TESIS DOCTORAL

UNIVERSIDAD DE EXTREMADURA

Departamento de Medicina Animal
Facultad de Veterinaria

EL DESARROLLO PRENATAL DEL ESTÓMAGO DE CABRA

Ángela María García González

Cáceres, 2013

UNIVERSIDAD DE EXTREMADURA

FACULTAD DE VETERINARIA

DEPARTAMENTO DE MEDICINA ANIMAL

ÁREA DE ANATOMÍA Y ANATOMÍA PATOLÓGICA COMPARADAS

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Los directores

Fdo. Eloy Redondo García

Fdo. Antonio Javier Masot Gómez-Landero

Fdo. Antonio Franco Rubio

Cáceres, 2013

Memoria presentada para optar al título de Doctor Internacional por la Universidad de Extremadura

Área de Anatomía y Anatomía Patológica Comparadas
Departamento de Medicina Animal
Facultad de Veterinaria
Universidad de Extremadura

D. **Eloy Redondo García**, Catedrático de Universidad del Departamento de Medicina Animal de la Facultad de Veterinaria de la Universidad de Extremadura,

INFORMA:

Que la Licenciada en Veterinaria **Ángela María García González**, ha realizado bajo mi dirección y asesoramiento el trabajo titulado **"El Desarrollo prenatal del estómago de cabra"** en la Unidad de Histología y Anatomía Patológica, con el que opta al título de Doctor con Mención Internacional en Veterinaria.

Dicho trabajo se conforma de seis publicaciones en revistas internacionales recogidas en base de datos S.C.I.-J.C.R. y ha sido informado favorablemente por tres expertos europeos en el área de Histología Veterinaria.

Por todo ello, y para que conste en su memoria de Doctorado, firmo el presente informe en Cáceres, a 2 de Septiembre de 2013.

Fdo. Eloy Redondo García

Área de Anatomía y Anatomía Patológica Comparadas
Departamento de Medicina Animal
Facultad de Veterinaria
Universidad de Extremadura

D. **Antonio Javier Masot Gómez-Landero**, Profesor Titular del Departamento de Medicina Animal de la Facultad de Veterinaria de la Universidad de Extremadura,

INFORMA:

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Por todo ello, y para que conste en su memoria de Doctorado, firmo el presente informe en Cáceres, a 2 de Septiembre de 2013.

Fdo. Antonio Franco Rubio

La realización de esta tesis ha sido posible gracias a:

❖ **Beca para la formación predoctoral de personal investigador (PRE08055),** vinculada al proyecto de investigación titulado "Cabritos destetados y melatonina, ¿una simbiosis esencial?, del Plan Nacional de I+D+I (AGL 2006-01107) en el marco de los Programas y las prioridades establecidas por el III Plan Regional de Investigación, Desarrollo e Innovación de Extremadura (Orden de 11 de junio de 2008 del D.O.E. nº 130 del 7 de julio de 2008 y Resolución de 29 de Octubre de 2008 del D.O.E. nº 221 del 14 de noviembre de 2008)

❖ **Ayuda para estancias breves en Centros de Investigación (Acción IV).** Plan de Iniciación a la investigación, Desarrollo Tecnológico e Innovación de la Universidad de Extremadura. 2010.

❖ **Ayuda Puente para Becarios Predoctorales (Acción III)**. Plan de Iniciación a la Investigación, Desarrollo Tecnológico e Innovación de la Universidad de Extremadura. 2012.

A mis padres, a mi hermana

por su apoyo en todas mis tareas emprendidas

"Cuando bebas agua, recuerda la fuente"

Proverbio chino

AGRADECIMIENTOS

AGRADECIMIENTOS

Han pasado más de cuatro años desde que comencé a trabajar en la Unidad de Histología y Anatomía Patológica y en el proyecto que ha dado lugar a esta Tesis Doctoral. Durante todo este tiempo son muchas las personas que han aportado su granito de arena en este trabajo, con el que se cierra una etapa importante de mi vida. Me gustaría aprovechar este capítulo para agradecer a todas aquellas personas que de una forma u otra, me han ayudado a recorrer este largo camino para obtener el título de Doctora.

A la primera persona que quiero agradecer este trabajo es al Dr. Eloy Redondo, director de esta tesis, en el que he encontrado un modelo a seguir de trabajo, entrega, constancia, lucha e ilusión en todo aquellos que hace. Le doy las gracias por haberme dado la oportunidad de formar parte de su grupo de investigación y por haber puesto toda su confianza en mí para el desarrollo de esta Tesis. Agradezco también a mis otros directores, Dr. Javier Masot y Dr. Antonio Franco, su tiempo, su ayuda y dedicación no solo en la realización de esta tesis sino también en mi formación docente.

Mi más sincero agradecimiento al resto de profesores integrantes de la Unidad de Histología y Anatomía Patológica, Dr. Antonio Gázquez, Dr. Vicente Roncero y Dr. Luis Gómez, quienes siempre se han mostrado atentos y dispuestos a prestarme su colaboración.

A la técnico de laboratorio, Doña Pilar Parra, por su inestimable ayuda en el corte y procesado de mis muestras y, lo más importante, por su amistad. A nuestra nueva técnico, Doña Diana Sánchez, que aunque hemos compartido poco tiempo, siempre ha estado dispuesta a ofrecerme su ayuda.

A los profesores, Dr. Francisco Serrano y Dr. Pedro Rodríguez, por su disposición y orientación en el estudio estadístico. Gracias por todas las horas que me habéis dedicado a resolver dudas y hacer que entendiera un poco mejor el mundo de la estadística.

Al personal del Servicio de Microscopía electrónica de la Universidad de Córdoba, por el buen trato recibido y por su ayuda en el procesado de las muestras. Quiero destacar la excelente labor del profesor Dr. Alfonso Blanco, quien sin yo pedírselo, se prestó inmediatamente a ayudarme con el manejo del microscopio electrónico y el fotografiado de mis muestras.

Me gustaría dar las gracias de una manera especial al profesor Dr. Aniceto Méndez, de la Facultad de Veterinaria de la Universidad de Córdoba, por acogerme desde el primer momento como a uno más de su grupo de trabajo. La dedicación y motivación que pone en cada necropsia o caso clínico que realiza, le hace estar siempre rodeado por un grupo de alumnos dispuestos a aprender. Durante mi estancia allí, tuve la oportunidad de pertenecer a uno de ellos, el llamado "Equipo de Aniceto" formado por Urso, Javi, Jose, Jesús e Irene. Gracias chicos, por hacer tan agradable mis días allí y especialmente por vuestra amistad.

Y si a alguien debo agradecerle la obtención del título de Doctor Internacional es a la Profesora Concepçao Peleteiro de la Faculdade de Medicina Veterinaria de la Universidade Técnica de Lisboa, quien sin conocerme de nada me abrió las puertas de su laboratorio para poder realizar mi estancia de doctorado. Gracias Profesora por el buen trato recibido y por su inestimable ayuda para los trâmites de esta tesis. También debo agradecer al Profesor Jorge Correa, su amabilidad, su agradable acogida y la formación recibida sobre oncología mamaria canina y felina. Al personal técnico del Laboratorio de Anatomía Patológica de la Universidad de Lisboa, María Augusta, Sandra, Rosario, Bruno y Doña Manuela, muito obrigada por sua atençao. De una forma especial, quiero dar las gracias a Hugo, Joao y Rute, por hacer que nunca me sintiera sola durante mi estancia allí.

No puedo olvidarme de mis compañeros de departamento, Javi, Sara y Chuchi, por hacer que el trabajo diario sea más llevadero y por todos los buenos momentos que me han hecho pasar en ese espacio tan reducido de nuestro "chiringuito", ahora quedará un sitio libre. Y a ti también, Edu, por hacer que la biblioteca no sea un lugar tan vacío y como no, por esas tonterías tuyas que siempre nos hacen reír. A Rafa, Gamito y Elisa gracias por vuestra amistad y por vuestra ayuda desde que me inicié en este mundo de la investigación.

A mis amigas de facultad, quienes siempre han estado en los buenos y malos momentos durante este tiempo. Gracias Anabel, Marta, Silvia, Laura, Elena, Lucía y Judit, por escucharme, soportarme y convertiros en mis mejores amigas. Siempre estarán en mí, aunque la vida nos disponga caminos diferentes.

A mi grupo de amigos de Alburquerque, pero en particular a Carmen y Almudena, porque siempre han estado apoyándome, animándome y atentas a todo lo que hacía. A Toni, gracias por tu ayuda con la portada y a Jose, gracias por haber resuelto más de una vez mis dudas.

A mi fiel e inseparable compañera y amiga de trabajo, Dana, que me hace sentir feliz simplemente con sus tonterías perrunas.

Y en último lugar, pero el más importante, se encuentran mis padres y mi hermana. Ellos son la base de todo este trabajo. Gracias por darlo todo por mí, por animarme, consolarme, ayudarme en cada cosa que hago y por enseñarme como vivir y afrontar la vida sin temor alguno para lograr todo aquello que me propongo.

A todos, muchas gracias.

ESTRUCTURA DE LA TESIS Y CERTIFICADOS

TESIS DOCTORAL COMO COMPENDIO DE PUBLICACIONES

La presente Tesis Doctoral está sustentada sobre 6 artículos científicos que se detallan a continuación y cuya copia íntegra se anexa en el apartado *6. Resultados y Discusión*.

1. Histomorphometric and immunohistochemical study of rumen goat during prenatal development.

Garcia A, Masot J, Franco A, Gazquez A, Redondo E.

Published on: *The Anatomical Record*, 2012; 295: 776-785.

Journal Ranking (JCR): **Anatomy & Morphology Q3** Impact Factor: **1.473**

Journal Ranking (SJR): **Histology Q1** Impact Factor: **0.634**

2. Histomorphometric and immunohistochemical study of omasum goat during prenatal development.

Garcia A, Masot J, Franco A, Gazquez A, Redondo E.

Published on: *Histology and Histopathology*, 2013; 28: 737-748.

Journal Ranking (JCR): **Pathology Q2** Impact Factor: **2.480**

Journal Ranking (SJR): **Histology Q1** Impact Factor: **0.769**

3. Histomorphometric and immunohistochemical study of reticulum goat during prenatal development.

Garcia A, Masot J, Franco A, Gazquez A, Redondo E.

Accepted for published on: *Histology and Histopathology*

Journal Ranking (JCR): **Pathology Q2** Impact Factor: **2.480**

Journal Ranking (SJR): **Histology Q1** Impact Factor: **0.769**

4. Histomorphometric and immunohistochemical study of abomasum goat during prenatal development.

Garcia A, Masot J, Franco A, Gazquez A, Redondo E.

Accepted for published on: *Histology and Histopathology*

Journal Ranking (JCR): **Pathology Q2** Impact Factor: **2.480**

Journal Ranking (SJR): **Histology Q1** Impact Factor: **0.769**

5. Immunohistochemical study of forestomach goat during prenatal development.

Garcia A, Masot J, Franco A, Gazquez A, Redondo E.

Accepted for published on: *Journal of Veterinary Science*

Journal Ranking (JCR): **Veterinary Science Q2** Impact Factor: **1.161**

Journal Ranking (SJR): **Veterinary (miscellaneous) Q2** Impact Factor: **0.389**

6. Histomorphometric study of stomach goat during prenatal development

Garcia A, Masot J, Franco A, Gazquez A, Redondo E.

Sent to: *Animal Science Journal*

Journal Ranking (JCR): **Agriculture, Dairy & Animal Science Q2** Impact Factor: **1.037**

Journal Ranking (SJR): **Veterinary (miscellaneous) Q2** Impact Factor: **0.437**

Anatomia Patológica

Maria da Conceição C. V. Peleteiro, Full Professor of Pathological Anatomy of the Faculty of Veterinary Medicine of the Technical University of Lisbon, and person responsible for Laboratory of Pathological Anatomy of the same Faculty,

certifies that,

The PhD Student **Angela Maria Garcia Gonzalez** (ID 07049979-L) spent three months (3 January 2011 - 8 April 2011) in our above mentioned Laboratory with the aim of obtaining the International Doctor mention. This student accompanied all the activities of the Laboratory, in particular anatomo-histopathological analysis of different species, cytological analyses and necropsies. She also performed immunohistochemical techniques to practice in her project of doctoral thesis.

Lisbon, 8th April of 2011

Prof. Maria da Conceição Peleteiro

Faculdade de Medicina Veterinária, Avenida da Universidade Técnica, Pólo do Alto da Ajuda,
1300-477 Lisboa, Portugal, Tel: 213652800, Fax: 213652897

Mário Soares de Pinho
Faculdade de Medicina Veterinária,
Av. da Universidade Técnica, 1300-477, Lisboa
Portugal

June 17, 2013

I have been asked to evaluate if the thesis entitled "El Desarrollo Prenatal del estómago de cabra" of A. Garcia Gonzalez fulfills the requirements of International Doctorate.

The thesis comprises a series of six research papers or manuscripts. Two of the papers have been published in prestigious scientific journals in the fields of Anatomy and Histology (*The Anatomical Record* 2012, 295: 776-785. *Histology and Histopathology*, 2013; 28: 737-748). Three other papers have been accepted for publication (two in *Histology and Histopathology* and one in *Journal of Veterinary Science*) and one manuscript has been submitted (*Journal of Veterinary Science*).

A. Garcia is the first author in all six articles showing that she has been the main contributor in all the experiments. The last author in all six papers is E. Redondo demonstrating his role as a supervisor of the candidate. There are several other authors in each paper; they were co-supervisor of the candidate and responsible for the laboratory work.

The six papers deal with several aspects of histology and structure of goat stomach during prenatal life. This includes the detection and distribution of neuroendocrine cells, glial cells and peptidergic innervation markers in each of the stomach compartments by immunohistochemical labeling. A histomorphometric analysis is included to complete the study of the growth and development of each of the tissue layers that make up the gastric wall.

So the PhD candidate should be complimented by the amount of research performed and published. Given the quality and quantity of the work, I would warmly recommend to be awarded the International Doctorate.

Sincerely,

MSPinho

Mário Soares de Pinho, DVM, Ph.D.
Auxiliary Professor

IOWA STATE UNIVERSITY
OF SCIENCE AND TECHNOLOGY

College of Veterinary Medicine
Department of Biomedical Sciences
Ames, Iowa 50011-1250
515 294-2440
FAX 515 294-2315

Dear Sir/Madam,

10 July 2013

To whom it may concern,

Re: Gracia Masot's PhD thesis for 'International Doctorate' award

I was invited to review Gracia Masot's PhD thesis for 'International Doctorate' award. Gracia's PhD thesis comprises of six peer-reviewed publications in international journals which are at different stages of publication. Two are published, three are accepted for publication (in press) and the sixth one is under review. Gracia's PhD work is focused on detailed histomorphometric and immunohistochemical study of goat rumen, reticulum, omasum and abomasum at different stages of development. The candidate has selected appropriate gestation periods for the study, experimental design and statistical analyses are entirely appropriate. The data presented/published are of high quality and she has discussed her results in the context of her own results quoting appropriate references. Some aspects of thesis also deal with physiological relevance of anatomical structure correlating with different stages of development which is an interesting aspect of this study. Using various immunohistochemical markers such as GFAP, vimentin and neuropeptides add further dimension to the proposed study. Overall, considering the quality of work presented in six manuscripts, without hesitation, I strongly recommend the thesis for the award of 'International Doctorate' to Gracia Masot.

Yours Sincerely,

T. Thippeswamy DVM, MVSc, PhD, PgCert HE&TL
Professor
Phone: +1 515 294 2571
Cell: +1 515 708 8444
E-mail: tswamy@iastate.edu

UNIVERSITÀ DEGLI STUDI DI MILANO

I have been asked to write a comment as an international reviewer for the doctoral thesis by Angela Garcia Gonzalez with the aim of evaluating if it fulfills the requirements of an International Doctorate.

The PhD thesis is entitled "El desarrollo prenatal del estomago de cabra", and it shows to be composed by six articles, two of which are published in "Anatomical Record" and "Histology and Histopathology", three have been accepted for publication in "Histology and Histopathology" and in "Journal of Veterinary Science", one has been submitted for publication to "Journal of Veterinary Science". All these are international journals, display international editorial boards and show I.F. values that are noteworthy in the fields of anatomy and morphology, histology, pathology, and veterinary science.

The doctoral thesis concerns with remarkable uniformity micro-anatomical studies of goat stomach during development, and utilizes histology, immunohistochemistry and electron microscopy to display results which are neat, elegant and further corroborated by quantitative reports (histometry). All the four compartments of the goat stomach are examined, not neglecting in the punctual description the presence and localization of the neuroendocrine as well as glial components.

The Candidate is the first Author in all the six articles, in such a way displaying her principal role in performing all parts of the scientific work.

I think that Angela Garcia Gonzalez fully deserves to obtain an International Doctorate.

Cinzia Domeneghini

Milan, July 25, 2013

Cinzia Domeneghini

Veterinary Anatomy, Professor

Dept. of Health, Animal Science and Food Safety

University of Milan - Italy

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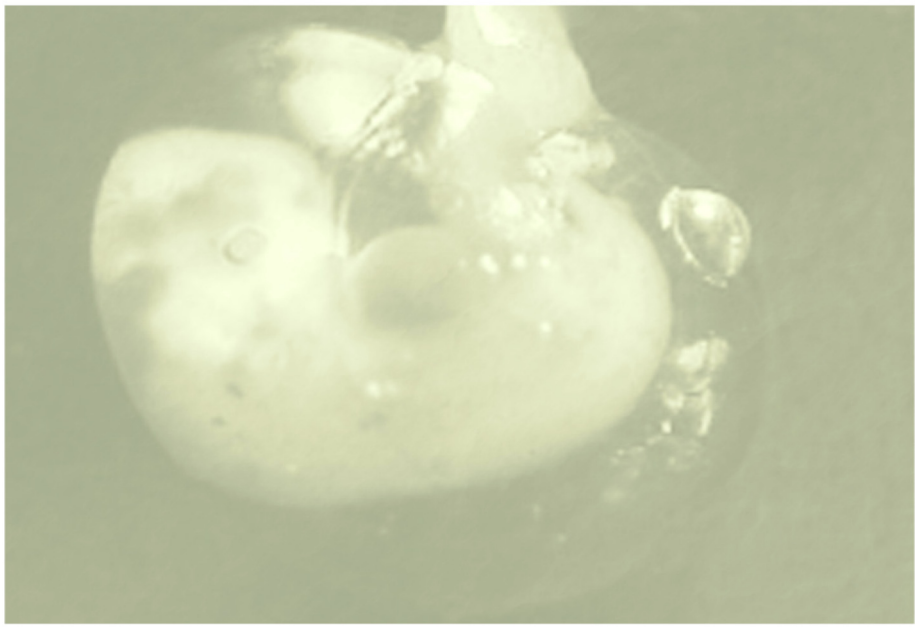

1. GRUPO INVESTIGADOR

1.1. GRUPO DE INVESTIGACIÓN DE LAUNIVERSIDAD DE EXTREMADURA

Investigador responsable	
Apellidos: GARCÍA GONZÁLEZ	Nombre: ÁNGELA MARÍA
Titulación académica: VETERINARIA	Grado: LICENCIATURA
Categoría profesional: BECARIA FPI	
Director de la Tesis Doctoral	
Apellidos: REDONDO GARCÍA	Nombre: ELOY
Titulación académica: VETERINARIA	Grado: DOCTOR
Categoría profesional: CU	Situación laboral: PLANTILLA
Co-directores de la Tesis Doctoral	
Apellidos: MASOT GÓMEZ-LANDERO	Nombre: ANTONIO JAVIER
Titulación académica: VETERINARIA	Grado: DOCTOR
Categoría profesional: TU	Situación laboral: PLANTILLA
Apellidos: FRANCO RUBIO	Nombre: ANTONIO
Titulación académica: VETERINARIA	Grado: DOCTOR
Categoría profesional: TU	Situación laboral: PLANTILLA
Otros investigadores	
Apellidos: GÁZQUEZ ÓRTIZ	Nombre: ANTONIO
Titulación académica: VETERINARIO	Grado: DOCTOR
Categoría profesional: CU	Situación laboral: PLANTILLA
Personal técnico de laboratorio	
Apellidos: PARRA	Nombre: PILAR
Titulación académica: LABORATORIO	Grado: F.P.
Categoría profesional: TÉCNICO	Situación laboral: PLANTILLA
Apellidos SÁNCHEZ ROSADO	Nombre: DIANA
Titulación académica: ENFERMERA	Grado: DIPLOMADA
Categoría profesional: TÉCNICO	Situación laboral: PLANTILLA

1.2. INVESTIGADORES DE OTRAS ENTIDADES

Universidad Técnica de Lisboa	
Departamento de Anatomía Patológica	
Apellidos: PELETEIRO	Facultad de Medicina Veterinaria
Titulación académica: VETERINARIA	Nombre: MARIA CONCEPÇAO
Categoría profesional: CU	Grado: DOCTOR

Universidad de Córdoba	
Departamento de Anatomía y Anatomía Patológica Comparadas	
Apellidos: BLANCO	Nombre: ALFONSO
Titulación académica: VETERINARIA	Grado: DOCTOR
Categoría profesional: CU	
Apellidos: MÉNDEZ SANCHEZ	Nombre: ANICETO
Titulación académica: VETERINARIA	Grado: DOCTOR
Categoría profesional: CU	

2. INTRODUCCIÓN

2.1. FINALIDAD DE LA INVESTIGACIÓN

La especie caprina, pese a su escaso censo en España, no ha dejado de tener trascendencia en la producción animal. Su rusticidad y hábitos de pastoreo le han permitido sobrevivir en áreas de difícil orografía y aprovechar los parajes más accidentados, donde ninguna otra especie puede prosperar.

La cabra es la especie animal mejor adaptada al pastoreo con gran variedad de vegetales y capaz de aprovechar los pastizales marginales **(Boyazoglu *et al.* 2005; Rancourt *et al.* 2006)**. El ramoneo es uno de los componentes importantes de su dieta, por lo que la cabra es considerada entre los rumiantes, la mejor usuaria de pastos pobres (**Gihad *et al.* 1980**).

La evolución de los rumiantes en la selección de alimentos tiene como resultados variaciones anatómicas en el estómago de las diferentes especies. Así, **Hofmann (1973),** clasificó los rumiantes en tres tipos morfofisiológicos según sus hábitos alimenticios: selectores de concentrados (*concentrate selectors*), consumidores de forraje (*grass/roughage eaters*) y de tipo intermedio (*intermediate type*). La cabra, animal objeto de este estudio, fue clasificada como animal de tipo intermedio, sin unos requerimientos específicos en la alimentación. Esta especie animal tiene una dieta mixta y es capaz de mostrar adaptaciones anatómicas en su estómago como consecuencia de cambios en la calidad del forraje. Los selectores de concentrados están equipados con un estómago pequeño, que se adapta perfectamente a la transformación de forraje fácilmente digerible. Los consumidores de forraje se caracterizan por poseer un estómago con gran capacidad y de mayor tamaño, adaptado al forraje menos digerible.

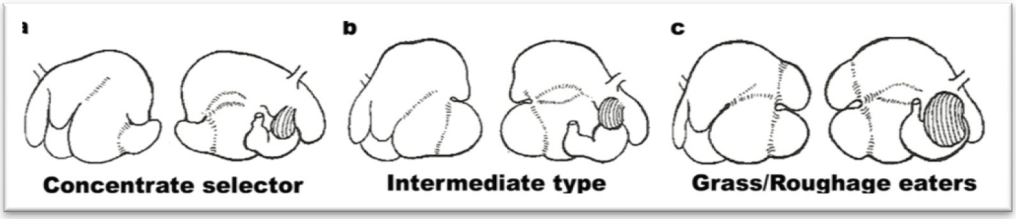

Imagen 1. Representación de la diferencia de tamaño del estómago de los rumiantes según sus hábitos alimenticios: selectores de concentrado (concentrate selector), comedores de hierba (grass eaters), animales de tipo intermedio (intermediate type) Hofmann (1989)

A pesar de estas variaciones morfológicas en su tracto digestivo, al nacimiento todos los rumiantes poseen un estómago que estructural y funcionalmente se asemeja al de no rumiante, sin funcionalidad de los compartimentos no glandulares (**Relling y Mattioli, 2002**). Su estómago requiere de un desarrollo adaptativo para poder realizar la digestión fermentativa y la rumia. Durante la vida prenatal, cada uno de los compartimentos gástricos que componen el estómago sufre una serie de cambios morfológicos para adaptarse a su funcionalidad postnatal.

La elevada capacidad de los rumiantes para convertir alimentos groseros en productos de gran valor nutritivo, ha impulsado en gran medida el estudio de la estructura y función del aparato digestivo de estos animales. A pesar de esto, el desarrollo prenatal del estómago de los rumiantes ha sido poco contemplado.

El grupo de investigación de Histología Veterinaria de la Universidad de Extremadura se ha centrado en el abordaje de la ontogénesis del estómago de los rumiantes. Son muchos los estudios realizados en diversas especies como el bovino (**Vivo *et al.* 1987, 1990**), el ovino (**Franco *et al.* 1989, 1992, 1993a, b, c, d, e; Regodon *et al.* 1996; Redondo *et al.* 1997**) y en rumiantes salvajes como el ciervo (**Franco *et al.* 2004a, b; 2011, 2012; Redondo *et al.* 2005, 2011; Masot *et al.* 2007a, b**). Sin embargo, la ontogénesis del estómago del cabra no había sido acometida hasta ahora. Por ello, la finalidad de este trabajo ha sido estudiar en la especie caprina y desde un punto de vista histofisiológico el desarrollo, la diferenciación y la configuración de los compartimentos gástricos durante la vida prenatal.

2.2. ANTECENDENTES Y ESTADO ACTUAL DEL TEMA

El estómago es considerado como la parte funcional del sistema digestivo, capaz de digerir y transformar los alimentos en nutrientes, para posteriormente ser absorbidos a través de la pared del intestino (**Al-Saffar, 2012**). El estómago de los rumiantes, de manera significativa adquiere gran importancia debido a su capacidad para la transformación de forrajes de baja calidad en productos de alto valor nutritivo (**Lombardi, 2005**). Esta característica se basa en la posibilidad de poder degradar los hidratos de carbono estructurales del forraje, como la celulosa, muy poco digestible para las especies no rumiantes.

Para satisfacer sus necesidades funcionales, el estómago de los rumiantes ha desarrollado varias particularidades morfológicas. Dicho órgano está dividido en cuatro compartimentos: rumen, retículo, omaso y abomaso. Los tres primeros forman los compartimentos no glandulares (*proventriculus*) mientras que el abomaso es glandular y funcionalmente análogo al estómago de las especies monosgástricas (**Nickel *et al.* 1973**). Cada uno de estos compartimentos posee unas características macroscópicas e histológicas únicas (**Schummer y Nickel, 1979**), que reflejan la adaptación morfológica y funcional de la ingesta, proceso y digestión del material vegetal.

Desde el punto de vista fisiológico, los compartimentos no glandulares juegan un importante papel en la digestión, ya que son el lugar donde los procesos físicos y bioquímicos relacionados con la rumia se llevarán a cabo, y en particular donde las bacterias simbióticas realizan su actividad de fermentación para digerir la celulosa (**Teixeira *et al.* 2009; Scala *et al.* 2011**). El abomaso, tiene como función la digestión de los alimentos mediante la secreción de jugos gástricos (**Aage *et al.* 2007**).

Histológicamente, los cuatro compartimentos gástricos poseen las mismas capas tisulares: mucosa, submucosa, túnica muscular y serosa. La mucosa del rumen, retículo y omaso está tapizada por un epitelio estratificado plano queratinizado (**Scala *et al.* 2011**) que cambia bruscamente en el abomaso a un

epitelio cilíndrico simple. El epitelio se asienta sobre una lámina propria de tejido conjuntivo fibroso, la cual sirve de sostén para las glándulas gástricas en el abomaso. La muscular de la mucosa está ausente en el rumen (**Kitamura *et al.* 2003**), y se aparece como haces aislados de fibras musculares lisas en el extremo superior de los pliegues reticulares, lo que produce una fusión de la lámina propia y la submucosa en ambos compartimentos. La submucosa está formada por tejido conjuntivo laxo con una rica red vascular y plexos nerviosos (plexo submucoso o de Meissner). La túnica muscular consta de dos capas de músculo liso, una circular interna y otra longitudinal externa (**Ramkrishna y Tiwari, 1979**), entre las cuales se encuentra el plexo mientérico o de Auerbach. La serosa está compuesta por el mesotelio y por tejido conjuntivo laxo en el que se pueden encontrar grasa, vasos sanguíneos, vasos linfáticos y nervios.

Imagen 2. Cabritos en lactación

El desarrollo del estómago de los rumiantes ha sido de interés con respecto a aspectos de morfogénesis e histogénesis en el pasado. Diversos estudios han sido llevados a cabo en las diferentes especies rumiantes domésticas, como ovino (**Wardrop, 1961a; Franco *et al.* 1992; 1993a,b,c,d,e**), bovino (**Warner, 1958; Arias *et al.* 1978; Kano *et al.* 1981; Vivo, 1987; Vivo *et al.* 1990; Stallcup *et al.* 1990**), así como rumiantes salvajes, como el búfalo (**Tiwari y Jamdar 1979; Phanchamukhi *et***

***al.* 1975; Panchamukhi y Srivastava 1979, 1980, 1982; Osman y Berg, 1981a,b, 1982a,b; Singh *et al.* 2007**) y el ciervo (**Franco *et al.* 2004a, b; Franco et al. 2011, 2012, Redondo *et al.* 2005, 2012; Masot *et al.* 2007a,b**). Sin embargo, son escasos los trabajos realizados sobre la histogénesis y desarrollo del estómago de la cabra (**Ramkrishna y Tiwari, 1979; Molinari y Jorquera, 1988, Nwaogu y Ezeasor, 2008**).

2.3. ORIGINALIDAD DEL TEMA

El análisis de la ontogénesis y desarrollo prenatal del estómago de la cabra ha sido poco considerado. Se trata de un trabajo innovador y original por dos motivos:

1º- Por la especie objeto de estudio, la cabra, debido a la estrecha relación con la economía de subsistencia, mediante la producción cárnica de cabritos y la producción láctea para la elaboración de quesos, además de su capacidad para la adaptación y el aprovechamiento de todos los recursos del medio.

2º La ausencia de datos sobre la estructura ontogénica y desarrollo de la víscera gástrica durante la vida intrauterina inducen a la conveniencia de la creación de una base de datos histológicos sobre el desarrollo prenatal del estómago de cabra, que podrían servir de base referencial para futuros estudios y para establecer comparaciones con otros estudios similares en otras especies rumiantes, tanto domésticas como salvajes.

2.4. OTROS GRUPOS DE INVESTIGACIÓN QUE TRABAJAN EN EL TEMA

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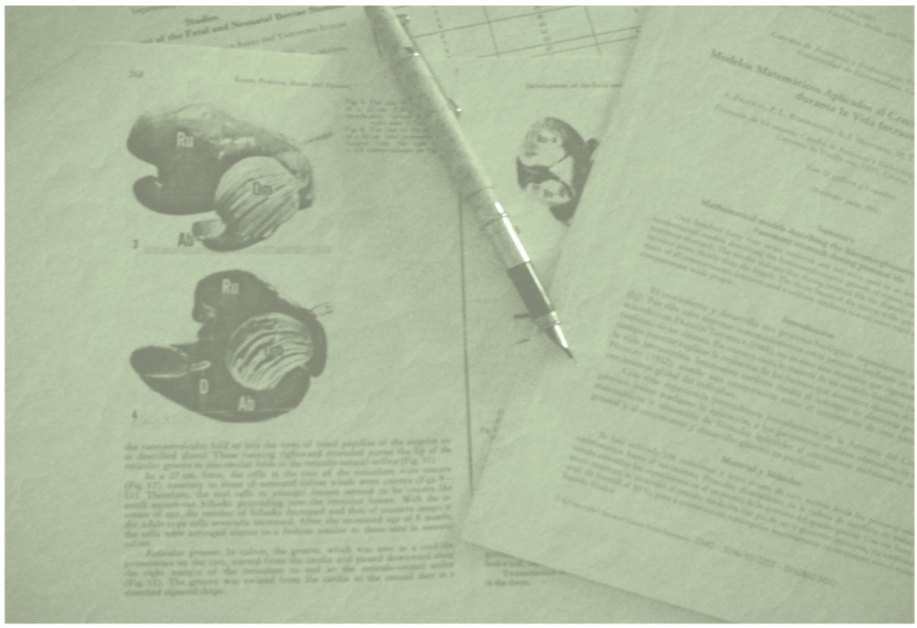
3. REVISIÓN BIBLIOGRÁFICA



3.1. DETERMINACIÓN DE LA EDAD FETAL EN ANIMALES DOMÉSTICOS

El desarrollo prenatal incluye el periodo comprendido entre la fecundación y el nacimiento. Este periodo involucra en los individuos una serie de cambios morfológicos tanto externos como internos, que servirán como criterios para determinar la edad de los individuos durante su vida prenatal.

El primer trabajo referente a la determinación de la edad embrionaria, fue realizado por **Nichols (1944)**, quien con un número escaso de especímenes de vacuno fijó unas tablas de crecimiento en base a la longitud corporal y su relación con el peso.

Posteriormente, **Green (1946), Postma (1947)** y **Mannely (1952)** se sumaron al estudio de la determinación de la edad embrionaria en ovino y bovino, respectivamente. Entre ellos, destacó **Postma (1947)** por establecer una estrecha correlación entre el periodo fetal y la longitud corporal en bovino, hasta una edad de 32 ± 4 semanas. Además, junto a estos parámetros, consideró otro criterio como la erupción del pelo y su crecimiento, lo que le permitió reducir el error máximo en la percepción de la edad a 1-2 semanas.

Kurnosov *et al.* (1960) realizaron un interesante trabajo sobre la sucesiva variación de los aspectos morfológicos externos en embriones y fetos bovinos.

Singh *et al*. (1963), obtuvieron unas tablas cronológicas del desarrollo prenatal del búfalo basadas en caracteres como peso, volumen, longitud corporal, circunferencia abdominal, longitud y anchura de la cabeza y longitud de la cola.

En 1969, **Gjesdal**, destacó por la introducción del uso de la radiografía para la determinación de la edad en fetos bovinos. Para ello, se basó en tres parámetros: osificación craneal, osificación del esqueleto, principalmente metacarpo y metatarso y aparición y crecimiento del pelo corporal.

Murillo Ferroll (1973) consideró tres periodos dentro de la gestación de la oveja para la determinación de la edad fetal: germinal (0 a 13 días), embrionario (13 a 34 días) y fetal (de 35 días al nacimiento). Así mismo, describió las características

morfológicas externas generales que presentaban los especímenes en cada uno de los periodos comprendidos.

El trabajo de **Evans y Sack (1973)** es considerado como la principal aportación a la determinación de la edad fetal en animales mamíferos. Estos autores, estudiaron el desarrollo prenatal en animales domésticos y de laboratorio y formularon una curva de crecimiento específica para cada especie. Utilizaron como criterio la longitud corporal, considerada la distancia entre el punto más alto de la cabeza y la base de la cola (*crown-rump*).

Thomsen (1973) estableció una correlación entre aspectos morfológicos (peso, longitud corporal y circunferencia cefálica) y la edad embrionaria en fetos bovinos, sin llegar a mejorar la clasificación de Evans y Sack.

Sivachelvan *et al.* (1996) crearon una guía cronológica de la edad fetal en oveja y cabra utilizando características anatómicas como el estado y transparencia de tegumento, visibilidad y prominencia de los vasos sanguíneos, desarrollo de los genitales externos, consistencia del *calvarium*, aparición del pelo y erupción de los dientes. Además compararon los cambios en el tamaño corporal para determinar las relaciones filogenéticas alométricas entre la oveja y la cabra.

Este mismo año, **Suzuki *et al.* (1996)** indicaron que la edad fetal en ciervo podría ser establecida a partir del peso fetal utilizando la siguiente ecuación T= (3 square root of W + 2.730)/0.091.

A partir de este momento y hasta la actualidad, los estudios sobre la determinación de la edad embrionaria están basados en la medida de distintos parámetros morfológicos mediante la utilización de ultrasonidos.

Doize *et al.* (1997) mediante ecografía transrectal establecieron que en oveja existía una mínima correlación entre el tamaño de los placentomas y la edad fetal, mientras que en cabra, el tamaño de placentoma podía ser usado como indicador válido de la edad de gestación.

Lee *et al.* (2005), estudiaron la relación de la edad de gestación en cabra con la medida de parámetros como el diámetro de la cabeza, órbita y tronco; así como el eje mayor y menor del corazón. De todos estos parámetros, observaron que el diámetro del tronco, el eje mayor y menor del corazón fueron los parámetros más efectivos para determinar la edad fetal.

Santiago-Moreno *et al.* (2005) en muflón y **Amer *et al.* (2008)** en cabra, mediante ecografía transrectal y transabdominal midieron la longitud corporal y el diámetro biparietal para determinar la edad fetal.

En cabra, **Karent *et al.* (2009)** y **Nwaogun (2010),** realizaron estimaciones de la edad embrionaria midiendo patrones como la longitud corporal, tamaño de placentomas, cordón umbilical y diámetro biparietal.

La longitud corporal ha sido el parámetro más utilizado para determinar la edad fetal en diferentes especies animales. Sin embargo, el uso de este criterio para determinar la edad fetal mediante técnicas de ultrasonidos como la ecografía puede tener limitaciones, debido a la reducción del campo de visión en fetos de edad avanzada.

3.2. COMPARTIMENTOS GÁSTRICOS DEL ESTÓMAGO DE LOS RUMIANTES

3.2.1. Anatomía

El estómago de los rumiantes ocupa casi el 75% de la cavidad abdominal y junto con su contenido representa alrededor del 30% del peso vivo del animal (**Luginbuhl *et al.* 1983**). Se encuentra dividido en cuatro compartimentos gástricos: rumen, retículo, omaso y abomaso. El rumen, junto con el retículo y el omaso, son los compartimentos que preceden al abomaso (compartimento glandular), razón por la cual son conocidos como compartimentos no glandulares.

El rumen es el compartimento más voluminoso del estómago y ocupa la mitad izquierda de la cavidad abdominal. Cranealmente contacta con el diafragma y caudalmente se extiende hasta la entrada de la pelvis (**Schwarze, 1970**). Se caracteriza por tener una morfología ovoide y aplanada lateralmente.

La superficie visceral está delimitada externamente por los surcos longitudinales derecho e izquierdo que subdividen el rumen en un saco dorsal (*Saccus dorsalis*) y otro ventral (*Saccus ventralis*). Estos sacos se continúan en dirección craneal o caudal en los profundos surcos craneal (*Sulcus cranialis*) y caudal (*Sulcus caudalis*). Un surco coronario de trayectoria dorsal y ventral, de conformación más débil, separan en cada uno de los sacos ruminales en un saco caudodorsal y caudoventral. Estos surcos que rodean externamente el rumen, se corresponden con proyecciones internas denominadas pilares ruminales (**May 1964; Habel, 1975; Nickel *et al.* 1979**).

La superficie interna del rumen está tapizada por numerosas papilas de morfología variada, cónicas, foliadas y en forma de lengüeta (**Wardrop, 1961a; Tamate *et al.* 1971; Schorr y Vollmerhaus, 1976**). El tamaño y la densidad de las papilas varían considerablemente según la dieta (**Wardrop, 1961b**; **Tamate *et al.* 1962; Ortega-Reyes *et al.* 1992**), la edad (**Klein *et al*. 1987; Franco *et al.* 1992; Amaral *et al.* 2005**) o hábitat del animal (**Rompala *et al.* 1990; Beharka *et al.* 1998**). Estas papilas aumentan la superficie ruminal y juegan un importante papel en la

absorción de los ácidos grasos volátiles, productos procedentes del metabolismo microbiano. El crecimiento de las papilas ruminales es estimulado por la producción de ácidos grasos volátiles (**Hofmann y Schnorr, 1982; Hofmann, 1989**). Por tanto, estas papilas son más densas y de mayor tamaño en el saco craneal, conocido como atrio ruminal, lugar donde se produce la fermentación más intensa, mientras que las más pequeñas se encuentran en el saco dorsal (**Kamler, 2001; Neiva *et al*. 2006**). La densidad de las papilas disminuye a medida que se acercan a los pilares ruminales. Las papilas ruminales permanecen poco desarrolladas mientras que el animal es lactante y aumentará rápidamente cuando el alimento sólido es incluido en su dieta y comienza la fermentación ruminal (**Brownlee, 1956; Sander *et al.* 1959**).

Imagen 3. Papilas ruminales distribuidas sobre la superficie interna del rumen de cabra

El retículo representa el compartimento más craneal del estómago de los rumiantes, tiene forma redondeada y ligeramente aplanado. Se encuentra separado del rumen por el pliegue rumino-reticular. Sin embargo, este pliegue no se extiende por la pared lateral derecha del rumen por lo que no existe una delimitación del retículo y rumen por ese lado. En consecuencia, esta separación anatómica incompleta y las similitudes funcionales del retículo y rumen, hacen que estos dos compartimentos sean considerados con una solo unidad funcional, la unidad rumino-reticular (**Habel, 1973, Kitamura *et al.* 1986**). En su región dorsal, el retículo

se comunica con el esófago a través del cardias y por la región ventral, con el omaso por medio del orificio retículo-omasal. El espacio proyectado desde el cardias hasta el orificio retículo-omasal constituye una estructura anatómica, el surco reticular, es una derivación que conecta el esófago con el abomaso (**Scala and Maruccio, 2012**). Las dimensiones de este surco oscilan entre los 15-20 cm de largo en los bovinos y entre los 7-12 cm en los pequeños rumiantes (**Krahmer, 1982**).

La superficie interna del retículo presenta un relieve en forma de panal y con aspecto de red que corresponde a las denominadas celdas reticulares (**Clauss, 2010**). Estas celdas, de cuatro a seis lados, se encuentran delimitadas por crestas reticulares primarias. Cada celda esta subdividida por crestas secundarias y terciarias y tanto las crestas como el suelo de las celdas están tapizadas por pequeñas papilas (**May, 1964; Habel, 1975; Nickel *et al.* 1979**). Se considera que las crestas reticulares y las celdas en nido de abeja, juegan un papel en el almacenamiento de agua, y ayudan a triturar los alimentos groseros mediante las contracciones. Estas estructuras también están directamente implicadas en el mecanismo de separación de partículas, al actuar como trampas que retienen las partículas más gruesas de alimentos y evitan su paso hacia el omaso (**Hofmann y Schnorr, 1982**) o permitir que las partículas de menor densidad sean dirigidas hacia el omaso durante las contracciones (**Reid, 1985**).

Imagen 4. Celdas reticulares en la mucosa del retículo de cabra

La altura de las crestas reticulares y la profundidad de las celdillas reticulares varía considerablemente entre las especies y representa una adaptación a la dieta de los rumiantes (**Clauss *et al.* 2010**). Así, **Hofmann (1973)** determinó que los herbívoros (*grazers*) tienen en general crestas reticulares de mayor altura que los animales selectores de concentrado (*concentrate selectors*). La razón funcional sugerida para esta observación es que las especies rumiantes de tipo herbívoros tiene un contenido ruminal más fluido, por lo que las crestas reticulares de mayor altura provocan un cierre más completo de la luz reticular que beneficia que el retículo pueda rápidamente volver a llenarse de contenido ruminal para seleccionar partículas. Por el contrario, los rumiantes de tipo selectores de concentrado, cuyo contenido ruminal es más viscoso, necesitan que el cierre de la luz reticular sea incompleto para retener parte del fluido y realizar la separación de partículas (**Clauss *et al.* 2010**).

El omaso se caracteriza por tener una forma ovoide, comprimida lateralmente y con dos curvaturas o extremidades, una mayor y otra menor (**Chandrasekar *et al*. 1993**). Este compartimento ocupa una posición profunda dentro de la cavidad abdominal y ninguna de sus caras está en contacto con la pared del abdomen, estando completamente cubierto por el hígado en el lado derecho. El omaso se comunica con el retículo y abomaso por los orificios retículo-omasal y el omaso-abomasal, respectivamente (**Harfoot, 1978**).

La característica más distintiva del interior de este compartimento es el gran número de láminas que se proyectan desde la curvatura mayor con los bordes libres paralelos hacia la curvatura menor. Estas láminas se extienden longitudinalmente y disminuyen gradualmente de tamaño desde el orificio reticulo-omasal al orificio omaso-abomasal (**Yamamoto *et al*. 1993, 1994; Dyce *et al.* 2002**). Son clasificadas de acuerdo a su tamaño en láminas de primer, segundo, tercer y cuatro orden. El área de superficie ofrecida por las láminas omasales sugiere que el omaso es un órgano de absorción (**Phillipson, 1982**). La superficie lateral de las láminas omasales está tapizada de papilas córneas que varían en tamaño y morfología según la región. **Garden y Scott (1971)** distinguieron tres formas diferentes de papilas:

papilas cónicas, las más comunes y situadas en la superficie lateral; papilas rugosas y largas en los bordes libres de las láminas y papilas de morfología unguliforme y de pequeño tamaño en las cercanías del orificio retículo-omasal.

