gestation), red deer (8-19 cm CRL, 97-135 days, 36-50% gestation); Group IV: sheep (20-31,5 cm CRL, 81-112 days: 54-75% gestation), red deer (21 to 33 cm C-R, 142-191 days, 51-75% gestation); Group V: sheep (32-40 cm CRL, 113-150 days: 76-100% gestation), red deer (36 to 40 cm C-R, 205-235 days, 76-100% gestation). E: Epithelium; Lp+sb: Lamina propria and submucosa; Tm: tunica muscularis. -, Non immunoreactivity; +, low immunoreactivity; ++, moderate immunoreactivity; +++, high immunoreactivity.

days (51% gestation) in deer and at 112 days (75% gestation) in sheep (Table 2, Fig. 3C,D). Immunoreactivity to VIM was detected in cells at 67 days (26% gestation) in deer and at 97 days (36% gestation) in sheep (Table 2, Fig. 3E,F). In both species, the localisation of the glial cells in the reticular mucosa during development affected the lamina propria, submucosa, tunica muscularis and serosa.

Discussion

At the first stage of development the stomach wall was already structured in two layers: the epithelium and the pluripotential blastemic tissue. Panchamukhi and Srivastava (1979), in buffalo and Fat El-Bab et al. (1983) in sheep, described the second layer as a submucosa from the first embryonic stages, contrary to that reported by Franco et al. (1992, 2004 a) in sheep and deer, respectively, who describe the submucosa as a distinct layer that will be derived from the pluripotential blastemic tissue (Franco et al., 1992, 1993a-c, 2004a,b; Redondo et al., 2005). In sheep at 33 days (22% gestation) the outline of the omasal compartment appeared, of an individualised form and separated from the rest of the compartments (Franco et al., 1993). The individualisation was observed in red deer in the final phases of this first phase, at 66 days- 25% gestation - (Franco et al., 2004c). The comparative study of the omasal differentiation with other species of ruminants showed that it occurs later in deer than in other species: cow – 30 days, 11% gestation - (Vivo et al., 1990, Vivo and Robina, 1991) and goat -28 days, 19%- (Molinari and Jorquera, 1988). The pseudostratified epithelium (Warner, 1958) lacked secretory capacity. Evidence of stratification, with the appearance of strata germinativum and stratum granulorum, was always earlier in deer (97 days, 36% gestation) than in sheep (72 days, 48% gestation). This stratification was completed with the appearance of the stratum lucidum-spinosum and stratum corneum. This completed stratification

began to be formed first in deer, at 142 days of prenatal life (51% gestation) and later in sheep (83 days, 55% gestation). The stratum lucidum-spinosum has only been described in buffalo by Osman and Berg (1981) at stages close to birth. Panchamukhi and Srivastava (1979) in buffalo and Vivo et al. (1990) in the cow also point out the stratified nature from the differentiation of the omasum, although without a fixed disposition of strata. The stratum corneum in sheep and red deer were similar (Franco et al., 1992, 1993c, 2004a,b). However, other authors describe it later: in sheep, Wardrop (1961) and Del Rio Ortega (1973) describe it at birth, while in goat Molinari and Jorquera (1988) refer to it at 30 cm (75% gestation). In buffalo Tiwari and Jamdar (1970) and Panchamukhi and Srivastava (1979), refer to the appearance of this stratum at 28.5 cm. In the cow it is described in stages of development close to birth (Warner, 1958; Asari et al., 1981; Vivo et al., 1990). The temporal order of appearance of the four order laminae was always earlier in sheep (primary at 21% of gestation, secondary at 26%, tertiary at 33% and quaternary at 40%) than deer (25%, 30%, 35% and 50% respectively). In sheep, there was a significant discrepancy in terms of the timing of the appearance of the smaller laminae. Del Rio Ortega (1973) placed them at 24, 28, 32 and 33% of gestation, respectively. Lubis and O'Shea (1978) placed them at 21, 25, 30 and 40% , and Fath-El Bab et al. (1983) at 21, 26, 33 and 52%. In cattle (Vivo et al., 1990; Vivo and Robina, 1991) and in buffalo (Osman and Berg, 1982) the process was earlier, the presence of the four sizes of laminae being calculated at around 20% of gestation. Most authors report four laminae in sheep (Del Rio Ortega, 1973; Lubis and O'Shea, 1978; Fath El-Bab et al., 1983; Franco et al., 1993a) in goat (Ramkrishna and Tiwari, 1979) and in cattle (Totzauer and Sinowatz, 1990; Vivo and Robina, 1991). However, in studies of goat (Ramkrishna and Tiwari, 1979), sheep (Wardrop 1961) and buffalo (Osman and Berg, 1982) the existence of a fifth order has been reported at birth. Omasal papillae