El abomaso es un saco alargado que se ubica a la derecha y ventralmente en la cavidad abdominal. Su cara parietal se relaciona con el suelo del abdomen y su cara visceral con el retículo y el omaso (**Sisson, 1974**).

La superficie interna del abomaso se dobla en crestas longitudinales o pliegues. Estos pliegues recorren craneocaudalmente y de forma oblicua toda la superficie del abomaso, comenzando al final del omaso donde son de mayor altura y disminuyendo hacia la parte pilórica. El número de pliegues puede variar entre especies, siendo de 13-14 en bovino, 13-15 en ovino y entre 16 y 17 en caprino (**Ellenberger y Baum, 1912**).

Imagen 5. Láminas omasales de diferentes tamaños tapizadas con papilas córneas en el omaso de cabra

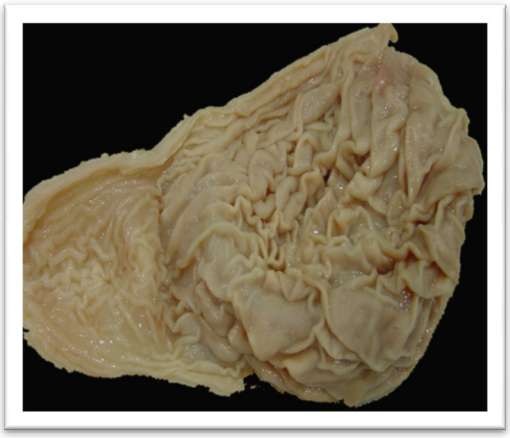


Imagen 6. Pliegues longitudinales en la mucosa abomasal

3.2.2. Histología

La estructura histológica de la pared de los compartimentos gástricos del estómago de los rumiantes comprende cuatro capas: mucosa, submucosa, muscular y serosa.

La mucosa se compone de un epitelio, una muscular de la mucosa y una lámina propia. El rumen, retículo y omaso se caracterizan por tener una mucosa tapizada por un epitelio estratificado plano queratinizado y aglandular (**Scala *et al.* 2011**). Este epitelio se organiza en cuatro capas definidas: estrato basal, estrato espinoso, granuloso y córneo (**Stevens y Marshall, 1970; Vallejo y Colmenares, 1972**). El estrato basal está formado por células cilíndricas dispuestas en una sola capa; el estrato espinoso por células de morfología poliédrica; el estrato granuloso, compuesto por células aplanadas con gránulos de queratohialina en su citoplasma y el estrato córneo, el más superficial, se encuentra constituido por células aplanadas carentes de núcleo y que pueden disponerse en un número de capas que oscila entre una y veinte (**Neiva *et al.* 2006; Scala *et al.* 2011**).

La mucosa de los compartimentos no glandulares (rumen, retículo y omaso) presenta complejas estructuras en su superficie, representadas por las papilas ruminales, crestas reticulares y láminas omasales (**Yamamoto *et al.* 1998).** Todas ellas se constituyen como evaginaciones de la lámina epitelial y están formadas por un esqueleto de tejido conjuntivo procedente de la lámina propia. La lámina omasal, además del tejido conectivo tiene en su eje central una delgada muscular de la mucosa. Autores como **Yamamoto *et al.* (1991)** describen en la lámina omasal tres capas musculares: una intermedia, procedente de la capa interna de la túnica muscular y dos capas laterales consideradas como ampliaciones de la muscular de la mucosa.

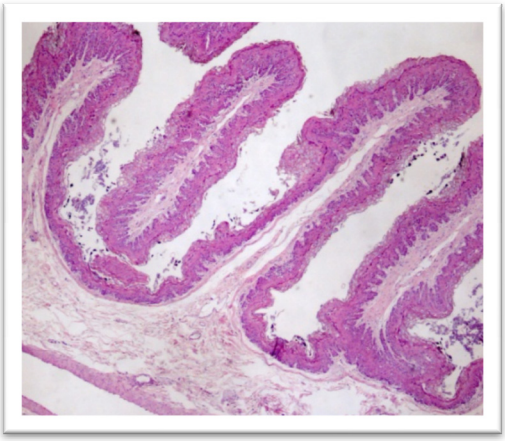


Imagen 7. Papilas ruminales distribuidas en la mucosa del rumen

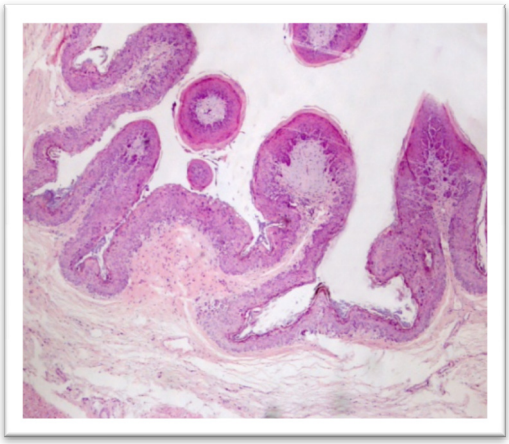


Imagen 8. Crestas reticulares en la mucosa del retículo

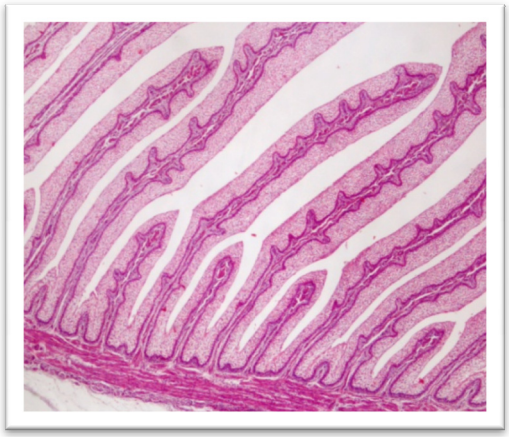


Imagen 9. Láminas omasales de diferentes tamaños en la mucosa omasal

El epitelio estratificado plano de los compartimentos no glandulares cambia bruscamente a simple cilíndrico y glandular en el abomaso. La superficie del abomaso está provista de pequeñas depresiones o invaginaciones denominadas criptas gástricas, que se continúan con las glándulas gástricas. La mucosa del abomaso se divide en tres regiones distintas, designadas de acuerdo a los diferentes tipos de glándulas presentes: cardias, fundus y píloro (**Age *et al*. 2008**). La región glandular cardial se sitúa en la abertura circular omaso-abomasal. Las glándulas cardiales son ramificadas, glomeruladas y tubulares, abriéndose en las criptas gástricas. La región glandular fúndica, está provisto de glándulas tubulares rectas y ramificadas. Estas glándulas constan de un cuello, cuerpo y base (**Aage *et al.* 2007**). En ellas se distinguen cuatro tipos celulares: células mucosas del cuello, células principales, células parietales y células argentafines. En la región glandular pilórica se encuentran glándulas ramificadas y más cortas que en el resto de las regiones.

El epitelio se asienta sobre una lámina propia, constituida por tejido conjuntivo fibroso con fibras de colágeno, elásticas y reticulares (**Kressin y Sommer, 1996; Age *et al*. 2008**).

La muscular de la mucosa es una fina capa de fibras musculares lisas. Esta muscular de la mucosa está ausente en el rumen (**Ramkrishna y Tiwari, 1979; Kitamura *et al.* 2003**), y en el retículo se encuentra situado en el extremo superior

de los pliegues reticulares como haces aislados de fibras musculares lisas. La inexistencia de la muscular de la mucosa en el rumen y su falta parcial en el retículo hace que no exista una separación definida entre la lámina propria y submucosa en estos dos compartimentos gástricos.

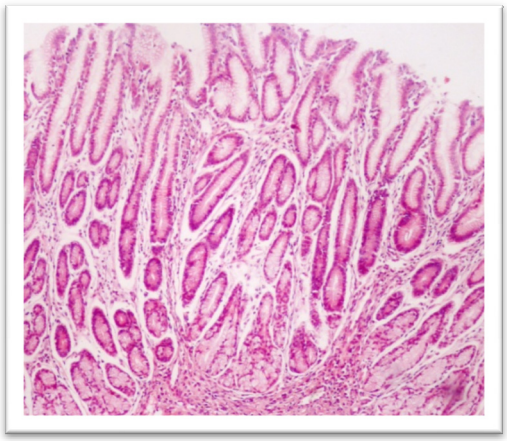


Imagen 10. Criptas y glándulas gástricas en la mucosa del abomaso

La mucosa descansa sobre una submucosa conformada de tejido conectivo fibroso, fibras de colágeno y proteínas de elastina al igual que la lámina propia.

La túnica muscular está constituida por dos fascículos de fibras musculares lisas: un fascículo interno con fibras musculares en disposición circular y un fascículo externo con fibras musculares orientadas longitudinalmente (**Ramkrishna y Tiwari, 1979**).

La serosa está compuesta por tejido conjuntivo laxo cubierta por un mesotelio. En esta capa se encuentran vasos sanguíneos, vasos linfáticos y nervios.

En general en la pared de cada uno de los compartimentos se localizan dos plexos nerviosos correspondientes a la inervación intrínseca del aparato digestivo. El primer plexo, plexo mienterico o de Auerbach se localiza entre los dos fascículos de fibras musculares lisas de la túnica muscular y controla la movilidad de la musculatura. El segundo plexo, denominado plexo submucoso o de Meissner se localiza en la capa submucosa y es el responsable de la sensibilidad de la zona

(**Furness y Costa, 1987**). En el rumen, la red de plexos submcuoso es irregular y los nervios se entrecruzan unos con otros por la pared reticular. El plexo submucoso reticular consiste en una red que se sitúa tanto en la pared como en las crestas reticulares. Y en el omaso, este plexo se divide en dos segmentos, uno sublaminar y otro intralaminar (**Yamamoto *et al*. 1995**).

3.2.3. Organogénesis e histogénesis

El estudio del estómago de los rumiantes ha sido objeto de interés para dilucidar diferentes aspectos referentes al origen de los compartimentos gástricos, así como para conocer la morfogénesis e histogénesis de cada uno de ellos.

Los primeros trabajos relativos al desarrollo anatómico prenatal del estómago de rumiantes fueron realizados por **Martín (1933)** y se refieren fundamentalmente al bovino.

Diversos estudios consideraron que los tres primeros compartimentos gástricos, el rumen, retículo y omaso se desarrollaban como dilataciones del esófago, cubiertas con el mismo tipo de epitelio estratificado escamoso encontrado en ese mismo órgano (**Ellenberger, 1884, Edelman, 1889, Zimmerman/Sal, 1894, Oppel, 1896**). El abomaso en los rumiantes se correspondía al estómago simple de los mamíferos. Sin embargo, **Stoss (1892)** y **Karl (1915)** en el ovino y **Zimmerl (1900)** en el bovino, sugieren el origen unitario de los cuatro compartimentos a partir de un primordio fusiforme.

En bovino, **Pernkopf (1931)** publicó datos que apoyaban la concepción del origen no esofágico de los tres primeros compartimentos gástricos. Afirmó que el revestimiento del estómago no era específico ni estaba asociado a una forma definida el estómago. Estos resultados fueron apoyados por **Lambert (1948)**, quien señaló la existencia de una dilatación ovoide en embriones bovinos de 0,7 cm. A partir de dicha dilatación, se desarrollaba el rumen, retículo, omaso y abomaso. Además, no observó ninguna constricción que separara el esófago del estómago, ni de la que pudiera diferenciarse el rumen, retículo y omaso.

Warner (1958), destacó que el esófago se mantuvo como un tubo uniforme sin expansiones durante el desarrollo del estómago de rumiantes y que no había ninguna contribución del esófago en la formación de cualquier parte del estómago de estas especies.

Debido a la similitud en el epitelio, **Tamate (1956)** dedujo que los tres primeros compartimentos gástricos se formaban a partir de una saculación esofágica. Esta misma aportación, fue realizada por **Schawrtz (1977)** en su libro "Embriología animal comparada" señalando que los tres segmentos anteriores del estómago de los rumiantes provenían del esófago mientras que el abomaso se correspondía con el verdadero estómago.

Mutoh y Wakuri (1988) señalaron en fetos caprinos que los compartimentos gástricos se desarrollaban en principio como un estómago simple a partir de una parte del esófago que se extendía dorsalmente.

El desarrollo embriológico de cada uno de los compartimentos gástricos es variable respecto a la longitud corporal de embriones y fetos de cada especie animal. En la especie caprina, la diferenciación de los cuatro compartimentos gástricos fue señalada por **Molinari y Jorquera (1988)** en un periodo comprendido entre 2,44 a 3,18 cm. **Mutoh y Wakuri (1988)** en esta misma especie, indicaron que el rumen, omaso y abomaso aparecían en embriones caprinos de 1,29 cm CRL, y el retículo en individuos de 1,33 cm CRL. El desarrollo fetal del estómago de bovino, fue relativamente rápido. Los cuatro compartimentos gástricos pudieron distinguirse a los 2,4 cm según lo descrito por **Church (1974**) y a los 1,3 cm CRL por **Vivo *et al.* (1990)**. La aparición de los cuatro compartimentos ocurrió a los 2,6 cm CRL en ovino (**Franco *et al.* 1992, 1993b, c, d**). En especies rumiantes salvajes como el búfalo, **Panchamukhi *et al.* (1975)** describieron los cuatro compartimentos gástricos claramente definidos en fetos de 2,5 cm CRL. En ciervo, esta diferenciación ocurría a los 4,5 cm CRL descrita por **Franco *et al.* (2004a, b)** en rumen y retículo, por **Redondo *et al.* (2005)** en omaso y por **Masot *et al.* (2007a)** en abomaso.

Los esbozos de los compartimentos gástricos antes de comenzar su desarrollo sufren una serie de desplazamientos: el rumen y el retículo giran para localizarse en posición dorsal e izquierda, el omaso, hacia la derecha y centralmente y el abomaso hacia la parte izquierda. Tras un periodo de desarrollo y diferenciación, principalmente de los sacos ciegos y de los surcos y pilares del rumen, el abomaso se vuelve a desplazar para situarse en el lateral derecho, girando a la par sobre su propio eje transversal. Tras esos movimientos, el estómago pluricavitario queda dispuesto en su típica forma de herradura y sólo necesita de su diferenciación para tomar la forma definitiva en el neonato (**Michel y Schwarze, 1970; Molinari y Jorquera, 1988; Vivo et al. 1990**).

Conjuntamente con el desarrollo externo, se produce el desarrollo de la mucosa de los compartimentos gástricos. Los estudios sobre la histogénesis de la mucosa se basan fundamentalmente en el desarrollo de las estructuras características de cada compartimento, representando una especificidad unida íntimamente a la función de cada uno de ellos.

En estudios sobre el epitelio de los compartimentos gástricos destaca **Warner (1958)**, quien describió en embriones bovinos de 1,9 cm CRL, un *epitelio embrionario* que consistía en 2 a 4 capas de células que era difícil determinar si se trataba de un epitelio estratificado verdadero o pseudoestratificado. En el abomaso, observó que el epitelio simple columnar aparecía en embriones de 5 cm CRL. **Fath El-Bab *et al.* (1983)** mostraron que el epitelio del estómago de fetos ovinos de 1,3 cm CRL era pseudoestratificado. Esto también fue descrito por **Asari *et al.* (1985)**, quienes observaron en el abomaso de fetos bovinos de 2,3 cm CRL un epitelio de tipo pseudoestratificado que cambiaba a epitelio simple columnar en embriones de 13-14 cm CRL.

La estratificación del epitelio en los estratos basal y granuloso fue descrita en bovino por **Arias *et al.* (1978)** a los tres meses de gestación. **Pachamukhi y Srivastava (1979, 1980, 1982)** y **Osman y Berg (1981a, b; 1982a)** en fetos de búfalos señalaron estos estratos a los 3,2 cm y a los 9 cm CRL, respectivamente. En oveja **(Franco *et al.* 1992, 1993c, d; Regodón *et al.* 1996)** a los 2,6 cm CRL y en

ciervo (**Franco *et al.* 2004a, 2004b; Redondo *et al.* 2005; Masot *et al*. 2007b**) a los 4,5 cm CRL fueron descritas dos zonas claramente definidas en la lámina epitelial, una basal oscura correspondiente al estrato basal y otra apical clara, que se correspondía al estrato granuloso.

Esta primera disposición en estratos se ve completada a lo largo del desarrollo prenatal por el estrato córneo y estrato espinoso. En caprino, **Molinari y Jorquera (1988)** señalaron su diferenciación en embriones de 28,3 cm CRL. En oveja, la aparición de estos dos estratos fue descrita al nacimiento por **Wardrop (1961a)** a los 10 cm CRL y 21 cm CRL por **Del Rio Ortega (1973)** y **Franco *et al.* (1992, 1993c, d),** respectivamente. En el vacuno, también fueron señalados al nacimiento por **Asari *et al.* (1981)** y **Vivo *et al.* (1990).** En rumiantes salvajes, como el ciervo, el estrato lúcido espinoso apareció a los 21 cm CRL en el rumen y omaso (**Franco *et al.* 2004a; Redondo *et al.* 2005**) y a los 36 cm CRL en el retículo (**Franco *et al*. 2004b**). En esta misma especie, el estrato córneo aparecía a los 36 cm CRL en el rumen y retículo (**Franco *et al*. 2004a, b**) y a los 8 cm CRL en el omaso (**Redondo *et al.* 2005**). En búfalo, **Tiwari y Jamdar (1979)** describieron el estrato espinoso y el córneo al nacimiento, pero sin mencionar un estrato lúcido. **Panchamukhi y Srivastaba (1979, 1980, 1982)**, en embriones de búfalo de 74 cm CRL, observaron un epitelio estratificado plano no cornificado similar al de adulto.

El desarrollo de la lámina epitelial de los compartimentos gástricos se acompañó de modificaciones en su superficie, con la aparición de los pilares y papilas ruminales, crestas reticulares, láminas omasales y pliegues del abomaso. **Michel y Schwarze, (1970)** observaron que las primeras estructuras en desarrollarse eran las láminas omasales, después los pliegues del abomaso, a continuación las crestas del retículo y finalmente las vellosidades del rumen.

Los pilares ruminales aparecieron como proyecciones papiliformes hacia la luz que dividían internamente al órgano en diversos sacos (**Habel, 1975; Nickel *et al.* 1979**). La formación de estos pilares fueron descritos en embriones de caprino en un periodo comprendido entre los 3,18 y 7,5 cm CRL por **Molinari y Jorquera (1988).** En ovino, los pilares fueron descritos a los 3 cm y a los 4,7 cm por **Del Rio**

Ortega (1973) y **Franco *et al.* (1992)**, respectivamente. En vacuno, **Vivo *et al.* (1990)** señalaron la aparición de estas estructuras en embriones de 2,5 cm. En especies salvajes, **Franco *et al.* (2004a)** en ciervo, observaron los pilares a los 4,5 cm CRL. En búfalo, **Osman y Berg (1981a)** identificaron la primera incidencia de la formación de los pilares en fetos de búfalo de 17 cm CRL.

Las papilas ruminales fueron descritas como evaginaciones del estrato basal del epitelio hacia la luz ruminal (**Franco *et al.* 1992; Franco *et al*. 2004a**). En caprino, **Ramkrishna y Tiwari (1979)** describieron las papilas ruminales a los 12,7 cm CRL. También en cabra, **Molinari y Jorquera (1988)** lo hicieron en el periodo comprendido entre 14,9 a 28,3 cm CRL. En ovino, **Del Río Ortega (1973)** señaló la diferenciación de las papilas a los 5 cm CRL siendo coincidentes con las crestas reticulares y los pliegues abomasales. **Fath El-Bab *et al.* (1983),** describió la formación de estas estructuras a los 30 cm CRL, involucrando en su desarrollo el estrato basal, la membrana basal y la lámina propia. **Franco *et al.* (1992)** observaron las papilas ruminales en esta misma especie a los 11,5 cm CRL. En vacuno, **Arias *et al.* (1978)** estudiaron la pared ruminal desde los 3 meses de edad fetal hasta los 60 días postnatales y señalaron que las papilas ruminales aparecían en embriones de 24 cm de longitud y sufrían un acelerado crecimiento hasta los 50-60 cm. Sin embargo, estos autores no describieron las papilas ruminales como entidades separadas e independientes hasta el nacimiento. **Amasaki y Daygo (1987)** también en bovinos, indicaron su diferenciación a los 37,5 cm. **Vivo *et al*. (1990)** describieron pequeñas papilas ruminales en fetos bovinos de 24 a 30 cm CRL. En ciervo, **Franco *et al.* (2004a),** señalaron la formación de las papilas ruminales a los 21 cm CRL. En búfalo, destacaron las observaciones de **Panchamukhi y Srivastava (1979),** quienes indicaron que las papilas ruminales empezaban a mostrarse a los 25 cm CRL, alcanzando la cuarta parte de la altura epitelial a los 32 cm CRL para llegar a la superficie a los 50 cm y sobresalir hacia la luz a los 74 cm CRL. También en búfalo, **Osman y Berg (1981a)** señalaron la diferenciación de las primeras papilas ruminales a los 17 cm CRL.

Las celdas reticulares fueron formadas como resultado del crecimiento y desarrollo de las crestas reticulares primarias (**Masot *et al.* 2007b**). En la especie caprina, **Ramkrishna y Tiwari (1979)** señalaron las primeras crestas a los 11,5 cm. **Molinari y Jorquera (1988),** también en cabra, indicaron la diferenciación de las crestas primarias en un periodo comprendido entre los 14,9 a 28,3 cm CRL y las crestas secundarias entre los 28,3 a 35 cm CRL. En ovino, **Fath El-Bab *et al.* (1983)** describieron las crestas primarias a los 24 cm, las secundarias a los 30 cm y las papilas en la superficie de las crestas primarias a los 24,5 cm CRL. **Franco *et al*. (1993c)** también en ovino, señalaron la diferenciación de las crestas primarias a los 15 cm CRL, las secundarias y las papilas córneas a los 21 cm CRL. En bovino, las crestas reticulares fueron observadas por primera vez en fetos de 15-16,5 cm CRL y la formación completa de las celdillas a los 62 a 95 cm (**Vivo *et al.* 1990**). En ciervo, **Franco *et al.* (2004b)** describieron las crestas primarias y secundarias a los 9,6 cm CRL y 21 cm CRL respectivamente. Además, indicaron que para la formación de las celdillas, las crestas reticulares primarias requerían dos tipos de crecimiento: "*crecimiento longitudinal desde la membrana basal hacia la superficie epitelial y crecimiento transversal o expansión de las crestas adyacentes para unirse unas con otras y formar las celdas*" (**Franco *et al.* 2004b; Masot *et al.* 2007b**). En búfalo, **Panchamukhi y Srivastava (1980)** señalaron a los 14 cm las crestas reticulares primarias, a los 25,5 cm las secundarias y además observaron crestas terciarias a los 50 cm CRL. En la misma especie, **Osman y Berg (1981b)** describieron las crestas reticulares a los 9 cm CRL, como evaginaciones de la zona basal de la lámina epitelial.

En relación a las láminas omasales, la mayoría de los autores describieron cuatro tamaños diferentes y por tanto cuatro órdenes distintos. Sin embargo, la cronología de aparición de los diferentes órdenes laminares varió entre las distintas especies animales. En cabra, **Ramkrishna y Tiwari (1979)** señalaron la aparición de las láminas de primer orden a los 14,6 cm y el resto de órdenes fue evidenciado a los 32,5 cm CRL. **Molinari y Jorquera (1988)** también en la misma especie, identificaron láminas de primer orden entre los 3,18 y 7,4 cm y las de segundo orden entre los

7,5 y 14,9 cm CRL. **Nwaogu *et al.* (2008)**, describieron láminas omasales de primer, segundo y tercer orden en fetos caprinos a mitad de gestación. En ovino, **Del Río Ortega (1973)** describió a los 3 cm CRL las primeras láminas, a los 5 cm las segundas, a los 6,5 cm las terceras y a los 7 cm las cuartas láminas omasales. Señaló además, la formación de las papilas córneas en la superficie de las láminas a los 13, 5 cm. **Lubis y O´shea (1978)** también centraron sus estudios en la mucosa omasal de fetos ovinos, distinguiendo cuatro categorías de láminas, en la que el primer orden aparecía en fetos de 2,5 cm, el segundo a los 3,5 cm, el tercero a los 4,4 cm y el cuarto a los 11 cm CRL. Años más tarde, **Fath El-Bath *et al.* (1993)** señalaron que el primer, segundo y tercer orden aparecían a los 8 cm CRL y el cuarto orden de lámina a los 19 cm CRL. Además describieron la formación de papilas córneas a los 39, 3 cm en todos los órdenes laminares, excepto en el cuarto. **Franco *et al.* (1993d)** describieron las primeras, segundas, terceras y cuartas lámina omasales a los 2,6 cm, 4, 7 cm y 11 cm CRL, respectivamente. **Vivo *et al.* (1990)** en el ganado vacuno determinaron en fetos de 3 a 3,3 cm CRL las láminas de primer orden; de 4,2 a 4,8 cm CRL las de segundo orden; de 5,5-6,5 cm CRL el tercer orden y entre los 9,5-11,5 cm el cuarto orden. En especies rumiantes salvajes, como el ciervo, **Redondo *et al.* (2005)** señalaron al igual que en otras especies domésticas, cuatro órdenes de láminas omasales, a los 4,5; 7,2; 8; 19 cm CRL, respectivamente. La existencia de un quinto orden de lámina omasal fue descrito al nacimiento en especies como cabra (**Toner *et al.* 1971**), oveja (**Wardrop, 1961a**) y vacuno (**Vivo *et al.* 1990**). En búfalo, **Osman y Berg (1982b)** observaron un quinto orden a los 32 cm CRL.

El abomaso, estructura correspondiente con el compartimento glandular del estómago de los rumiantes, mostró características idénticas al estómago simple de las especies monogástricas (**Nickel *et al*. 1973**). El desarrollo histológico del abomaso en fetos caprinos fue estudiado por **Kamel *et al.* (1987),** quienes describieron en embriones de 8 cm CRL un abomaso con pliegues y tapizado por un epitelio columnar pseudoestratificado. **Lee *et al.* (1994)** en cabras nativas de Corea, identificaron la transformación de un epitelio simple columnar a los 13 cm CRL. En ovino, **Franco *et al.* (1993b)**, observaron un cambio de epitelio pseudoestratificado

a epitelio simple cilíndrico a los 13,5 cm. **Xiao-guang (2010),** señaló en fetos ovinos de 3 cm CRL un epitelio simple columnar en el compartimento glandular. La transición de un epitelio pseudoestratificado a epitelio simple cilíndrico fue descrita también en fetos bovinos de 13-14 cm CRL por **Asari *et al*. (1985).** En esta misma especie, **Vivo *et al.* (1990)**, indicaron esta transformación epitelial en el periodo entre 9,5-11,5 cm CRL. En cuanto a las especies salvajes, **Masot *et al.* (2007a)** describieron en abomaso de ciervo un epitelio pseudoestratificado a los 4,5 cm CRL, que pasaba a ser epitelio simple cilíndrico a los 8 cm CRL. En búfalo, **Singh *et al.* (2007)** señalaron la presencia de un epitelio estratificado columnar en fetos de 5,5 cm que se transformaba en epitelio simple columnar en la región fúndica y pilórica entre los 11,2 y 14,7 cm CRL.

En relación a la diferenciación de las glándulas gástricas, en la especie caprina **Kamel *et al.* (1987)** describieron su formación a los 25 cm CRL y a los 33 cm CRL diferenciaron en ellas las células parietales, principales y mucosas profundas. **Molinary y Jorquera (1988)**, situaron su aparición en embriones de cabra en el periodo comprendido entre 28,3 a 35 cm CRL. **Lee *et al.* (1994)** describieron la formación de las criptas y glándulas gástricas a los 90 días de gestación y además diferenciaron entre células mucosas, parietales y principales en esta misma edad. En ovino, **Franco *et al.* (1993b)** señalaron que las glándulas gástricas empezaban a aparecen a los 15 cm CRL. **Xiao-guang (2010)** también en la especie ovina, describió el desarrollo de las glándulas a los 30 cm CRL. En fetos bovinos, **Asari *et al.* (1985),** identificaron las glándulas gástricas a los 16-18 cm CRL y las células mucosas y principales a los 43-45 cm y 58-65 cm CRL, respectivamente. **Vivo *et al.* (1990),** observaron las glándulas en fetos bovinos de 62-95 cm CRL. En la especie cervuna, **Masot *et al.* (2007a)** describieron las glándulas gástricas a los 21 cm CRL.

La diferenciación de la lámina propia, submucosa y túnica muscular fue descrita a partir del tejido blástico pluripotencial en fetos bovinos a los 4,2-4,7 cm (**Vivo *et al.* 1990**), en fetos ovinos de 6 cm (**Franco *et al.* 1992, 1993b, c, d**) y en fetos de ciervo de 4,5 cm (**Franco *et al.* 2004a, b; Redondo *et al.* 2005; Masot *et al.* 2007a**). La lámina propia formada por células de pequeño tamaño y forma alargada

y fusiforme aparecía mezclada con la submucosa al no existir muscular de la mucosa en el rumen (**Osman y Berg, 1981a; Franco *et al.* 1992; Franco *et al.* 2004a**) y en el retículo (**Franco *et al.* 1993c; 2004b**). La submucosa aparecía más fibrosa y delgada que la lámina propia (**Ramkrishna y Tiwari, 1979**).Esta lámina propia se introducía en los lugares invaginados de la zona basal de la lámina epitelial para formar el núcleo central de las papilas ruminales (**Osman y Berg, 1981a; Franco *et al.* 1992**), crestas reticulares (**Franco *et al.* 1993c, 2004b**) y láminas omasales (**Franco *et al.* 1993d, Redondo *et al.* 2005**). En el interior de las láminas omasales también fueron halladas fibras musculares lisas, que conformaban la muscular de la mucosa (**Redondo *et al.* 2005**).

La muscular de la mucosa fue descrita como una capa constituida por fibras musculares lisas procedentes del fascículo interno de la túnica muscular que aparecía en el retículo de búfalo a los 50 cm CRL (**Osman y Berg, 1981b**), en oveja a los 32 cm CRL (**Franco *et al.* 1993c**) y en ciervo a los 36 cm CRL (**Franco *et al.* 2004b**). En el omaso y abomaso de ciervo, la muscular de la mucosa fue diferenciada a los 7,2 cm CRL (**Redondo *et al*. 2005; Masot *et al.* 2007a**). En oveja, esta capa fue descrita en el omaso y abomaso en individuos de 7 cm CRL (**Franco *et al.* 1993b, d**).

La túnica muscular fue descrita como una capa de mioblastos en las primeras etapas embrionarias, y a medida que avanzaba el periodo de gestación aparecía constituida por dos fascículos: uno interno con fibras orientadas en dirección circular y otro externo con dirección longitudinal (**Vivo *et al.* 1990; Franco *et al.* 1992; Redondo *et al.* 2005; Masot *et al.* 2007a**).

Cada uno de los compartimentos del estómago de los rumiantes apareció cubierto por una serosa. Esta lámina fue descrita en especies rumiantes como el vacuno (**Vivo *et al.* 1990**), el ovino (**Franco *et al.* 1992, 1993b, c, d**) y el ciervo (**Franco *et al*. 2004a, b; Redondo *et al.* 2005; Masot *et al*. 2007a)** como una capa de tejido conjuntivo laxo cubierto por un mesotelio, en la que se podría encontrar vasos sanguíneos, nervios e incluso algunos adipocitos.

3.3. ANÁLISIS INMUNOHISTOQUÍMICOS

La utilización de técnicas inmunohistoquímicas ha abierto una nueva era para la histología e histopatología veterinarias. Estas técnicas son usadas fundamentalmente en el diagnóstico de patrones tumorales y para conocer la función de las células en relación a su origen y potencial de diferenciación. Además, el estudio inmunohistoquímico permite conocer la expresión celular relacionada con el desarrollo embriológico y la maduración de tejidos, de gran interés en la organogénesis y morfogénesis.

La aplicación de estas técnicas inmunohistoquímicas en el estudio histomorfogénico del estómago de rumiantes permite conocer la naturaleza, origen, presencia y evolución durante la vida intrauterina de los diferentes tipos celulares que conforman las estructuras tisulares de cada compartimento gástrico.

En el siglo XIX, Heidenkaium y Pavlov, describieron que la actividad funcional del tracto gastrointestinal estaba regulada por dos mecanismos: nervioso y hormonal. **Ceccarelli *et al.* (1995)** señalaron también que el proceso digestivo estaba estrechamente relacionado con el funcionamiento del sistema neuroendocrino difuso. Por tanto, la marcación inmunohistoquímica de células nerviosas, endocrinas y células neuroendocrinas tiene un gran interés para conocer la funcionalidad del estómago.

3.3.1. Marcadores de células endocrinas

En el aparato digestivo se encuentran células endocrinas entremezcladas con células nerviosas, capaces de elaborar diversas hormonas. Estos tipos celulares se engloban dentro del Sistema Neuroendocrino Difuso (SNED). Estas células son capaces de captar los precursores de aminas, lo mismo que descarboxilar a los aminoácidos, por lo que reciben el nombre de células APUD, acrónimo de inglés "*Amine Precursor Uptake and Decarboxylation*", término introducido por **Pearse *et al.* (1976).** Las células APUD producen más de 35 péptidos. Algunos de estos

péptidos reguladores gástricos están contenidos exclusivamente en células de tipo endocrino (péptido inhibitorio gástrico, gastrina, glucacón, insulina), mientras que otros han sido identificados en células neuronales (sustancia P, colecistiquinina y somatostatina).

Los péptidos hormonales gastrointestinales tienen especial importancia durante la vida prenatal y neonatal, y son indicadores de la participación de algunos tipos de células endocrinas en la regulación del crecimiento y morfogénesis del tracto gastrointestinal (**Larsson 1977, 2000; Guilloteau *et al.* 2006**).

La mucosa del estómago está provista de diferentes tipos de células endocrinas. Entre ellas, destacan la gastrina, una de las hormonas más importante en el sistema endocrino gastrointestinal. La gastrina fue descubierta por **Edkins** en **1905** y determinada su configuración molecular por **Gregory y Tracy (1964)**. Además, fue catalogada como el más potente estimulante de secreción ácida del estómago (**Jain y Samuelson, 2006**). Esta hormona es sintetizada y almacenada en las células G que se localizan en la parte pilórica del estómago y en menor medida es producida en el intestino delgado y grueso (**Konda *et al*. 1999**).

Anderson *et al.* (1975) determinaron que existía asociación entre la subida de los niveles de gastrina en sangre con determinadas parasitosis gástricas como *Ostertagia circumcita* en la oveja. En fetos de ratón, **Larsson (1977)** detectó gastrina y somatostatina mediante inmunofluorescencia a los 19 y 20 días de gestación en el antro pilórico. Utilizando esta misma técnica, **Tobe *et al.* (1979)** estudiaron la distribución de gastrina en órganos digestivos de perro, gato y hombre detectando células inmunorreactivas frente a la gastrina sólo en el antro pilórico y duodeno.

Bunnet y Harrison (1979), determinaron la presencia y localización de células productoras de gastrina en el tracto digestivo de ovinos adultos mediante inmunofluorescencia indirecta (IFI) y utilizando como anticuerpo primario gastrina de cerdo obtenida en conejo. La distribución de dichas células fue similar a la descrita anteriormente por **McGuigan (1968)** en el estómago de porcino y

humanos. Se detectaron además células específicas inmunorreactivas en las zonas más profundas de las criptas de Lieberkühn de intestino delgado.

Shulkes *et al.* (1981) abordaron en sus investigaciones la ontogénesis de la hormona gastrina, a partir de plasma sanguíneo de fetos ovinos con edades comprendidas entre los 100 días de gestación hasta el nacimiento y 16 días postparto. Encontraron niveles bajos de 8±1 fm/ml a los 101-110 días; ascendieron de forma constante hasta los 38±5fm a los 141-145 días y 90±13 fm/ml a los 1-5 días postparto; para finalmente descender a los 6-10 días postnacimiento, cuando se detectaron valores de 38±5 fm/ml. En **1982**, estos autores concluyeron que la gastrina era estable en el contenido gástrico fetal, pero rápidamente se metabolizada cuando el contenido gástrico se hacía más ácido, por otro lado, establecieron que las concentraciones de esta hormona en plasma siempre fueron más altas en el jugo gástrico debido precisamente al hecho anterior.

En porcinos adultos, **Rawdon y Andrew (1981)** determinaron la presencia de células productoras de gastrina en la región pilórica, duodeno e íleon mediante la técnica de la peroxidada-antiperoxidasa (PAP). Destacaron la región pilórica como la zona con una mayor concentración de células, siendo por el contrario el intestino, la zona con menor número de células.

Haciendo referencia a la distribución de células endocrinas gastro-intestinales en ovinos adultos, destaca el estudio de **Caligansan *et al.* (1984),** quienes mediante análisis inmunohistoquímicos, señalaron la existencia de nueve tipos diferentes de células endocrinas, de las cuales las productoras de somatostaina, gastrina y glucagón fueron localizadas en el abomaso. Dichas células (de morfología oval o piramidal) productoras de gastrina, eran más numerosas en la región pilórica, donde se localizaron diseminadas a lo largo de toda la superficie mucosa, menos numerosas en duodeno, más escasa en yeyuno y apenas existente en íleon.

Ito et al. (1985) determinaron la existencia de distintas células endocrinas en las vellosidades intestinales productores de gastrina, secretina, somatostatina y colecistoquinina mediante la técnica PAP.

Kitamura *et al.* (1985), estudiaron la distribución regional de las principales células endocrinas en el tracto gastrointestinal de 12 bovinos Holstein, clasificados en cuatro grupos de edad postnacimiento: 3 días, 1-2 meses, 3-4 meses y más de 1 año. Para ello, obtuvieron muestras de fondo, cardias y píloro abomasales y de las distintas asas intestinales. Utilizaron la técnica PAP y el antisuero específico gastrina humana sintética (GP-1304) y somatostatina para el análisis inmunohistoquímico. Establecieron, en términos generales, una relación inversa entre la edad y el número de células endocrinas detectadas, y detectaron que en el abomaso era la región pilórica donde quedaban confinadas las células productoras de gastrina, apareciendo éstas en menor número que las de somatostatina en vacas adultas, al contrario que ocurría en terneras.

Alison (1989), detectó las primeras células productoras de gastrina a los 14 días de gestación en el intestino delgado de embriones porcinos y a los 16 días en la región pilórica. **Avila *et al.* (1989),** investigando el papel que desempeñaba la gastrina en el desarrollo del tracto gastrointestinal de la oveja, durante la última mitad de gestación obtuvieron mediante análisis de radioinmunoensayo (RIA), niveles de gastrina de 19 pmol/l en sangre de fetos a los 135 días de gestación.

Kitamura *et al.* (1990) identificaron mayor número de células de gastrina en la porción inicial del intestino delgado (duodeno proximal); y un escaso número de células fueron encontradas en la porción terminal de un mamífero insectívoro (*Suncus murinus*).

Franco *et al.* (1993a) midieron concentraciones de gastrina en plasma y realizaron un estudio inmunohistoquímico en fetos de oveja. En plasma, las concentraciones de gastrina fueron detectadas a los 69 días, incrementando sus niveles hasta el nacimiento, mientras que en tejidos, las células productoras de gastrina aparecieron a los 69 y 77 días de gestación en duodeno y antro pilórico

respectivamente. Además, no identificaron células inmunorreactivas a gastrina en el cardías, ciego, colon y recto.

En 1999, **Lucini *et al.*** estudiaron la distribución de las células endocrinas en diferentes edades en el búfalo. Identificaron células de gastrina a los 5 meses de edad en el intestino delgado. Además, señalaron que las poblaciones de células endocrinas disminuían a medida que aumentaba la edad de los individuos.

Dall´Aglio *et al.* (1999) identificaron células positivas frente a gastrina, serotonina, somatostatina y colecistoquinina en el tracto gastrointestinal de fetos de gamos entre las 11 y 17 semanas de vida prenatal.

Soehartono *et al*. (2002) investigaron los posibles cambios en células endocrinas en el abomaso de terneros vagotomizados. Hallaron que estas células disminuían en los animales vagotomizados en comparación con los animales del grupo control. Además sugirieron que el nervio vago tenía gran influencia en la regulación intrínseca del sistema mediante el control de células endocrinas. **Agungpriyodo *et al.* (2000)**, detectaron mediante el método de avidita-biotina peroxidada células de gastrina en el píloro y en menor cantidad en el duodeno de *Babyrousa babyrussa* (Suidae).

Kitamura *et al.* (2001), estudiaron la aparición y desarrollo de las células productoras de proquimosina y pepsinógeno en el abomaso de ovejas desde edades fetales hasta adultos mediante inmunohistoquímica. La proquimosina aparecía por primera vez en las glándulas gástricas en fetos de 100 días de gestación. La intensidad y distribución de proquimosina aumentaba gradualmente con el avance de la gestación y su inmunorreavitividad fue más intensa en corderos de 3 días de edad.

En el estudio comparativo entre oveja y ciervo, **Franco *et al*. (2011)** señalaron la presencia de células neuroendocrinas en el rumen de fetos de ciervo de 8 cm CRL (97 días, 35% de gestación) y en fetos ovinos de 20 cm CRL (81 días, 54% gestación). A estas mismas edades fueron detectados en el retículo de las mismas especies (**Franco *et al.* 2012**). En el omaso de ciervo, **Redondo *et al*. (2011)**

identificaron células neuroendocrinas marcadas con enolasa específica neuronal (NSE) a los 4,5 cm CRL (67 días, 25% de gestación). **Masot *et al.* (2007a)** en el abomaso de ciervo indicaron la presencia de células neuroendocrinas mediante NSE a los 4,5 cm (67 días, 25% de gestación) y las células productores de gastrina fueron observadas a los 21cm CRL (142 días, 50% de gestación), incrementando su número a medida que avanzaba la desarrollo.

3.3.2. Marcadores de células gliales

Además de células nerviosas, en el sistema nervioso entérico se localizan células de la glía, que son elementos de soporte en los ganglios entéricos, similares a los astrocitos del sistema nervioso central (**Gabella 1971**) y diferentes de las células Schwann del sistema nervioso periférico (**Geboes y Colins, 1998**). Estas células gliales son positivas a la proteína ácida fibrilar glial-GFAP (**Scharrer 1990; Von Boyer *et al.* 2004**) y vimentina-VIM (**Lazarides, 1980**), siendo esta última proteína, un marcador de células mesenquimales (**Franke *et al.* 1979**).

Jessen y Mirsky (1983) estudiaron las células gliales mediante la marcación con GFAP y VIM en ratones recién nacidos. Detectaron inmunorreactividad para ambos marcadores en ganglios miéntericos y submucosos del intestino. Sólo inmunorreactividad para VIM fue encontrada en el tejido conectivo de la pared intestinal, incluida la serosa, submucosa y mucosa. Estos autores basándose en la morfología y estructura molecular sugirieron que las células gliales entéricas y los astrocitos podrían compartir funciones que no eran llevadas a cabo ni por las células de Schwann ni por las células satélites.

Nada y Kawana (1988) en el colon y recto de rata encontraron inmunorreactividad frente a GFAP en los plexos mientéricos y con menos frecuencia en los plexos submucosos. La inmunorreactividad frente a S-100 fue localizada en todas las capas del intestino y en los nervios perivascular de la submucosa. La variedad en la localización de estas dos proteínas hizo suponer que existían dos tipos de células de soporte, un primer tipo llamado células de glía, que podrían ser

originadas a partir de los nervios centrales y positivos a GFAP y S-100 y un segundo tipo, llamado células de Schwann, que podrían ser originadas de las crestas neurales y positivas frente a S-100.

Balaskas y Gabella (1998) en embriones de pollo, detectaron inmunorreactividad frente a GFAP en células ganglionares de proventrículo, duodeno e íleon a los 4,5 días de incubación.

Franco *et al.* (2011) y **Franco *et al.* (2012)** en el estudio comparativo de ciervo y oveja en los compartimentos ruminal y reticular, observaron células gliales positivas a GFAP a los 21 cm CRL (142 días, 50% de gestación) en ciervo y a los 31.5 cm (112 días, 75% gestación) en oveja. Estos mismos autores detectaron la presencia de células gliales inmunorreactivas a VIM en etapas más tempranas de gestación, a los 4 (67 días ,25% gestación) en el rumen y retículo de ciervo y a los 8 cm (52 días, 35% ,5 cm gestación) en el rumen y retículo de oveja. En el omaso de ciervo, **Redondo *et al.* (2005)** señalaron células gliales positivas a GFAP y VIM a los 4,5 cm CRL (67 días, 25% de gestación), al igual en el abomaso de ciervo (**Masot *et al.* 2007a**).

3.3.3. Marcadores de inervación peptidérgica

Los mecanismos nerviosos que intervienen en las funciones motoras de los compartimentos gástricos están controlados no sólo por el sistema nervioso central, sino por un elaborado sistema nervioso entérico (**Ruckebusch, 1989**), a través de la combinación de neurotransmisores de excitación e inhibición (**Kitamura *et al.* 1986; Groenewald, 1994; Brookes, 2001; Pfannkuche *et al.* 2002; Pfannkuche *et al.* 2004**).

Los neuropéptidos actúan como neurotransmisores en las neuronas entéricas (**Cooke 1986; Mawe *et al.* 1989; Scharrer 1990**). La marcación mediante técnicas inmunohistoquímicas de estos neuropéptidos permite el estudio de la compleja estructura del sistema nervioso entérico.

Con el objetivo anterior, **Jessen *et al.* (1980)** estudiaron la anatomía y funcionalidad de los plexos ganglionares del sistema nervioso entérico mediante la detección de péptido intestinal vasoactivo (VIP) y sustancia P (SP) mediante técnicas inmunohistoquímicas. Abundantes fibras nerviosas positivas a VIP fueron detectadas en las células ganglionares del plexo mientérico, capa longitudinal y circular de la túnica muscular y escasas fibras en la submucosa. También fueron detectadas fibras nerviosas positivas a SP en el plexo mientérico y con mayor densidad que para VIP.

Lindh *et al.* (1986) utilizaron anticuerpos como neuropeptido Y (NPY), somatostatina (SOM), VIP y polipéptido HI para detectar neuronas peptidérgicas en los ganglios mientéricos. De todos ellos, encontraron que un 65% de las células ganglionares eran positivas a NPY, mientras que las células inmunorreactivas a SOM representaban el 25%.

Kitamura *et al.* (1986) estudiaron la distribución de los nervios en el surco reticular y en los compartimentos ruminal y reticular de ternero y de adultos mediante inmunodetección por SP, VIP, leucina encefalina (LENK), polipéptido liberador de gastrina (GRP), SOM y enolasa neuronal específica (NSE). Inmunorreactividad frente a NSE, SP, VIP, LENK y SOM fue detectada en fibras y cuerpos nerviosos, mientras que GRP fue localizada solo en fibras nerviosas. Además, la inmunorreactividad fue mayor en terneros que en animales adultos. En cuanto a la localización, el surco reticular fue la región con mayor densidad de fibras y cuerpos nerviosos, con respecto al rumen y retículo. **Kitamura *et al.* (1993)**, determinaron fibras nerviosas y cuerpos nerviosos inmunurreactivos frente a sustancia P en la lámina propia de rumen, retículo y omaso, así como en las papilas ruminales, reticulares y láminas omasales de terneros y adultos. Además fueron detectadas, aunque con poca frecuencia, fibras nerviosas intraepiteliales en rumen y retículo.

Wathuta (1986) detectó fibras nerviosas inmunorreactivas frente a VIP, SP y bombesina distribuidas en la mucosa, submucosa, túnica muscular y plexos de los

compartimentos gástricos no glandulares, abomaso e intestino delgado y grueso de oveja.

Timmermans *et al.* (1990) detectaron fibras nerviosas inmunorreactivas frente a NPY y VIP en el plexo submucoso, muscular de la mucosa y lámina propia del intestino del cerdo.

Yamamoto *et al.* (1993), identificaron en el omaso de oveja, fibras nerviosas inmunorreeactivas frente a SP y encefalina (ENK) en la capa muscular externa, mientras que fibras positivas frente a VIP, NPY y GFAP fueron observadas en la capa muscular interna de la túnica muscular. Fibras nerviosas positivas frente a estos péptidos también fueron observadas en las papilas y láminas omasales.

Groenewald (1994) determinó la funcionalidad de los ganglios mientéricos en el rumen, retículo, omaso y abomaso de cordero mediante la presencia de VIP, SOM, NPY, SP, ENK, neurotensina y calcitonina. La inmunorreactividad fue de moderada a alta para SOM, SP y VIP en el rumen, retículo, omaso y baja en el abomaso, mientras que para calcitonina, NPY y ENK fue de moderada a baja en todos los compartimentos gástricos.

Balemba *et al*. (1999) examinaron el sistema nervioso entérico en yeyuno e íleon de terneros mediante VIP. Detectaron inmunorreactvidad en las fibras nerviosas de los plexos nerviosos, así como en el fascículo interno de la túnica muscular. Además, fue observada inmunorreactividad frente a VIP en los plexos nerviosos, tejido conectivo perivascular, fascículo interno muscular y pilares del rumen. En el abomaso, se identificó su presencia en los plexos nerviosos pero no en el tejido conectivo perivascular.

Pfannkuche *et al*. (2002) estudiaron la inervación intrínseca en las capas musculares del abomaso en bovinos mediante la inmunodetección de acetilcolintransferasa (ChAT), óxido nitrico (NOS) y NSE. Concluyeron que la inervación colinérgica excitatoria era predominante en ambas capas musculares del abomaso, pero que el fascículo muscular circular recibe inervación colinérgica excitatoria e inhibitoria nitrogénica, mientras que el fascículo longitudinal era

inervado por vías colinérgicas. Finalmente concluyeron que el patrón de inervación era diferente a la musculatura gástrica de monogástricos.

Zitare *et al.* (2013) señalaron que NPY jugaba un importante papel en las funciones del sistema digestivo de ciervo. Identificaron este neuropeptido en la túnica muscular del rumen y del intestino de dicha especie.

Münnich *et al.* (2008) analizaron las diferencias en la inervación ruminal intrínseca entre especies rumiantes de diferentes hábitos alimenticios (cabra, gamo, oveja y vaca) mediante análisis inmunohistoquímico con ChAT, NOS, VIP, NPY, SP, calbidina (CALB) y SOM. Los resultados sugirieron que el rumen de vaca y oveja bajo control colinérgico era más fuerte que el del resto de las especies.

La inmunodetección de neuropeptidos durante la vida prenatal fue llevada a cabo por **Wathura y Harrison (1987)** en el tracto digestivo de la oveja. Estos autores detectaron inmunorreactivdad para VIP a los 70 días de gestación en los plexos del esófago y preestómagos e inmunorreactividad para SP a los 50 días de gestación en los plexos miéntericos y submucosos de los compartimentos no glandulares y abomaso.

En fetos bovinos, **Stallcup *et al.* (1990)** observaron una reacción inmunohistoquímica de NADPH2R en epitelio, lámina propia y muscular del rumen, mientras que fosfatasa alcalina fue confirmada en epitelio y vasos sanguíneos de la lámina propia ruminal.

También en especies rumiantes salvajes como el ciervo, **Franco *et al.* (2004a)** detectaron en rumen fibras nerviosas inmunorreactivas frente a VIP y NPY a los 8 cm CRL (97 días, 35% gestación). En las mismas edades, **Franco et al. (2004b)** detectaron estos péptidos en el retículo de ciervo. En omaso de esta misma especie, **Redondo *et al.* (2005)** detectaron VIP y NPY a los 21 cm (142 días, 50% gestación), al igual que en el abomaso (**Masot *et al.* 2007a**).

3.4. ANÁLISIS ESTRUCTURALES MEDIANTE MICROSCOPÍA ELECTRÓNICA DE BARRIDO

El primer trabajo encontrado sobre la estructura de los compartimentos gástricos mediante microscopía electrónica de barrido fue llevado a cabo por **Gardner y Scott (1971).** Estos autores estudiaron la superficie del omaso en la oveja y observaron que las laminas omasales variaban en tamaño, y estaban adjuntas a la curvatura mayor. Los bordes libres de estas láminas contenían papilas con tres morfologías diferentes: largas con forma cónica y punta afilada y queratinizadas, pequeñas de forma cónica con punta redondeada y otras en forma de clavija. Además, en la superficie de estas papilas fueron visibles desprendimientos celulares procedentes de la capa córnea.

Este mismo año, **Scott *et al*. (1973)** centraron sus investigaciones en la morfología papilar de rumen, retículo y omaso en ovejas adultas. En rumen, las papilas mostraban distinta morfología, variando desde pequeñas lengüetas hasta grandes estructuras alargadas y foliadas, quedando delimitadas por profundos surcos más marcados en uno de los dos lados de la papila. Por otro lado, la superficie reticular mostraba un aspecto a modo de panel debido a las gruesas paredes de las crestas que delimitan las celdillas. En el interior de estas celdillas se hallaban papilas, con un aspecto más foliado que las papilas ruminales. Por último, en el omaso se evidenciaron papilas cónicas, a lo largo de la superficie de las láminas, si bien también podían detectarse papilas con morfología más triangular y de mayor tamaño que las observadas en retículo.

Posteriormente, **McGavin *et al.* (1976)** relacionaron la interacción entre la dieta alimenticia y la morfología de las papilas ruminales en bovinos. De tal forma, que analizaban muestras ruminales de 20 terneros neonatos que eran alimentados, con dieta láctea; y cuatro diferentes tipos de raciones, compuesta de alimentos groseros. Ocho de los animales fueron sacrificados a las cuatro semanas de vida y el resto a las seis. En el primer grupo, aparecían papilas delgadas y con morfología a

modo de lengua, mientras que en el segundo, las papilas eran de menor tamaño, redondeadas y con signos de paraqueratosis.

En este sentido, **Hentges *et al.* (1966)** observaron que al añadir harina obtenida a partir de cítricos a la dieta, las papilas ruminales aparecían de menor tamaño y con un tinte más oscuro de lo habitual. **Nockels *et al.* (1966)** detectaron papilas ruminales irregulares en morfología, de longitud mayor de lo normal y exceso de pigmentación o disminución en grosor del estrato granuloso, en situaciones anormales de la dieta en ovinos.

Amasaki y Daygo (1988) analizaron mediante microscopía electrónica de barrido los cambios que sucedían en la mucosa ruminal de fetos bovinos a lo largo del desarrollo. Estos autores observaron las papilas ruminales por primera vez al quinto mes de gestación, participando en su formación la lámina propia. También, observaron gránulos de queratohialina en las células más superficiales de la mucosa a partir del quinto mes de gestación.

Franco *et al.* (1989) analizaron mediante microscopía electrónica de barrido, la evolución estructural del complejo rumino-reticular ovino relacionada con la edad (neonato y adulto) y subsecuentemente con la dieta, "*base determinante del crecimiento de papilas ruminales y crestas reticulares*".

Agungpriyono *et al.* (1995) en su estudio sobre el estómago del ratón venado (*Tragalus javanicus*) observaron en la superficie reticular cresta reticulares primarias y secundarias. En la región basal de estas crestas se encontraban grandes papilas cónica corneas. Las papilas ruminales mostraban una morfología en forma de lengüeta.

Yamamoto *et al.* (1998) en cabra observaron que las papilas ruminales estaban distribuidas por todas la superficie del rumen. Estas papilas tenían morfología foliada o filiforme.

En 2004, **Graham y Simmons**, estudiaron la superficie epitelial del rumen en bovino. En las papilas se observaron células en proceso de desprendimiento procedentes del estrato córneo. Concluyeron que la capa más externa de células

queratinizadas es probable que actuara como una barrera de permeabilidad epitelial.

El-Gendy *et al.* (2010) estudiaron el omaso de cabras adultas mediante microscopía electrónica de barrido. Describieron la superficie del omaso cubierta de papilas distribuidas por toda la región. En el orifico reticulo-omasal se observaron papilas largas, cónicas y queratinizadas, mientras que en la parte craneal del omaso las papilas eran de menor tamaño y en forma de gancho. El tamaño y la densidad de la distribución de las papilas descendía a medida que las láminas se acercaban hacia el orifico omaso-abomasal.

Garcia *et al.* (2012) examinaron mediante microscopía electrónica de barrido el rumen en fetos caprinos desde los primeros estadios embrionarios hasta el nacimiento. Observaron que en edades tempranas de gestación (38 días) la mucosa ruminal aparecía lisa y sin fenómenos de descamación y no fue hasta los 50 días de gestación cuando se hacían evidentes las papilas ruminales. Un año más tarde, **García *et al.* (2013)** también en la especie caprina estudiaron la mucosa omasal. Describieron que a los 38 días de gestación podían observarse pequeños pliegues longitudinales en la superficie de dicho compartimento, los cuales se correspondían con las láminas omasales de primer orden. A los 85 días de gestación, los cuatro órdenes de láminas omasales eran visibles en toda la superficie.

3.5. ANALISIS MATEMÁTICOS. APLICADOS AL CRECIMIENTO Y DESARROLLO FETAL

La realización de análisis morfométricos resulta esencial para todo trabajo basado en la evolución en el tiempo de cualquier tipo de elemento. En este sentido, cabe destacar la importancia que tiene la morfometría para estudios como éste, en el que se describe el desarrollo del órgano gástrico desde las primeras etapas embrionarias hasta el nacimiento.

El crecimiento y el desarrollo son procesos biológicos íntimamente unidos y dependientes del tiempo. Por ello, cabe expresar matemáticamente estos procesos mediante modelos matemáticos, que de acuerdo con **Thornley y France (2007),** no es sino una ecuación o un conjunto de ecuaciones que representa el comportamiento de un sistema.

Las curvas de crecimiento reflejan la interrelación entre los impulsos de un individuo a crecer y madurar en todas las partes del cuerpo y el entorno en que estos impulsos se expresan. El término, curva de crecimiento por lo general evoca una curva sigmoidea que representa una secuencia de medidas en relación al tiempo (**Fizthugh, 1976**). Se trataría de un modelo dinámico en el que el factor tiempo permite explicar la evolución de los sucesos que acontecen durante el transcurso de la vida intrauterina.

Fizthugh (1976) señaló que los objetivos principales de las curvas de crecimiento eran descriptivos y predictivos. Además, las características específicas de los datos y objetivos determinaban el método de elección de la curva de crecimiento. Las bases principales para la elección de la curva eran tres: interpretación biológica de los parámetros, bondad del ajuste para los datos reales y dificultad computacional que variaba con la elección de la función.

Una característica común de los modelos de crecimiento es que cada uno de ellos utiliza dos parámetros biológicamente relevantes. El primer parámetro establecía la posición individual (o grupo) en la población general del tamaño a una

edad (**Brinks *et al.* 1964; Taylor y Craig, 1965; Brown *et al.* 1973**). El segundo parámetro se refiere a la tasa de crecimiento en relación con el tamaño corporal. Además, algunos como **Brody (1945)** nombran un tercer parámetro que se utilizaba para dividir la curva de crecimiento en dos etapas: aceleración e inhibición durante las cuales la velocidad de crecimiento incrementaba o descendía, respectivamente.

El crecimiento de los rumiantes en su fase embrionaria según **Roux (1990),** es de tipo exponencial, ajustándose a la función $W= a\ e^{t}$, en la que *W* es el peso esperado al tiempo *t*, *a* la estimación del peso inicial (huevo fecundado) y *t* el tiempo transcurrido de gestación.

El crecimiento global de un individuo, desde la concepción a su estado adulto, adopta una curva sigmoidea (**Sotillo y Vigil, 1978**). Sin embargo, se han propuesto diferentes modelos, entre los que destaca **Brody (1945),** quien propone la siguiente ecuación para la fase inicial del crecimiento: $W= a\ e^{kt}$, donde *W* es el peso esperado al tiempo *t*, *a* la estimación del peso inicial, *k* la constante específica del modelo y *t* el tiempo. Para la fase de crecimiento retardado propone la fórmula $W= a\ (1-ce^{-kt})$, en la que *W* es el peso esperado al tiempo *t*, *c* y *k* constantes, *a* estimación del peso final y t tiempo.

Este modelo ha resultado ser francamente fecundo, al dar lugar a otros derivados. Así **Von Bertalanffy (1957)** propone un modelo similar: $W = a\ (1-c\ e^{-kt})^{a}$ siendo *W* el peso esperado al tiempo *t*, *a*, *c* y *k*, constantes y *t* el tiempo; que puede simplificarse en la siguiente expresión: $W = (1-e^{-t})^{a}$.

Los modelos revisados hasta el momento siguen un modelo de desarrollo en función del tiempo, cuyos parámetros pretenden tener una explicación fisiológica. Sin embargo, existe una familia de modelos de tipo polinómico que responde a la fórmula genérica:

$$W = a_0 + a_1\ t + a_2 t^2 + a_3\ t^3 + \ldots . a_n\ t^n$$

Estos modelos son de tipo empírico, y con difícil explicación fisiológica de los parámetros obtenidos. En cualquier caso, el peso inicial $W_0 = a_0$, ya que si t=0 el resto de la expresión pierde validez; los puntos de inflexión a lo largo de la curva, a

su vez, vienen determinados por el signo y valor de los parámetros a_1, a_2, ….a_n en su interacción con el tiempo. Este tipo de modelo, de acuerdo con **Causton y Venus (1981),** es de fácil ajuste en contraposición con los previamente estudiados.

Respecto al orden del polinomio utilizado, **Hurd (1977)** indica que no debe superarse el cuarto orden si se quieren evitar ajustes no deseables por su extremada sensibilidad a los cambios en los datos experimentales y, en definitiva, la obtención de respuestas espúreas. **Fitzhugh (1976)** señaló previamente que el modelo polinómico realizaba siempre el mejor ajuste, sin embargo, los parámetros estimados no eran biológicamente interpretativos.

Huxley (1932) propuso el concepto de alometría, siendo una comparación entre el crecimiento de una parte frente al crecimiento global del individuo. Dicha alometría se expresa a través del coeficiente de alometría k, obtenido de la siguiente fórmula: $W = a\ t^k$, en la que *W* es el peso de la parte, *a* y *k* constantes y *t* el tiempo. De esa forma, cuando los ritmos de crecimiento (parte y todo) son equiparables, se habla de crecimiento isométrico (k= 1). Por otro lado, cuando la parte crece más velozmente que el todo se habla de alometría positiva ($k > 1$), en el caso contrario, alometría negativa ($k<1$).

Si bien existen autores que, mediante la aplicación de programas matemáticos, correlacionan dos o más variables. Sin embargo, son escasos los trabajos que presentan y aplican series completas de datos cuantitativos al crecimiento embrionario a lo largo de todo el periodo gestacional, y ninguno considera el crecimiento en grosor de las distintas capas tisulares que integran la víscera gástrica en los rumiantes **(Vivo, 1987).**

Church *et al.* (1962), a partir de 10 corderos de edades comprendidas entre 7 y 34 días, y sacrificados a intervalos de una semana, establecieron análisis de correlación entre los pesos de los compartimentos gástricos a las distintas edades consideradas. Mostraron que el crecimiento en los rumiantes está altamente correlacionado con el crecimiento reticular (r^2= 0,96), de forma significativa con el crecimiento omasal (r^2= 0,77) mientras que y con el abomaso esta relación es más

baja (r^2= 0,38). Por otro lado, la regresión del crecimiento gástrico con la edad, indica que el crecimiento durante los periodos observados (7-34 días) fue esencialmente lineal y apuntan que las ecuaciones de predicción para el crecimiento reticular y ruminal son respectivamente: y= 0,42x + 8,22 e y= 3,03x + 4,55, en la que *y* son miligramos de peso y *x* los días.

Goedloed (1972) en sus investigaciones sobre el crecimiento corporal embrionario en ratas, propuso un modelo de crecimiento cuya curva consistía en la asociación de dos rectas. La primera de estas rectas, de gran pendiente, cubre un periodo de crecimiento exponencial, probablemente debido al constante ritmo de división celular, no viéndose influida la velocidad de crecimiento por los cambios de situación durante este periodo. Aunque llega un momento en que se suceden, de forma secuencial ciertos cambios en el ritmo de crecimiento, que provocan una disminución en la pendiente.

Sbarbati (1979) estimó, mediante técnicas morfométricas, el incremento de volumen que sufrían el estómago e intestino con el tiempo en embriones de ratón. Señaló la existencia de un ritmo constante hasta el nacimiento en el crecimiento específico del intestino, mientras que el específico del estómago decrecía de forma constate con el tiempo.

En relación con la víscera gástrica **Fukaya *et al.* (1979),** realizaron un estudio morfométrico en fetos bovinos y neonatos basado en la observación de sus moldes obtenidos mediante la introducción, vía esofágica, de resinas sintéticas. Las medidas fueron conseguidas a partir de la longitud de distintos ángulos y planos trazados a través del eje longitudinal de rumen, omaso y abomaso. Entre sus conclusiones destacamos que "la correlación existente entre el volumen ruminal y el peso corporal fue positiva". Esto sugiere la posibilidad de estimar inversamente el volumen ruminal, con sólo conocer el tamaño corporal de espécimen a considerar.

Asari *et al.* (1981) abordaron el desarrollo morfométrico del estómago en fetos bovinos y neonatos, haciendo especial referencia a los cambios acontecidos en el volumen y peso del abomaso. Encontraron que en los primeros periodos de gestación, el rumen era el mayor de los cuatro compartimentos, tanto en volumen

como en peso. A los seis meses de gestación el volumen porcentual de rumen y abomaso eran inversos y era al nacimiento cuando el abomaso muestraba, en términos porcentuales, mayor peso y volumen.

En **1982**, **Abdallah *et al*.** describieron las diferencias existentes entre búfalo y bóvidos frisones adultos, en el desarrollo y crecimiento de los componentes del tracto alimentario. Indicaron, en primer lugar, que el incremento de peso en la canal se acompañaba con un descenso en la proporción de tejido gastro-intestinal. Por otro lado, cuando aumentaba el peso de éste, el aumento se refería a todos sus componentes, excepto el rumen e intestino delgado, que permanecían constates. Por último, concluyeron que el búfalo, presentaba un peso mayor de rumen y retículo y menor de omaso e intestino grueso que el vacuno.

Franco *et al.* (1993e) estudiaron el crecimiento embrionario durante el periodo gestacional del estomago de oveja, considerando el grosor de las distintas capas tisulares que integran la víscera gástrica. Efectuaron 30 medidas de cada una de las capas (lámina epitelial, lámina propia-submucosa, túnica muscular, serosa y grosor total de la pared) de cada uno de los compartimentos gástricos. El modelo utilizado dependió de la curva real de crecimiento. Inicialmente probaron el modelo mutiltiplicativo y = aXb y el exponencial y = EXP (a+bx), utilizando como criterio de ajuste un coeficiente de determinación $r^2 > 0,70$. En aquellos en los que no hubo un buen ajuste, se realizaró un ajuste por tramos, utilizando un modelo lineal y = a + bx. Por otro lado, en los casos en los que el proceso siguió una curva sigmoidea, utilizaron un modelo polinómico.

Finalmente, **Franco *et al.* (2004a, b)** en rumen y retículo y **Redondo *et al.*, (2005)** en omaso, realizaron estudios similares en el ciervo. En dichos trabajos, efectuaron 50 medidas de cada una de las capas tisulares. Los modelos matemáticos utilizaron fueron los mismos que para el estudio en la especie ovina, anteriormente descrita. En los compartimentos no glandulares, estos autores observaron que la lámina epitelial aumentaba de grosor, en contra de lo que sucedió en el abomaso, donde el epitelio mostró una regresión en su crecimiento (**Masot *et al.* 2007a**). La lámina propia - submucosa y serosa, siguieron un proceso

involutivo a lo largo de todo el periodo prenatal. La túnica muscular, en todos los compartimentos gástricos, mostró un ritmo de crecimiento constante durante la vida prenatal.

3.5.1. Factores que influyen en el crecimiento y desarrollo fetal

El neonato es el resultado de una serie de procesos ordenados que transforman un óvulo unicelular en un organismo típico de la especie. (**Hafez, 1963**). Entre estos procesos destacan el crecimiento (incremento del número y tamaño de células y tejidos) y desarrollo fetal (cambios en la estructura y función de células y tejidos) (**Wu *et al.* 2006**).

Estos conceptos biológicos están influenciados por fáctores genéticos, maternales y mediambientales entre otros (**Redmer *et al.* 2004; Gootwine, 2005**).

Factores genéticos

La tasa de crecimiento prenatal podría estar determinada hasta en un 30-70% por factores genéticos, entre los que incluyen los genes propios fetales, genes paternales y genes que regulan el ambiente uterino (**Hafez, 1963**). El tamaño fetal podría reflejar un conflicto de intereses entre el padre y la madre (el interés del padre es que su descendiente no sea pequeño y el interés de la madre es que sobreviva a ella). Este conflicto es mediado por la expresión en el feto y en la placenta de genes heredados del padre y de la madre. Sin embargo, **Lush *et al.* (1934)** y **Venge (1950)** señalaron que un 50 a 70% de la variabilidad del tamaño fetal era debido a factores maternales.

Factores no genéticos

El ambiente en que crece el feto también influye en las vías metabólicas de éste y en la distribución de los nutrientes, y puede determinar cambios en el crecimiento y desarrollo prenatal. Entre estos factores se incluyen tamaño y edad

maternal, nutrición materna, tamaño de camada, tamaño de placenta, temperatura y hormonas.

- Tamaño y edad fetal: el tamaño maternal parece estar positivamente correlacionado con el crecimiento prenatal rápido. Este fenómeno ha sido demostrado en caballos (**Walton y Hamond, 1938**), vaca (**Jotbert y Hammond, 1958; King y Donald, 1955**), ovejas (**Hunter, 1956; Dickinson *et al.* 1962**), conejos (**Venge, 1950**) y ratones (**Bateman, 1954**). Aunque el efecto de la edad en el tamaño fetal no ha sido muy investigado, **Dickinson (1960)** señaló que las hembras jóvenes primerizas que aún no habían alcanzado su tamaño adulto y continuaban creciendo tenían una competencia con el feto por los nutrientes, lo que provocaba que el crecimiento del feto fuera menor y el de la madre también.

- Nutrición materna: el crecimiento fetal es dependiente del plano nutricional de la madre y de sus reservas energéticas. Una restricción alimenticia de la madre puede provocar una reducción en el crecimiento fetal (**Wallace, 1948**). Sin embargo, una condición corporal demasiado alta de las hembras puede conllevar también a la disminución del crecimiento fetal, debido a que el exceso de grasa dificulta la expansión del útero grávido.

- Número de embriones: el aumento en número de embriones implantado tiene su efecto en la tasa de crecimiento prenatal, debido a una competencia de fetos en el útero. **Eckstein *et al.* (1955),** indicaron que la influencia del número de fetos en el tamaño fetal se manifestaba a través de un efecto local y un efecto general. El efecto local hacía referencia a la influencia del crecimiento fetal determinado por la presencia de otros fetos en el mismo útero, mientras el efecto general se refería a la influencia de otros fetos en el útero.

- Tamaño de placenta: el periodo de máximo crecimiento fetal es precedido por un periodo de crecimiento máximo de la placenta (**Hafez, 1963**). Además, el

crecimiento fetal se ve influido por los cotiledones y la vascularización. En el caso de los rumiantes, el número de cotiledones disminuye al aumentar el número de fetos

- Sexo del feto: los machos tienen mayor competencia por el crecimiento que las hembras (**Dickinson, 1960**). Además, **Donald y Purser (1956)** señalaron que en embarazos de gemelos de oveja, la competencia de mayor crecimiento estaba en ventaja para el feto masculino.

- Temperatura ambiente: el estrés térmico por calor provoca una reducción en el crecimiento fetal. El grado de reducción es proporcional a la duración de la exposición (**Hafez, 1963**). Autores como, **Yeates and Parer (1962**) señalaron que un exceso de calor puede producir enanismo, con proporciones no adecuadas a la reducción del tamaño.

- Hormonas: destacan por su influencia sobre el crecimiento fetal la insulina. **Fowden (2003)** demostraron mediante modelos experimentales en roedores que las células β pancreáticas productoras de insulina tenían un importante papel en el crecimiento y desarrollo fetal. Además la secreción de insulina por parte de estas células era esencial para la captación de glucosa por parte de los tejidos fetales. La melatonina, es otra de las hormonas que tiene un efecto sobre el crecimiento cerebral del feto. Estudios como el de **Nagai *et al.* (2008)** revelaron que la administración de esta hormona en hembras gestantes de rata impedía el daño oxidativo cerebral y la reducción del crecimiento fetal.

4. OBJETIVOS

OBJETIVOS

4.1. OBJETIVOS GENERALES

Los objetivos generales propuestos para este trabajo de investigación fueron los siguientes:

1. La descripción de los fenómenos histofisiológicos más relevantes que acontecen en el estómago de cabra durante la vida prenatal.
2. La creación de una base de datos histológicos, morfométricos e inmunohistoquímicos del desarrollo prenatal del estómago de cabra que puedan servir de referencia para futuros estudios y establecer comparaciones con estudios similares en otras especies rumiantes.

4.2. OBJETIVOS ESPECÍFICOS

Los objetivos específicos de este estudio de investigación fueron los siguientes:

1. La descripción de la secuencia histogénica de los diversos compartimentos gástricos (rumen, retículo, omaso y abomaso) desde los primeros estadios embrionarios hasta el nacimiento, con especial referencias a las características morfológicas de sus estructuras parietales.
2. El análisis estructural mediante microscopía electrónica de barrido de las superficies mucosas de los distintos compartimentos gástricos, desde las primeras etapas de desarrollo prenatal hasta el final de la gestación.
3. La determinación mediante técnicas inmunohistoquímicas de la presencia durante la vida intrauterina de células neuroendocrinas (sinaptofisina- SPY, enolasa no neuronal- NNE), células glíales (proteína glial fibrilar ácida- GFAP, vimentina- VIM) y marcadores de inervación peptidérgica simpática y parasimpática (péptido intestinal vasoactivo-VIP, neuropeptido Y-NPY) en todos los compartimentos gástricos y la marcación de células productoras de gastrina en el abomaso exclusivamente.
4. El análisis histomorfométrico del crecimiento y desarrollo de los estratos tisulares que componen la pared gástrica (epitelio, lámina propia y submucosa, túnica muscular y serosa), y su ajuste a modelos matemáticos con el fin de obtener fórmulas aplicables a dicho crecimiento y desarrollo gástrico.

GENERAL OBJETIVES

The general objectives for this research were the following:

1. The description of the most outstanding histophysiological phenomena that take place in the stomach of a goat during its prenatal life.

2. The creation of a histological, morphometric and immunohistochemical database of the prenatal development of a goat stomach which can serve as a reference for future studies and also can make comparisons with similar studies in other ruminant species.

SPECIFIC OBJETIVES

The specific objectives of this research study were the following:

1. The description of the histological sequence of the different gastric compartments (rumen, reticulum, omasum and abomasum) from the early embryonic stages to birth, with special references to the morphological features of its parietal structures.

2. The structural analysis by scanning electron microscopy of the mucosal surface of the different gastric compartments from the early stages of the prenatal development to the end of the gestation.

3. The determination by immunohistochemical analysis of the presence during the intrauterine life of neuroendocrine cells (synaptophysin-SPY, non-neuronal enolase-NNE), glial cells (glial fibrillary acidic protein-GFAP, vimentin-VIM) and peptidergic markers of sympathetic and parasympathetic innervations (vasoactive intestinal peptide-VIP, neuropeptide Y-NPY) in all the gastric compartments and the branding of gastrin-producing cells cells in the abomasum only and exclusively.

4. The histomorphometric analysis of the growth and development of the tissue layers that compose the gastric wall (epithelium, lamina propria and submucosa, tunica muscularis and serosa) and its adjustment to mathematical models in order to obtain applicable formulas to this said gastric development and growth.

5. MATERIAL Y MÉTODOS

5.1. MATERIAL

5.1.1. Material biológico

El material biológico estuvo constituido por 140 embriones y fetos caprinos de diferentes razas, en distintas fases del desarrollo, desde las primeras etapas embrionarias hasta el nacimiento. Los embriones y fetos procedieron en su totalidad del matadero Municipal de Plasencia (Cáceres). Los fetos fueron recogidos en el momento de sacrificio de las madres, siguiendo el proceso habitual de la cadena del matadero y siendo extraídos los especímenes en el momento de la apertura del aparato genital.

Los embriones y fetos caprinos fueron destinados para el estudio de su estómago. Para ello, fueron realizados análisis macroscópicos, análisis histomorfológicos mediante tinciones de hematoxilina–eosina, tricrómico de Masson y tinción de Gomori, así como técnicas inmunohistoquímicas y análisis estructural mediante microscopía electrónica de barrido. Además, fueron realizados estudios morfométricos en la pared compartimental en cada uno de los estratos tisulares constituyentes de dicha pared para posteriormente ajustar su crecimiento a modelos matemáticos aplicados.

Para su estudio, los especímenes fueron clasificados en cinco grupos de gestación según su longitud corporal (distancia desde la cabeza hasta la base de la cola-*crown-rump length [CRL]*) y su edad de gestación de acuerdo a los estudios previos realizados en oveja (**Franco *et al.* 1992, 1993a,b,c**) y ciervo (**Franco *et al.* 2004a, b; Redondo *et al.* 2005; Masot *et al.* 2007**). Los grupos de gestación fueron los siguientes:

- *Grupo I*: 1,5 a 4,3 cm CRL, 13-38 días, 0-25% gestación
- *Grupo II*: 4,4 a 8 cm CRL, 39-52 días, 25-35% gestación
- *Grupo III*: 9 a 17,5 cm CRL, 53-75 días, 35-50% gestación
- *Grupo IV*: 18 a 32 cm CRL, 76- 112 días, 50-75% gestación
- *Grupo V*: 33 a 47 cm CRL, 113-150 días, 75-100% gestación

5.1.2. Material de laboratorio para microscopía óptica

Reactivos:

- Formol tamponado al 10%.
- Alcohol etílico absoluto.
- Alcohol etílico de 96º.
- Alcohol etílico de 70º.
- Xileno.
- Parafina.
- Agua destilada.
- Reactivos para la tinción de hematoxilina-eosina.
- Reactivos para la tinción de Tricrómico de Masson.
- Reactivos para la tinción de Gomori o Reticulina.

Instrumental:

- Procesador de tejidos *Leica,* Mod. *TP 1020.*
- Dispensador de parafina *Selecta Dispenser,* Mod. *496*.
- Microtomo *Leica,* Mod. *2255*.
- Estufa *Carbolite eurotherm*.
- Baño termoestático de recogida de cortes *Selecta*, Mod. *101932*.
- Portaobjetos.
- Cubreobjetos.
- Balanza de precisión *AND*, Mod. *FX-320*.
- Cinta métrica.
- Microscopio *Nikon*, Mod. *Eclipse 80i* y *Nikon* Cámara Digital *DXMI200F.*
- Estereomicroscopio *Nikon*, Mod. *SMZ 1500*.
- Tijeras y bisturí.

5.1.3. Material de laboratorio para microscopía electrónica de barrido

Reactivos:

- Glutaraldehído 25.
- Fosfato monosódico.
- Fosfato potásico.
- Glicina 2%.
- Sucrosa 2%.
- Glutamato sódico 2%.
- Ácido tánico.
- Tetróxido de osmio.
- Acetona.
- Agua destilada.

Instrumental:

- Bisturí.
- Eppendor y Pipetas.
- Estufa *Carbolite Eurotherm*.
- Aparato Punto crítico *Balzers*, Mod. *CPD 030*.
- Microscopio electrónico de barrido *JSM-6300*.
- Metalizador de muestras.

5.1.4. Material de laboratorio para análisis inmunohistoquímico

Reactivos:

- Kit UltraVision ONE Detection System *Thermo Scientific.*
- Anticuerpos primarios (*Tabla 3*).
    - Sinaptofisina
    - Enolasa no neuronal
    - Proteína glial fibrilar ácida

  - Vimentina
  - Neuropeptido Y
  - Péptido intestinal vasoactivo
  - Gastrina
- Agua destilada.
- Tampón fosfato salino (PBS) 1%.
- Citrato 25%.
- Hematoxilina de Mayer.

Instrumental:

- Estufa *Carbolite Eurotherm.*
- Microondas *Moulinex Compact*.
- Eppendor y pipetas.
- Portas ICH *Microscope Slides K8020*.

5.1.5. Material para el análisis morfométrico

- Microscopio *Nikon*, Mod. *Eclipse 80i.*
- Programa NIS-Elements BR 2.30.
- Programa Microfsoft® Excel 2007.
- Programa estadístico Statistical Six Sigma, 2006.
- Programa estadístico R® version 2.12.1.

5.2. MÉTODOS

5.2.1. Análisis laboratoriales

El total de 140 embriones y fetos caprinos proceden en su totalidad del matadero de Plasencia (Cáceres) fueron utilizados para este estudio. Para su obtención, las madres fueron sacrificadas por el proceso habitual en la cadena del matadero, siendo extraídos los especímenes en el momento de la apertura del aparato genital. Inmediatamente después de su extracción, los especímenes fueron fijados mediante su inmersión en formaldehido al 10%.

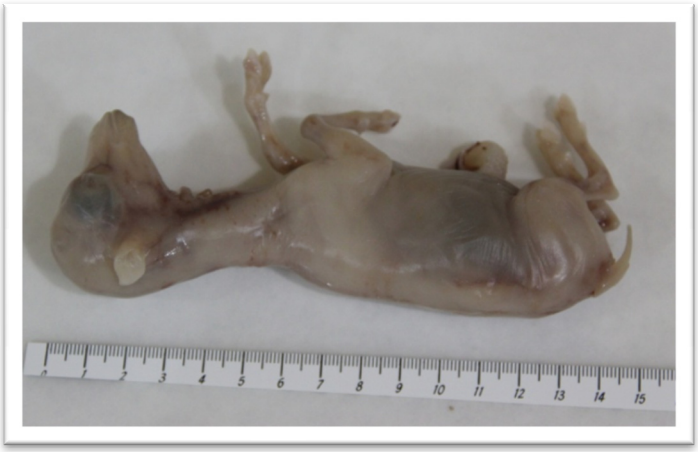


Imagen 11. Medición de fetos con cinta métrica desde la cabeza hasta la base de cola

La edad gestacional de cada embrión y fetos fue estimada utilizando como criterio principalmente la longitud corporal, considerada la distancia entre el punto más alto de la cabeza cabeza y la base de la cola (*crown-rump length-CRL*) siguiendo el criterio establecido por **Evans y Sack (1973)** en oveja, al tener la misma duración gestacional que la cabra. También fueron utilizados los criterios establecidos por **Sivalchelvan *et al.* (1996)** en cabra.

Los fetos fueron clasificados en cinco grupos de gestación de acuerdo a su tamaño y edad gestacional. Estos grupos y el número de especímenes correspondientes a cada uno ellos quedan detallados en la *Tabla 1*.

Tabla 1. Distribución de los especímenes por grupos según la edad de gestación

	% de Gestación	Días	CRL (cm)	Nº de especímenes
GRUPO I	0-25%	0-38	3 a 4,3	17
GRUPO II	25-35%	39-52	4,4 a 8	25
GRUPO III	35-50%	53-75	8,1 a 17,5	41
GRUPO IV	50-75%	76-112	18 a 32	35
GRUPO V	75-100%	113-150	33 a 47	22
				140

La extracción del paquete gástrico de los individuos pertenecientes al grupo I y II fue efectuada mediante la realización de dos cortes transversales, uno en el ángulo caudal de la escápula y otro inmediatamente craneal a los huesos coxales. De las tres secciones obtenidas, fue el segmento central el escogido para actuar sobre él posteriormente.

En los grupos III, IV y V, compuestos todos ellos por especímenes en periodo fetal, fueron practicadas disecciones directas con el fin de extraer el órgano gástrico completo, mediante una necropsia reglada según la metodología propuesta por **Gázquez *et al.* (1989)**. Una vez extraído el estómago, se procedió a la identificación de cada uno de los compartimentos gástricos a función de sus características morfológicas externas y la obtención de muestras de los referidos compartimentos de acuerdo a las siguientes pautas:

- De rumen: sacos dorsal y ventral, hacia sus niveles medios.
- De retículo, de la zona media craneal.
- De omaso, centro de su cara parietal.
- De abomaso, de cardias, fondo-cuerpo y región pilórica.

En cada uno de los compartimentos gástricos, las muestras fueron tomadas mediante cortes perpendiculares a la pared del compartimento con el fin de asegurar una homogeneidad y corrección de los datos morfométricos.

Las muestras así obtenidas fueron fijadas mediante inmersión en formol al 10%, para el análisis histológico e inmunohistoquímico, mientras que otras se fijaron en glutaraldehído 5% para el análisis estructural mediante microscopía electrónica de barrido.

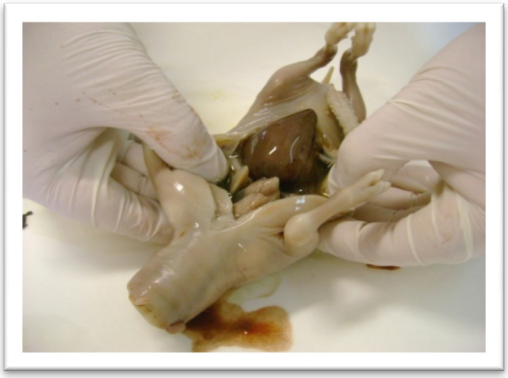


Imagen 12. Disección de los especímenes para la obtención de la víscera gástrica

Imagen 13. Paquete gástrico extraído de uno de los fetos.

5.2.1.1. Análisis macroscópicos

El estudio macroscópico fue realizado mediante una lupa estereomicroscópica con el objeto de determinar el tamaño, la forma, la coloración y la consistencia de la superficie mucosa de cada uno de los compartimentos gástricos.

5.2.1.2. Análisis histológicos

El estudio histológico consistió en el análisis mediante microscopia óptica de cada uno de los compartimentos gástricos, a partir de secciones fijadas en formol, incluidas en parafina y teñidas con hematoxilina-eosina.

Para la obtención de preparaciones histológicas, las muestras obtenidas mediante cortes transversales de la pared parietal de cada compartimento fue colocada en casetes, convenientemente cerrados e inmersos en formol 10% durante 24 horas y a temperatura ambiente. Transcurrido dicho periodo estas muestras fueron sometidas a un procesamiento que implicó una deshidratación

constante de la muestra por inmersión sucesiva en escalas ascendentes de alcoholes, para terminar la deshidratación completa mediante xilol y embebimiento de parafina de las estructuras tisulares durante un periodo de 24 horas.

Una vez procesadas las muestras, se procedió a la obtención del bloque mediante dos pletinas metálicas. Este bloque permaneció en congelación hasta que la parafina quedó totalmente solidificada. A partir de este bloque se realizaron cortes con el microtomo con un grosor de 5 micras. Estas secciones obtenidas fueron pegadas en un porta para continuar con las tinciones oportunas.

Imagen 14. Piezas cortadas transversalmente de la pared compartimental e introducidas en casetes

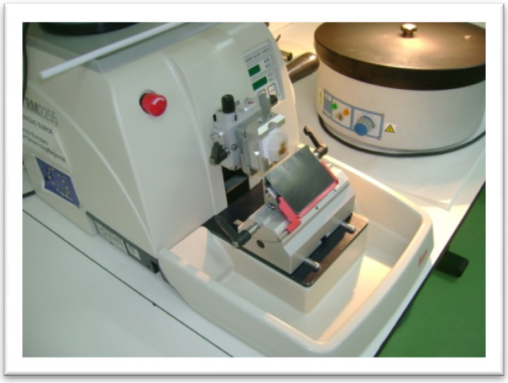


Imagen 15. Corte de bloque de parafina con microtomo

Histomorfogénesis generalizada por hematoxilina-eosina.

Las preparaciones montadas en su porta respectivos fueron teñidas manualmente con hematoxilina-eosina según la técnica usual laboratorial. Estas muestras fueron destinadas al análisis histogénico de cada uno de los compartimentos gástrico y toma de datos morfométricos. La metodología utilizada para la tinción de hematoxilina-eosina se describe a continuación:

- Desparafinado e hidratación.
- Lavado en agua destilada (5 minutos).
- Hematoxilina de Carazzi (10-15 minutos).
- Lavado con agua corriente hasta eliminar el colorante.

- Eosina al 1,25% (3 minutos).
- Deshidratación en escala ascendente de alcoholes y xilol.
- Montaje con Eukit.

Tinciones especiales asociadas al desarrollo gástrico

Una vez comprobada la morfología estructural característica de los diversos tejidos y células que integran los compartimentos gástricos, se procedió a la aplicación de técnicas especiales encaminadas a destacar algún constituyente histológico específico.