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appeared as lateral evaginations of connective tissue from the stratum basale of laminae, oriented toward the epithelial surface, in accordance with their histophysiological function. (ie, in such a way that with the movement of folds they acted on the food, channelling it into interlaminae spaces (Franco et al., 1993a). The temporal order of appearance in the four order laminae was always earlier in sheep (in primary laminae at 45% of gestation, in secondary at 53%, in tertiary at 76% and quaternary at 80%) than deer (45%, 50%, 85% and 95% respectively). These conical papillae were observed in sheep at 42% of gestation (Del Rio Ortega, 1973), at 46% (Lubis and O'Shea, 1978) and at 82% (Fath-El Bab et al., 1983); in goat, at 45% (Ramkrishna and Tiwari, 1979) and in cattle at 32% (Totzauer and Sinowatz 1990). In both species, the presence of mucopolysaccharides could be related to the gradual adaptation of the omasal mucosa to its function as chemical protector in postnatal life, acting as a shock absorbing buffer system in the neutralisation of the acid compounds that are produced from reticular fermentation, and of the acid compounds found in the amniotic fluid, since the swallowing of this is a normal occurrence during gestation (Franco et al., 1992, 1993c). In both cases, they gradually decreased throughout prenatal development until birth and were localised around the tegumentary mucosa in the deepest layers of the epithelium, without affecting the stratum corneum. Acid mucopolysaccharides, mucins, and mucoid compounds were not found during development.

The muscular layer and the muscularis of the mucosa were observed at similar stages of gestation (28% gestation in sheep and 26% in deer). In sheep (Del Rio Ortega, 1973) and in the cow (Vivo et al., 1990) placed the muscular layer at earlier stages of development (around 18% gestation). In ruminants, the appearance of the muscularis of the mucosa was placed in perinatal stages in buffalo (Panchamukhi and Sravastava, 1980) or during postnatal development (Wardrop, 1961; Kitamura et al., 2003).

The presence of neuroendocrine cells (NNE-positive) in the omasal mucosa was not detected until 97 days (36% gestation) in deer and 81 days (54% gestation) in sheep. In both species, these cells were located in the lamina propria and submucosa, close to the epithelium and in the thickness of the intermuscular connective tissue in the myenteric plexus. Similar opinions were reported by Kitamura et al. (1986) in cattle and Groenewald (1994) in sheep. The presence of GFAP-positive cells occurred at around 142 days (51% gestation) in deer and at 112 days (75% gestation) in sheep. Immunoreactivity to VIM was detected in cells at 67 days (26% gestation) in deer and at 52 days (35% gestation) in sheep. The comparative study of immunostaining of both glial cell markers proved that VIM is a marker of more primitive glial cells, a fact previously reported in the prenatal development of sheep pineal gland (Franco et al., 1997). In both species, the localisation of the glial cells in the reticular mucosa

during development affected the lamina propria, submucosa, tunica muscularis and serosa. Similar results have been reported for the small intestine of human fetuses (Fekete et al., 1995).

From the analysis of the results obtained, we can conclude that the omasum in both species during fetal life undergoes gradual adaptations prior to the intense changes that will be produced at the moment of birth. When we compare the two species, although in deer the individualisation of the omasum occurs later in its first differentiation from the gastric outline, it is earlier in terms of the appearance of a complete stratification of the epithelial layer and of the specific adaptations of its mucosa – omasal laminae. The same can also be said with regard to the earlier appearance and more rapid decrease of neutral mucopolysaccharides and in its neuroendocrine nature (early appearance of neuroendocrine and glial cells).

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