La primera tinción especial realizada fue la técnica del Tricrómico de Masson. Fue utilizada como complemento de la H-E, dado su alto carácter diferenciador; así como para la tinción específica de fibras de colágeno del tejido conectivo, que aparecen teñidas de verde, mientras que los núcleos lo hacían de negro y de rojo los citoplasmas, en fibra muscular lisa y fibras intercelulares. El procedimiento a seguir fue el siguiente:

- Desparafinado e hidratación
- Lavado con agua destilada
- Hematoxilina de Harris (20 minutos)
- Lavado con agua destilada
- Solución A (10 minutos)
- Lavado con agua destilada
- Solución B (5 minutos)
- Escurrido sin lavar
- Solución C (4-10 minutos)
- Solución D (30 minutos)
- Solución E (5 minutos)
- Deshidratación en escala ascendiente de alcoholes y montaje

Solución A: solución de fucsina ácida y escarlata de Biebrich

- escarlata de Biebrich, acuosa 1% ..... 90 ml
- fucsina ácida, acuosa 1% ..... 10 ml
- ácido acético ..... 1 ml

Solución B: solución de ácido fosfomolibdico-fosfotungstico

- ácido fosfomolíbdico ..... 5 ml
- ácido fostofúngstico ..... 5 ml
- agua destilada ..... 200 ml

Solución C: solución azul de anilina

- azul de anilina ..... 2,5 gr
- ácido acético ..... 2 ml
- agua destilada ..... 100 ml

Solución D: solución verde claro

- verde Saturno .....5 g
- ácido acético ..... 2 ml
- agua destilada ..... 250 ml

Solución E: solución de ácido acético 1%

En segundo lugar, fue realizada la técnica Van Giesson para observar la configuración estructural de la fibra muscular lisa y la distribución del componente fibrilar del tejido conectivo (colágeno y elástico), que se teñía de rojo; el tejido muscular y epitelio cornificado lo hacían de amarillo y los núcleos de azul o negro. La técnica fue realizada del siguiente modo:

- Desparafinado e hidratación
- Hematoxilina férrica de Harris (5 minutos)
- Lavado con agua destilada
- Tinción con la solución de Van Gieson (1 minuto)
- Deshidratado y montaje

Hematoxilina férrica formada por:

- Solución A

- hematoxilina ..... 1 g
- alcohol 96º ..... 99 ml

- Solución B
  - cloruro férrico ..... 4 ml
  - ácido clorhídrico..... 1 ml
  - agua destilada ..... 95 ml

Solución Van Gieson:

- fucsina ácida acuosa 1% ..... 2,5 ml
- solución satura de ácido pícrico ..... 97,5 ml

Para la tinción de las fibras de reticulina fue utilizada la tinción de Gomori o Reticulina. La técnica realizada fue la siguiente:

- Desparafinado e hidratado.
- Lavado con agua corriente.
- Permanganato potásico 1% (2 minutos).
- Agua destilada (5 minutos).
- Ácido oxálico 5% (hasta que se descolore).
- Lavado con agua destilada.
- Alumbre férrico amónico 2% (2 minutos).
- Agua destilada (5 minutos).
- Solución de plata amoniacal (3 minutos).
- Lavado con agua destilada.
- Formol 10% (3 minutos).
- Lavado con agua destilada.
- Cloruro de oro 1/500 (10 minutos).
- Lavado con agua destilada.
- Hiposulfito sódico 5% (1 minuto).
- Lavado con agua destilada.
- Deshidratado y montaje.

5.2.1.3. Análisis de microscopía electrónica de barrido

Previamente a la fijación de las muestras para el análisis estructural mediante microscopía electrónica de barrido (MEB), se procedió a un lavado exhaustivo de cada una de ellas realizando los siguientes pasos:

- Lavado con agua corriente
- Lavado con agua con detergente
- Lavado con agua destilada (5 minutos).
- Lavado con PBS (5 minutos)

Cada uno de los lavados se realizó dos veces.

Posteriormente se llevó a cabo el protocolo de MEB que a continuación se expone:

1. Fijación del rumen por inmersión en glutaraldehído al 5%.

Se preparó de la siguiente manera en el momento del uso.

- Glutaraldehído al 25%
- Solución Tampón

Se parte de dos soluciones:

Solución A (ácida): sodio dihidrógeno fosfato ($NaH_2PO_4H_20$)

Solución B (básica): potasio dihidrógeno fosfato (KH_2P0_4)

Para preparar la solución tampón se mezclaron ambas soluciones en la siguiente proporción:

- solución A: 7 partes.
- Solución B: 3 partes.

De esta forma quedó una solución tampón 0,1M, pH 7,4.

2. Lavado de las muestras con agua destilada para eliminar el glutaraldehído. Este lavado se hizo en 3 pases de 5 minutos cada uno.

3. Preparación de la solución A, compuesta por:

- glicina 2%.
- sucrosa 2%.
- glutamato sódico 2%.

Esta solución A sirvió como previa preparación de la muestras para la fijación con tetróxico de osmio.

4. Solución A durante 1 hora y media.

5. Lavado las muestras con agua destilada (3 pases de 10 minutos cada uno).

6. Solución B (ácido tánico) durante 1 hora y media.

7. Lavado con agua destilada (varios lavados durante 1 horas).

8. Solución C (tetróxido de osmio 2%) durante toda la noche.

9. Lavado con agua destilada (3 pases de 10 minutos cada uno).

10. Deshidratación de las muestras, mediante la utilización de los siguientes compuestos:

- Acetona 30% (1 pase de 15 minutos).
- Acetona 50% (1 pase de 15 minutos).
- Acetona 70% (2 pases de 15 minutos).
- Acetona 90% (2 pases de 15 minutos).
- Acetona 100% (2 pases de 30 minutos).

11. Punto crítico: desecación física por $C0_2$ en la que las muestras sufre los siguientes procesos:

- Enfriamiento de la muestra hasta alcanzar Tª de 10 ºC.
- Inyección de CO_2.
- Calentamiento de la muestra hasta alcanzar una Tª de 40 ºC.

12. Sombreado con oro.

Finalmente las muestras fueron observadas y fotografiadas con el microscopio electrónico de barrido a 30 Kv y una magnitud de 10 a 800 x.

Imagen 16. Punto crítico utilizado en el análisis estructural.

Imagen 17. Microscopio electrónica de barrido utilizado para el análisis estructural

5.2.1.4. Análisis inmunohistoquímicos

El kit comercial Ultravision ONE Detected System HRP polymer system & DAB Plus Chromogen (*Thermo Scientific*), basado en la conjugación de un polímero con peroxidasa de rábano fue utilizado para el análisis inmunohistoquímico.

Para el análisis imunohistoquímico fueron seleccionados seis individuos de cada grupo de gestación, correspondiendo dos de cada uno de ellos a tres estadios de desarrollo prenatal por grupo. Estos estadios de desarrollo y sus correspondientes edades de gestación se detallan en la *Tabla 2.*

Tabla 2. Estadios de desarrollo y número de especímenes seleccionados en el análisis inmunohistoquímico

	CRL (cm)	Edad gestacional	Nº de especímenes
Grupo I	3,5	35	2
	4	36	2
	4,3	38	2
Grupo II	5	39	2
	6	46	2
	7,5	50	2
Grupo III	13,5	64	2
	15	68	2
	17	70	2
Grupo IV	19	75	2
	22	87	2
	30	95	2
Grupo V	33	113	2
	36	120	2
	42	150	2
			30

El protocolo a seguir para la realización de la técnica inmunohistoquímica fue el siguiente:

- Desparafinado (20 minutos en estufa a 60º y 20 minutos en xilol).
- Hidratación (paso sucesivos de alcohol 100, 90, 80, 70).
- Lavado con tampón fosfato salino (PBS) (2 veces durante 1 minutos).
- Pretatamiento con citrato (0,01M citrato, 5 minutos a 800 vatios en microondas).
- Lavado con PBS (4 lavados de 1 minuto cada uno).
- Bloqueo de la peroxidasa endógena con solución de peróxido de hidrógeno-metano al 3% (**Hydrogen Peroxide Block**) (10-15 minutos).
- Lavado en PBS (2 lavados de 1 minuto cada uno).
- Bloqueo de uniones inespecíficas (**Ultra V Block**) (5 minutos).
- Lavado con PBS (2 lavados de 1 minuto cada uno).
- Incubación con el anticuerpo primario específico (*Tabla 3*).
- Lavado en PBS (2 lavados de 1 minuto cada uno).

- Incubación con el polímero (**UltraVision ONE HRP Polymer**), (30 minutos en oscuridad).
- Lavado con PBS (2 lavados de 1 minuto cada uno).
- Revelado con solución sustrato de diaminobencidina (**DAB Plus Chromogen + DAB Plus Substrate**) (15 minutos).
- Lavado con agua destilada (2 lavados de 1 minuto cada uno).
- Tinción con la solución de Hematoxilina de Carazzi (4 minutos).
- Lavado con agua corriente (7-8 veces hasta eliminar todo el colorante).
- Deshidratado y aclarar a través de alcohol etílico al 95%, alcohol etílico absoluto y xileno, 2 cambios cada uno, durante 2 minutos cada uno.
- Montar con *Eukit*.

La especificidad de la reacción fue empleados controles positivos correspondientes a cada marcador y controles negativos en los que el anticuerpo primario fue sustituido por PBS.

Las secciones de inmunohistoquímicas fueron analizadas mediante el programa morfométrico NIS- Element B.R. La superficie inmunarcada fue medida para cada uno de los estratos tisular (epitelio, lámina propia y submucosa, tunica muscular y serosa) y pared parietal.

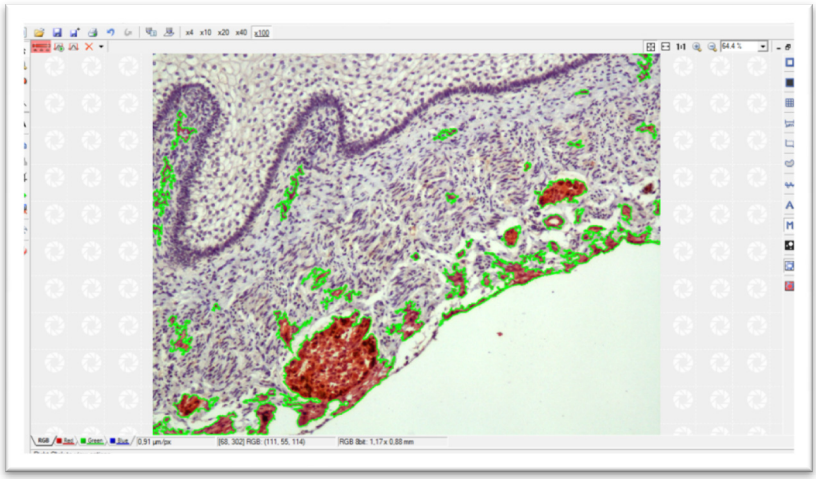


Imagen 18. Medidas efectuadas para el análisis inmunohistoquímico en cada uno de los estratos tisulares de la pared ruminal con el programa NIS Element B.R.

Tabla 3. Anticuerpos primarios utilizados en el análisis inmunohistoquímico.

Anticuerpo	Tipo	Origen	Reactividad	Dilución	Control Positivo
NPY	pAc	AbD serotec	Fibras y cuerpos nerviosos parasimpáticos	1/50[c]	Cerebro
VIP	pAc	AbD serotec	Fibras y cuerpos nerviosos simpáticos	1/50[c]	Cerebro
GFAP	pAc	Thermo Scientific	Células gliales	Preparado[c]	Cerebro
VIM	mAc	Thermo Scientific	Células gliales	Preparado[c]	Cerebro
NNE	pAc	AbD serotec	Células neuroendocrinas	1/100[c]	Páncreas
SPY	mAc	Thermo Scientific	Células neuroendocrinas	1/10[c]	Páncreas
Gastrina	pAc	Thermo Scientific	Células productoras de gastrina	1/50[c]	Estómago

pAc: anticuerpo policlonal**; mAc:** anticuerpo monoclonal**; c:** pretratamiento con citrato

5.2.2. Análisis morfométricos

El estudio histomorfométrico fue llevado a cabo usando preparaciones histológicas teñidas con H-E. Las secciones histológicas fueron observadas a través de un microscopio equipado con cámara, para la captura de imágenes digitales. Estas imágenes fueron analizadas mediante el programa de morfometría NIS-Element B.R. 2.30.

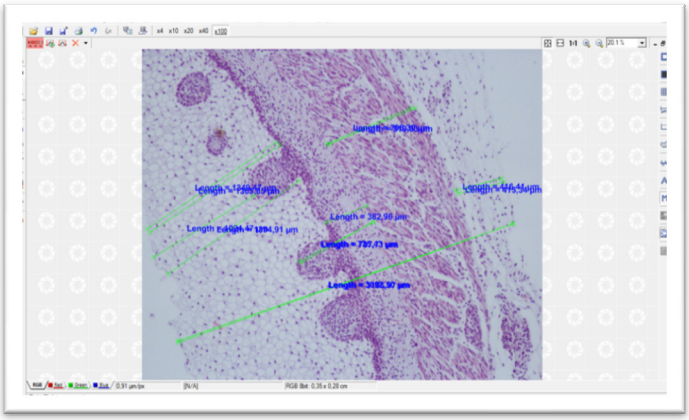


Imagen 19. Medidas efectuadas en cada uno de los estratos tisulares de la pared ruminal con el programa NIS Element B.R.

Para el estudio fueron seleccionados tres estadios de desarrollo dentro de cada grupo de gestación. De cada uno de los estadios de gestación seleccionados fueron elegidos cuatro especímenes, tanto hembras como macho. Esto supuso el análisis de doce especímenes por grupos de gestación y un total de 60 fetos analizado (42% del total). Los estadios de desarrollo seleccionados en cada uno de los grupos de gestación quedan recogidos en la *Tabla 4*.

Tabla 4. Estadios de desarrollo y número de especímenes seleccionados para el análisis morfométrico.

	CRL (cm)	Edad gestacional	Nº de especímenes
Grupo I	3,5 3,8 4	35 37 38	4 4 4
Grupo II	5 6 7,5	44 50 52	4 4 4
Grupo III	13,5 15 17	64 69 70	4 4 4
Grupo IV	19 22 30	75 87 95	4 4 4
Grupo V	33 36 42	113 120 150	4 4 4
			60

Las variables estudiadas en el análisis morfométrico fueron el grosor de cada estrato tisular (epitelio, lámina propia y submucosa, túnica muscular y serosa) y el grosor total de la pared parietal. Fueron realizadas 100 mediciones en cada estrato tisular y pared total de cada uno de los individuos seleccionados en los grupos de gestación.

5.2.3. Análisis estadísticos

El análisis estadístico de los datos se llevo a cabo con los siguientes programas:

- Microsoft Excel 2007.
- Statistical Six Sigma 2006.
- R® versión 2.12.1.

El análisis estadístico de los datos fue realizado mediante el programa estadístico Statisitica Six Sigma (2006). Los datos fueron sujetos un análisis de varianza factorial (ANOVA), con grupo y sexo como factores. En aquellos casos, en los que el análisis de varianza reveló diferencias significativas fueron realizados un post-hoc (Tukey) para probar las diferencias significativas entre las medias. Un valor de $p<0,05$ fue considerado significativo.

El crecimiento de cada estrato tisular (lámina epitelial, lámina propia y submucosa, túnica muscular y serosa) y de la pared parietal de cada compartimento gástrico fue ajustado a un modelo matemático. Estos modelos fueron creados mediante un programa estadístico (Statistica Six Sigma, 2006). Las gráficas representaron los promedios de los valores reales de crecimiento de cada estrato tisular y pared total junto con la línea de ajuste matemático. La bondad del ajuste de cada gráfica se midió utilizando el coeficiente lineal de correlación (*r*). En todos los casos, la variable independiente fue la longitud corporal (longitud cráneo-caudal, en centímetros) y la variable dependiente, el espesor de cada estrato tisular y pared total, expresados en micrómetros.

Los modelos matemáticos elegidos fueron para el ajuste del crecimiento de las capas tisulares y pared total de cada uno de los compartimentos gástricos fueron los siguientes:

- Lineal $(y= a+b\ x)$
- Polinomial $(y = a +bx + cx^2)$
- Logarítmico $y = a + log\ 10(x)$
- Exponencial $y = a \cdot exp\ (x)$

El estudio del crecimiento relativo de cada uno de los estratos tisulares frente al crecimiento global de la pared parietal fue realizado mediante regresiones simples a partir de la ecuación exponencial de $y= a \cdot x^b$ de Huxley (1932). Esta fórmula fue transformada en regresión lineal a través de logaritmos decimales quedando de la siguiente manera: *Log y = log a + b log (X),* donde la variable independiente (*x*) estuvo representado por el grosor de la pared compartimental (todo) y la variable dependiente (*y*) por el grosor de cada estrato tisular del compartimento (parte). El valor de "*a*" es la ordenada en el origen y "*b*" la pendiente de la recta de regresión, correspondiente al coeficiente de alometría. Este coeficiente de alometría fue el indicador de la tasa de crecimiento relativo de Y en relación X.

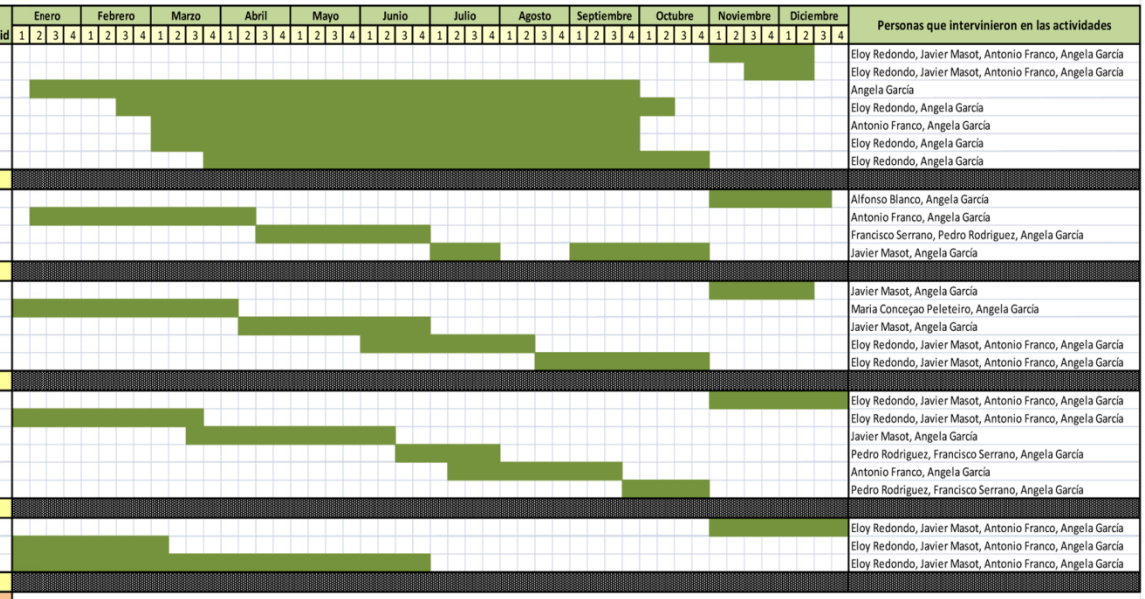
En el análisis inmunohistoquímico, las medidas efectuadas fueron expresadas en micrómetros cuadrados (μm^2) mostrados como media ± error estándar. El análisis estadístico de los datos fue efectuado mediante un análisis de varianza (ANOVA) y prueba de post-hoc (Tukey) para establecer las diferencias entre estratos tisulares y grupos de gestación. La densidad inmunorreactiva fue clasificada en cuatro categorías según la superficie inmunomarcada.

- *no inmunoreactividad* (0 μm^2)
- *baja immunorreactividad,* (<200 μm^2)
- *moderada inmunorreactividad* (entre 200 y 400 μm^2)
- *intensa immunorreactividad* (más de 400 μm^2)

5.3. CRONOGRAMA DE ACTIVIDADES

FECHA DE INICIO DEL PROYECTO	1/11/2008
COORDINADOR DEL PROYECTO	ELOY REDONDO GARCÍA
TÍTULO DEL PROYECTO	EL DESARROLLO PRENATAL DEL ESTÓMAGO DE CABRA

FASE	Nº	ACTIVIDADES	PERIODO Inicio	PERIODO Fin	% cumplid	Personas que intervinieron en las actividades
2008/2009	1	Definición de objetivos del estudio	03/11/2008	19/12/2008	2,5	Eloy Redondo, Javier Masot, Antonio Franco, Angela García
	2	Elaboración diseño experimental	17/11/2008	19/12/2008	2	Eloy Redondo, Javier Masot, Antonio Franco, Angela García
	3	Recogida de embriones y fetos	08/01/2009	30/09/2009	7	Angela García
	4	Catalogación de embriones y fetos	20/02/2009	09/10/2009	4,5	Eloy Redondo, Angela García
	5	Apertura de embriones y fetos	02/03/2009	30/09/2009	4	Antonio Franco, Angela García
	6	Análisis macroscópicos y fotografiado	02/03/2009	30/09/2009	5	Eloy Redondo, Angela García
	7	Análisis micróscopicos y fotografiado	29/03/2009	30/10/2009	5	Eloy Redondo, Angela García
	% de avance: 30					
2009/2010	1	Análisis estructural y fotografiado	03/11/2009	22/12/2009	7	Alfonso Blanco, Angela García
	2	1ª Análisis histomorfométrico	07/01/2010	16/04/2010	5	Antonio Franco, Angela García
	3	1º Análisis estadístico	19/04/2010	30/06/2010	4	Francisco Serrano, Pedro Rodriguez, Angela García
	4	1ª Análisis inmunohistoquímico	01/07/2010	29/10/2010	4	Javier Masot, Angela García
	% de avance: 20					
2010/2011	1	1ª Análisis inmunohistoquímico	02/11/2010	22/12/2010	2	Javier Masot, Angela García
	2	Estancias doctorado	03/01/2011	08/04/2011	4	Maria Conceçao Peleteiro, Angela García
	3	2º Análisis inmunohistoquímico	25/04/2011	10/06/2011	4	Javier Masot, Angela García
	4	Elaboración 1º artículo	06/06/2011	12/08/2011	4	Eloy Redondo, Javier Masot, Antonio Franco, Angela García
	5	Elaboración 2º artículo	15/08/2011	31/10/2011	3	Eloy Redondo, Javier Masot, Antonio Franco, Angela García
	% de avance: 20					
2011/2012	1	Elaboración 3º artículo	02/11/2011	30/12/2011	3	Eloy Redondo, Javier Masot, Antonio Franco, Angela García
	2	Elaboración 4º artículo	02/01/2012	16/03/2012	3	Eloy Redondo, Javier Masot, Antonio Franco, Angela García
	3	Análisis histomorfométrico inmunohistoquímica	19/03/2012	08/06/2012	3	Javier Masot, Angela García
	4	Estudio estadístico (inmunohistoquímica)	11/06/2012	27/07/2012	4	Pedro Rodriguez, Francisco Serrano, Angela García
	5	2º Análisis histomorfométrico	16/07/2012	21/09/2012	3	Antonio Franco, Angela García
	6	Estudio estadístico y modelización matemática	24/09/2012	31/10/2012	4	Pedro Rodriguez, Francisco Serrano, Angela García
	% de avance: 20					
2012/2013	1	Elaboración 5º artículo	01/11/2012	31/12/2012	3	Eloy Redondo, Javier Masot, Antonio Franco, Angela García
	2	Elaboración 6º artículo	01/07/2012	08/03/2013	3	Eloy Redondo, Javier Masot, Antonio Franco, Angela García
	3	Elaboración memoria final	07/01/2013	29/06/2013	14	Eloy Redondo, Javier Masot, Antonio Franco, Angela García
	% de avance: 20					
Total	% AVANCE TOTAL: 100					



FECHA DE CIERRE DEL PROYECTO	30/6/2013		
PERSONAS QUE DIRIGEN EL PROYECTO	ELOY REDONDO GARCÍA	JAVIER MASOT GÓMEZ-LANDERO	ANTONIO FRANCO RUBIO

6. RESULTADOS Y DISCUSIÓN

6. RESULTADOS Y DISCUSIÓN

Artículo 1


AR
The Anatomical Record
Advances in Integrative Anatomy and Evolutionary Biology

Histomorphometric and Immunohistochemical Study of the Goat Rumen During Prenatal Development

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ABSTRACT

This study sought to chart the ontogenesis of the goat rumen by histomorphometric examination, scanning electron microscopy and immunohistochemical analysis. A total of 140 goat embryos and fetuses were used, from the first stage of prenatal life until birth. The appearance of the rumen from the primitive gastric tube was observed at 35 days of prenatal life (CRL 3 cm, 23% gestation). By 38 days (CRL 4.3 cm CRL, 25% gestation) the ruminal wall comprised three layers: an internal epithelial layer, a middle layer of pluripotential blastemic tissue and an external layer or serosa. Ruminal pillars were visible at 46 days (CRL 6 cm, 30% gestation), and by 76 days (CRL 18 cm, 50% gestation) ruminal papillae were starting to appear. Under scanning electron microscopy, by 50 days (CRL 7.7 cm, 33% gestation) small ruminal papillae were observed protruding from the surface. Finally, neuroendocrine cells (synaptophysin, SYP) were detected at 53 days (CRL 9 cm CRL, 35% gestation), while glial cell markers (glial fibrillary acidic protein—GFAP, and vimentin-VIM) were found at 108 days (CRL 31 cm, 72% gestation) and 39 days (CRL 4.4 cm, 26% gestation), respectively. Neuropeptide Y (NPY) and vasoactive intestinal polypeptide (VIP) were detected immunohistochemically at 113 days (CRL 33 cm, 75% gestation) and 120 days (CRL 35 cm, 80% gestation), respectively. In conclusion, histomorphogenesis of the rumen in goats was similar to that reported in deer, but rather slower than observed for sheep or cattle. Anat Rec, 295:776–785, 2012.

Key words: *Capra hircus*; forestomach; histochemistry; histology; prenatal development

The goat is among the ruminant species best able to make use of marginal pasturelands (Boyazoglu et al., 2005; Rancourt et al., 2006), and is highly adapted to grazing over a wide range of vegetation (El-Gendy et al., 2010). Browse appears to be an important component of its diet, and it is widely regarded as the best user of poor roughage (Gihad et al., 1980). Morphological analysis of the goat digestive tract has led to its being classed as an intermediate feeder, situated between the concentrate selectors at one extreme and the grass-roughage eaters at the other (Hofmann 1973, 1989).

This ability to make use of grazing land is linked to the particular morphology of the ruminant stomach, which comprises four compartments: rumen, reticulum, omasum, and abomasum. The rumen, reticulum and omasum form the forestomach (*proventriculus*), whose tunica mucosa is lined by a squamous, keratinized stratified epithelium (Ramkrishna and Tiwari, 1979;

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Scala et al., 2010). The tunica mucosa of the abomasum, by contrast, has a simple, glandular, prismatic epithelium similar to that found in the stomach of monogastric species (Franco et al., 1993b; Masot et al., 2007a). Each compartment has its own distinct morphological features and resulting physiological peculiarities, but all four are jointly involved in the digestive process; the rumen is perhaps the most important compartment in physiological terms, in that it plays a key role in the breakdown and subsequent microbial digestion of cellulose, an essential nutrient for ruminants.

Although a number of studies have addressed the histological organization of the forestomach in domestic ruminants (Habel, 1963; Schnorr and Vollmerhaus, 1967; Hofmann, 1973; Hofman and Schnorr, 1982), little research has focused on the prenatal development of the stomach. The present authors have examined the ontogenesis of the stomach in sheep (Franco et al., 1989, 1992, 1993a,b,c; Redondo et al., 1996, 1997) and in red deer (Franco et al., 2004a,b, 2011; Redondo et al., 2005, 2011; Masot et al., 2007a,b). However, the literature contains few studies dealing specifically with the goat.

The aim of this study was to describe the morphology of the goat rumen during prenatal development, using structural, histomorphometric, and immunohistochemical methods including scanning electron microscopy and light microscopy.

MATERIAL 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 five sequential groups, according to major histomorphogenic characteristics (Franco et al., 1992, 2011): 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 from the municipal slaughterhouse in Caceres (Spain). In order to obtain embryos and fetuses at various stages of development, caesarean sections were performed on females at the time of slaughter, in accordance with the provisions of Spanish Royal Decree 54/1995, on the protection of animals at slaughter or sacrifice. 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).

Sampling and Processing

Once the rumen had been separated, small pieces of tissue were dissected from the medial region of the dorsal sac and from the cranial sac. Tissues for histological examination were fixed in 4% buffered formaldehyde for 24 hr, routinely processed and embedded in paraffin. Sections 5 μm thick were stained with Hematoxylin-Eosin (H&E), Masson's Trichrome and Gomori's reticulin.

Histomorphometric Analysis

Specimens for morphometric analysis 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. Ten specimens were selected for each group for histology and morphometric study. 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, were selected 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. One hundred measurements were made in each tissue layer (epithelium, lamina propria + submucosa, tunica muscularis, serosa and total thickness of the rumen wall) of each of the selected individuals from each group.

The results are shown as mean ± SE. Data was subjected to analysis of variance (ANOVA). Wherever ANOVA revealed significant differences, a post-hoc (Turkey) 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, CRL, in centimetres) 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

ExtrAvidin Peroxidase Staining (EAS) was performed on deparaffinized tissue from the dorsal and ventral sac of the rumen in order to detect neuroendocrine cell markers (synaptophysin SYP) and the glial cell markers glial fibrillary acidic protein (GFAP) and vimentin (VIM). Tissues were deparaffinized, hydrated, and treated sequentially with 0.5% hydrogen peroxide for 30 min in order to block endogenous peroxidase activity. Nonspecific 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 antihuman SYP (Sigma/Aldrich Química, Madrid, Spain, no. S5768); 1:400 monoclonal antihuman GFAP (Sigma/Aldrich Química, Madrid, Spain, no. G-3893) and 1:20 monoclonal antihuman VIM (Sigma/Aldrich Química, Madrid, Spain, no. V-5255) for 3 hr at 20°C. Biotinylated goat anti-mouse IgG (1:200 dilution) (Sigma/Aldrich Química, Madrid, Spain no. B7151) was then added to sections for 30 min. Sections were finally incubated with diluted (1:50) ExtrAvidin-Horseradish Peroxidase (Sigma/Aldrich Química, Madrid, Spain no.

E2886) for 1 hr at 20°C. After the diaminobenzidine reaction, nuclear counterstaining with Mayer hematoxylin was applied. The specificity of the staining reaction was determined in control experiments involving either substitution of the primary antibody by PBS or normal mouse serum 1:100, or omission of both primary and secondary antibodies; prior absorption of the primary antibody (overnight preincubation of the primary antisera with the respective peptide, 50–100 μM) was also carried out. Next, the antibody/peptide mixture was applied to sections in an identical manner and at the same concentration as that of the primary antibody.

Scanning Electron Microscopy

Small pieces of rumen were fixed in 2.5% buffered glutaraldehyde for 24 hr, dehydrated through graded ethanol and amyl acetate, and dried in a critical-point dryer. Sections were covered with coating materials including gold and examined at various tilt angles, at a magnification of 10 to 800x.

RESULTS

Gross Findings

At 75 days (CRL 17.5 cm, 50% gestation), the surface of the rumen was smooth, soft and whitish in color. By 113 days (CRL 33 cm, 75% gestation), conical ruminal papillae were evident on the surface; thereafter, papilla protrusion became increasingly marked until birth.

Ruminal Histomorphogenesis

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

At 35 days (CRL 3 cm, 23% gestation), the rumen was apparent as a single cavity in the primitive gastric tube. The ruminal wall comprised an internal epithelial layer and an external layer of pluripotential blastemic tissue (Fig. 1A). The stratified epithelium (108 ± 12 μm) comprised cylindrical cells with basally located nuclei and apical cytoplasm. The pluripotential blastemic tissue (129 ± 4 μm) of mesenchymal character was composed of stellate cells grouped in several irregularly distributed layers, together with abundant ground substance.

At 38 days (CRL 4.3 cm, 25% gestation; Fig. 1B), la serosa appeared as a new tissue layer appeared, so the ruminal wall comprised three layers: an internal epithelial layer, a middle layer of pluripotential blastemic tissue and an external layer, or serosa. The stratified epithelial layer (136 ± 12 μm) was formed by two bands: a darker, basal area rich in nuclei and a lighter apical band of cytoplasm. The pluripotential blastemic tissue (147 ± 9 μm) contained clusters of longitudinally-arranged spindle-shaped cells. The serosa (51.57 ± 2 μm) was formed by a single layer of flat cells (mesothelium) supported by loose connective tissue.

Group II (CRL 4.4–8 cm CRL, 39–52 days, 25–35% gestation).

This developmental stage (Fig. 1C,D) was marked by a considerable increase in epithelial thickness (157.33 ± 7 μm). The basal area contained clusters of basophilic cells forming a basal layer. At 46 days (CRL 6 cm, 30% gestation), small papilliform projections were observed growing from the epithelial layer into the lumen to form rudimentary pillars. These pillars would later provide the internal division of the rumen into sacs. All wall tissue strata were involved in pillar formation.

At 50 days (CRL 7.7 cm, 33% gestation), the pluripotential blastemic tissue was highly vascularized, and the first signs of differentation into lamina propria and submucosa were observed. A rudimentary tunica muscularis was visible, composed of two layers of myoblasts, a circular internal layer and a longitudinal external layer (Fig. 1D).

The serosa (35.84 ± 3 μm) comprised a highly cellular subserosa underlying a mesothelial layer.

Group III (CRL 9–17.5 cm, 53–75 days, 35–50% gestation).

At 53 days (CRL 9 cm, 35% gestation), the ruminal wall was formed by four distinct layers: epithelium, lamina propria + submucosa, tunica muscularis and serosa (Fig. 1E,F).

The epithelial layer (224 ± 9 μm, Fig. 1E) was thinner in apical areas of the ruminal pillars. The stratified epithelium displayed two clearly differentiated bands: a narrow basal band of oval cells with basophilic cytoplasm forming the stratum basale, and a thicker apical band of polyhedral cells with light cytoplasm forming the stratum granulosum. This early epithelial stratification was accompanied by slight evaginations of the stratum basale into the stratum granulosum to form rudimentary ruminal papillae.

The lamina propria, composed of connective tissue rich in stellate cells, was visible within elevations of the epithelial stratum basale, forming the nascent papillae. The submucosa, adjacent to the tunica muscularis, contained fewer cell elements and a larger amount of ground substance. These two layers had a joint thickness of 81 ± 11 μm.

The tunica muscularis comprised two interwoven bundles of smooth muscle fiber: an internal circular bundle and an external longitudinal bundle. Muscularis thickness was greater in the area of the ruminal pillars (46.57 ± 6 μm), for which it provided muscular support (Fig. 1F).

The serosa comprised a flat epithelium supported by loose connective tissue rich in reticulin fibers and ground substance. Some blood vessels were visible.

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

By this stage (Fig. 1G), the epithelium (498 ± 12 μm) comprised four layers. The stratum basale was composed of strongly basophilic cells, while the overlying stratum granulosum comprised polyhedral cells with light-staining cytoplasm. The stratum spinosum was superficial to the stratum granulosum. Finally, the stratum corneum—formed by a single layer of flat cells—was in contact with the lumen.

At 76 days (CRL 18 cm, 50% gestation), ruminal papillae were more developed than in earlier groups, appearing as small elevations of the basal area towards the ruminal lumen, involving the lamina propria and the submucosa. By this stage, ruminal papillae had reached half the height of the epithelial layer (Fig. 1G).

The lamina propria and the submucosa were highly vascularized, and their joint thickness was 68.79 ± 6 μm.

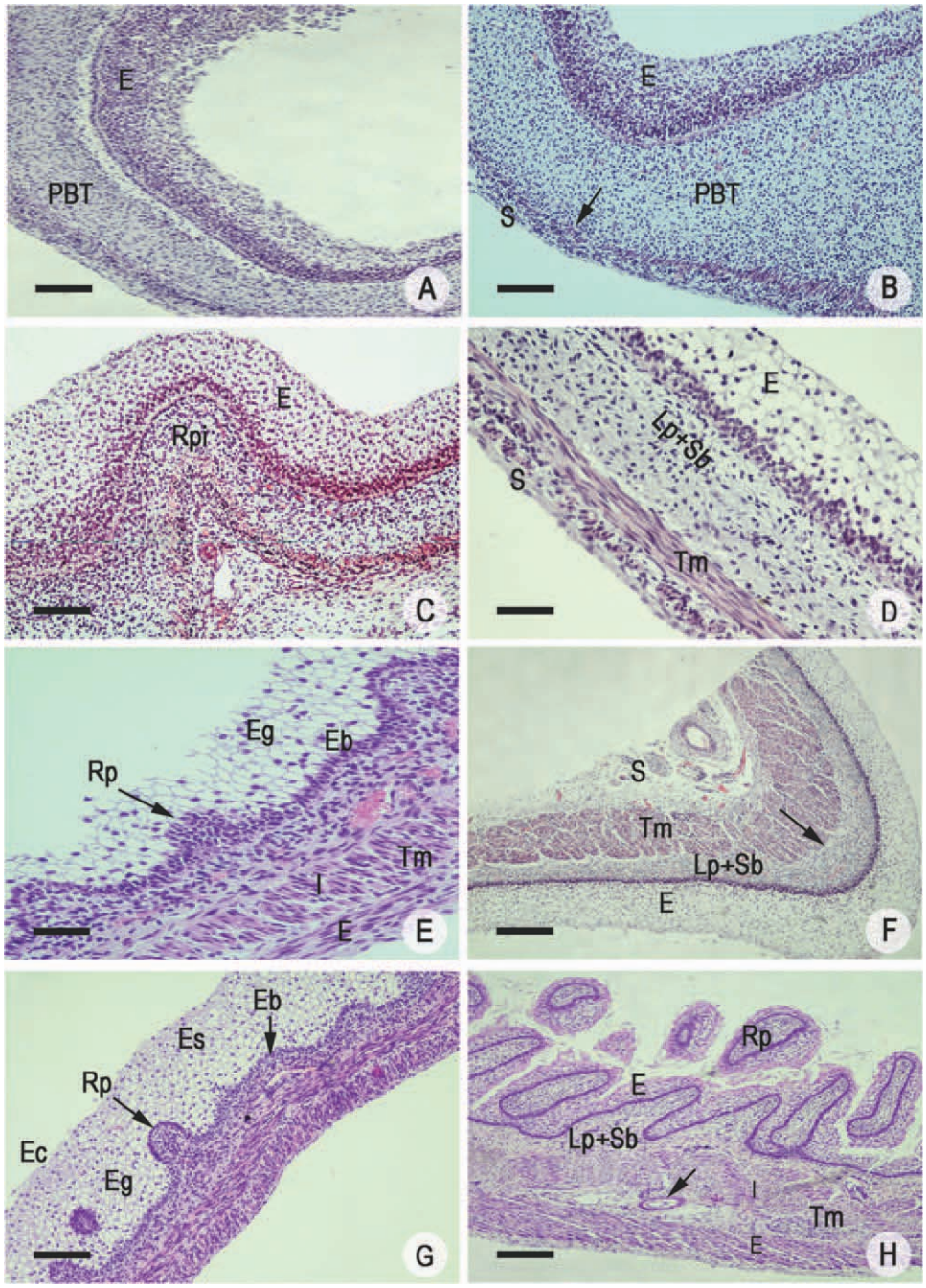

Fig. 1. Histomorphogenesis of goat rumen (35-150 days, 1-100% gestation). **A**: Photomicrograph of a section of the rumen appearing as a single cavity of the early stomach (35 days, 23% gestation). The wall comprises two layers: epithelium (E) and pluripotential blastemic tissue (PBT). HE. Bar: 20 μm. **B**: Photomicrograph of a section of the ruminal wall (38 days, 25% gestation). The wall is composed of three layers: epithelium (E), pluripotential blastemic tissue (PBT) with longitudinally arranged spindle-shaped myoblastic cells (arrow) which are starting to become differentiated, and an external layer or serosa (S). HE. Bar: 30 μm. **C**: Photomicrograph of a section of the ruminal wall (46 days, 30% gestation). Outline of ruminal pillars (Rpi) as the epithelial layer (E) projects towards the ruminal lumen. H-E. Bar: 25 μm. **D**: Photomicrograph of a section of the ruminal wall (50 days, 33% gestation). The wall is composed of four layers: epithelium (E), lamina propria and submucosa (Lp+Sb), tunica muscularis (Tm) and serosa (S). Histodifferentiation of lamina propia + submucosa and tunica muscularis from pluripotential blastemic tissue. H-E. Bar: 40 μm. **E**: Photomicrograph of a section of the ruminal wall (53 days, 35% gestation). Epithelium stratified into two distinct areas: a basal area or stratum basale (Eb), and an apical area or stratum granulosum (Eg). Considerable development of the tunica muscularis (Tm), comprising an internal circular bundle (I) and an external longitudinal bundle (E). H-E. Bar: 35 μm. **F**: Photomicrograph of a section of the ruminal wall (64 days, 43% gestation). Tunica muscularis (Tm) entering pillars to form the muscular body (arrow). Thinning of epithelium and lamina propria + submucosa (Lp+Sb). Numerous blood vessels in serosa (S). H-E. Bar: 20 μm. **G**: Photomicrograph of a section of the ruminal wall (76 days, 50% gestation). Stratified epithelium: stratum basale (Eb), stratum granulosum (Eg), stratum spinosum (Es) and stratum corneum (Ec). Ruminal papillae (Rp) visible as evaginations of the stratum basale towards the stratum granulosum, reaching half the height of the epithelium. H-E. Bar: 30 μm. **H**: Photomicrograph of a section of the ruminal wall (150 days, 100% gestation). Lamina propia and submucosa (Lp+Sb) visible within papilla, forming the papillary body. Numerous blood vessels (arrow) between the internal (I) and external bundles (E) of the tunica muscularis (Tm) Ruminal papillae (Rp) reaching the apical third of the epithelium (E). H-E. Bar: 35 μm.

TABLE 1. Morphometrical analysis of tissue layer thickness in goat rumen during prenatal development (μm)

	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: 35–50% 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)
Epithelium	105 ± 18	128 ± 19*	139 ± 34*	450 ± 96*	443 ± 137*
Lp + Sb	Pbt[a]	pbt	133 ± 11	71 ± 31**	72 ± 22**
Tm	pbt	pbt	60 ± 34	104 ± 18**	171 ± 68**
Serosa	53 ± 8	32 ± 12*	27 ± 11*	22 ± 9*	23 ± 12*
Wall	340 ± 48	305 ± 32	300 ± 67	664 ± 92**	679 ± 53**

Lp + Sb, lamina propria + submucosa; Tm, tunica muscularis; pbt, pluripotential blastic tissue.
[a]No statistical comparison was made of pluripotential blastemic tissue, since that structure later gives; rise to others, including lamina propia-submucosa and tunica muscularis.
*$P < 0.005$ vs. Group I; **$P < 0.005$ vs. Group III.

The tunica muscularis was thicker than at earlier stages (85.23 ± 9 μm), the increase being most pronounced in the internal bundle. Blood vessels were observed within intermuscular and perimuscular connective tissue.

Finally, the serosa (23.27 ± 3 μm) displayed no differences with respect to earlier stages of development.

Group V (CRL 33–47 cm, 113–150 days, 75–100% gestation).

The epithelium was composed of four tissue layers similar to those observed in Group IV. By this developmental stage, however, undulations of the epithelial surface were discernible, coinciding with the tips of the most developed ruminal papillae. At 113 days (CRL 33 cm, 75% gestation), ruminal papillae were both longer and thicker, reaching the apical third of the epithelium. At 150 days (CRL 47 cm, 100% gestation; Fig. 1H), these fully-developed papillae had reached the epithelial surface.

The lamina propria was composed of dense, fibrous connective tissue. It was now visible within each ruminal pillar forming the papillary skeleton. The submucosa was composed of elastin and collagen fibers, and there was no clear boundary between it and the lamina propria. Both layers contained abundant blood vessels; joint thickness was 51.45 ± 3 μm.

The tunica muscularis (265 ± 12 μm) was composed of two layers of smooth muscle tissue. The fibers of the inner layer were arranged in a circular pattern, while those of the outer layer were arranged longitudinally. Numerous blood vessels were visible between the two layers of muscle fiber.

The very slender serosa (27 ± 4 μm) was composed of mesenchymal loose connective tissue with a mesothelial lining, containing varying amounts of adipocytes, blood vessels, and nerve tissue.

Morphometric Observations

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

Mean epithelial growth in Group I was significantly lower than in Groups II–V. Growth of the lamina propria and submucosa in Group III differed significantly from that observed in Groups IV and V ($P = 0.003$). There was also a significant difference in tunica muscularis growth between Groups III/IV and V ($P = 0.004$). Mean growth of the serosa was significantly greater in Group I than in Groups II–V ($P = 0.004$). Finally, total ruminal wall thickness differed significantly between Groups IV and V ($P = 0.003$).

Mathematical growth models for each tissue stratum, with the corresponding growth equations and correlation coefficients, are shown in Fig. 2A–D. The epithelial layer displayed a rapid growth rate until 25 cm (107 days, 71% gestation), coinciding with the greatest degree of stratification. Thereafter, the growth rate stabilized from 25 cm to 47 cm (150 days, 100% gestation). The growth rate of the lamina propria and submucosa declined from the early embryonic stages until birth. The tunica muscularis continued to grow rapidly until birth, with phases of accelerated growth at 13 cm (64 days, 42% gestation) and 25 cm (107 days, 71% gestation). The serosa maintained a steady rate of growth until birth.

Immunohistochemical Observations

The results of immunohistochemical staining of the rumen during prenatal development—for synaptophysin (SYP), glial fibrillary acidic protein (GFAP), vimentin (VIM), neuropeptide Y (NPY), and vasoactive intestinal polypeptide (VIP)—are shown in Table 2.

Neuroendocrine cells were first detected by SYP staining at 53 days (CRL 9 cm, 35% gestation) in the lamina propria, submucosa, tunica muscularis, myenteric plexus and intervascular and perivascular connective tissue. By 76 days (50% gestation) staning intensity was greater in these layers, and at 113 days CRL (33 cm, 75% gestation) neuroendocrine cells (SYP+) were observed in the epithelial layer (Fig. 3A,B). Positive staining for GFAP was detected from 68 days (CRL 10 cm, 45% gestation) until birth in lamina propria, submucosa, tunica muscularis and serosa (Fig. 3C,D). The same layers stained positive for VIM at 39 days (CRL 4.4 cm, 26% gestation; Fig. 3E,F). Positive staining for NPY and VIP was observed at 113 days (CRL 33 cm, 75% gestation) and 120 days (CRL 35 cm, 80% gestation), respectively, in both the lamina propria and the submucosa (Fig. 3G,H).

Scanning Electronic Microscopy

At 38 days (CRL 4.3 cm, 25% gestation), the ruminal wall was visible as a smooth surface displaying no evidence of keratinization or desquamation (Fig. 4A). Cells were well defined, with clear outlines and intercellular borders (Fig. 4B).

Small ruminal papillae started to protrude from the surface at 50 days (CRL 7.7 cm, 33% gestation; Fig. 4C), acquiring clearer outlines by 64 days (Fig. 4D).

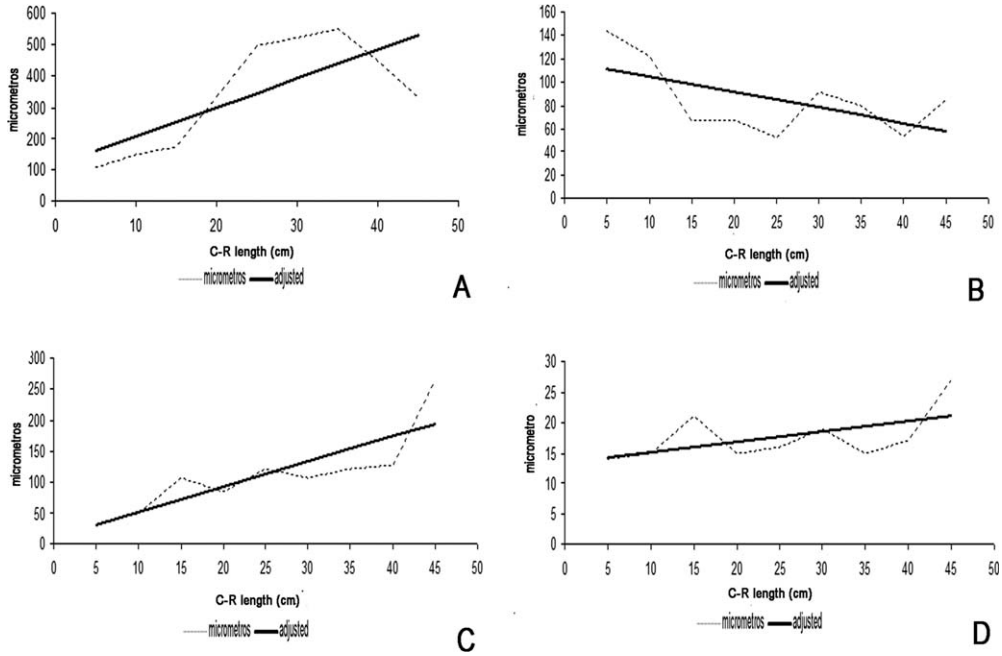

Fig. 2. Mathematical growth models for rumen tissue layers. **A:** Mathematical model of rumen growth (epithelium). $y = 9.16x + 116.33$, $r^2 = 0.54$. **B:** Mathematical model of rumen growth (lamina propria and submucosa). $y = -1.31x + 117.28$, $r^2 = 0.34$. **C:** Mathematical model of rumen growth (tunica muscularis). $y = 4.08x + 10.36$, $r^2 = 0.71$. **D:** Mathematical model of rumen growth (serosa). $y = 0.16x + 13.5$, $r^2 = 0.31$.

TABLE 2. Immunohistochemical analysis of goat rumen during prenatal development

	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: 35–50% 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	LP-S	TM	S	E	LP-S	TM	S	E	LP-S	TM	S	E	LP-S	TM	S	E	LP-S	TM	S
SYP	−	−	−	−	−	−	−	−	−	+	+	−	−	++	++	−	+	++	++	−
GFAP	−	−	−	−	−	−	−	−	−	−	−	−	−	++	++	++	−	++	++	++
VIM	−	−	−	−	−	+	+	+	+	+	+	+	−	++	++	++	−	+++	+++	+++
NPY	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	++	−	−
VIP	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−

−, no reactivity; +, low reactivity; ++, high reactivity; +++, strong reactivity; E, epithelium; LP-S, lamina propia-submucosa; TM, tunica muscularis; S, serosa.

At 81 days (CRL 24 cm, 54% gestation), ruminal papillae were circular in shape and varied in length (Fig. 4E). By 113 days (CRL 33 cm, 75% gestation) papillae had started to assume a conical shape and were of uniform size (Fig. 4F).

Finally, immediately before birth (150 days, CRL 47 cm, 100% gestation), ruminal papillae were leaf-shaped (Fig. 4G) and showed signs of surface keratinization (Fig. 4H).

DISCUSSION

Differentiation of the goat rumen took place at 35 days (23% gestation); this is somewhat later than the 28 days (19% gestation) reported by Molinari and Jorquera (1988) in goats. Rumen differentiation in sheep takes place at a similar stage, reported by Del Río Ortega (1973) and Franco et al. (1992) at 34 days (22% gestation) and 32 days (22% gestation), respectively, although other authors, including Fath El-Bab et al. (1983), have indicated an even later development, at around 97 days (65% gestation). In red deer, Franco et al. (2004a) observed this differentiation at 60 days (25%), while in cattle (Vivo et al., 1990) it occurs rather earlier, at 30 days (11% gestation).

With regard to the mucosa, the transition from an epithelium of "embryonic type" (Warner, 1958) toward a stratified epithelium with uniform distribution of stratum basale and stratum granulosum was evident at 53 days (35% gestation). The stratified squamous epithelium observed here was also observed by Ramkirshna and Tiwari (1979). Similar findings have been reported in sheep (53 days, 35% gestation; Franco et al., 1992) and in deer (97 days, 36% gestation; Franco et al.,

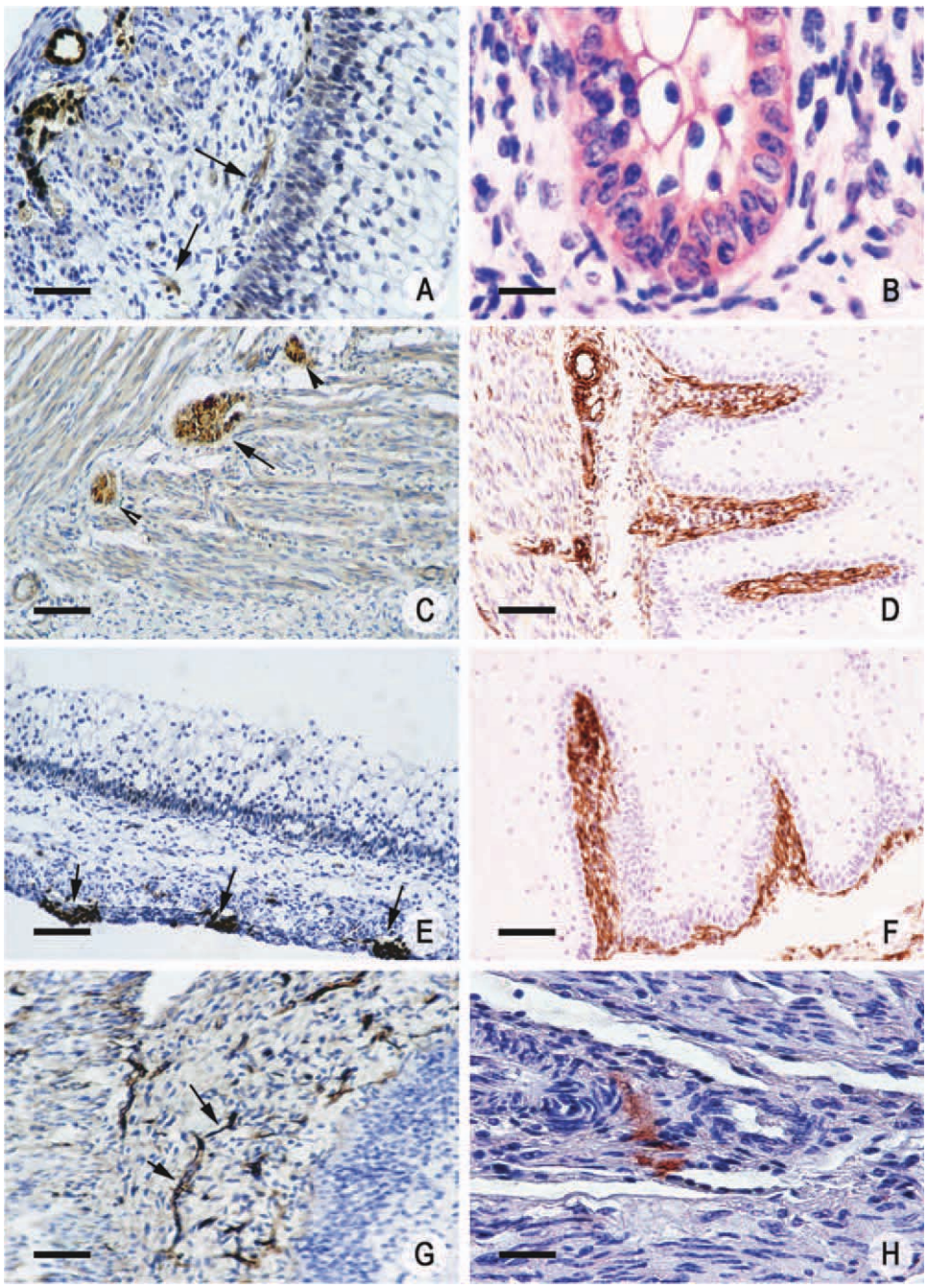

Fig. 3. Immunohistochemical findings in goat rumen (35–150 days, 23–100%). **A**: Photomicrograph of a section of the ruminal wall (53 days, 35% gestation). SYP+ staining (arrow, SYP) in lamina propria + submucosa, myenteric plexus and intervascular and perivascular connective tissue. EAS. Bar: 30 μm. **B**: Photomicrograph of a section of the ruminal wall (113 days, 75% gestation). SYP+ staining in epithelial cells. EAS. Bar: 40 μm. **C**: Photomicrograph of a section of the ruminal wall (C: 69 days, 46% gestation). GFAP+ cells (arrow) in lamina propia + submucosa and myenteric plexus. EAS. Bar: 25 μm. **D**: Photomicrograph of a section of the ruminal wall (90 days, 60% gestation). GFAP+ staining within the papillary body, in intervascular connective tissue in the lamina propria-submucosa, and within the myenteric plexus. EAS. Bar: 25 μm. **E**: Photomicrograph of a section of the ruminal wall (39 days, 26% gestation). VIM+ cells (arrow) in connective tissue of lamina propia-submucosa, myoblastic fibers and serosa. EAS. Bar: 25 μm. **F**: Photomicrograph of a section of the ruminal wall (80 days, 53% gestation). VIM+ staining within the papillary body. EAS. Bar: 25 μm. **G**: Photomicrograph of a section of the ruminal wall (113 days, 75% gestation). NPY+ staining (arrow) in the lamina propria and submucosa. EAS. Bar: 30 μm. **H**: Photomicrograph of a section of the ruminal wall (120 days, 80% gestation). VIP+ staining in the connective tissue of lamina propria and submucosa. EAS. Bar: 30 μm.

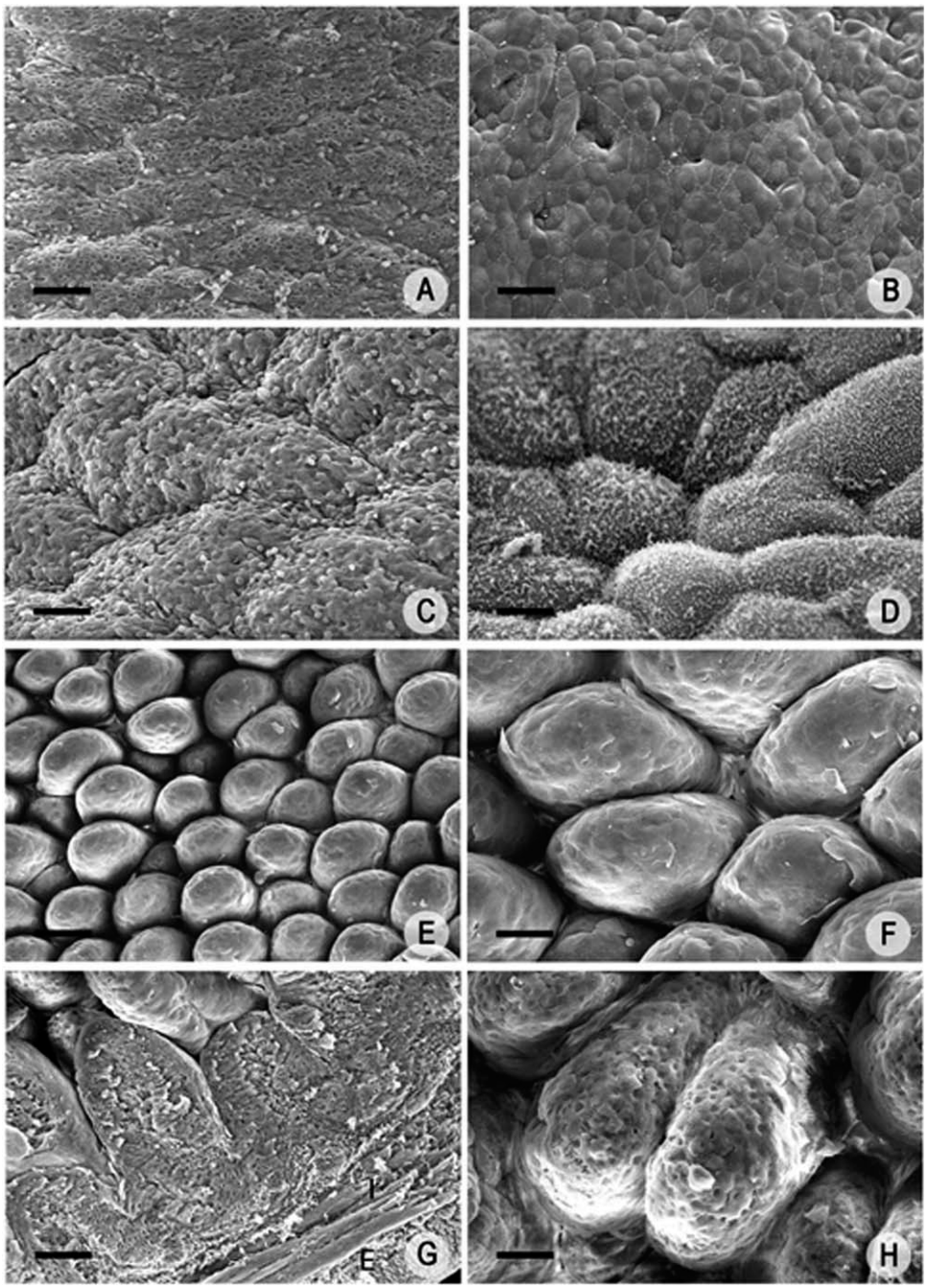

Fig. 4. Scanning electron microscopy of the goat rumen (38–150 days, 25–100%). **A**: Photomicrograph of the ruminal wall (38 days, 25% gestation). Smooth surface, with no evidence of keratinization or desquamation. Bar: 200 μm. **B**: Photomicrograph of the ruminal wall (38 days, 25% gestation). Well-defined cells with clear outlines and intercellular borders. Bar: 50 μm. **C**: Photomicrograph of the ruminal wall (50 days, 33% gestation). Incipient ruminal papillae starting to protrude into the epithelial surface. Bar: 300 μm. **D**: Photomicrograph of the ruminal wall (64 days, 42% gestation). Differentiation of ruminal papillae, separated by clear boundaries. Bar: 100 μm. **E**: Photomicrograph of the ruminal wall (81 days, 54% gestation). Circular ruminal papillae of varying lengths. Bar: 200 μm. **F**: Photomicrograph of the ruminal wall (113 days, 75% gestation). Conical ruminal papillae of uniform size. Bar: 75 μm. G. Photomicrograph of the ruminal wall (150 days, 100% gestation). Development of lamina propria + submucosa. Tunica muscularis composed of internal (I) and external (E) bundles. Bar: 100 μm. **H**: Photomicrograph of the ruminal wall (150 days, 100% gestation). Leaf-shaped ruminal papillae, showing signs of surface keratinization. Bar: 150 μm.

2004a). However, Panchamukhi and Srivastave (1979) in buffalo and Vivo et al. (1990) in cattle report epithelial stratification without distribution into strata. The stratum corneum and the stratum spinosum were visible at 79 days (53% gestation). The stratum lucidum has hitherto only been reported in buffalo by Osman and Berg (1981) at a late stage in prenatal rumen development. There is some agreement regarding the appearance of

the stratum corneum, which has been observed in sheep at 81 days (54% gestation; Franco et al., 1992) and in deer at 142 days (45% gestation; Franco et al., 2004a). However, other authors report the appearance of this stratum at a later stage: in goats, it was noted by Molinari and Jorquera (1988) at 104 days (70% gestation), while in sheep it has been observed by Wardrop (1961) and Del Rio Ortega (1973) only at birth. The stratum corneum has been reported shortly before birth in buffalo (Tiwari and Jamdar, 1970; Panchamukhi and Srivastava, 1979) and in cattle (Warner, 1958; Asari et al., 1981; Vivo et al., 1990).

Epithelial stratification coincided with two structural modifications: the appearance of ruminal pillars and papillae. Pillars became visible at 46 days (30% gestation), a finding similar to that reported in sheep by Del Rio Ortega (1973) and Franco et al., (1992) and in deer by Franco et al., (2004a), who observed ruminal pillars at 39 days (26% gestation), 42 days (28%) and 67 days (27% gestation), respectively. By contrast, Vivo et al., (1990) noted pillar formation at an earlier stage, 44 days (16% gestation). Ruminal papillae appeared as evaginations of the stratum basale at 76 days (50% gestation). This differs markedly from the 136 days (91% gestation) reported in goats by Molinari and Jorquera (1988) and at 39.5 cm CRL (126 days, 84% gestation) observed in goats by Ramkrishna and Tiwari (1979); papillae have been observed shortly before birth in cattle (Arias et al., 1978; Amasaki and Daigo, 1987; Vivo et al., 1990). By contrast, opinions differ with regard to the timing of its appearance in sheep: Del Rio Ortega (1973), Fath El-Bab et al., (1983) and Franco et al., (1992) report papillae at 64 days (43% gestation), 103 days (69% gestation) and 61 days (42% gestation), respectively. In deer, they have been observed at 142 days (45% gestation) by Franco et al., (2004a).

The differentiation of lamina propria + submucosa and tunica muscularis from pluripotential blastemic tissue was first observed at 50 days (33% gestation), i.e. rather later than in other species: in sheep, differentiation is reported at 33 days (22% gestation; Franco et al., 1992), in cattle at 33 days (19% gestation; Vivo et al., 1990) and in deer at 60 days (25% gestation; Franco et al., 2004a).

Growth and differentiation of the serosa took place at a fairly steady rate from the earliest stages of gestation to birth; similar findings have been reported for sheep (Franco et al., 1992).

Morphometric results showed accelerated growth of the epithelial layer and the tunica muscularis at 107 days (71% gestation), coinciding with greater stratification of the epithelium and involvement of the tunica muscularis in the formation of the muscle body of the ruminal pillars. By contrast, the growth-rate of the lamina propia and submucosa declined from the early embryonic stages until birth, perhaps due to constraints on expansion resulting from the active growth of the epithelial layer and the tunica muscularis. The serosa maintained a steady rate of growth until birth. Similar morphometric findings have been reported in sheep and in deer by Franco et al., (1992, 2004a).

Neuroendocrine (SYP positive) cells were detected at 53 days (35% gestation) in the lamina propria, the submucosa and the tunica muscularis, and at 113 days (75% gestation) in the epithelium. Similar results are noted for deer by Franco et al., (2004a). By contrast, Franco et al. (2011) detected these neuroendocrine cells at a later stage (81 days; 54% gestation) in sheep, and at 97 days (36% gestation) in goats, i.e. a similar stage that observed here. GFAP-positive glial cells were observed at 68 days (45% gestation) and VIM-positive cells at 39 days (26% gestation). GFAP is widely considered a valid label for the detection of astrocyte development (Valentino et al., 1983) but VIM has been identified as an early glial cell marker in the prenatal development of the pineal gland (Franco et al., 1997). GFAP+ glial cells were detected earlier here than in sheep and deer, where they have been reported at 112 days (75% gestation) and 142 days (51% gestation), respectively (Franco et al., 2011). Finally, VIP+ and NPY+ cells were observed during late stages of development, at 113 days (75% gestation) and 120 days (80% gestation), respectively, as also reported for deer by Franco et al., (2004a). None of the authors consulted detected the presence of neuropeptides in the epithelial layer, although they have been reported in other rumen and omasum tissue in sheep (Vergara-Eseras et al., 1990), in the abomasum of lambs (Groenewald, 1994) and in the stomach of cows (Kitamura et al., 1986).

The results of scanning electron microscopy suggested that age was a decisive factor in the structural development of ruminal papillae. Here, papillae were first observed at 50 days (33% gestation); in the bovine foetus, Amasaki and Daigo (1988) detected papillae at a later stage, in the fifth month of gestation. The changes noted here in papillary morphology during development have also been reported in sheep by Scott et al. (1973).

Overall, the results obtained in this study confirm that prenatal development of the goat rumen is similar to that reported in deer, but somewhat slower than that recorded for sheep and cattle.

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LITERATURE CITED

Amasaki H, Daigo M. 1987. Prenatal development of subepithelial vasculature related to appearance of ruminal papillae in the cow rumen. Anat Anzeiger 164:139–147.

Amasaki H, Daigo M. 1988. Morphogenesis of the epithelium and the lamina propria of the rumen in bovine fetuses and neonates. Anat Histol Embryol 17:1–6.

Arias JLR, Cabrera R, Valencia A. 1978. Observation on the histological development of the rumen papillae of cow. Morphological changes due to age. Anat Histol Embryol 7:140–151.

Asari M, Fukaya K, Kano Y, Eguichi Y. 1981. Development of the fetus and neonatal bovine stomach. Anat Histol Embryol 10: 264–274.

Boyazoglu J, Hatziminaoglou I, Morand-Fehr P. 2005. The role of the goat in society: past, present and perspectivas for the future. Small Ruminat Res 60:13–23.

Del Rio Ortega S. 1973. Desarrollo prenatal del estómago de la oveja. Doctoral thesis, Facultad de Veterinaria, Zaragoza, Spain.

El-Gendy A, Darbalah E. 2010. Macroscopic and microscopic anatomical features of the omasum Baladi goats. J Biol Sci 10:596–607.

Evans HE, Sack WO. 1973. Prenatal development of domestic and laboratory mammals: growth curves, external features and selected references. Anat Histol Embryol 2:11–45.

Fath El-Bab MR, Schwart R, Ali AMA. 1983. Micromorphological studies on the stomach of sheep during prenatal life. Anat Histol Embryol 12:139–53.

Franco A, Vivo JM, Guillen MT, Regodón S, Robina A. 1989. Evolución parietal del retículo ovino de raza merina desde los 68 días de gestación hasta el nacimiento. Histol Med 5:57–58.

Franco A, Regodon S, Robina A, Redondo E. 1992. Histomorphometric analysis of the rumen of the sheep during development. Am J Vet Res 53:1209–1217.

Franco A, Robina A, Regodon S, Vivo JM, Masot AJ, Redondo E. 1993a. Histomorphometric analysis of the omasum of sheep during development. Am J Vet Res 54:1221–1229.

Franco A, Robina A, Guillen MT, Mayoral AI, Redondo E. 1993b. Histomorphometric analysis of the abomasum of sheep during development. Anat Anz 175:119–125.

Franco A, Robina A, Regodon S, Vivo JM, Masot AJ, Redondo E. 1993c. Histomorphometric análisis of the reticulum of the sheep during development. Histol Histopathol 8:547–556.

Franco A, Regodón S, Masot AJ, Redondo E. 1997. A combined immunohistochemical and electron microscopic study of the second cell type in the developing sheep pineal gland. J Pineal Res 22:130–136.

Franco A, Masot AJ, Gómez L, Redondo E. 2004a. Morphometric and immunohistochemical study of the rumen of red deer during prenatal development. J Anat 204:501–513.

Franco A, Redondo E, Masot AJ. 2004b. Morphometric and immunohistochemical study of the reticulum of red deer during prenatal development. J Anat 205:277–289.

Franco A, Masot AJ, Redondo E. 2011. Ontogenesis of the rumen: a comparative analysis of the merino sheep and Iberian red deer. Anim Sci J 82:107–116.

Gihad EA, El-Bedawy TM, Mehrez AZ. 1980. Fibre digestibility by goat and sheep. J Dairy Sci 63:1701–1706.

Groenewald HB. 1994. Neuropeptides in the myenteric ganglia and nerve fibres of the forestomach and abomasums of grey, white and black karakul lambs. Onderstepoort J Vet Res 61:207–213.

Habel RE. 1963. Carbohydrates phosphatise an esterase in the mucosa of the ruminant forestomach during postnatal development. Am J Vet Res 24:199–211.

Hofmann RR. 1973. The Ruminant Stomach. Stomach structure and feeding habits of East African game ruminants. East African Literature Bureau, Nairobi.

Hofmann RR. 1989. Evolutionary steps of ecophysiological adaptation and diversification of ruminats—a comparative view of their digestive system. Oecologia 78:443–457.

Hofmann R, Schnorr B. 1982. Die funktionelle Morphologie des wiederkaeuer Magens, Ferdinand Enke Verlag, Stuttgart.

Kitamura N, Yamada J, Yamashita T. 1986. Immunohisochemical study on the distribution of neuron-specific enolase and peptide-containing nerves in the reticulo-rumen and the reticular groove of the cattle. J Compar Neurol 248:223–234.

Masot AJ, Franco A, Redondo E. 2007a. Morphometric and immunohistochemical study of the abomasum of red deer during prenatal development. J Anat 211:376–386.

Masot AJ, Franco A, Redondo E. 2007b. Comparative análisis of the forestomach mucosa in red deer during prenatal development. Revue Méd Vét 7:397–409.

Molinari E, Jorquera B. 1988. Intrauterine development stages of the gastric compartments of the caprine *Capra hircus*. Anat Histol Embryol 17:121–137.

Osman AHR, Berg. 1981. Studies on the histogenesis of the tunica mucosa of the stomach of the Egyptian Water buffalo (*Bos Bubalis*) 1. Histogenesis of the ruminal mucosa. Anat Anz 149: 232–240.

Panchamukhi BG, Srivastava HC. 1979. Histogenesis of the rumen of the buffalo (*Bubalus bubalis*) stomach. Anat Histol Embryol 8: 97–105.

Ramkrishna V, Tiwari GP. 1979. Histological and histochemical observations on the forestomach of goat during pre-natal life. Acta anat 103:292–300.

Rancourt M, Fois N, Lavin MP, Tchakerian E, Vallerand F. 2006. Mediterranean sheep and goats production: an uncertain future. Small Ruminat Res 62:167–179.

Redondo E, Regodon S, Masot AJ, Franco A, Lignereux Y. 1997. The gastrin and CCK cells in the gastrointestinal tract of the sheep during development. A differential study. Rev Med Vet 6: 511–518.

Redondo E, Franco A, Masot AJ. 2005. Morphometric and immunohistochemical study of the omasum of red deer during prenatal development. J Anat 206:543–555.

Redondo E, Masot J, Garcia A, Franco A. 2011. Ontogenesis of the omasum: a comparative analysis of the Merino sheep and Iberian red deer. Histol Histopathol 26:1135–1144.

Regodón S, Franco A, Masot AJ, Redondo E. 1996. Estudio ontogénico comparativo de la lámina epitelial de los compartimentos gástricos no glandulares en ovinos merinos. Anat Histol Embryol 25: 233–241.

Scala G, Corona M, Maruccio L. 2010. Structural, histochemical and immunocytochemical study of the forestomach mucosa in domestic ruminants. Anat Histol Embryol 40:47–54.

Sivachelvan MN, Ghali Ali M, Chibuzo GA. 1996. Foetal age estimation in sheep and goats. Small Ruminat Res 19:69–76.

Schnorr B, Vollmerhaus J. 1967. Feinstruktur des Pansenepithels von Ziege und Rind. Zentralbl Veterinärmed A 14:789–818.

Scott A, Gardner JC. 1973. Papillar form in the forestomach of the sheep. J Anat 116:255–267.

Tiwari GP, Jamdar NM. 1970. Studies of the gross and histological structure and development on the forestomach of Indian water buffalo calf in early postnatal life with reference to normal feeding. 2. Reticulum. Indian J Anim Sci 57:335–340.

Valentino KL, Jones EG, Kane SA. 1983. Expression of GFAP immunoreactiviy during development of long fiber tracts in the rat CNS. Devel Brarin Res 9:317–336.

Vergara-Esteras P, Harrison FA, Brown D. 1990. The localization of somatostain-like immunoreactivity in the alimentary tract of the sheep with observations of the effect of an infection with the parasite *Haemonchus contortus*. Exp Physiol 75:779–789.

Vivo JM, Robina A, Regodon S, Guillen MT, Franco A, Mayoral AI. 1990. Histogenetic evolution of bovine gastric compartments during prenatal period. Histol Histopathol 5:461–476.

Wardrop JD. 1961. Some preliminary observations on the histological development on the forestomach of the lamb. I. Histological changes due to age in the period from 46 days of foetal life to 77 days of postnatal life. J Agric Sci 57:335–341.

Warner ED. 1958. The organogenesis and early histogneesis of the bovine stomach. Am J Anat 102:33–63.

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Histomorphometric and immunohistochemical study of the goat omasum during prenatal development

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Summary. This work studies the morphological changes taking place in the goat omasum during prenatal development, using scanning electron microscope, light microscopy and immunohistochemical analysis. A total of 140 goat embryos and fetuses were used, from the first stages of prenatal life until birth. Differentiation of the omasum as a separate compartment of the primitive gastric tube was observed at 35 days of prenatal life ([crown-rump length (CRL)] 3 cm, 23% gestation). By 38 days (CRL 4.3 cm, 25% gestation) the omasal wall comprised three layers: an internal epithelial layer, a middle layer of pluripotential blastemic tissue and an external layer or serosa. Omasal laminae appeared in the following order: primary at 38 days (CRL 4.3 cm, 25% gestation), secondary at 50 days (CRL 7.7 cm, 33% gestation), tertiary at 59 days (CRL 12 cm, 39% gestation) and quaternary at 64 days (CRL 13.5 cm, 43% gestation). Neuroendocrine cells were detected by synaptophysin (SYP) at 52 days (CRL 8 cm, 35% 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. Sympathetic and parasympathetic nerve fibers and nerve bodies were detected via neuropeptide Y (NPY) and vasoactive intestinal polypeptide (VIP) at 95 days (CRL 20 cm, 63% gestation). In conclusion, prenatal development of the omasum - like that of the rumen - appears to take place somewhat earlier in goats than in sheep or cattle, but at a similar stage to that reported in deer.

Key words: Forestomach, Goat, Immunohistochemisty, Prenatal development

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Introduction

Although all ruminants share the ability to digest grass, and transform it into products of great nutritional value, Hofmann (1973) categorized them by their feeding habits into grass-roughage eaters, intermediate feeders and concentrate selectors. The goat is classed as an intermediate feeder, able to make use of marginal pasturelands. These differences in feeding habits are linked to differences in digestive-tract anatomy and histology among ruminant species (Hofmann and Schnorr, 1982).

Morphological examination of the ruminant digestive tract suggests that the forestomach - and particularly the omasum - is structurally adapted to feeding habits (Hofmann and Schnorr, 1982). The omasum contains laminae of varying sizes, which are composed of a thin muscular sheet enclosed by connective tissue and covered by a non-glandular mucosa whose surface is studded with papillae (Yamamoto et al., 1994). Omasal laminae are classified as first-, second-, third- or fourth-order, as a function of their size (Yamamoto et al., 1994; Dyce et al., 2002). The conical papillae studding the surface of the laminae vary in size and shape, depending on species and breed: different morphometric findings have been reported for cattle, sheep (Stafford and Stafford 1993), goats (Yamamoto et al., 1994; Green and Baker, 1996), deer (Mathiesen et al., 2000; Lentle et al., 1998; Kamler, 2001) and musk oxen (Clauss et al., 2006). The relationship between omasal structure and function has long been a matter of debate. While some authors have suggested that its cornified laminae serve as a grinding mill (Ellenberger, 1881), others prefer to see the omasum as a sieve that allows only small particles to pass on to the lower digestive tract (Bost, 1970). More recently, however, it has been widely accepted that the omasum is primarily an organ for the absorption of volatile fatty acids, minerals and electrolytes, due to the surface area provided by its laminae (Phillipson, 1982).

Although there has been considerable research into the ruminant stomach, focusing particularly on cattle (Vivo et al., 1990), sheep (Wardrop, 1961; Franco et al., 1992, 1993a-c; Redondo et al., 1997; Regodón et al., 1996), and deer (Franco et al., 2004a,b, 2011; Redondo et al., 2005, 2011; Masot et al., 2007a,b), few studies have addressed the development of the forestomach in goats (Mcsweeny, 1988; Ramkirshna and Tiwari, 1979; Green and Baker, 1996; Nwaogu and Ezseaor, 2008; El-Gendy et al., 2010; Garcia et al., 2012).

Ruminant animals are born with a stomach that is structurally and functionally similar to non-ruminant animals. During prenatal life, the stomach of ruminant animals suffers some morphological changes to suit its function in postnatal life. In the omasum, the morphological changes are the growth and development of the omasal laminae covered with conical papillae in order to play a role in the digestive process. For this reason, the aims of the present study were as follows: (1) to describe the histological evolution of goat omasum during prenatal life; (2) to determine the morphometric changes in the omasal wall during intrauterine life; (3) to detect by immunohistochemistry the presence of neuroendocrine cells with synaptophysin (SYP), the glial cell with glial fibrillary acidic protein (GFAP) and vimentin (VIM) and markers of peptidergic innervation such as neuropetide Y (NPY) and vasoactive intestinal peptide (VIP); (4) to analyze the surface of the omasal mucosa by 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 a 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).

Sampling and processing

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

Morphometric analysis

Ten specimens were selected for each group for histology and morphometric analysis. In group I of these 10 individuals, 2 belonged to the age of 13 days, 3 of 35 days and 5 of 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. Specimens 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 the 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 (epithelium, lamina propria and submucosa, tunica muscularis and serosa) 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 correctness of fit of this adjustment was measured using the rate of determination, r^2. In all cases, embryo body length (crown-rump length, CRL, in centimetres) was used as the independent variable; the thickness of each tissue stratum (epithelium, lamina propria and 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

deparaffinized tissue from the omasum to detect the neuroendocrine cell marker with synaptophysin (SYP), the glial cell marker with glial fibrillary acidic protein (GFAP) and vimentin (VIM) and markers of peptidergic innervation such as neuropetide Y (NPY) and vasoactive intestinal peptide (VIP). Tissues were deparaffinized and hydrated. Recovery of antigens was performed with citrate and microwave. Blocking of endogenous peroxidase activity was done 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) 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's 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. No staining was found in structures on the sections which served as absorption controls.

Scanning electron microscopy

For scanning electron microscopy (SEM) small pieces of omasum 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 at various tilt angles, at a magnification of 10 to 800x.

Results

Gross findings

At 35 days (CRL 3 cm, 23% gestation), the omasum was observed as a separate compartment of the primitive gastric tube, appearing as an oval, laterally-compressed cavity lined by a beige mucosa with small surface elevations corresponding to rudimentary omasal laminae. By 75 days (CRL 17.5cm, 50% gestation), primary, secondary, tertiary and quaternary laminae had started to protrude into the omasal cavity. At 112 days (CRL 32 cm, 75% gestation), conical papillae were apparent as small prominences arising from the lateral surfaces of laminae. Finally, by 150 days (CRL 47 cm, 100% gestation), all four orders of laminae were clearly apparent; they varied in length, and their surfaces were studded with numerous conical papillae.

Omasal histomorphogenesis

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

At 35 days (CRL 3 cm, 23% gestation), the omasum became apparent as a separate compartment of the primitive gastric tube. The omasal wall comprised an internal epithelial layer and an external layer of pluripotential blastemic tissue (Fig. 1a).

At 38 days (CRL 4.3 cm, 25% gestation), the omasal wall comprised three layers: an internal epithelial layer, a middle layer of pluripotential blastemic tissue and an external layer, or serosa. Rudimentary primary omasal papillae were visible as small protrusions from the omasal wall.

The stratified epithelium (163.58±5 μm) was divided into lighter luminal zones composed of globose cells with rounded apical nuclei, and inner darker zones comprising cylindrical germinal cells with ovoid basal nuclei.

The pluripotent blastemic tissue layer (936.36±11 μm) was composed of abundant ground substance interspersed with pluripotent mesenchymal stem cells.

The external serosa (507.14±7 μm) was formed by a slender layer of connective tissue covered by flat cells.

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

This stage of prenatal development was marked by histological changes affecting each stratum of the omasal wall.

The stratified epithelium (236.28±11 μm) was clearly divided into a stratum basale formed by 2-4 layers of germinal cells with dark cytoplasm and a central nucleus, and a thicker external stratum granulosum comprising 6-10 layers of globose cells with light cytoplasm (Fig. 1b).

Primary omasal laminae were larger and more numerous. By 50 days (CRL 7.7 cm, 33% gestation), secondary omasal laminae had become visible as small elevations between primary laminae, growing out from the wall towards the lumen (Fig. 1b). Both primary and secondary laminae appeared as outfoldings of the epithelial stratum basale.

The middle layer of blastemic tissue (853.14±16 μm) was formed by highly-cellular connective tissue and sparse ground substance, and the first signs of differentiation into lamina propria and submucosa were

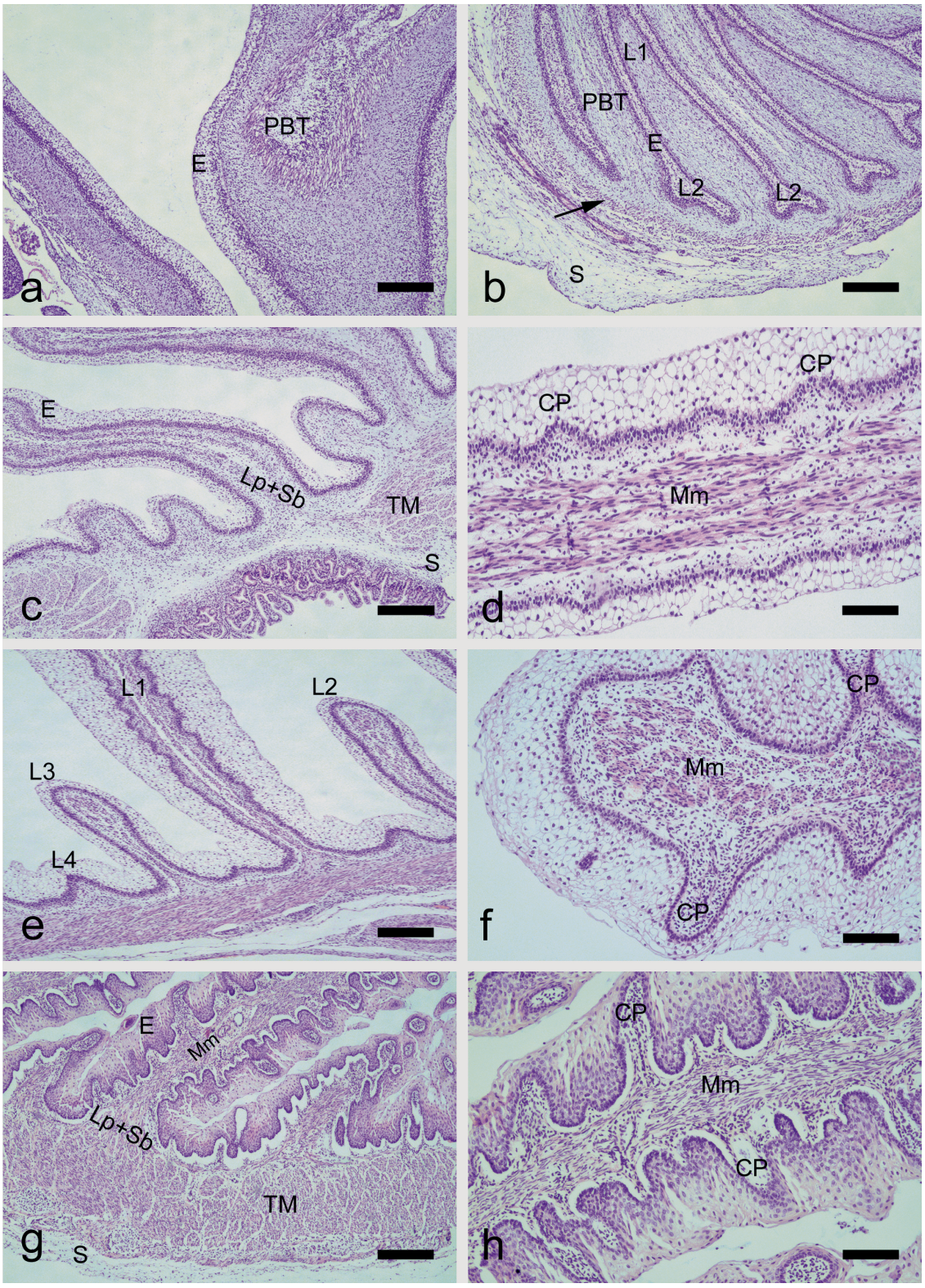

Fig. 1. Histomorphogenesis of goat omasal wall (35 to 150 days, 23-100% gestation). **a.** Photomicrograph of section of the differentiated omasum (35 days, 23% gestation). The wall comprises two layers: epithelium (E) and pluripotential blastemic tissue (PBT). **b.** Photomicrograph of section of omasal wall (52 days, 35% gestation). The wall is composed of three layers: epithelium (E), pluripotential blastemic tissue (PBT) with spindle-shaped myoblastic cells (arrow) and serosa (S). Primary (L1) and secondary laminae (L2) are visible. **c.** Photomicrograph of section of omasal wall (64 days, 35% gestation). The wall comprises four layers: epithelium (E), lamina propria and submucosa (Lp+Sb), tunica muscularis (TM), and serosa (S). Myoblastic fibers of the tunica muscularis (TM) infiltrating laminae to constitute muscularis mucosae. **d.** Photomicrograph of section of omasal laminae (70 days, 46% gestation). Muscularis mucosae (Mm) constituting the skeleton of omasal laminae. Conical papillae (CP) visible in primary laminae. **e.** Photomicrograph of section of the omasal wall (80 days, 53% gestation). Four orders of laminae now visible: primary (L1), secondary (L2), tertiary (L3) and quaternary (L4). **f.** Photomicrograph of section of omasal laminae (101 days, 67% gestation). Fully-developed muscularis mucosae (Mm) occupying the center of laminae. Larger conical papillae (CP) visible within stratified epithelium. **g.** Photomicrograph of section of omasal wall (113 days, 75% gestation). Wall formed by epithelium (E), lamina propria and submucosa (Lp+Sb), tunica muscularis (TM) and serosa (S). Conical papillae visible in primary, secondary and tertiary laminae. **h.** Photomicrograph of section of omasal laminae (150 days, 100% gestation). Muscularis mucosae (Mm) visible within omasal laminae. H-E. Bar: a, b, 30 μm; c, e, f, 25 μm; d, h, 20 μm.

observed. Both tissue layers protruded towards the epithelium, becoming involved in the formation of omasal laminae. By 50 days (CRL 7.7 cm, 33% gestation), a rudimentary tunica muscularis was visible in the form of a slender layer of myoblasts enclosed by blastemic tissue.

The serosa (420.72±7 μm) comprised a subserosa of loose connective tissue underlying a mesothelial layer of flat cells.

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

At this stage, the omasal wall was formed by four distinct layers: mucosa (composed of epithelium and lamina propria), submucosa, tunica muscularis and serosa.

The epithelium (422.8±12 μm) comprised the stratum basale and stratum granulosum. An outer stratum corneum - formed by a single layer of flat, anucleate cells - was also visible.

The lamina propria and submucosa (598.15±13 μm) were composed of fibroblast-rich connective tissue interspersed with sparse ground substance. The tunica muscularis (379±18 μm) comprised a circular internal layer and a longitudinal external layer. By 64 days (CRL 13.5 cm, 43% gestation), the muscularis mucosae was visible at the centre of omasal laminae, arising from the inner circular layer of smooth muscle fibers of the tunica muscularis (Fig. 1c).

Primary and secondary laminae were taller and thicker. At 59 days (CRL 12 cm, 39% gestation), tertiary laminae were apparent in the spaces between primary and secondary laminae. By 64 days (CRL 13.5 cm, 43% gestation), quaternary laminae were also visible (Fig. 1e). These laminae were composed of all tissue layers (epithelium, lamina propria, submucosa and muscularis) except the serosa.

At 70 days (CRL 15 cm, 47% gestation), conical papillae started to arise from the surface of omasal laminae (Fig. 1f). These were first apparent as elevations of the epithelial stratum basale in primary laminae, and by 76 days (CRL 16 cm, 51% gestation) were also visible in secondary laminae.

The serosa (184±12 μm) displayed no differences with respect to earlier stages of development.

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

During this stage, the omasal wall was composed of the same tissue layers observed earlier.

The epithelium (579.08±17 μm) comprised four strata: a stratum basale underlying a stratum granulosum, as noted earlier, and an overlying stratum spinosum composed of elongated cells; finally, the stratum corneum - formed by flat, anucleate cells - was in contact with the lumen.

Abundant conical papillae, visible in primary and secondary laminae, were filled with connective tissue from the lamina propria and the submucosa (376.18±15 μm). Both layers were apparent inside omasal laminae and conical papillae, forming the skeleton.

Both layers of the tunica muscularis were thicker (752.36±21 μm); the internal fiber layer had thickened sufficiently to give rise to the muscularis mucosae. At 101 days (CRL 21 cm, 67% gestation), a fully-formed muscularis mucosae was visible within omasal laminae.

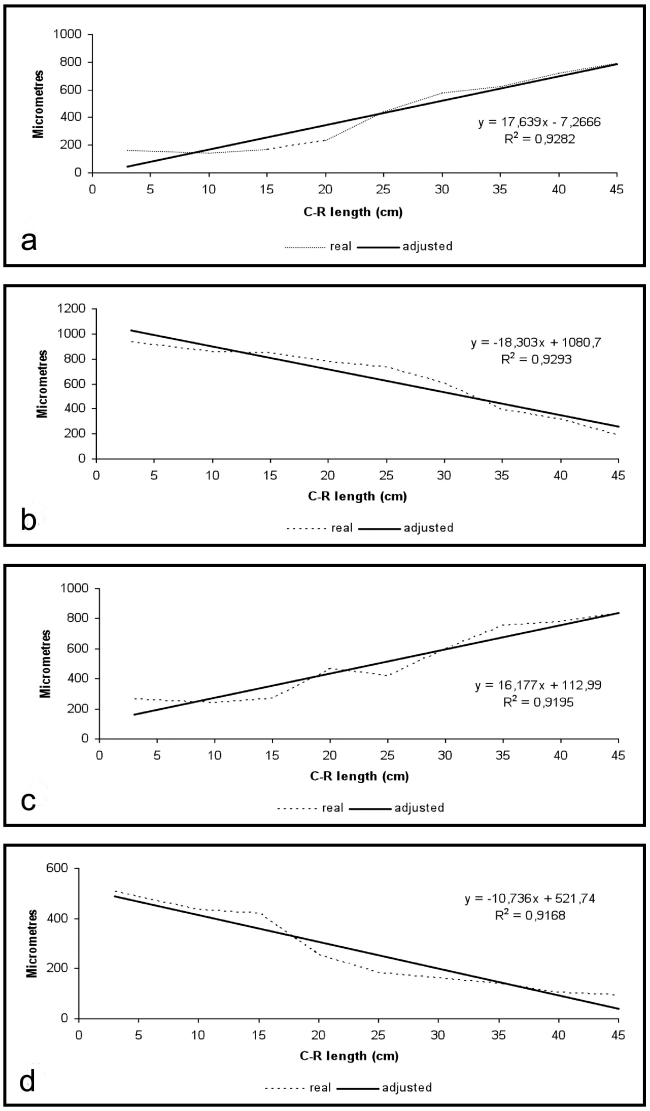

Fig. 2. Mathematical growth models for omasal tissue layers. **a.** Mathematical model of omasal growth (epithelium). **b.** Mathematical model of omasal growth (lamina propria and submucosa). **c.** Mathematical model of omasal growth (tunica muscularis). **d.** Mathematical model of omasal growth (serosa).

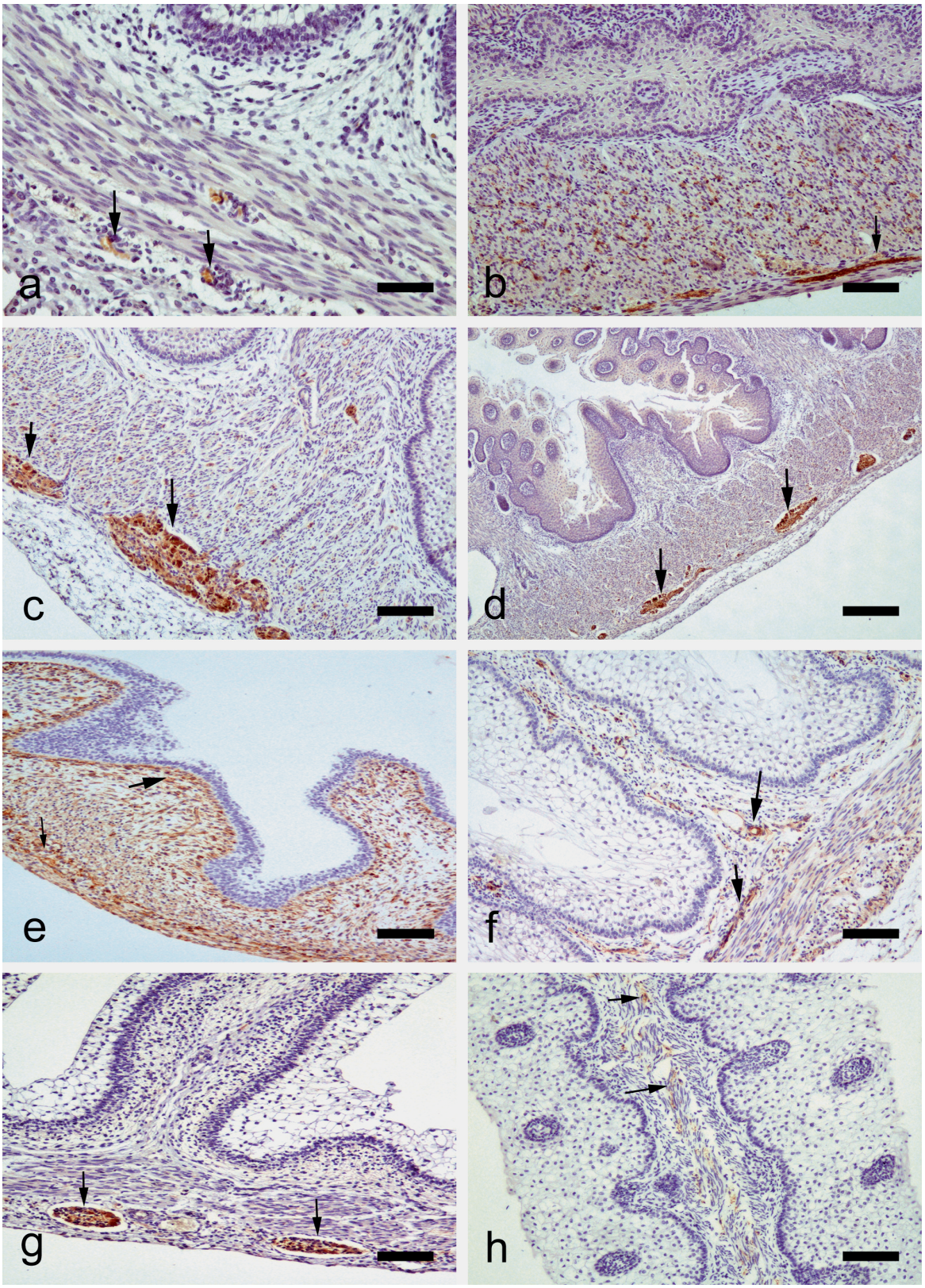

Fig. 3. Immunohistochemical findings in goat omasum. **a.** Photomicrograph of section of omasal wall (53 days, 35% gestation). SYP+ staining in lamina propria + submucosa, tunica muscularis and myenteric plexus (arrows). **b.** Photomicrograph of section of omasal wall (70 days, 75% gestation). SYP+ staining in lamina propria + submucosa, tunica muscularis and myenteric plexus (arrow). **c.** Photomicrograph of section of omasal wall (70 days, 46% gestation). GFAP+ cells (arrow) in lamina propria + submucosa, tunica muscularis and myenteric plexus. **d.** Photomicrograph of section of omasal wall (113 days, 60% gestation). GFAP+ staining in lamina propria-submucosa, tunica muscularis and myenteric plexus (arrow). **e.** Photomicrograph of section of omasal wall (38 days, 26% gestation). VIM+ cells (arrow) in mesenchymal layer of pluripotential blastemic tissue. **f.** Photomicrograph of section of omasal wall (84 days, 53% gestation). VIM+ staining in myenteric plexus, tunica muscularis and intervascular connective tissue in lamina propria-submucosa of laminae (arrow). **g.** Photomicrograph of section of omasal wall (95 days, 75% gestation). VIP+ staining (arrow) in lamina propria + submucosa and myenteric plexus. **h.** Photomicrograph of section of omasal wall (113 days, 80% gestation). NPY+ staining in connective tissue of lamina propria and submucosa of laminae (arrow). HRP. Bar: a, g, h, 20 μm; b-d, 30 μm; e, f, 25 μm.

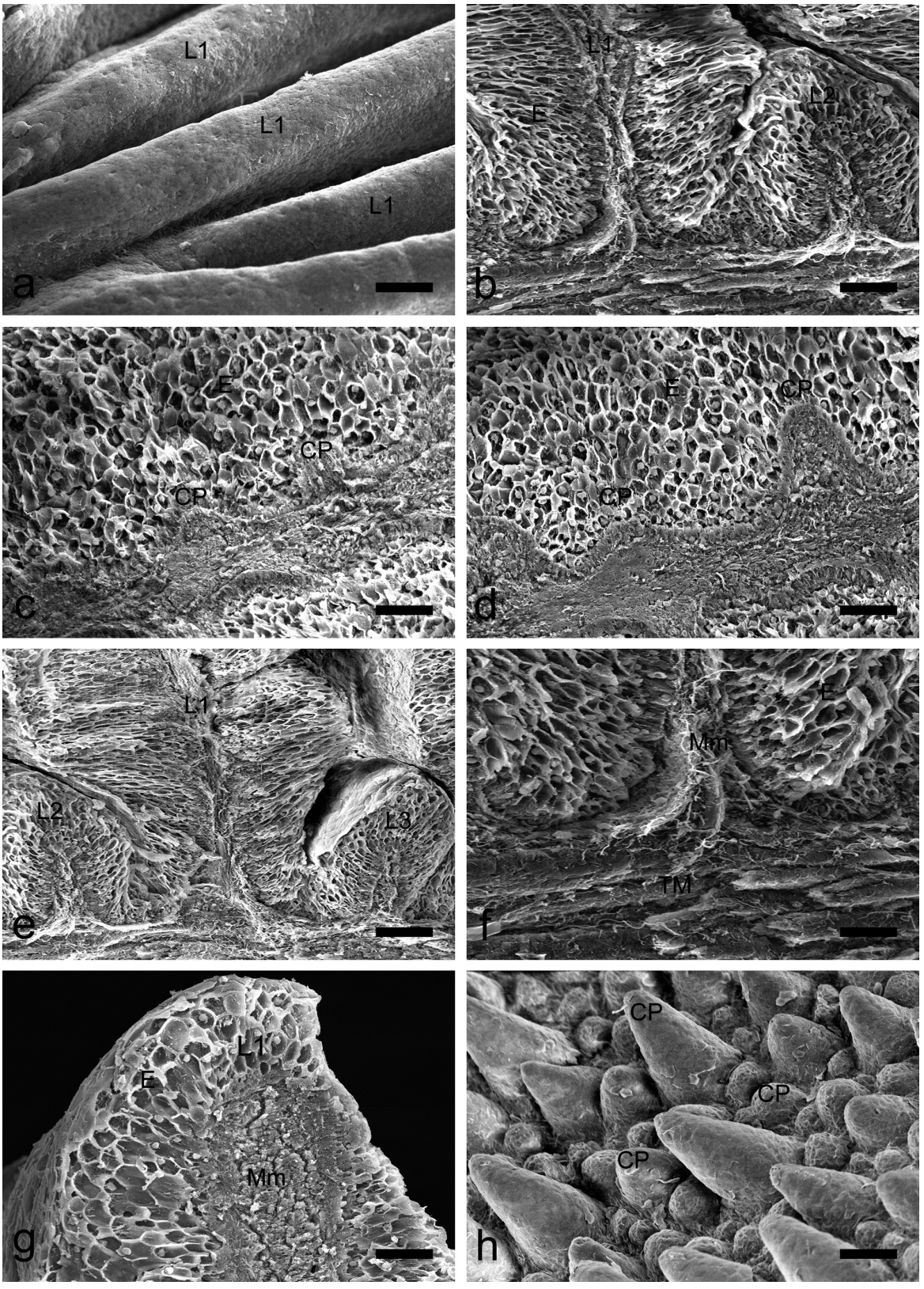

Fig. 4. Scanning electron microscopy of the goat omasum (38 to 150 days, 25-100% gestation). **a.** Photomicrograph of section of omasal wall (38 days, 25% gestation). Superficial longitudinal folds were observed at the surface of the omasum. These constituted the primary omasal laminae (L1). **b.** Photomicrograph of section of omasal wall (52 days, 35% gestation). Tunica muscularis (TM) and lamina propria-submucosa (Lp+Sb) visible in omasal wall. Secondary laminae were visible between primary laminae. **c.** Photomicrograph of section of omasal wall (70 days, 46% gestation). Conical papillae (CP) infiltrating epithelium (E) of omasal lamina. **d.** Photomicrograph of section of omasal wall (85 days, 55% gestation). Increase in size and number of conical papillae (CP) in omasal lamina. **e.** Photomicrograph of section of omasal wall (100 days, 66% gestation). Secondary (L2), tertiary (L3) laminae visible in spaces between primary laminae (L1). **f.** Photomicrograph of section of omasal wall (112 days, 74% gestation). Inner layer of muscular fiber of tunica muscularis (TM) infiltrating omasal lamina to form muscularis mucosae (Mm). **g.** Photomicrograph of section of primary omasal laminae (L1) (125 days, 82% gestation). Muscularis mucosae (Mm) occupying inside of omasal lamina. **h.** Photomicrograph of section of omasal wall (150 days, 100% gestation). Conical papillae (CP) of different sizes on the surface of the primary laminae. SEM. Bar: a-d, 6 μm; e, f, 4 μm; g, h, 2 μm.

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

By this late stage of prenatal development, all tissue layers were fully evident in the omasal wall. The epithelium was thicker (719.15±16 μm), and the stratum corneum comprised a larger number of layers of flat cells.

Four orders of omasal laminae of varying thickness were apparent, all studded with numerous conical papillae (Fig. 1g).

The skeleton of omasal laminae and conical papillae was formed by the lamina propria and submucosa (316±27 μm), composed of connective tissue, together with the muscularis mucosae.

The tunica muscularis (839.43±23 μm) comprised two layers of smooth muscle fibers, an inner circular layer and an outer longitudinal layer. The muscularis mucosae was fully developed, and occupied virtually the whole thickness of omasal laminae (Fig. 1h).

The whole stomach compartment was lined by an external serosa (94.88±7 μm) formed by a subserosa of connective tissue and an overlying mesothelium.

Morphometric observations

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

Mean epithelial growth in group I was significantly lower than in groups II to V (P= 0.004). Pluripotential blastemic tissue started to decline in thickness as the first signs of differentiation into lamina propria + submucosa and tunica muscularis were observed. Growth of the lamina propria and submucosa in group III differed significantly from that observed in groups IV and V (P=0.003). The tunica muscularis increased in thickness throughout prenatal development, a significant difference in growth-rate being noted between groups III/IV and V (P=0.003). The serosa became more slender as fetal development progressed: mean serosa thickness was significantly greater in group I than in groups II to V (P=0.002).

Mathematical growth models were constructed for each tissue stratum, with the corresponding growth equations and correlation coefficients. The epithelial layer grew progressively throughout prenatal development, and reached a maximum thickness at CRL 30 cm (105 days, 70% gestation) with the formation of the four epithelial strata: basale, granulosum, spinosum, and corneum (Fig. 2a). The growth rate of the lamina propria and submucosa declined from the early embryonic stages until birth (Fig. 2b). The tunica muscularis grew progressively throughout development. The greatest thickness of the tunica muscular was reached at CRL 35 cm (120 days, 80% gestation; Fig. 2c). The growth rate of the serosa diminished steadily from the early stages until birth (Fig. 2d).

Immunohistochemical observations

The results of immunohistochemical staining of the

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

	Group I	Group II	Group III	Group IV	Group V
E	151±5	280±8[a]	442±13[a]	639±21[a]	791±33[a]
Lp + Sb	pbt*	pbt	602±14	396±24[b]	316±37[b]
TM	pbt	pbt	419±22	778±27[b]	946±41[b]
S	420±6	269±12[a]	139±13[a]	103±8[a]	95±7[a]

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 pluripotent blastic tissue, which will later give rise to the lamina propria-submucosa and tunica muscularis was 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.

Table 2. Immunohistochemical findings in the omasum 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	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	++	+	+

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 acidic protein, VIM: vimentine, VIP: vasoactive intestinal peptide, NPY: neuropeptide Y. -, non immunoreactivity; +, low immunoreactivity; ++, moderate immunoreactivity; +++, high immunoreactivity.

omasum during prenatal development for synaptophysin (SYP), glial fibrillary acidic protein (GFAP), vimentin (VIM), neuropeptide Y (NPY), and vasoactive intestinal polypeptide (VIP) are shown in Table 2.

Neuroendocrine cells were first detected by SYP staining at 52 days (CRL 8 cm, 35% gestation; fig. 3a), in the lamina propria, submucosa, tunica muscularis and myenteric plexus. Positive-staining cells were more abundant at these sites by 64 days (CRL 13.5 cm, 43% gestation; fig. 3b).

Cells staining positive to GFAP were first observed at 64 days (CRL 13.5 cm, 43% gestation), in myenteric and submucosal plexuses, and scattered throughout the lamina propria-submucosa and tunica muscularis (Fig. 3c); they were more abundant at these locations by 113 days (Fig. 3d). VIM-positive glial cells were observed at 38 days (CRL 4.3 cm, 25% gestation) in the lamina propria, submucosa, tunica muscularis and serosa (Fig. 3e). By 101 days (CRL 21 cm, 67% gestation), VIM-positive cells were observed in myenteric plexuses and scattered throughout the lamina propria and submucosa of omasal laminae (Fig. 3f).

Sympathetic and parasympathetic nerve fibers and nerve bodies were detected by immunolabeling with neuropeptides NPY and VIP at 95 days (CRL 22.5 cm, 63% gestation). These were observed in the lamina propria and submucosa, tunica muscularis and serosa (Figs. 3g, h). The number of positive-staining cells increased as prenatal development progressed.

Scanning electron microscopy

During the early embryonic stages, the omasum displayed superficial longitudinal folds arising from the epithelium towards the omasal lumen; these constituted the primary omasal laminae (Fig. 4a).

At 50 days (CRL 7.7 cm, 33% gestation), primary omasal laminae were longer, and small folds were starting to develop in the spaces between them, to form secondary laminae (Fig. 4b).

Midway through gestation, at 70 days (CRL 15 cm, 47% gestation), the first conical papillae emerged on the lateral surface of omasal laminae (Fig. 4c). Thenceforth, papillae grew in size and number, horizontal to the omasal wall (Fig. 4d).

As development progressed, tertiary and quaternary laminae gradually arose from the omasal wall. By 85 days (CRL 20 cm, 57% gestation) all four orders of laminae were visible (Fig. 4e).

At 112 days (CRL 32 cm, 75% gestation), the two smooth muscle fiber layers forming the tunica muscularis were clearly visible. The inner layer gave rise to the muscularis mucosae, which gradually penetrated omasal laminae (Fig. 4f). The fully-developed muscularis mucosae extended along the inside of the omasal lamina, constituting the skeleton (Fig. 4g).

By the final stages of prenatal development, the omasal surface comprised four orders of omasal laminae of varying sizes, primary to quaternary (Fig. 4h).

Discussion

Differentiation of the goat omasum took place at 35 days (23% gestation); this is somewhat later than the 28 days (19% gestation) reported by Mutoh and Wakuri (1989) in goats. Omasum differentiation in sheep takes place at a similar stage, reported at 33 days (22% gestation) by Franco et al. (1993a), whereas in cattle this differentiation has been observed at 30 days (11% gestation) by Vivo et al. (1990). In wild ruminants, including deer (Redondo et al., 2005), it takes place at around 67 days (25% gestation), i.e. at a similar stage as that noted here.

During the early stages of embryo development, rudimentary primary laminae were visible as slight elevations of the omasal wall, which comprised an internal epithelium, a middle layer of pluripotent blastemic tissue, and an external serosa. A similar structure has been reported in the Nigerian goat fetus by Nwaogu and Ezeasor (2008) at 60 days (40% gestation). Primary omasal laminae were observed here at 38 days (25% gestation), somewhat later than in sheep, where they have been reported at 33 days (22% gestation) by Franco et al., (1993a), at 21% gestation by Lubis and O'Shea (1978) and Fath-El Bab et al., (1983), and at 24% gestation by Del Rio Ortega (1973).

Primary laminae were observed here at 38 days (25% gestation), secondary laminae at 50 days (33% gestation), tertiary laminae at 59 days (39% gestation) and quaternary laminae at 64 days (43% gestation). These timings differ slightly from those reported for other species. In sheep the four orders of laminae were noted at 24, 28, 32 and 33% gestation, respectively (Del Rio Ortega, 1973), at 21, 26, 33, and 52% (Fath-El Bab, 1983) and at 21, 26, 33 and 40% gestation (Franco et al., 1993a). In cattle, Vivo et al., (1990) observed all four orders of laminae at a rather earlier stage of prenatal development, i.e. around 20% gestation. Emergence of the four omasal laminae in deer has been reported by Redondo et al. (2005) at 25, 30, 35 and 50% gestation, indicating timing similar to that observed here. Other studies in goats noted the presence of all four orders midway through gestation (Nwaogu and Ezeasor, 2008).

Although most studies report laminae of four different sizes in the omasal wall, a number of authors - including Wardrop (1961) in sheep, Ramkrishna and Tiwari (1979) in goats, Osman and Berg (1982) in buffalo, and Vivo et al., (1990) in cattle - highlight a fifth laminar size during prenatal development.

Conical papillae, apparent as small lateral outfoldings of the omasal laminar surface, were observed at 70 days (46% gestation). Similar histological findings have been reported in goats by Ramkrishna and Tiwari (1979), in Nigerian goats by Nwaogu and Ezeasor (2008), in sheep by Franco et al. (1993a) and in deer by Redondo et al. (2005), at 45%, 50%, 45% and

50% gestation, respectively.

The differentiation of lamina propria + submucosa from pluripotent blastemic tissue was first observed at 50 days (33% gestation); there was no histological separation between these two layers, as also reported in the goat rumen (García et al., 2012). Similar timings are reported in sheep (Franco et al., 1993a) and in deer (Redondo et al., 2005).

Differentiation of the tunica muscularis from pluripotent blastemic tissue also took place at 50 days (33% gestation). By 60 days (40% gestation), two layers of smooth muscle fibers were apparent: an inner, circular layer and an outer longitudinal layer. Ramkrishna and Tiwari (1979) observed a fully-formed tunica muscularis in goats at 67 days (45% gestation). Findings for other species differ: in sheep, Duncan and Phillipson (1955) and Franco et al. (1993a) report tunica muscularis formation at an earlier point in development (39 days, 26% gestation), while Redondo et al. (2005) noted it in deer at 67 days (25% gestation).

The muscularis mucosa was observed in omasal laminae at 64 days (43% gestation). The findings of the present study agree with those reported in goats by Ramkrishna and Tiwari (1979), who observed the muscularis mucosae at 67 days (45% gestation), and in cattle by Totzauer and Sinowatz (1990), who reported it at 120 days (43% gestation). By contrast, it has been observed later in sheep (79 days, 53% gestation) by Franco et al. (1993a) and at an earlier stage of development in deer (90 days, 35% gestation) by Redondo et al. (2005).

In our study, omasal lamina was formed by an intermediate layer of smooth muscle and two layers of connective tissue on both sides. According to our observations and those previously referenced by Franco et al. (1993a) in sheep and Franco et al. (2004a,b) in deer, this intermediate layer of smooth fibers corresponded to the muscularis mucosa and originated from the inner layer of the tunica muscularis.

However, some authors have argued that the muscularis mucosa does not arise solely from the internal muscle layer (Gerneke, 1989; Bacha and Wood, 1990; Banks, 1993). Our results do not agree with Yamamoto et al. (1991), who described in the omasal laminae three layers of smooth muscle fibers: an intermediate layer and two lateral layers, but without mentioning that this intermediate layer constituted the muscularis mucosa.

The histological characteristics of the serosa, comprising a subserosa of loose connective tissue with an overlying mesothelium, are similar to those reported in the goat rumen by Garcia et al. (2012).

Immunohistochemical examination of the goat omasum revealed SYP-positive neuroendocrine cells at 53 days (35% gestation) in the lamina propria, the submucosa and the tunica muscularis. Similar findings have been reported for the deer omasum by Redondo et al., (2005), for the sheep rumen and reticulum by Franco et al., (2004a,b) and for the goat rumen by García et al., (2012).

VIM-positive glial cells were detected at 38 days (25% gestation) in the lamina propria, submucosa, tunica muscularis and serosa. Differentiation of tissue layers became more marked as development progressed, allowing more precise location of VIM-positive cells. At 101 days, these cells were also observed in myenteric and submucosal plexuses. GFAP-positive glial cells were first detected somewhat later than VIM-positive cells (64 days, 43% gestation), and in the same locations. A similar time-lag in the immunohistochemical detection of glial cells has also been reported by Redondo et al. (2011) in a comparative study of the omasum in deer and sheep. The difference in glial-cell labeling reflects the fact that VIM is an earlier marker, as noted by Franco et al. (1997) in a study of prenatal pineal-gland development in sheep.

The sympathetic and parasympathetic nerve fibers and nerve cells were detected by neuropeptide Y and vasoactive intestinal peptide (VIP), respectively. Both were detected at 90 days in the lamina propria, submucosa, tunica muscularis and serosa, and also in myenteric plexuses. Wathuta (1986) has also reported VIP-positive nerve fibers in the forestomach, abomasum and small and large intestines of sheep at a similar stage of development. Yamamoto et al. (1994) detected the neuropeptides VIP and NPY in nerve fibers, especially in the inner layer of the tunica muscularis in the omasum of adult sheep. VIP and NPY positivity has also been reported in the myenteric neurons of sheep rumen, reticulum and omasum by Groenewald (1994). In deer, Redondo et al. (2005) observed nerve fibers in the omasum at an earlier stage of development (142 days, 51% gestation).

Structurally, primary omasal laminae were apparent during the earliest stages of prenatal life. As development progressed, the formation and growth of further laminae of varying sizes was observed. Four orders of laminae were recorded here; similar findings have been reported by other authors both in the European goat (Schummer and Nickel, 1979) and in Zarabi goats (El-Gendy et al., 2010). However, other researchers have observed only 3 orders in the omasum of the African goat (Green and Baker, 1996), and 5 orders in the Australian goat (McSweeny, 1988). Inter-breed differences in the number of omasal laminae may reflect the influence of locally-available foliage in different regions (Hofmann, 1973). Omasal laminae were studded with small conical papillae. Here, papillae were structurally apparent at 70 days (47% gestation). These papillae were observed with blunt or pointed conical tips. These have also been reported in goats (Green and Backer, 1996), sheep (Scott and Gardner, 1973) and cattle (Yamamoto, 1994).

Overall, histological, structural and immunohistochemical examination suggests that prenatal development of the omasum - like the rumen - is somewhat faster in the goat than in sheep and cattle, but takes place at a similar pace to that reported in deer.

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References

Bacha W.J. and Wood L.M. (1990). Digestive system. In: Colour atlas of veterinary histology. Lea & Febiger. Philadelphia. pp 111-150.

Banks W.J. (1993). Digestive system I. Alimentary canal. In: Applied veterinary histology. 3rd ed. Mosby Year Book (ed). Philadelphia. pp 326-359.

Bost J. (1970). Omasal physiology. In: Physiology of digestion and metabolism in the ruminant. Phillipson A.T. (ed). Newcastle upon Tyne. Oriel. pp 52-65.

Clauss M., Hofmann R.R., Hummel J., Adamczewski J., Nygren K., Pitra C., Streich W.J. and Reese S. (2006). Macroscopic anatomy of the omasum of free-ranging moose (*Alces alces*) and muskoxen (*Ovibos moschatus*) and a comparison of the omasal surface area in 34 ruminant species. J. Zool. 270, 346-358.

Del Rio Ortega S. (1973). Desarrollo prenatal del estómago de la oveja. Doctoral thesis. Facultad de Veterinaria. Zaragoza, Spain.

Duncan D.L. and Phillison A.T. (1955). The development of motor responses in the stomach of the foetal sheep. J. Exp. Biol. 28, 32-40.

Dyce K.M., Sack W.O. and Wensing C.J.B. (2002). The abdomen of ruminants. In: Textbook of veterinary anatomy. 3rd ed. WB Saunders Co. (ed). Philadelphia. pp 666-690.

El-Gendy S.A.A. and Derbalah A. (2010). Macroscopic and microscopic anatomy of the omasum of the Baladi goat. J. Biol. Sci. 10, 596-607.

Ellenberger W. (1881). Zur Anatomie und Physiologie des dritten Magens der Wiederkäuer. Archiv für wissenschaftliche und praktische Tierheilkunde. 7, 17-58.

Evans H.E. and Sack W.O. (1973). Prenatal development of domestic and laboratory mammals: Growth curves, external features and selected references. Anat. Histol. Embryol. 2, 11-45.

Fath El-Bab M.R., Schwart R. and Ali A.M.A. (1983). Micro-morphological studies on the stomach of sheep during prenatal life. Anat. Histol. Embryol. 12, 139-53.

Franco A., Regodon S., Robina A. and Redondo E. (1992). Histomorphometric analysis of the rumen of the sheep during development. Am. J. Vet. Res. 53, 1209-1217.

Franco A., Robina A., Regodon S., Vivo J.M., Masot A.J. and Redondo E. (1993a). Histomorphometric analysis of the omasum of sheep during development. Am. J.Vet. Res. 54, 1221-1229.

Franco A., Robina A., Guillen M.T., Mayoral A.I. and Redondo E. (1993b). Histomorphometric analysis of the abomasum of sheep during development. Anat. Anz. 175, 119-125.

Franco A., Robina A., Regodon S., Vivo J.M., Masot A.J. and Redondo E. (1993c). Histomorphometric analysis of the reticulum of the sheep during development. Histol. Histopathol. 8, 547-556.

Franco A., Regodón S., Masot A.J. and Redondo E. (1997). A combined immunohistochemical and electron microscopic study of the second cell type in the developing sheep pineal gland. J. Pineal. Res. 22, 130-136.

Franco A., Masot A.J., Gómez L. and Redondo E. (2004a). Morphometric and immunohistochemical study of the rumen of red deer during prenatal development. J. Anat. 204, 501-513.

Franco A., Redondo E. and Masot A.J. (2004b). Morphometric and immunohistochemical study of the reticulum of red deer during prenatal development. J. Anat. 205, 277-289.

Franco A., Masot A.J. and Redondo E. (2011). Ontogenesis of the rumen: a comparative analysis of the merino sheep and Iberian red deer. Anim. Sci. J. 82, 107-116.

Garcia A., Masot J., Franco A., Gazquez A. and Redondo E. (2012). Histomorphometric and immunohistochemical study of the goat rumen during prenatal development. Anat. Rec. 295, 776-785.

Gerneke W.H. (1989). The digestive system. In: Veterinary histology, 2nd ed. Onderstepoort. pp 131-166.

Green E.D. and Baker C. (1996). The surface morphology of the omasum of the African goat. J. S. Afr. Vet. Assoc. 67, 117-122.

Groenewald H.B. (1994). Neuropeptides in the myenteric ganglia and nerve fibres of the forestomach and abomasums of grey, white and black karakul lambs. Onderstepoort. J. Vet. Res. 61, 207-213.

Hofmann R.R. (1973). The ruminant stomach. Stomach structure and feeding habits of East African game ruminants. East African Literature Bureau, Nairobi.

Hofmann R. and Schnorr B. (1982). Die funktionelle Morphologie des wiederkaeuer Magens, Ferdinand Enke Verlag, Stuttgart.

Kamler J. (2001). Morphological variability of forestomach mucosal membrane in red deer, fallow deer, roe deer and mouflon. Small. Rum. Res. 41, 101-107.

Lentle R.G., Stafford K.J. and Henderson I.M. (1998). Omasal anatomy in New Zealand fallow deer: an exploratory multivariate analysis. Aust. J. Agric. Res. 43, 1429-1440.

Lubis D. and O'Shea J.D. (1978). Development of the omasum in sheep. Acta. Anat. 100, 400-410.

Masot A.J., Franco A. and Redondo E. (2007a) Morphometric and immunohistochemical study of the abomasum of red deer during prenatal development. J. Anat. 211, 376-386.

Masot A.J., Franco A. and Redondo E. (2007b). Comparative analysis of the forestomach mucosa in red deer during prenatal development. Revue. Méd. Vét. 7, 397-409.

Mathiesen S.D., Vader M.A., Readergard V.B., Sormo W., Haga O.E., Tyler N.J. and Hofmann R.R. (2000) Functional anatomy of the omasum in high arctic svalbard reindeer (*Rangifer tarandus ptatyhynchus*) and Norwegian reindeer (*Rangifer tarandus tarandus*). Acta. Vet. Scand. 41, 25-40.

McSweeney C.S. (1988). A comparative study of the anatomy of the omasum in domesticated ruminants. Aust. Vet. J. 65, 205-207.

Mutoh K. and Wakuri H. (1989). Early organogenesis of the caprine stomach. Nippon. Juigaku. Zasshi. 51, 474-484.

Nwaogu C.I. and Ezeasor N.D. (2008). Studies on the development of omasum in west African dwarf goats (*Capra hircus*). Vet. Res. Commun. 32, 543-552.

Osman A.H. and Berg R. (1982). Studies on the histogenesis of the tunica mucosa of the stomach of the Egyptian water buffalo (*Bos bubalus L.*) 4. Histogeneis of the omasal mucosa. Anat. Anzeiger. 151, 467-471.

Phillipson A.T. (1982). Ruminant digestion. In: M.J. Swenson Duke's Physiology of domestic animals. 9th edn. Comstock Publishing Associates (ed). London. pp 277.

Ramkrishna V. and Tiwari G.P. (1979). Histological and histochemical observations on the forestomach of goat during pre-natal life. Acta Anat. 103, 292-300.

Redondo E., Regodon S., Masot A.J., Franco A. and Lignereux Y.

(1997). The gastrin and CCK cells in the gastrointestinal tract of the sheep during development. A differential study. Rev. Med. Vet. 6, 511-518.

Redondo E., Franco A. and Masot A.J. (2005). Morphometric and immunohistochemical study of the omasum of red deer during prenatal development. J. Anat. 206, 543-555.

Redondo E., Masot J., Garcia A. and Franco A. (2011). Ontogenesis of the omasum: a comparative analysis of the Merino sheep and Iberian red deer. Histol. Histopathol. 26, 1135-1144.

Regodon S., Franco A., Masot A.J. and Redondo E. (1996). Estudio ontogénico comparativo de la lámina epitelial de los compartimentos gástricos no glandulares en ovinos merinos. Anat. Histol. Embryol. 25, 233-241.

Schummer A. and Nickel R. (1979). The viscera of the domestic animals. 2nd ed. Verlag Paul Parey (ed). Berlin.

Scott A. and Gardner I.C. (1973). Papillar form in the forestomach of the sheep. J. Anat. 116, 255-267.

Sivachelvan M.N., Ghali Ali M. and Chibuzo G.A. (1996). Foetal age estimation in sheep and goats. Small. Rum. Res. 19, 69-76.

Stafford K.J. and Stafford Y.M. (1993). The anatomy of the omasum of some Zambian game species. Anat. Histol. Embryol. 22, 342-347.

Totzauer I. and Sinowatz F. (1990). Fetal development of the omasum of cattle (*Bos taurus*). Tierarztl. Prax. 18, 577-583.

Vivo J.M., Robina A., Regodon S., Guillen M.T., Franco A. and Mayoral A.I. (1990). Histogenetic evolution of bovine gastric compartments during prenatal period. Histol. Histopathol. 5, 461-476.

Wardrop J.D. (1961). Some preliminary observations on the histological development on the forestomach of the lamb. I. Histological changes due to age in the period from 46 days of foetal life to 77 days of postnatal life. J. Agric. Sci. 57, 335-341.

Wathuta E.M. (1986). The distribution of the vasoactive intestinal polypeptide-like, substante P-like and bombesin-like immunoreactivity in the digestive system of the sheep. Exp. Physiol. 71, 615-631.

Yamamoto Y., Kitamura N., Yamada J. and Yamashita T. (1991). Muscular architecture in the omasal laminae of cattle and sheep. Vet Res Comm 15, 249-256.

Yamamoto Y., Kitamura N., Yamada J., Andren A. and Yamashita T. (1994). Morphological study of the surface structure of the omasal laminae in cattle, sheep and goats. Anat. Histol. Embryol. 232, 166-176.

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Dear Doctors,

We are pleased to inform you that the paper entitled "Histomorphometric and immunohistochemical study of the goat reticulumduring prenatal development" (authors: A. Garcia, J. Masot, A. Franco, A. Gazquez and E. Redondo) is acceptable for publication in HISTOLOGY AND HISTOPATHOLOGY, but it is necessary that you prepare the paper according to the editor´s and referee´s comments.

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Histomorphometric and immunohistochemical study of the goat reticulum during prenatal development

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Short running title: goat reticulum during prenatal development

Summary. This study sought to describe the morphological changes taking place in the goat reticulum during prenatal development, using histomorphometric and immunohistochemical techniques. A total of 140 goat embryos and foetuses were used, from the first stages of prenatal life until birth. Differentiation of the reticulum as a separate compartment of the primitive gastric tube was observed at 35 days of prenatal life (23% gestation). By 38 days (25% gestation) the reticular wall comprised three layers: an internal epithelial layer, a middle layer of pluripotential blastemic tissue and an external layer or serosa. Primary reticular crests were visible at 59 days (38% gestation) as evaginations of the epithelial stratum basale, marking the earliest histological differentiation of future reticular cells. Secondary reticular crests were observed at 87 days (61% gestation). Corneum papillae first became apparent on the lateral surface of primary reticular crests at 101 days (64% gestation). The muscularis mucosae was visible by 101 days (64% gestation) in primary reticular crests. Neuroendocrine cells were detected by synaptophysin at 64 days (43% gestation), while glial cell markers (glial fibrillary acidic protein and vimentin) were observed at 64 days (43% gestation) and 38 days (25% gestation), respectively. The peptidergic innervation markers such as neuropeptide Y and vasoactive intestinal polypeptide were detected at 75 days (50% gestation). In conclusion, prenatal development of the reticulum – like that of the rumen – appears to take place somewhat earlier in goats than in sheep or cattle, but at a similar rate to that reported in deer.

Key words: *Capra hircus* · goat histomorphometric · immunohistochemistry · reticulum · prenatal development

Introduction

Although all ruminants share the ability to transform low-quality forage into products of great nutritional value, due to the structure and function of their digestive tract (Lombardi, 2005), differences in their feeding habits led Hofmann (1973) to assign them to three groups: grazers, concentrate selectors and intermediate feeders. The goat is classified as an intermediate feeder, able to make use of marginal pasturelands. These differences in feeding habits reflect differences in digestive-tract anatomy and histology among ruminant species (Hofmann and Schnorr, 1982).

In order to meet its functional needs, the ruminant's stomach has developed four separate compartments, each with its own morphological particularities. A distinctive feature of the reticulum, the second gastric compartment, is the arrangement of the internal mucosa in a network of crests forming a honeycomb-like pattern (Clauss et al., 2010). The height of the reticular crests represents a dietary adaptation in ruminants (Clauss et al., 2010). Historically, reticular crests and honeycomb cells have been suspected to play a role in water storage, to help grind coarse particle separation mechanism either by acting as traps that hold larger food particles and prevent their passage towards the omasum (Hofmann and Schnorr, 1982), or by acting as sedimentation traps that catch small dense particles and direct them towards the omasum during contractions (Reid, 1985; Mathison et al., 1995; Okine et al., 1998). However, more recently, Clauss et al., (2010) suggested that the reticular honeycomb structures do not separate particles by acting as traps either for small or for large particles.

Moreover, reticulum with the rumen is considered as a one functional chamber (Kitamura et al. 1986; Koch and Berg, 1990). They take part in mixing and fermentation of the ingesta and they need the specific motility patters (Pfannkuche et al., 2004) that are controlled by the neurons of enteric nervous system by releasing excitatory and inhibitory neurotransmitters (Kitamura et al., 1986; Groenewald, 1994; Brookes, 2001; Pfannuke et al., 2002; Münnich et al., 2008). These specific motility patterns can be recorded in the reticulum before birth, although they disappear in newborn (Ruckesbusch et al., 1983).

Although there has been considerable research into the organisation of the forestomach in cattle (Vivo et al., 1990), sheep (Wardrop, 1961; Franco et al., 1992,

1993a,b,c; Redondo et al., 1997; Regodon et al., 1996), and deer (Franco et al., 2004a,b; 2011, 2012; Redondo et al., 2005; 2011; Masot et al., 2007a,b). Several studies have addressed the prenatal development of stomach compartments in goats (Mcsweeny, 1988; Ramkirshna and Tiwari, 1979; Nwaogu and Ezseaor, 2008; El-Gendy et al., 2010; Garcia et al., 2012), and fewhave dealt specifically with the reticulum (Franco et al., 1993c, 2004b, 2012).

The primitive digestive tract in ruminant animals is structurally and functionally similar to that of non-ruminant animals (Relling and Mattioli, 2002). During prenatal life, however, the ruminant stomach undergoes certain morphological changes in order to meet functional demands in postnatal life (Relling and Mattioli, 2002). The major morphological changes in the reticulum are the growth and development of the reticular ribs or ridges to form reticular cells, which play a key role in the digestive process. The aims of the present study were as follows: (1) to describe the histological development of the goat reticulum during prenatal life; (2) to determine morphometric changes taking place in the reticular wall over the same period; (3) to detect immunohistochemical techniques for the detection of neuroendocrine cells using synaptophysin (SYP), of glial cells using glial fibrillary acidic protein (GFAP) and vimentin (VIM), and of peptidergic innervation markers neuropeptide Y (NPY) and vasoactive intestinal peptide (VIP) using immunohistochemical techniques; (4) to despict the surface of the reticular mucosa 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 size and prenatal age: 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 goat females. These pregnant females were slaughtered by the usual process in the slaughterhouse specify. Embryos and foetuses were obtained after opening the abdominal cavity uterus and placenta. 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 age classifications methods previously reported for sheep and goat (Evans and Sack 1973; Franco et al., 1992; Sivachelvan et al., 1996), and deer (Franco et al., 2004a, b, 2012).

Sampling and processing

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

Morphometric analysis

Ten specimens were selected for each group for histomorphometric 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. Histological sections were viewed through a microscope (NIKON Eclipse 80i) equipped with a digital video camera (NIKON DXMI200F). Digital images were analyzed using the Nis-Element 2.30 software package. Variables studied were height of various tissue strata (epithelium, lamina propia and submucosa, tunica muscularis and serosa) and total wall thickness. One hundred measurements were made in each tissue layer (epithelium, lamina propria + submucosa, tunica muscularis and serosa) of each of the selected individuals from each group.

Statistical analysis

The measurements are expressed in μm and 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 Microsoft Office Excell® (2003) 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 propia + 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 reticulum to detect the neuroendocrine cell marker with synaptophysin (SYP), the glial cell marker with glial fibrillary acidic protein (GFAP) and vimentin (VIM) and markers of peptidergic innervation as neuropetide Y (NPY) and vasoactive intestinal peptide (VIP). Tissues were

deparaffinized and hydrated. Recovery of antigens was performed in microwave with buffer citrate solution 0,01M pH 6, which was heated for 5 minutes at 800 watts of power. 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 antiserum: 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) 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. No staining was found in structures on the sections which served as absorption controls.

Scanning electron microscopy

Small pieces of reticulum 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

In the earliest embryonic stages, the reticulum was observed as a rounded cavity lined by a soft and smooth whitish mucosa (Fig. 1A). At 75 days (50% gestation), primary reticular crests were visible in the form of small surface elevations of the mucosa. By 113 days (75% gestation), clearly-delimited reticular cells gave the reticular surface an irregular appearance (Fig. 1B).

Reticular histomorphogenesis

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

The reticulum became apparent as a separate compartment of the primitive gastric tube at 35 days (CRL 3 cm, 23% gestation). The reticular wall comprised an internal epithelial layer and an external layer of pluripotential blastemic tissue and external layer, or serosa (Fig. 2A).

The stratified epithelium (358.04 ± 59 μm) was divided into a darker basal zone composed of germinal cells with central nuclei, and a lighter external zone comprising globose cells with apical nuclei.

The pluripotential blastemic tissue layer (731.11 ± 88 μm) was composed of abundant amorphous ground substance interspersed with star-shaped mesenchymal cells.

The reticular wall was lined by an external serosa (107.64 ± 12.91 μm) comprising a layer of submesothelial loose connective tissue covered by a mesothelium composed of flat cells.

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

This stage of prenatal development was marked by histological changes affecting each stratum of the reticular wall.

The stratified epithelium (487.32 ± 56 μm) was clearly divided into a stratum basale formed by 3 layers of germinal cells with basophilic cytoplasm and a central

nucleus, and a thicker stratum granulosum in contact with the reticular lumen and comprising 6-8 layers of globose cells with light cytoplasm (Fig. 2B).

Pluripotential blastemic tissue (467.54 ± 16.53 μm) was composed of amorphous ground substance interspersed with abundant fibroblast-like mesenchymal cells. The appearance of fibroblasts led to the differentiation of this tissue into lamina propria and submucosa at 50 days (CRL 7.7 cm, 33% gestation).

By this stage, the tunica muscularis was distinguishable from pluripotential blastemic tissue, in the form of a layer of longitudinally-arranged myoblasts (Fig. 2B).

The serosa (119.79 ± 22 μm) comprised a subserosa of fibrous loose connective tissue underlying a mesothelial layer of flat cells.

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

At 53 days (CRL 9 cm, 35% gestation), the reticular wall was formed by four distinct tissue layers: epithelium, lamina propria + submucosa, tunica muscularis and serosa (Fig. 2C).

The stratified epithelium (583.17 ± 68.79 μm) was still divided into two layers, the stratum basale and stratum granulosum, with morphological features similar to those observed at the earlier stage, although thicker – particularly in the case of the stratum granulosum.

The lamina propria and submucosa (252.21 ± 53.25 μm) were composed of abundant cell elements, including fibroblasts, collagen fibers and reticulin fibers interspersed with sparse ground substance.

The tunica muscularis (413.94 ± 31 μm) was formed by two layers: an internal layer of obliquely-arranged smooth muscle fibers and an external layer of longitudinally-arranged muscle fibers.

At 59 days (CRL 10 cm, 38% gestation) primitive primary reticular crests were visible as small protrusions from the epithelial stratum basale (Fig. 2C). By 64 days (CRL 13.5 cm, 43% gestation), crests were increasingly prominent and more regularly spaced over the epithelium (Fig. 2D).

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

Mid-way through the gestation period, the histological structure of the reticular wall was broadly similar to that of earlier stages, although some differences were apparent.

The epithelium (1125.27 ± 20.93 μm) now comprised four layers: a stratum basale underlying a stratum granulosum, as noted earlier, together with an overyling stratum spinosum composed of condensed cells with dark cytoplasm, and finally the outermost stratum corneum, formed by flat, anucleated cells.

The increasingly-prominent primary reticular crests continued to grow towards the reticular lumen. Since crest growth involved the lamina propria but not the submucosa (Fig. 2E), a spatial separation was apparent between the two layers (255.38 ± μm). The lamina propria was thinner, and contained a greater number of fibroblast-like cells, than the submucosa.

The tunica muscularis (876.19 ± 12.22 μm) comprised two full-developed fiber layers; a longitudinally-arranged external layer and a slanting internal layer.

At 87 days (CRL 22 cm, 61% gestation), the lateral surface of primary reticular crests was studded with small corneum papillae, formed from epithelial stratum basale germinal cells (Fig. 2E). At 101 days (CRL 27.5 cm, 67% gestation), secondary reticular crests were – like their primary counterparts – visible as evaginations of the stratum basale, though never attaining the same height as primary crests (Fig. 2F). At this stage, a layer of smooth muscle fibers was observed in the uppermost area of primary reticular crests, to form the muscularis mucosae.

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

At 113 days (CRL 33 cm, 75% gestation), all tissue layers were evident in the reticular wall. The stratified epithelium (2015.92 ± 40 μm) was composed of four fully-developed strata, in order outwards to the lumen: basale, granulosum, spinosum and corneum (Fig. 2G)

The skeleton of primary and secondary reticular crests, and of corneum papillae, was formed by the lamina propria, comprising fibroblasts and collagen fibers and containing abundant blood vessels. The submucosa was composed of sparse spindle-shaped and star-shaped cells interspersed among abundant amorphous ground

substance. The two layers had a joint thickness of 480.68 ± 37.63 μm. The fully-developed muscularis mucosae occupied the upper area of primary reticular crests. By the end of gestation, the muscularis mucosae was visible as a band of muscle tissue joining one crest to another to form a continuous layer of muscle tissue.

The tunica muscularis (1255.86 ± 83.94 μm) comprised two layers of smooth muscle fibers, identical in arrangement and morphological features to those observed at earlier stages.

Reticular crests displayed two growth patterns in forming reticular cells, growing longitudinally from the stratum basale towards the epithelial surface in order to lengthen crests, and transversally (horizontally) to fuse adjacent crests (Fig. 2H). By the end of gestation, the reticular wall was composed of fully-developed reticular cells.

The external serosa (177.02 ± 8 μm) was formed by a subserosa of fibrous loose connective tissue covered by a mesothelial layer of flat cells, characteristic similar to early prenatal development stages.

Morphometric observations

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

Mean epithelial growth in group I was significantly lower than in groups II to V (P=0.003). Growth of the lamina propria and submucosa in group III differed significantly from that observed in groups IV and V (P=0.004). Tunica muscularis thickness in group III was also significantly different from that recorded in groups IV and V (P=0.002). The serosa was thicker in group V than in groups I to IV (P=0.004).

Mathematical growth models were constructed for each tissue stratum, with the corresponding growth equations and correlation coefficients. The epithelial layer grew progressively throughout prenatal development, due to the formation of the four epithelial strata: basale, granulosum, espinosum, and corneum (Fig. 3A). The growth rate of the lamina propria and submucosa declined from differentiation until 20 cm CRL (81 days, 54% gestation); thereafter, growth increased due to the involvement of these layers in the formation of reticular crests (Fig. 3B). The tunica muscularis grew progressively throughout prenatal development. The greatest thickness of the tunica muscularis was reached at 30 cm CRL (104 days, 69% gestation) with the development

of its two muscle-fiber layers (Fig. 3C). The growth rate of the serosa diminished steadily from the early stages, but from 30 cm CRL (104 days, 69% gestation) increased progressively (Fig. 3D).

Immunohistochemical observations

The results of immunohistochemical staining of the reticulum for synaptophysin (SPY), glial fibrillary acidic protein (GFAP), vimentin (VIM), neuropeptide Y (NPY) and vasoactive intestinal polypeptide (VIP) during prenatal development are shown in Table 2.

Neuroendocrine cells were first detected by SYP staining at 53 days (CRL 9 cm, 35% gestation), in the lamina propria, submucosa, tunica muscularis and serosa (Fig. 4A). At 75 days (CRL 17.5 cm, 50% gestation), positive-staining cells were additionally detected in myenteric plexuses (Fig. 4B).

Glial cells staining positive to GFAP were first observed at 64 days (CRL 13.5 cm, 43% gestation) in myenteric and submucosal plexuses, and scattered throughout the lamina propria, submucosa, tunica muscularis and serosa (Fig. 4C); they were more abundant at these locations by 113 days (CRL 33 cm, 75% gestation; Fig. 4D). VIM-positive glial cells were observed at 38 days (CRL 4.3 cm, 25% gestation) in pluripotential bastemic tissue and serosa (Fig. 4E). By 113 days (CRL 33 cm, 75% gestation), they were also detected in myenteric and submucosal plexuses, and scattered throughout the lamina propria, tunica muscularis and perivascular connective tissue (Fig. 4F).

The peptidergic innervation markers NPY and VIP were detected at 76 days (CRL 18 cm, 50% gestation) in the lamina propria, submucosa, tunica muscularis, serosa and myenteric plexuses (Fig. 4G, H).

Scanning electron microscopy

During the early embryonic stages (38 days, CRL 4.3. cm, 25% gestation) the reticular wall was visible as a smooth surface displaying no evidence of keratinization or desquamation. Cells were clearly visible and well defined (Fig. 5A).

Primary reticular crests were first observed at 64 days (CRL 13.5 cm, 43% gestation) in the form of small elevations of the reticular wall mucosa (Fig. 5B). At 75 days (CRL 17.5 cm, 50% gestation), crests displayed greater development (Fig. 5C).

By 113 days (CRL 33 cm, 75% gestation) incipient corneum papillae were observed as small protuberances on reticular crest surfaces (Fig. 5D).

Secondary reticular crests were visible at 120 days (CRL 35 cm, 80% gestation); they were smaller than primary crests (Fig. 5E).

By 150 days (CRL 47 cm, 100% gestation), the reticular wall was composed of fully-developed reticular cells formed by primary and secondary crests, and corneum papillae of varying sizes (Fig. 5F).

Discussion

Differentiation of the goat reticulum from the primitive gastric tube took place at 35 days (23% gestation), i.e. at the same stage as that of the rumen (García et al., 2012). This is somewhat later than the 28 days (19% gestation) reported by Molinari and Jorquera (1988), and the 15 days (10% gestation) recorded by Mutoh and Wakuri (1989). Reticulum differentiation in sheep takes place at a similar stage, being reported at 33 days (22% gestation) by both Del Rio Ortega (1973) and Franco et al., (1993c). In cattle, it occurs rather earlier, Vivo et al., (1990) reporting differentiation at 30 days (11% gestation), while in deer it has been observed at 67 days (25% gestation) by Franco et al., (2004b), i.e. at a similar stage to that recorded in goats.

Differentiation of the embryonic stratified reticular epithelium into stratum basale and stratum granulosum was observed at 53 days (35% gestation). These two strata have also been detected at this stage of development in the goat rumen (García et al., 2012). Similar findings have also been reported at 35% gestation in sheep (Franco et al., 1992, 1993c). By contrast, the appearance of these two epithelial strata in deer has been recorded at the earlier stage of 67 days (25% gestation) by Franco et al., (2004b). Stratification of the epithelium concluded with the differentiation of the stratum spinosum and the stratum corneum at 75 days (50% gestation). Presence of all four strata has been reported in deer at 142 days, i.e. also at 50% gestation (Franco et al., 2004b). According to Franco et al., (1993c), full differentiation of epithelial strata in sheep takes place at 83 days (55% gestation), although Del Rio Ortega (1973) recorded it considerably earlier, at 57 days (38% gestation), while Wardrop (1961) reported it at birth. The present findings contrast with those of Molinari and Jorquera (1988), who noted the presence of all four strata in the goat reticulum only at a later stage (104 days).

Primary reticular crests, the first stage in the differentiation of reticular cells, were first observed at 59 days (38% gestation) as small surface elevations of the epithelial stratum basale. Primary crests have been recorded in deer at 117 days (40% gestation) by Franco et al., (2004b). Again, the present findings differ from those reported in goats by Molinari and Jorquera (1988), who noted the first appearance of primary reticular crests at a later stage of between 70 and 100 days' gestation. In the sheep reticulum, primary crests have been observed by Del Rio Ortega (1973), Fath El-Bab et al., (1983) and Franco et al., (1993c) at 72 days (48% gestation), 95 days (63%

gestation) and 69 days (45% gestation), respectively. In buffalo, primary crests were first observed at 70 days/22% gestation by Panchamuki and Serivastava (1980), and at 200 days/62% gestation by Osman and Berg (1981).

Secondary reticular crests appeared at 87 days (58% gestation). Franco et al., (2004b) report their presence in deer at a similar stage (142 days 50% gestation). In sheep, both Del Rio Ortega (1973) and Franco et al., (1993c) observed secondary crests at 82 days (55% gestation), while Fath El-Bab et al., (1983) reported them at a later stage (70% gestation). The present findings contrast from those reported in goats by Molinari and Jorquera (1988), who observed secondary reticular crests only at 69% gestation. In buffalo, they appear earlier than in goats, at 40% gestation (Panchamuchi and Serivastava, 1980).

By 101 days (64% gestation) corneum papillae were visible as elevations of the epithelial stratum basale on the lateral surface of primary reticular crests; similar findings have been reported in deer at 142 days/50% gestation (Franco et al., 2004b). Papillae have been observed in sheep at 63% gestation (Fath El-Bab et al., 1983), although Franco et al., (1993) recorded them only at a later stage of 55% gestation, while Del Rio Ortega (1973) reported their appearance only at birth. Other studies in goats report conflicting results: Ramkrishna and Tiwari (1979) observed papillae at an earlier stage (11.5 cm, 44% gestation), while Molinari and Jorquera (1988) detected them only close to birth.

The muscularis mucosae was first observed in the upper area of primary reticular crests at 101 days (67% gestation), deriving from the inner smooth muscle fiber layer of the tunica muscularis. In other ruminant species, it appears at a slightly later stage: 113 days/75% gestation in sheep (Franco et al., 1993c), and 205 days/75% gestation in deer (Franco et al., 2004b); in both cases, it derives from the internal bundle of the tunica muscularis.

Differentiation of the lamina propria and submucosa from pluripotential blastemic tissue took place at 50 days (33% gestation), as also reported for the goat rumen (García et al., 2012). No spatial separation was observed between the two tissue layers, but a difference in genesis was apparent due to the involvement of the lamina propria in the formation of reticular crests.

Differentation of the tunica muscularis – formed by 2 or 3 layers of myoblasts – from pluripotential blastemic tissue was observed at 50 days (33% gestation). By 60 days (40% gestation), the tunica muscularis comprised two muscle fiber layers; an

internal obliquely-arranged layer and an external longitudinal layer. Similar findings have been reported for sheep at 50 days (33% gestation) by Del Rio Ortega (1973), although both Duncan and Philipson (1955) and Franco et al., (1993c) recorded tunica muscularis differentiation at 34 and 33 days of gestation, respectively. Franco et al., (2004b) observed a primitive tunica muscularis in deer at an earlier stage of 67 days (25% gestation).

The serosa, formed by a subserosa of loose connective tissue and an overlying mesothelium, was histologically similar to that reported in the goat rumen by Garcia et al., (2012).

Neuroendocrine (SYP-positive) cells were detected at 53 days (35% gestation) in lamina propria, submucosa, tunica muscularis, serosa and myenteric plexuses, as also reported for the goat rumen (García et al., 2012). These cells were also observed at 97 days (35% gestation) in deer, but were detected at a later stage of 81 days/54% gestation in sheep (Franco et al., 2012).

VIM-positive glial cells were detected at 38 days (25% gestation) in pluripotential blastemic tissue and serosa. By 113 days (75% gestation), these cells were also observed in myenteric and submucosal plexuses, lamina propria, tunica muscularis and perivascular tissue. GFAP-positive glial cells were detected in the same locations but at a later stage of 64 days (43% gestation). A similar difference in the timing of glial-cell positivity to these two antibodies has been reported for the goat rumen (García et al., 2012) and also by Franco et al., (2012) in a comparative study of reticulum ontogeny in sheep and deer. The difference confirms that VIM is an earlier glial-cell marker, a finding reported by Franco et al., (1997) in a study of the prenatal development of the sheep pineal gland. Teixeira et al., (1998) also detected positive-staining glial cells in reticular papillae, suggesting that some functions of the reticular mucosa may be regulated intrinsically by the submucosal plexuses.

The peptidergic innervation markers NPY and VIP were detected at 76 days (50% gestation) in lamina propria and submucosa, tunica muscularis, serosa and myenteric plexuses. Similar findings are reported in deer by Franco et al., (2004b), who detected these markers at 142 days (50% gestation). VIP has also been observed in reticular myenteric plexuses in 3-day-old calves (Kitamura et al., 1986). In goat rumen, by contrast, García et al., (2012) detected NPY and VIP only at later stages. NPY and VIP are neuropeptides that act as putative neurotransmitters in the enteric neurones (Cooke, 1986; Mawe et al., 1989; Scharrer, 1990) and they are need in the reticulum

motility (Kitamura et al., 1986; Groenewald, 1994; Münnich et al., 2008). The presence of these neuropeptides in fetal reticulum suggests a possible functional role for NPY and VIP during prenatal life and puts forward the suggestion of specific motility patterns can be recorded in the reticulum before birth as reported by Ruckebusch et al., (1983).

Under scanning electron microscopy, the reticulum wall was visible as a smooth surface during the early stages of prenatal development, a finding also reported for the goat rumen (García et al., 2012). The primary reticular crest appeared as protrusions of the mucosa as observed in histological sections, having a correlation between histological images and electron microscopy. However, these primary crests were first observed by scanning electron microscopy at later ages than for histological sections. The structures forming reticular cells and the reticular wall gradually became visible as gestation advanced, as also observed for the rumen by García et al., (2012).

Differences in age of appearance of the reticular structures between ruminant species can originate in different feeding habits of the different species. According to their feeding habits, ruminants were classified into: grazers, concentrate selectors and those of a intermediate type (Hofmann, 1989). These differences in feeding habits are reflecting in distinct anatomical properties of the forestomach. Furthermore, 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. The goat was classified as animal of intermediate type (Hofmann and Schnorr, 1982) and has ability to survive under adverse foraging conditions. Our results indicate that prenatal development of the goat reticulum is similar to the deer. This coincidence can be explained because both species have a maternal diet similar (Garcia Gonzalez and Cuartas, 1992). However, the reticulum of sheep and goat has a later prenatal development that goat, despite being classified as grazers (Hofmann, 1989), these animals digest low quality forage, so you do not need an early development of forestomach to adapt to the environment.

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References

Brookes S.J. (2001). Classes of enteric nerve cells in the guinea-pig small intestine. Anat. Rec. 262, 58-70.

Clauss M., Hofmann R.R., Streich W.J., Fickel J. and Hummel J. (2010). Convergence in the macroscopic anatomy of the reticulum in wild ruminant species of different feeding types and a new resulting hypothesis on reticular function. J. Zool. 281, 26-38.

Cooke H.J. (1986). Neurobiology of the intestinal mucosa. Gastroenterology. 90, 1057-1081.

Del Rio Ortega S. (1973). Desarrollo prenatal del estómago de la oveja. Doctoral thesis, Facultad de Veterinaria, Zaragoza.

Duncan D.L. and Phillison A.T. (1955). The development of motor responses in the stomach of the foetal sheep. J. Exp. Biol. 28, 32-40.

El-Gendy S.A.A. and Derbalah A. (2010). Macroscopic and microscopic anatomy of the omasum of the Baladi goat. J. Biol. Sci. 10, 596-607.

Evans H.E. and Sack W.O. (1973). Prenatal development of domestic and laboratory mammals: Growth curves, external features and selected references. Anat. Histol. Embryol. 2, 11-45.

Fath El-Bab M.R., Schwart R. and Ali A.M.A. (1983). Micromorphological studies on the stomach of sheep during prenatal life. Anat. Histol. Embryol. 12, 139-153.

Franco A., Regodon S., Robina A. and Redondo E. (1992). Histomorphometric analysis of the rumen of the sheep during development. Am. J. Vet. Res. 53, 1209-1217.

Franco A., Robina A., Regodon S., Vivo J.M., Masot A.J. and Redondo E. (1993a). Histomorphometric analysis of the omasum of sheep during development. Am. J. Vet. Res. 54, 1221-1229.

Franco A., Robina A., Guillen M.T., Mayoral A.I. and Redondo E. (1993b). Histomorphometric analysis of the abomasum of sheep during development. Anat. Anzeiger. 175, 119-125.

Franco A., Robina A., Regodon S., Vivo J.M., Masot A.J. and Redondo E. (1993c). Histomorphometric analysis of the reticulum of the sheep during development. Histol. Histopathol. 8, 547-556.

Franco A., Regodon S., Masot A.J. and Redondo E. (1997). A combined immunohistochemical and electron microscopic study of the second cell type in the developing sheep pineal gland. J. Pineal. Res. 22, 130-136.

Franco A., Masot A.J., Gómez L. and Redondo E. (2004a). Morphometric and immunohistochemical study of the rumen of red deer during prenatal development. J. Anat. 204, 501-513.

Franco A., Redondo E. and Masot A.J. (2004b). Morphometric and immunohistochemical study of the reticulum of red deer during prenatal development. J. Anat. 205, 277-289.

Franco A., Masot A.J. and Redondo E. (2011). Ontogenesis of the rumen: a comparative analysis of the merino sheep and Iberian red deer. Anim. Sci. J. 82, 107-116.

Franco A., Masot A.J., García A. and Redondo E. (2012). Ontogenesis of the reticulum with special reference to neuroendocrine and glial cells: a comparative analysis of the Merino sheep and Iberian red deer. Anat. Histol. Embryol. 41, 362-373.

Garcia A., Masot J., Franco A., Gazquez A. and Redondo E. (2012). Histomorphometric and immunohistochemical study of the goat rumen during prenatal development. Anat. Rec. 295, 776-785.

Garcia-Gonzalez R. and Cuartas P. (1992). Food habits of *Capra pyrenaica*, *Cervus elaphus* and *Dama dama* in the Cazorla Sierra (Spain). Mammalia. 56, 195-202.

Groenewald H.B. (1994). Neuropeptides in the myenteric ganglia and nerve fibres of the forestomach and abomasums of grey, white and black Karakul. Onderstepoort. J. Res. 61, 207-213.

Hofmann R.R. (1973). The Ruminant Stomach. Stomach structure and feeding habits of East African game ruminants. East African Literature Bureau, Nairobi.

Hofmann R. and Schnorr B. (1982). Die funktionelle Morphologie des wiederkaeuer Magens, Ferdinand Enke Verlag, Stuttgart.

Kitamura N., Yamada J. and Yamashita T. (1986). Immunohistochemical study on the distribution of Neuron-Specific Enolase and Peptide-Containing nerves in the reticulorumen and the reticular groove of cattle. J. Comp. Neurol. 248, 223-234.

Lombardi G. (2005). Optimum management and quality pastures for sheep and goat in mountain areas. Options mediterraneennes, Series A, No. 67, pp. 19-29.

Mathison G.W., Okine E.K., Vaage A.S., Kaske M. and Milligan L.P. (1995). Current understanding of the contribution of the propulsive activities in the forestomach to the flow of digesta. In: Von Engelhardt W, Leonhard-Marek S, Breeves G,

Giesecke D (eds) Ruminant physiology: digestion, metabolism, growth and reproduction. Stuttgart: Ferdinand Enke Verlag. pp 23-41.

Masot A.J., Franco A. and Redondo E. (2007a). Morphometric and immunohistochemical study of the abomasum of red deer during prenatal development. J. Anat. 211, 376-386.

Masot A.J., Franco A. and Redondo E. (2007b). Comparative analysis of the forestomach mucosa in red deer during prenatal development. Revue. Méd. Vét. 7, 397-409.

Mawe G.W., Schemann M., Wood J.D. and Gehrson M.D. (1989). Immunocytochemical analysis of potential neurotransmitters present in the myenteric plexus and muscular layers of the corpus of the guinea pig stomach. Anat. Rec. 224, 431-442.

McSweeney C.S. (1988). A comparative study of the anatomy of the omasum in domesticated ruminants. Aust. Vet. J. 65, 205-207.

Molinari E. and Jorquera B. (1988). Intrauterine development stages of the gastric compartments of the goat (*Capra hircus*). Anat. Histol. Embryol. 17, 121-137.

Münnich J., Gäbel G. and Pfannkuche H. (2008). Intrinsic ruminal innervation in ruminants of different feeding types. J. Anat. 213, 442-451.

Mutoh K. and Wakuri H. (1989). Early organogenesis of the caprine stomach. Nippon. Juigaku. Zasshi. 51, 474-484.

Newburg D.S. and Walker W.A. (2007). Protection of the neonate by the innate immune system of developing gut and of human milk. Pediatr. Res. 61, 2-8.

Nwaogu C.I. and Ezeasor N.D. (2008). Studies on the development of omasum in west African dwarf goats (*Capra hircus*). Vet. Res. Commun. 32, 543-552.

Okine E.K., Mathison G.W., Kaske M., Kenelly J.J. and Christopherson R.J. (1998). Current understanding of the role of the reticulum and reticulo-omasal orifice in the control of digesta passage from the ruminoreticulum of sheep and cattle. Can. J. Anim. Sci. 78, 15-21.

Osman A.H.R. and Berg R. (1981). Studies on the histogenesis of the tunica mucosa of the stomach of the Egyptian water buffalo (*Bos bubbalis*) 2. Histogenesis of the reticular mucosa. Anat. Anzeiger. 150, 516-520.

Panchamukhi B.G. and Srivastava H.C. (1980). Histogenesis of the reticulum of the buffalo (*Bubbalus bubalis*) stomach. Indian. J. Anim. Sci. 50, 1064-1070.

Pfannkuche H., Schemann M. and Gabel G. (2002). Ruminal muscle of sheep is innervated by non-polarized pathways of cholinergic and nitrergic myenteric neurones. Cell. Tissue. Res. 309, 347-354.

Ramkrishna V. and Tiwari G.P. (1979). Histological and histochemical observations on the forestomach of goat during pre-natal life. Acta. Anat. 103, 292-300.

Redondo E., Regodon S., Masot A.J., Franco A. and Lignereux Y. (1997). The gastrin and CCK cells in the gastrointestinal tract of the sheep during development. A differential study. Rev. Med. Vet. 6, 511-518.

Redondo E., Franco A. and Masot A.J. (2005). Morphometric and immunohistochemical study of the omasum of red deer during prenatal development. J. Anat. 206, 543-555.

Redondo E., Masot A.J., Garcia A. and Franco A. (2011). Ontogenesis of the omasum: a comparative analysis of the Merino sheep and Iberian red deer. Histol. Histopathol. 26, 1135-1144.

Regodon S., Franco A., Masot A.J. and Redondo E. (1996). Estudio ontogénico comparativo de la lámina epitelial de los compartimentos gástricos no glandulares en ovinos merinos. Anat. Histol. Embryol. 25, 233-241.

Reid C.S.W. (1985). The progress of solid feed residues through the rumino-reticulum: the ins and outs of particles, in: Ruminants physiology: concepts and consequences. Baker, S.R., Gawthorne, J.M., Mackintosh, J.B., Purser, D.B. (Eds). Perth: University of Western Australia Press. pp 79-84.

Relling A.E. and Mattioli G.A. (2002). Fisiologia Digestive y Metabolica de los Rumiantes, EDUL ed. Universidad Nacional de La Plata, Buenos Aires.

Ruckebusch Y., Dardillat C. and Guilloteau P. (1983). Development of digestive functions in the newborn ruminant. Ann. Rec. Vet. 14, 360-374.

Scharrer B. (1990). The neuropeptide saga. Amer. Zool. 30, 887-895.

Sivachelvan M.N., Ghali Ali M. and Chibuzo G.A. (1996). Foetal age estimation in sheep and goats. Small. Rum. Res. 19, 69-76.

Teixeira A.F., Wedel T., Krammer H.J. and Kuhnel W. (1998). Structural differences of the enteric nervous system in the cattle forestomach revealed by whole mount immunohistochemistry. Anat. Anz. 180, 393-400.

Vivo J.M., Robina A., Regodon S., Guillen M.T., Franco A. and Mayoral A.I. (1990). Histogenetic evolution of bovine gastric compartments Turing prenatal period. Histol. Histopathol. 5, 461-476.

Wardrop J.D. (1961). Some preliminary observations of the histological development on the forestomach of the lamb. I. Histological changes due to age in the period from 46 days of foetal life to 77 days of postnatal life. J. Agric. Sci. 57, 335-341.

Legends of Figures

Fig. 1. Gross anatomy of the reticular mucosa.

(A) Photograph of reticular mucosa (39 days, 25% gestation). Reticular mucosa was observed as a smooth surface.

(B) Photograph of reticular mucosa (113 days, 75% gestation). Reticular cells were visible and completely formed. Corneum papillae within reticular cells.

Fig. 2. Histomorphogenesis of goat reticular wall (35 to 150 days, 23-100% gestation).

(A) Photomicrograph of section of the differentiated reticulum (35 days, 23% gestation). Two layers of reticular wall were visible: epithelium (E) and pluripotential blastemic tissue (PBT). H-E. Scale Bar: 30 µm.

(B) Photomicrograph of section of reticular wall (50 days, 33% gestation). The wall is composed of three layers: epithelium (E), pluripotential blastemic tissue (PBT) with spindle-shaped myoblastic cells (arrow) and serosa (S). Epithelial layer composed of stratum basale (Eb) and stratum granulosum (Eg). H-E. Scale Bar: 30 µm.

(C) Photomicrograph of section of reticular wall (59 days, 40% gestation). The wall comprises four layers: epithelium (E) with stratum basale (Eb) and stratum granulosum (Eg), lamina propria and submucosa (Lp+Sb), tunica muscularis (TM), and serosa (S). Primary reticular crests (Cr1) visible as evaginations of the epithelial stratum basale. H-E. Scale Bar: 30 µm.

(D) Photomicrograph of section of reticular wall (64 days, 43% gestation). Growth of the primary crest (Cr1) involving the lamina propria (Lp + Sb). Tunica muscularis (TM) formed by an internal bundle of obliquely-arranged muscle fibers and an external bundle of longitudinal fibers. H-E. Scale Bar: 25 µm.

(E) Photomicrograph of section of reticulum wall (87 days, 61% gestation). Corneum papillae (PC) visible in primary reticular crests (Cr1). H-E. Scale Bar: 25 µm.

(F) Photomicrograph of section of reticulum wall (101 days, 67% gestation). Primary (Cr1) and secondary crests (Cr2) visible. Fusion of reticular crests to form reticular cells (RC). Presence of the muscularis mucosae (Mm) in the primary reticular crest. Tunica muscularis visible (TM), formed by two smooth muscle fiber bundles and the serosa (S) H-E. Scale Bar: 25 µm.

(G) Photomicrograph of section of reticular wall (113 days, 75% gestation). Wall formed by epithelium (E), lamina propria and submucosa (Lp+Sb), tunica muscularis

(TM); primary (Cr1) and secondary crests (Cr2) visible. Muscularis mucosae (Mm) visible within primary crests (Cr1). H-E. Scale Bar: 30 µm.

(H) Photomicrograph of a transversal section of reticulum wall (150 days, 100% gestation). Fully-formed reticular cells visible (RC). H-E. Scale Bar: 30 µm.

Fig 3. Mathematical models of prenatal growth of reticular wall tissue layers in goats: y = thickness of each tissue layer (micrometers); x = crown-rump length (centimeters) and r^2 = linear coefficient of determination.

(A) Mathematical model of epithelium growth.

(B) Mathematical model of lamina propria and submucosa growth.

(C) Mathematical model of tunica muscularis growth.

(D) Mathematical model of serosa growth.

Fig. 4. Immunohistochemical findings in goat reticulum during prenatal development.

(A) Photomicrograph of section of reticulum wall (53 days, 35% gestation). SYP-stained cells in tunica muscularis and serosa (arrow) and a low inmunoreactivity in lamina propria+submucosa. HRP. Scale Bar: 20 µm.

(B) Photomicrograph of section of reticulum wall (75 days, 50% gestation). SYP-stained cells in lamina propria + submucosa, tunica muscularis and myenteric plexus (arrow) and a low immunoreactivity in lamina propia + submucosa. HRP. Scale Bar: 25 µm.

(C) Photomicrograph of section of reticulum wall (64 days, 43% gestation). GFAP-stained glial cells in lamina propria + submucosa, tunica muscularis and myenteric and submucosal plexuses (arrows) and a low inmunoreactivity in lamina propria+submucosa. HRP. Scale Bar: 30 µm.

(D) Photomicrograph of section of reticulum wall (113 days, 75% gestation). GFAP-stained glial cells in tunica muscularis and myenteric plexus (arrows) and low immunoreactivity in lamina propria+submucosa. HRP. Scale Bar: 30 µm.

(E) Photomicrograph of section of reticulum wall (38 days, 25% gestation). VIM-stained glial cells in mesenchymal layer of pluripotential blastemic tissue and serosa. HRP. Scale Bar: 20 µm.

(F) Photomicrograph of section of reticulum wall (113 days, 75% gestation). VIM-stained glial cells in myenteric and submucosal plexuses, tunica muscularis and perivascular connective tissue (arrow). HRP. Scale Bar: 30 µm.

(G) Photomicrograph of section of reticulum wall (75 days, 50% gestation). NPY-stained cells in lamina propria + submucosa and myenteric plexus (arrows). HRP. Scale Bar: 20 µm.

(H) Photomicrograph of section of reticulum wall (75 days, 50% gestation). VIP-stained cells in lamina propria + submucosa (small arrows), tunica muscularis and myenteric plexus (arrow). HRP. Scale Bar: 25 µm.

Fig. 5. Scanning electron microscopy of the goat reticulum (38 to 150 days, 25-100% gestation).

(A) Photomicrograph of section of reticulum wall (38 days, 25% gestation). Smooth surface displaying no evidence of keratinisation or desquamation. SEM. Scale Bar: 8 µm.

(B) Photomicrograph of section of reticulum wall (64 days, 43% gestation). First primary reticular crests (Cr1) protruding from the surface. SEM. Scale Bar: 6 µm.

(C) Photomicrograph of section of reticulum wall (75 days, 50% gestation). Primary reticular crests (Cr1) growing from surface. SEM. Scale Bar: 6 µm.

(D) Photomicrograph of section of reticulum wall (113 days, 75% gestation). Corneum papillae (PC) and muscularis mucosae (Mm) visible in the primary reticular crests (Cr1). SEM. Scale Bar: 4µm.

(E) Photomicrograph of section of reticulum wall (120 days, 80% gestation). Fusion of primary reticular crests (Cr1) to form reticular cell. SEM. Scale Bar: 2 µm.

(F) Photomicrograph of section of reticulum wall (150 days, 100% gestation). Fully-formed reticular cells (RC) and presence of desquamation in the corneum papillae (PC). SEM. Scale Bar: 2 µm.

Table 1. Thickness of the tissue layer in the reticulum of goat during prenatal development (µm).

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

Table 1. Thickness of the tissue layer in the reticulum of goat during prenatal development (μm).

	Group I	Group II	Group III	Group IV	Group V
E	368.53±5	442.35±8[a]	564.12±13[a]	1228.28±21[a]	2338.12±33[a]
Lp + Sb	pbt[*]	pbt	263.33±14	343.67±24[b]	578.86±37[b]
TM	pbt	pbt	484.77±22	1119.23±27[b]	1664.12±41[b]
S	111.31±6[c]	115.29±12[c]	95.44±13[c]	107.92±8[c]	181.36±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: 35-50% 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; [b]P<0.005 vs Group III; [c]P<0.005 vs Group V.

Table 2. Immunohistochemical findings in the reticulum 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	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	++	+	+

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: vimentin, VIP: vasoactive intestinal peptide, NPY: neuropeptide Y. -, non immunoreactivity; +, low immunoreactivity; ++, moderate immunoreactivity; +++, high immunoreactivity.

Figures

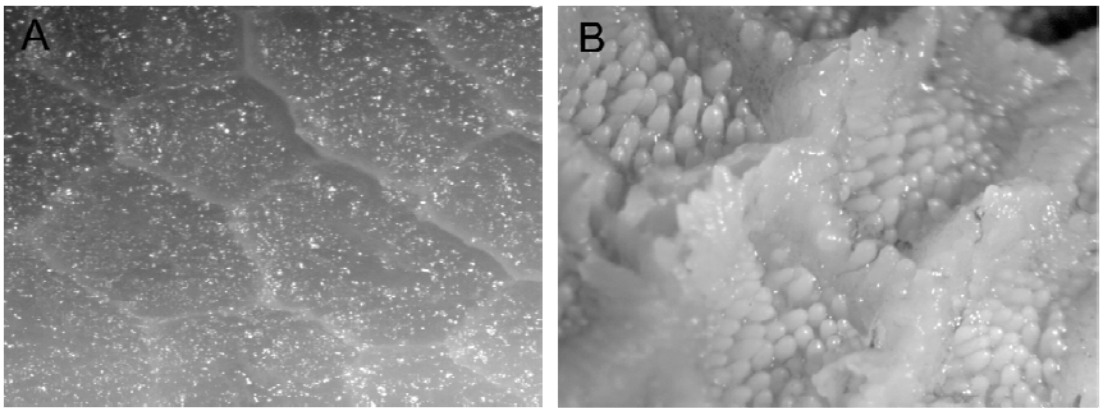
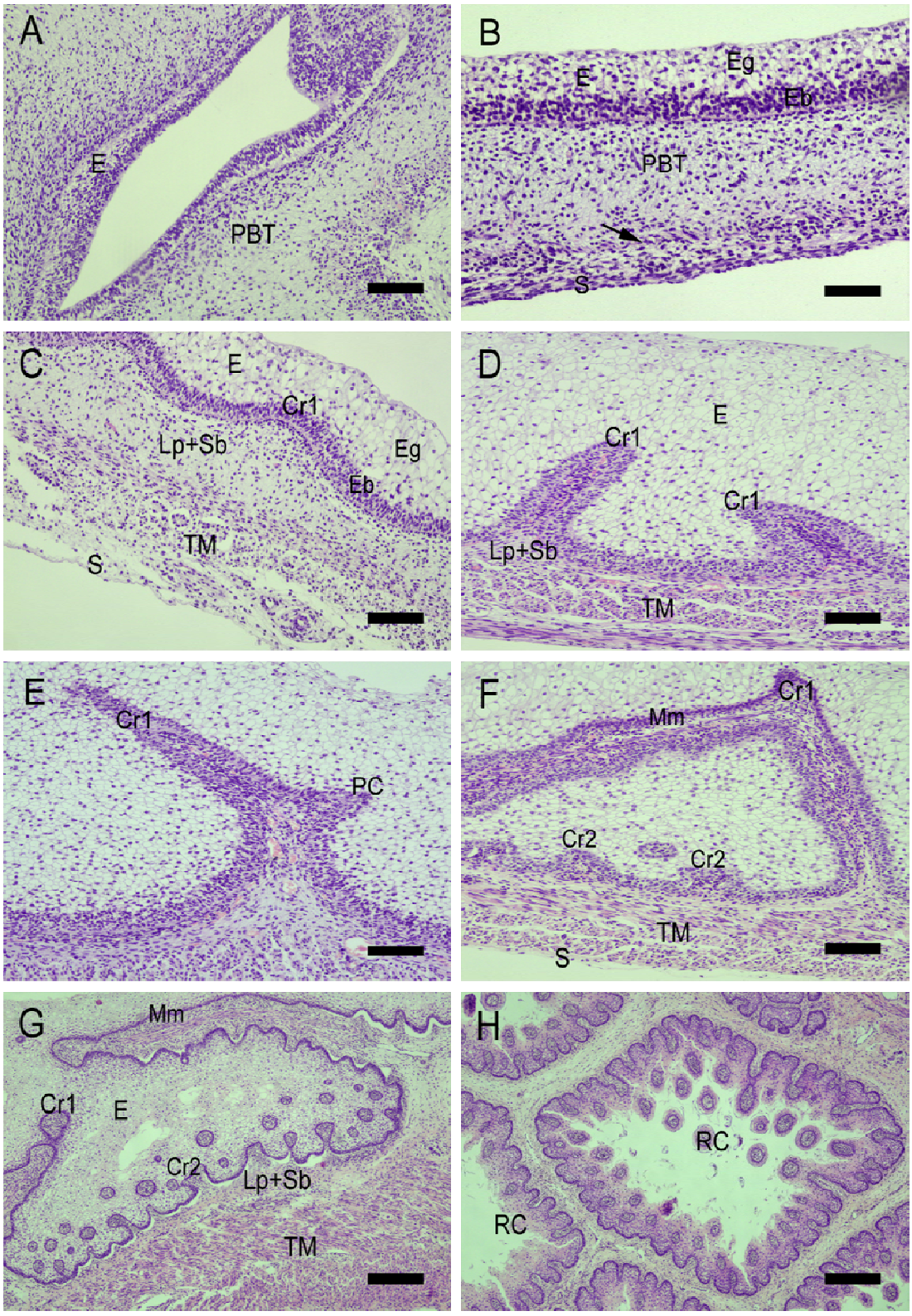


Figure 1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B
E
Eg
Eb
PBT
S
C
E
Cr1
Lp+Sb
Eg
Eb
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S
D
E
Cr1
Cr1
Lp+Sb
TM
E
Cr1
PC
F
Cr1
Mm
Cr2
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TM
S
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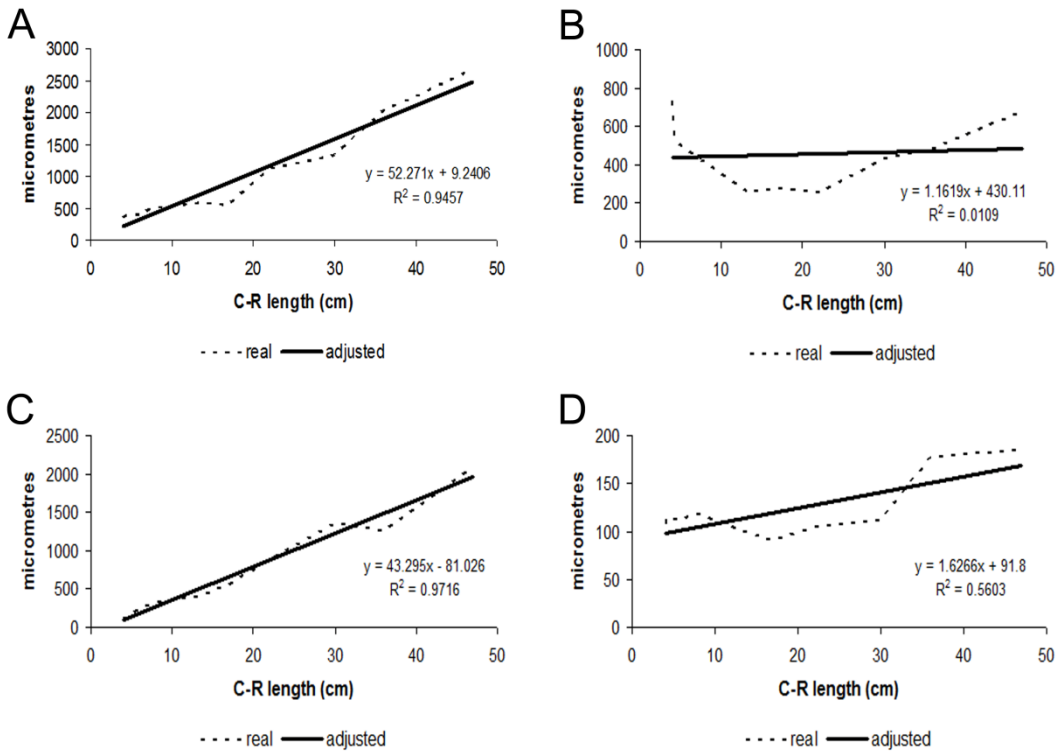
Cr1
E
Cr2
Lp+Sb
TM
H
RC
RC

Figure 2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R2 = 0.9457
real
adjusted
B
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C-R length (cm)
y = 1.1619x + 430.11
R2 = 0.0109
real
adjusted
C
micrometres
C-R length (cm)
y = 43.295x - 81.026
R2 = 0.9716
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adjusted
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micrometres
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R2 = 0.5603
real
adjusted

Figure 3

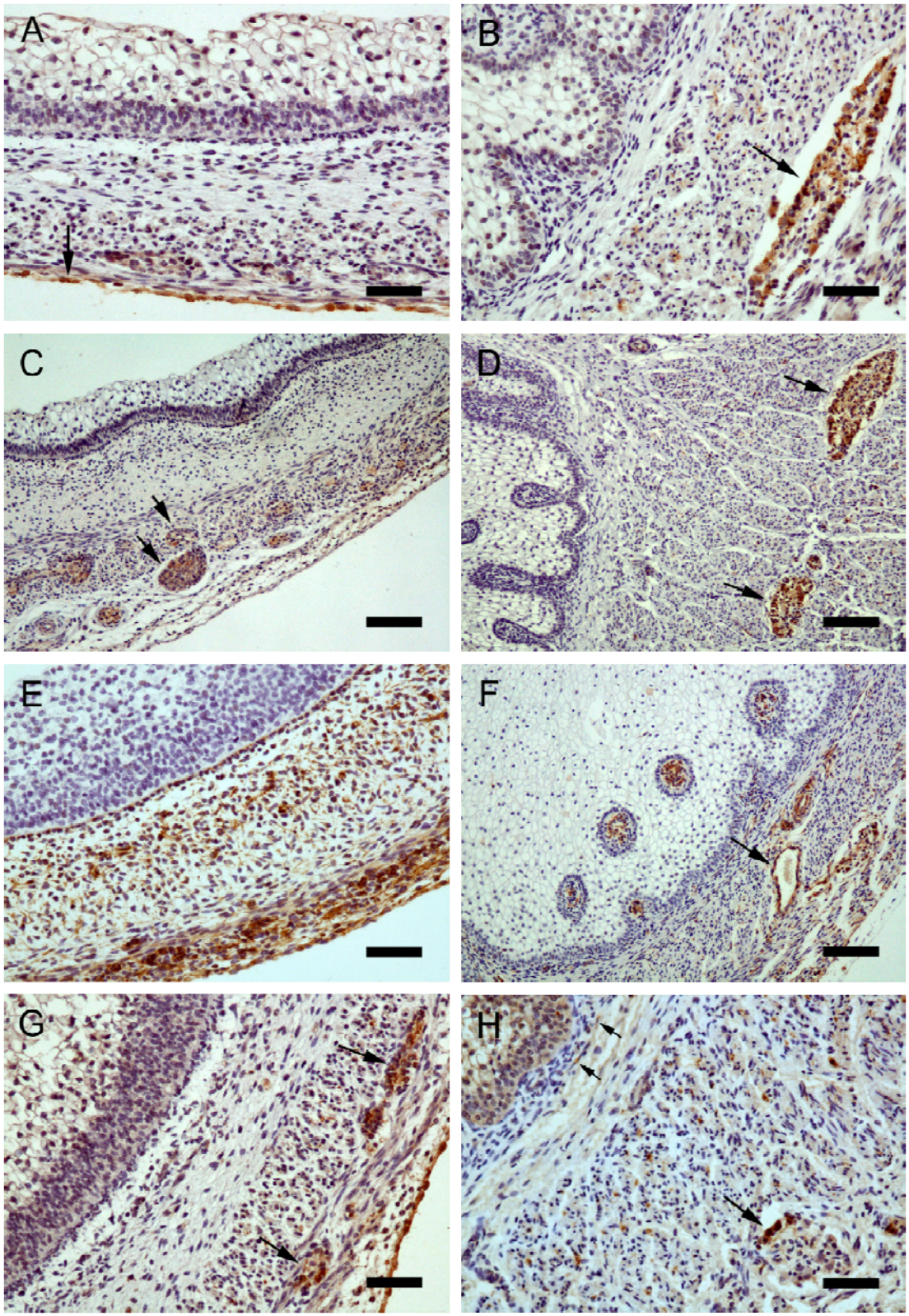

Figure 4

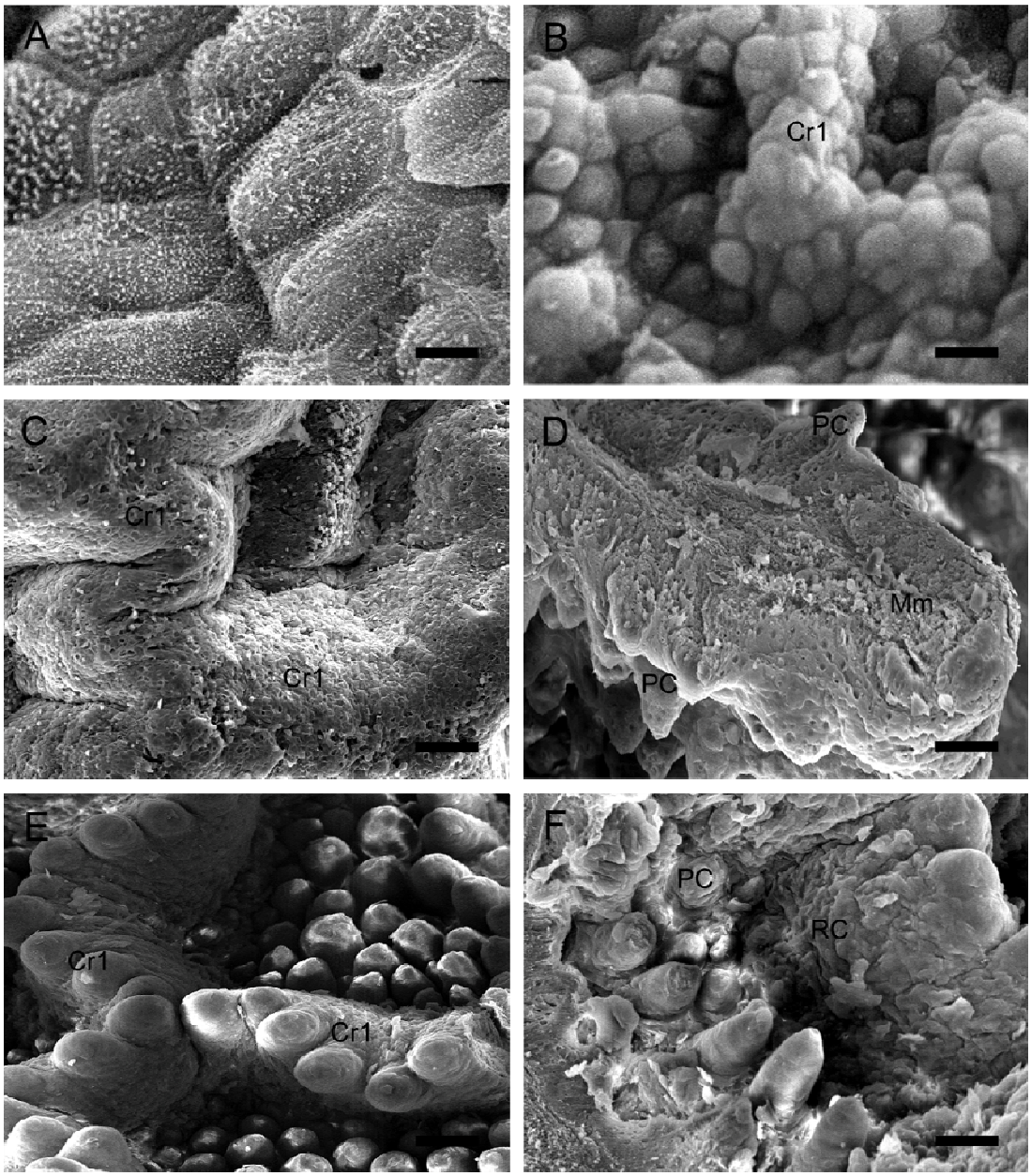

Figure 5

Artículo 4


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Dear Doctors,

We are pleased to inform you that the paper entitled "Histomorphometric and immunohistochemical study of the goat abomasums during prenatal development" (authors: A.García, J.Masot, A.Franco, A.Gázquez and E.Redondo) is acceptable for publication in HISTOLOGY AND HISTOPATHOLOGY, but it is necessary that you prepare the paper according to the editor's and referee's comments.

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Histomorphometric and immunohistochemical study of the goat abomasum during prenatal development

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Short running title: goat abomasum during prenatal development

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, Capra hircus, immunohistochemistry, prenatal development

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,b,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 et al., 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 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 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).

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, 3b).

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 (1987). 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. (2007) 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 those of Molinari and Jorquera (1987),

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 11^{th} and 17^{th} 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.

Acknowledgements

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References

Age H.M., Bhosale N.S., Kadam S.D. and Kpadnis P.J. (2007). Histomorphological study of abomasums in cattle. Indian J. Anim. Res. 41, 192-195.

Al-Saffar F.J. (2012). Effect of different diets on number and distribution of gastrin cells in the mucosa of goat´s abomasums. Asian J. Anim. Sci. 6, 110-121.

Age H.M., Bhosle N.S. and Kapadnis P.J. (2008). Microscopic study of abomasum in sheep. Indian J. Anim. Res. 42, 134-135.

Asari M., Ashige H., Wahui S., Fakaya K. and Kano Y. (1985). Histological development of bovine abomasum. Anat. Anz. 159, 1-11.

Boyazoglu J., Hatziminaoglou I. and Morand-Fehr P. (2005). The role of the goat in society: past, present and perspectivas for the future. Small. Ruminat. Res. 60, 13-23.

Dall´Aglio C., Gargiulo A.M., Pedini V. and Ceccarelli P. (1999). Presence and distribution of neuroendocrine cells in the gastroenteropacnreatic endocrine system of fallow deer foetuses. Anat. Histol. Embryol. 28, 331-336.

Del Rio Ortega S. (1973). Desarrollo prenatal del estómago de la oveja. Doctoral thesis, Facultad de Veterinaria, Zaragoza, Spain.

El-Gendy, S.A.A. and Derbalah, A. (2010). Macroscopic and microscopic anatomy of the omasum of the Baladi goat. J. Biol. Sci. 10, 596-607.

Evans H.E. and Sack W.O. (1973). Prenatal development of domestic and laboratory mammals: Growth curves, external features and selected references. Anat. Histol. Embryol. 2, 11-45.

Fath El-Bab M.R., Schwart R. and Ali A.M.A. (1983). Micromorphological studies on the stomach of sheep during prenatal life. Anat. Histol. Embryol. 12, 139-53.

Franco A., Vivo J.M., Guillen M.T., Regodon S. and Robina A. (1989). Evolución parietal del retículo ovino de raza merina desde los 68 días de gestación hasta el nacimiento. Histol. Med. 5, 57-58.

Franco A., Regodon S., Robina A. and Redondo E. (1992). Histomorphometric analysis of the rumen of the sheep during development. Am. J. Vet. Res. 53, 1209-1217.

Franco A., Robina A., Regodon S., Vivo J.M., Masot A.J. and Redondo E. (1993a). Histomorphometric analysis of the omasum of sheep during development. Am. J.Vet. Res. 54, 1221-1229.

Franco A., Robina A., Guillen M.T., Mayoral A.I. and Redondo E. (1993b). Histomorphometric analysis of the abomasum of sheep during development. Anat. Anz. 175, 119-125.

Franco A., Robina A., Regodon S., Vivo J.M., Masot A.J. and Redondo E. (1993c). Histomorphometric analysis of the reticulum of the sheep during development. Histol. Histopathol. 8, 547-556.

Franco A., Regodon S., Gazquez A., Masot A.J. and Redondo E. (1993d). Ontogeny and distribution of gastrin cells in the gastrointestinal tract of the sheep. Eur. J. Histochem. 37, 83-90.

Franco A., Regodon S., Masot A.J. and Redondo E. (1997). A combined immunohistochemical and electron microscopic study of the second cell type in the developing sheep pineal gland. J. Pineal. Res. 22, 130-136.

Franco A., Masot A.J., Gómez L. and Redondo E. (2004a). Morphometric and immunohistochemical study of the rumen of red deer during prenatal development. J. Anat. 204, 501-513.

Franco A., Redondo E. and Masot A.J. (2004b). Morphometric and immunohistochemical study of the reticulum of red deer during prenatal development. J. Anat. 205, 277-289.

Franco A., Masot A.J. and Redondo E. (2011). Ontogenesis of the rumen: a comparative analysis of the merino sheep and Iberian red deer. Anim. Sci. J. 82, 107-116.

Franco A., Masot A.J., García A. and Redondo E. (2012). Ontogenesis of the reticulum with special reference to neuroendocrine and glial cells: a comparative analysis of the Merino sheep and Iberian red deer. Anat. Histol. Embryol. 41, 362-373.

Garcia A., Masot J., Franco A., Gazquez A. and Redondo E. (2012). Histomorphometric and immunohistochemical study of the goat rumen during prenatal development. Anat. Rec. 295, 776-785.

Garcia-Gonzalez R. and Cuartas P. (1992). Food habits of *Capra pyrenaica*, *Cervus elaphus* and *Dama dama* in the Cazorla Sierra (Spain). Mammalia. 56, 195-202.

Gihad E.A., El-Bedawy T.M. and Mehrez A.Z. (1980). Fiber digestibility by goats and sheep. J. Dairy. Sci. 63, 1701-1706.

Groenewald H.B. (1994). Neuropeptides in the myenteric ganglia and nerve fibres of the forestomach and abomasums of grey, white and black karakul lambs. Onderstepoort. J. Vet. Res. 61, 207-213.

Hofmann R.R. (1973). The Ruminant Stomach. Stomach structure and feeding habits of East African game ruminants. East African Literature Bureau, Nairobi.

Lee J.H., Huh C.K., Kim C.S. and Kwak S.D. (1994). Development of abomasums of fetuses and neonates in Korean native goats. Korean J. Vet. Res. 34, 219-227.

Lombardi G. (2005). Optimum management and quality pastures for sheep and goat in mountain areas. Options mediterraneennes, 67.

Molinari E. and Jorquera B. (1988). Intrauterine development stages of the gastric compartments of the goat (*Capra hircus*). Anat. Histol. Embryol. 17, 121-137.

Masot A.J., Franco A. and Redondo E. (2007a) Morphometric and immunohistochemical study of the abomasum of red deer during prenatal development. J. Anat. 211, 376-386.

Masot A.J., Franco A. and Redondo E. (2007b). Comparative analysis of the forestomach mucosa in red deer during prenatal development. Revue. Méd. Vét. 7, 397-409.

McSweeney C.S. (1988). A comparative study of the anatomy of the omasum in domesticated ruminants. Aust. Vet. J. 65, 205-207.

Mutoh K. and Wakuri H. (1989). Early organogenesis of the caprine stomach. Nippon. Juigaku. Zasshi. 51, 474-484.

Newburg D.S. and Walker W.A. (2007). Protection of the neonate by the innate immune system of developing gut and of human milk. Pediatr. Res. 61, 2-8.

Nickel R., Schummer A. and Seiferle E. (1973). The Viscera of the Domestric Mammals. Berlin. Verlage. Paul Parey.

Nwaogu C.I. and Ezeasor N.D. (2008). Studies on the development of omasum in west African dwarf goats (*Capra hircus*). Vet. Res. Commun. 32, 543-552.

Otani H., Yoneyama T., Hashimoto R., Hatta T. and Tanaka O. (1993). Ultrastructure of the developing stomach in human embryos. Anat. Embryol. 187, 145-151.

Ramkrishna V. and Tiwari G.P. (1979). Histological and histochemical observations on the forestomach of goat during pre-natal life. Acta. Anat. 103, 292-300.

Rancourt M., Fois N., Lavin M.P., Tchakerian E. and Vallerand F. (2006). Mediterranean sheep and goats production: an uncertain future. Small. Ruminat. Res. 62, 167-179.

Redondo E., Regodon S., Masot A.J., Franco A. and Lignereux Y. (1997). The gastrin and CCK cells in the gastrointestinal tract of the sheep during development. A differential study. Rev. Med. Vet. 6, 511-518.

Redondo E., Franco A. and Masot A.J. (2005). Morphometric and immunohistochemical study of the omasum of red deer during prenatal development. J. Anat. 206, 543-555.

Redondo E., Masot J., Garcia A. and Franco A. (2011). Ontogenesis of the omasum: a comparative analysis of the Merino sheep and Iberian red deer. Histol. Histopathol. 26, 1135-1144.

Regodon S., Franco A., Masot A.J. and Redondo E. (1996). Estudio ontogénico comparativo de la lámina epitelial de los compartimentos gástricos no glandulares en ovinos merinos. Anat. Histol. Embryol. 25, 233-241.

Sivachelvan M.N., Ghali Ali M. and Chibuzo G.A. (1996). Foetal age estimation in sheep and goats. Small. Rum. Res. 19, 69-76.

Vivo J.M., Robina A., Regodon S., Guillen M.T., Franco A. and Mayoral A.I. (1990). Histogenetic evolution of bovine gastric compartments during prenatal period. Histol. Histopathol. 5, 461-476.

Wardrop J.D. (1961). Some preliminary observations on the histological development on the forestomach of the lamb. I. Histological changes due to age in the period from 46 days of foetal life to 77 days of postnatal life. J. Agric. Sci. 57, 335-341.

Yu H.G., Nam J.O., Miller N.L.G., Tanjoni I. and Waslh C., Shei L., Kim L., Chen X.L., Toma A., Kim S.T. and Schlaepfer D.D. (2011). P190RhoGEF (Rgnef) promotes colon carcinoma tumor progression via interaction with focal adhesion hinase. Cancer Res. 71, 360-370.

Legends of figures

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. Bar: 25 µm.

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. Bar: 25 µm.

c. Photomicrograph of section of abomasal wall at 64 days (43% gestation). Simple columnar epithelium visible (E). H-E. Bar: 20 µm.

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. Bar: 30 µm.

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. Bar: 20µm.

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. Bar:30 µm.

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. Bar: 20 µm.

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. Bar: 30 µm.

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).

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. Bar: 25 µm.

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. Bar: 25 µm.

c. Photomicrograph of section of abomasal wall (38 days, 25% gestation). VIM-immunoreactive glial cells in mesenchymal layer of pluripotential blastemic tissue. HRP. Bar: 30 µm.

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. Bar: 25 µm.

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. Bar: 25 µm.

f. Photomicrograph of section of abomasal wall (75 days, 50% gestation). VIP-immunoreactivite lamina propria + submucosa, tunica muscularis, serosa and myenteric plexus (arrows). HRP. Bar: 20 µm.

g. Photomicrograph of section of abomasal wall (150 days, 100% gestation). Gastrin-immunoreactive cells visible in gastric glands (arrows). HRP. Bar: 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. Bar: 8 µm.

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. Bar: 8 µm.

c. Photomicrograph of section of abomasal wall (64 days, 43% gestation). Abomasal folds have increased in size (Af). SEM. Bar: 6 µm.

d. Photomicrograph of section of abomasal wall (75 days, 50% gestation). Abomasal folds (Af) larger and more numerous. SEM. Bar: 6 µm.

e. Photomicrograph of section of abomasal wall (113 days, 75% gestation). Gastric pits (arrows) visible as small depressions on the surface of folds. SEM. Bar: 6 µm.
f. Photomicrograph of section of abomasal wall (150 days, 100% gestation). Abomasal mucosa showing fully-developed folds and mucous residue. SEM. Bar: 4µm.

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

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

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[ab]	215.78±7[ab]

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.

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.

Figures

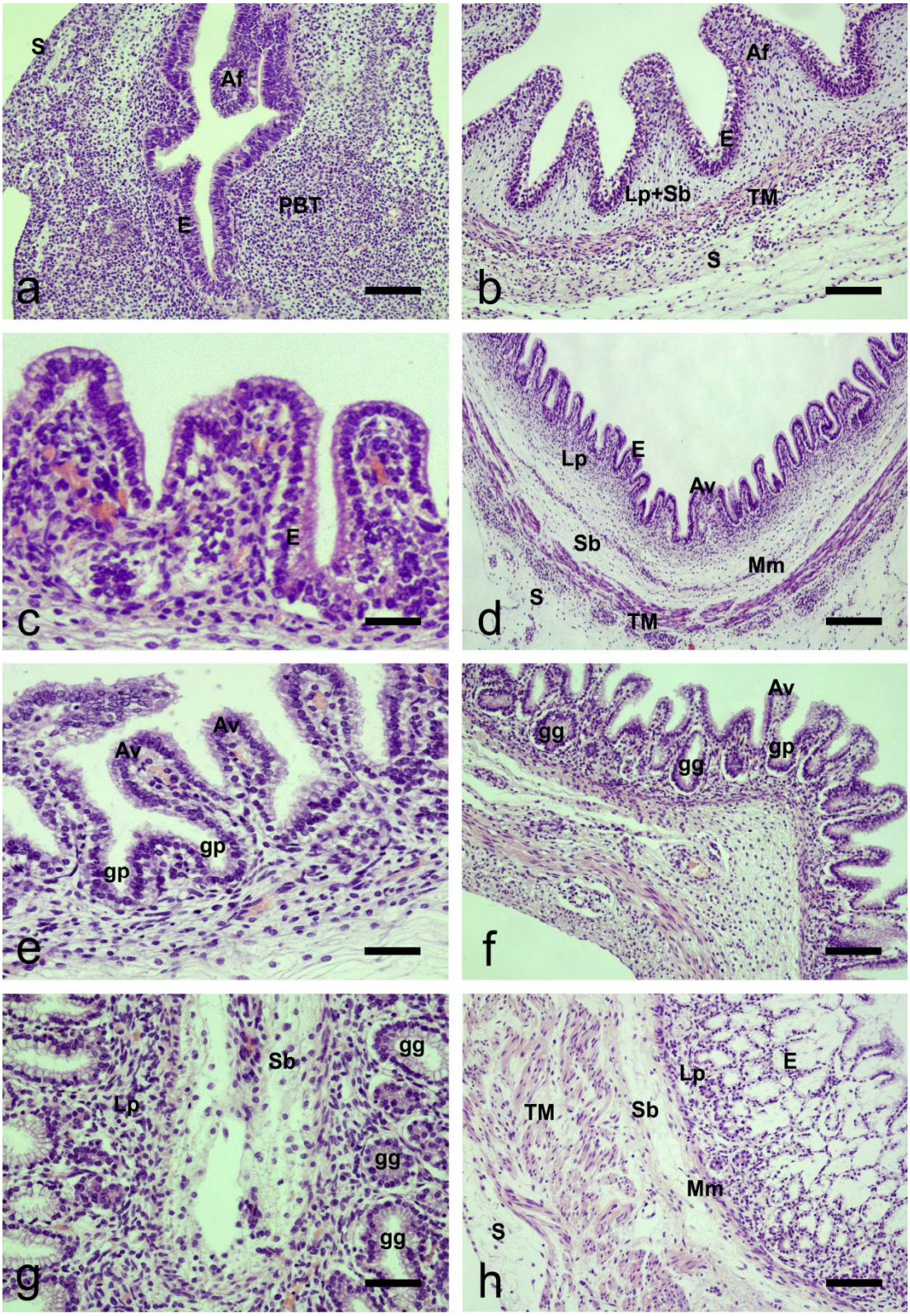
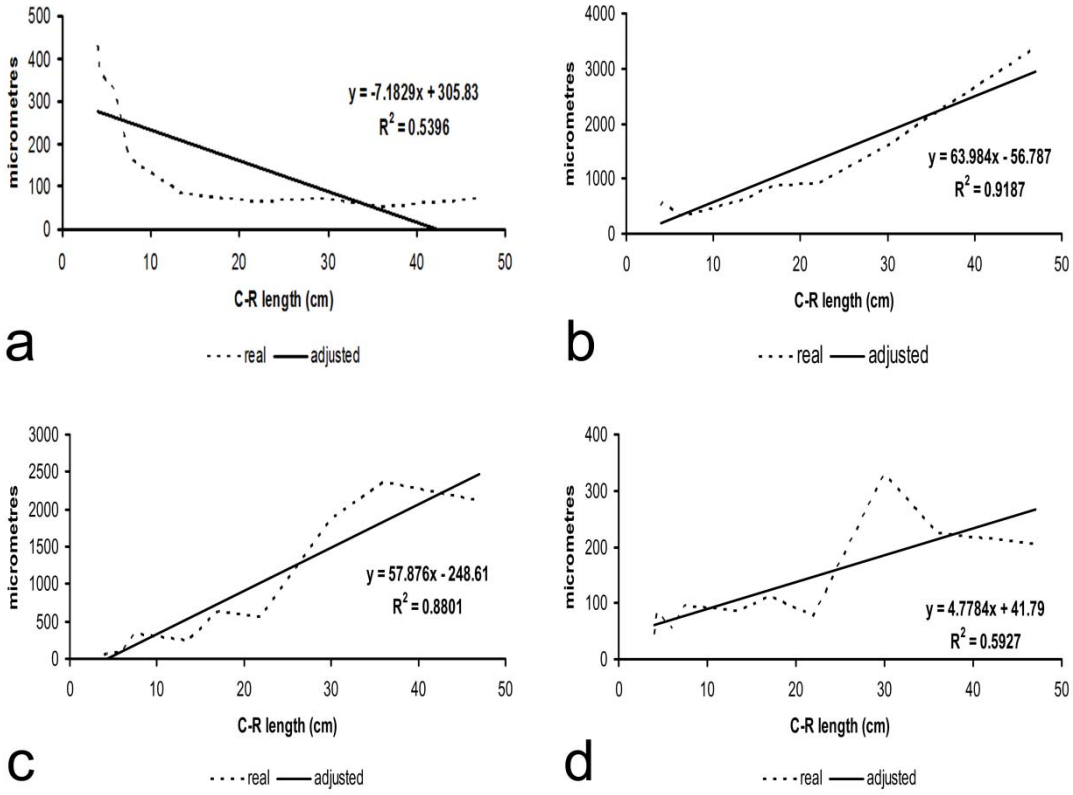


Figure 1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R2 = 0.5396
a
real
adjusted
y = 63.984x - 56.787
R2 = 0.9187
b
y = 57.876x - 248.61
R2 = 0.8801
c
y = 4.7784x + 41.79
R2 = 0.5927
d

Figure 2

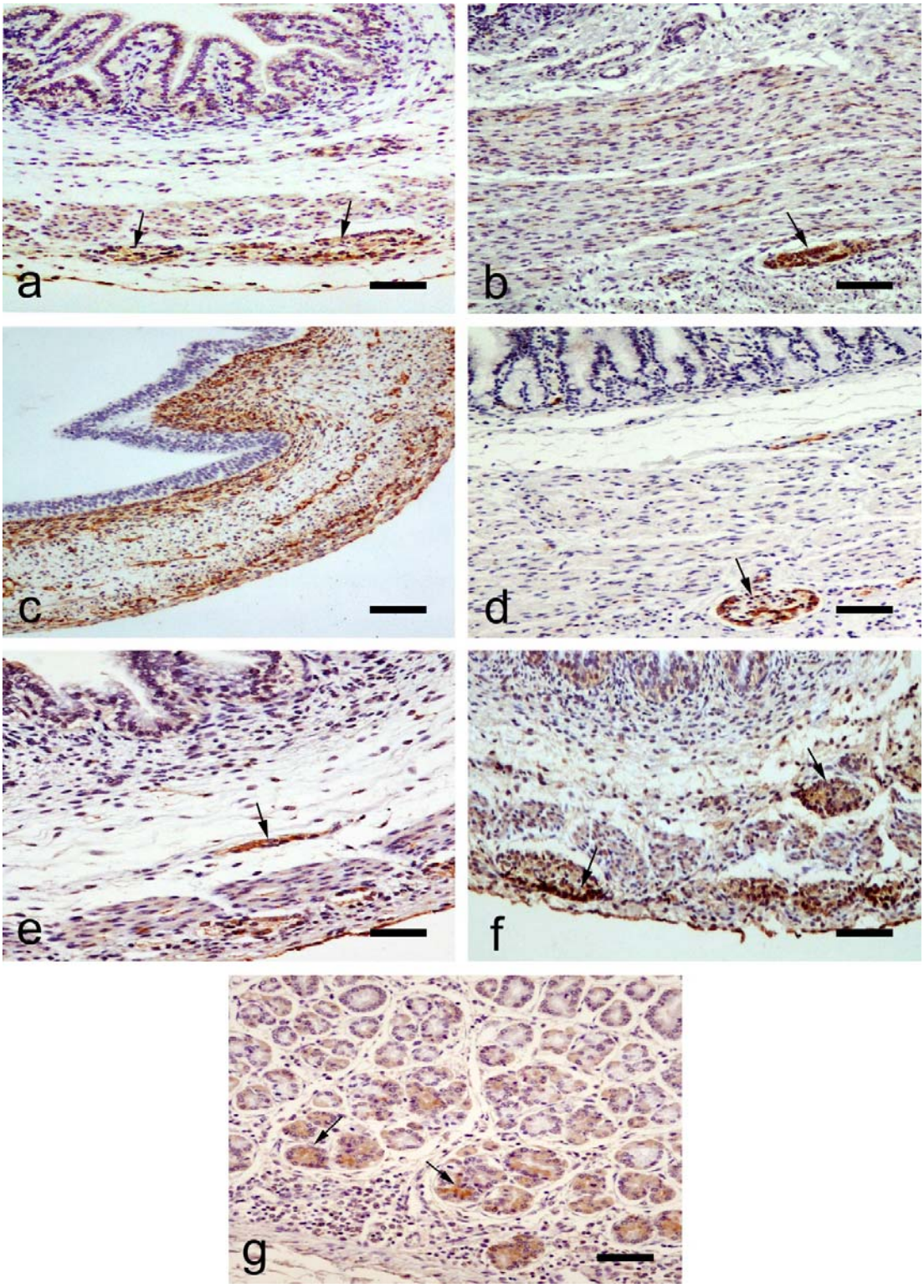

Figure 3

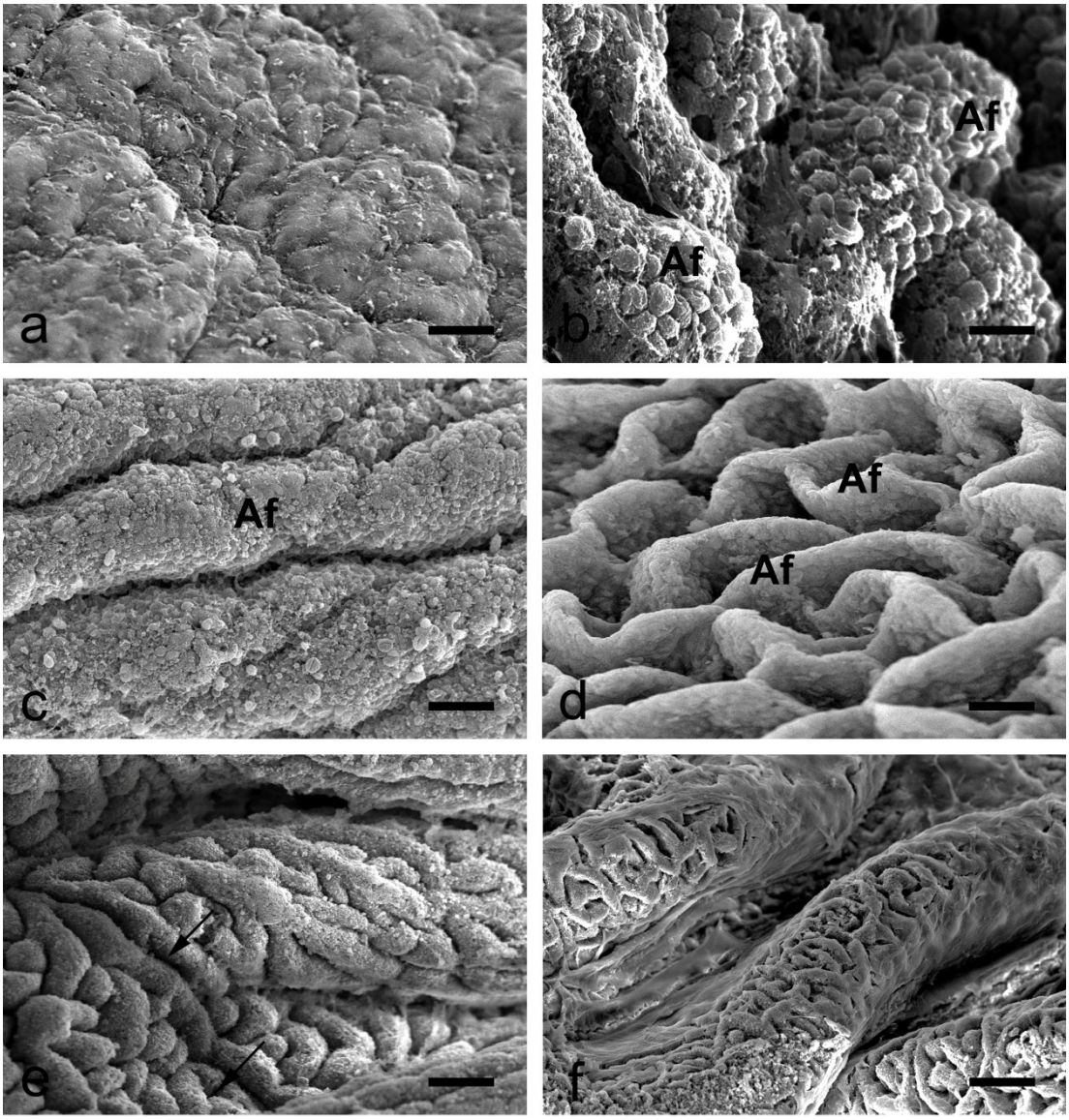

Figure 4

Artículo 5

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- Manuscript Title : Immunohistochemical study of the goat forestomach during prenatal development
- Abstract :
  This paper reports on the detection and distribution of immunoreactivity to synaptophysin (SPY), non-neuronal enolase (NNE), glial fibrillary acidic protein (GFAP), vimentin (VIM), neuropeptide Y (NPY) and vasoactive intestinal peptide (VIP) in the goat forestomach during prenatal life. A total of 140 embryos and fetuses were used, from the first stage of prenatal life until birth. Synaptophysin immunoreactivity was detected at 53 days' gestation in lamina propria-submucosa, tunica muscularis, serosa and myenteric plexuses in all cases. Immunoreactivity to non-neuronal enolase was observed at 64 days' gestation in the same locations, and additionally in the epithelial layer. Glial cells were detected at 64 days by glial fibrillary acidic protein and at 39 days by vimentin. Positive staining for neuropeptide Y and vasoactive peptide intestinal were observed at 113 days, 75 days and 95 days in the rumen, reticulum and omasum, respectively, in both cases in the lamina propria-submucosa, tunica muscularis and myenteric plexuses. These findings point to a possible function of the fetal goat forestomach in preparation for its postnatal function. Compared to other ruminant species, these cell types were detected earlier than in sheep, but at around the same stage as in deer.
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It is our great pleasure to inform you that your revised manuscript, JVS-13-048.R1, entitled "Immunohistochemical study of the goat forestomach during prenatal development " is now accepted by our journal, Journal of Veterinary Science.

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Sincerely,

Immunohistochemical study of the goat forestomach during prenatal development

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Abstract

This paper reports on the detection and distribution of immunoreactivity to synaptophysin (SPY), non-neuronal enolase (NNE), glial fibrillary acidic protein (GFAP), vimentin (VIM), neuropeptide Y (NPY) and vasoactive intestinal peptide (VIP) in the goat forestomach during prenatal life. A total of 140 embryos and fetuses were used, from the first stage of prenatal life until birth. Synaptophysin immunoreactivity was detected at 53 days' gestation in lamina propria-submucosa, tunica muscularis, serosa and myenteric plexuses in all cases. Immunoreactivity to non-neuronal enolase was observed at 64 days' gestation in the same locations, and additionally in the epithelial layer. Glial cells were detected at 64 days by glial fibrillary acidic protein and at 39 days by vimentin. Positive staining for neuropeptide Y and vasoactive peptide intestinal were observed at 113 days, 75 days and 95 days in the rumen, reticulum and omasum, respectively, in both cases in the lamina propria-submucosa, tunica muscularis and myenteric plexuses. These findings point to a possible function of the fetal goat forestomach in preparation for its postnatal function. Compared to other ruminant species, these cell types were detected earlier than in sheep, but at around the same stage as in deer.

Keywords: forestomach; goat; immunohistochemistry; prenatal development

Introduction

The ruminant stomach is particularly remarkable for its ability to transform low-quality forage into products of great nutritional value [22]. It is subdivided into four compartments: rumen, reticulum, omasum and abomasum. Each compartment is characterized by certain unique gross and histological features [37] reflecting its morphological and functional adaption to the ingestion, processing and digestion of plant material. Before passing into the abomasum, the last and only truly secretory part of the ruminant stomach, ingesta are previously exposed to the actions of the other three forestomach compartments [40], which play a major role in the bacterial digestion of cellulose, acting as fermentation chambers and at the same time ensuring propulsive peristaltic movements, contractions and the separation of fluids and solids [41]. These motor functions require not only coordinated control of the central nervous system, but also the presence of an elaborate enteric nervous system [34].

The enteric nervous system is composed of ganglionate plexuses containing neurons and glia, situated between the smooth muscle layers of the digestive wall [14]. The functioning of enteric neurons depends on the presence of neurotransmitters [4, 21, 24]. Neuropeptides such as acetylcholine, susbstance P, neuropeptide Y and vasoactive intestinal peptide act as putative neurotransmitters in the enteric neurons [4, 24, 36].

Glial cells are the non-neuronal elements of the enteric nervous system. They are similar to astrocytes of the central nervous system, and most of them are positive for glial fibrillary acidic protein [23, 35, 42] and vimentin [20]. They regulate and maintain enteric neuronal function and support intestinal barrier function [41]

The enteric nervous system also contains endocrine cells capable of secreting hormones. These play a key role in the overall regulation of digestive processes such as nutrient absorption and homeostasis of the carbohydrate metabolism [5].

Immunohistochemical studies have detected neuropeptides [15, 17, 18, 25, 29, 30, 31, 43], glial cells [40] and endocrine cells [2] in the forestomach of adult ruminants. However, there has been little research into the prenatal development of these cell types in the stomach of ruminants such as sheep [43] and deer [8, 9, 10, 11, 32, 33], and virtually none in goats.

The present study used immunohistochemical techniques to chart the presence and distribution of the neuroendocrine cell markers synaptophysin (SPY) and non-neuronal enolase (NNE), the glial cell markers glial fibrillary acidic protein (GFAP) and vimentin (VIM), and the peptidergic innervation markers neuropeptide Y (NPY) and vasoactive intestinal peptide (VIP) in the goat forestomach during prenatal development.

Material and Methods

Animals

A total of 140 goat (*Capra hircus*) embryos and fetuses, ranging from the earliest 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 a municipal slaughterhouse in Caceres (Spain), from pregnant goat females. These pregnant females were slaughtered by the usual process in the slaughterhouse specify. Embryos and fetuses were obtained after opening the abdominal cavity uterus and placenta. 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 age classification methods previously reported for sheep and goats [6, 39]. Six specimens were selected from each group for immunohistochemical analysis. In group I, 2 fetuses belong to the age of 35 days, 2 in 36 days and 2 for 38 days; in group II, 3 fetuses of 39 days, 2 of 46 days and 2 for 50 days were selected; in group III, 2 fetuses of 64 days, 3 of 68 days and 2 of 70 were chosen; in group IV, 2 fetuses of 75 days, 2 of 87 days and 2 of 95 days were selected; in group V, 2 fetuses of 113 days, 2 of 120 days and 2 of 150 days were chosen.

Sampling and processing

Forestomach was extracted and small pieces of tissue were dissected for analysis. Tissue samples were washed in phosphate-buffered saline (PBS) and fixed with 4% buffered formaldehyde for 24 hours, routinely processed and embedded in paraffin; sections 5µm thick were cut from the resulting block.

Immunohistochemical analysis

The UltraVision One HRP polymer system (polymer conjugated with horseradish peroxidase) was performed on tissue from forestomach to detect the neuroendocrine cell markers SYP and NNE, glial cell markers GFAP and VIM, and peptidergic innervation markers NPY and VIP. Tissue were deparaffinized and hydrated. For antigen retrieval, sections were performed in microwave-heated in buffer citrate solution 0.01M pH 6, for 5 minutes at 800 watts of power. Endogenous peroxidase activity was blocked by 0.5% hydrogen peroxide for 30min. Non-specific tissue binding sites were blocked by incubation in 1% normal goat serum for 30 min. Samples were incubated for 30 min at room temperature with the following primary antibodies: 1:10 mouse monoclonal anti-SPY (Thermo Scientific, MA1-35810); anti-non neuronal enolase (AbD Serotec, 6880-0410); ready-to-use rabbit polyclonal anti-GFAP (Thermo Scientific, 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). Sections were then incubated with horseradish peroxidase-conjugated polymer (Thermo Scientific, UltraVision ONE HRP Polymer, TL-015-PHJ) for 30 minutes at room temperature, without exposure to light.

Diaminobenzidine was applied to tissues (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, sections were contrasted with Mayer's hematoxylin. The specificity of the staining reaction was determined in control experiments in which the primary antiserum was replaced by PBS.

Statistical analysis

Immunolabeled sections were analyzed using the Nis-Element 2.30 software package. The immunostaining surface was measured for various tissue strata (epithelium, lamina propria and submucosa, tunica muscularis, serosa and myenteric plexus) and for the whole wall. Optimal intervals were performed statistically and four immunoreactivity density categories were established [17]: *no immunoreactivity*: no surface staining; *low immunoreactivity*: stained surface less than 200 μm^2; *moderate immunoreactivity*: stained surface between 200 and 400 μm^2; *intense immunoreactivity*: stained surface over 400 μm^2.

Measurements, expressed in μm^2, 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.

Results

Forestomach histomorphogenesis

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

At 35 days (CRL 3 cm, 23% gestation), rumen, reticulum and omasum became separate compartments of the primitive gastric tube. Its wall was comprised of three layers: an internal stratified epithelium layer, a middle layer of pluripotencial blastemic tissue and an external layer or serosa.

At 38 days (CRL 4.3 cm, 25% gestation), rudimentary primary omasal laminae were visible as small protrusion from the omasal wall.

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

The stratified epithelium was divided into two bands: a stratum basal formed by 2-3 layers of germinal cells with basophilic cytoplasm and a stratum granulosum, comprising 5-6 layers globose cells with light cytoplasm.

At 46 days (CRL 6 cm, 30% gestation), small papilliform projections were observed in ruminal wall to from rudimentary pillars.

At 50 days (CRL 7.7 cm, 33% gestation), the lamina propria and submucosa were differentiated from pluripotencial blastemic tissue. By this stage, the tunica muscularis was distinguishable from pluripopotencial blastemic tissue, in the form of a layer of longitudinal-arranged myoblasts Moreover, secondary omasal laminae began to appear as small elevations between primary laminae. Primary omasal laminae were larger and more numerous, at this age of gestation.

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

The wall of three gastric compartments was formed by four layers: mucosa (composed by epithelium and lamina propria), submucosa, tunica muscularis and serosa.

At 53 days (CRL 9 cm, 35% gestation) slight evaginations of the epithelium were visible to form rudimentary ruminal papillae.

The lamina propria and submucosa were composed of fibroblast with spare ground substance. No separate was apparent between the two layers. The tunica muscularis was formed by two layers: an inner circular layer and an outer longitudinal layer.

At 59 days (CRL 10 cm, 38% gestation), primary reticular crests were visible and tertiary omasal laminae were apparent in the spaces between primary and secondary laminae. The quaternary laminae were also visible at 64 days (CRL 13.5 cm, 43% gestation). At this stage, the muscularis mucosae was observed at the centre of omasal laminae, arising from the inner circular layer of smooth muscle fibers of the tunica muscularis. At 70 days (CRL 15 cm, 47% gestation), conical papillae started to arise from the surface of primary omasal laminae.

The serosa was comprised a subserosa of fibrous loose connective tissue underlying a mesothelial layer of flat cells.

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

In this group, the stratified epithelium comprised four layers: stratum basale was formed of strongly-basophilic cells; stratum granulosum comprised polyhedral cell with light-staining cytoplasm; stratum spinosum was superficial to the stratum granulosum and stratum corneum formed by a single layer of flat cells.

At 76 days (CRL 18 cm, 50% gestation), ruminal papillae were more developed than in earlier groups, appearing as small elevations of the basal area towards the ruminal lumen. At this age gestational, conical papillae were observed in the secondary laminae in omasum.

At 87 days (CRL 22 cm, 61% gestation), the lateral surface of primary reticular crest was studded with small corneum papillae. At 101 days (CRL 27.5 cm, 67% gestation), secondary reticular crest were visible. At this stage, a layer of smooth muscle fibers was observed in the uppermost area of primary reticular crest to form the muscularis mucosae.

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

Ruminal, reticular and omasum mucosa were covered by a plane stratified epithelium, which was divided into stratrum basale, granulosum, spinosum and corneum.

The lamina propria and submucosa were composed of fibroblast and collagen fibers and among ground substance. There was no clear separation between it and the lamina propria. The muscularis mucosa was fully developed and occupied the centre of omasal laminea and the top in reticular crests.

The tunica muscularis comprised two layers of smooth muscle fibers an inner circular layer and an outer longitudinal layers.

Ruminal papillae were both longer and thicker, reaching the apical third of the epithelium. Reticular crests displayed two growth patterns in forming reticular cells; a longitudinal and transversal growth. In omasum, four orders of omasal laminae of varying thickness were apparent, all studded with numerous conical papillae

Each compartments gastric was lined by an external serosa formed by a subserosa of connective tissue and overlying mesothelium.

Immunohistochemical observations

Immunoreactivity for synaptophysin, non-neuronal enolase, glial fibrillary acidic protein, vimentin, neuropeptide Y and vasoactive intestinal peptide was observed in all forestomachs of embryos and fetuses. The total immunostained surface (μm^2) in the rumen, reticulum and omasum is shown in Tables 1, 2, 3, respectively. The staining intensity for each antibody in each stratum of the goat forestomach is summarized in Figure 1. Staining distribution is illustrated in Figures 2, 3 and 4 for the rumen, reticulum and omasum, respectively.

Synaptophysin (SPY)

Rumen. SPY immunoreactivity was observed at 53 days (CRL 9 cm, 35% gestation) and distributed unevenly, staining being more intense in tunica muscularis than lamina propria-submucosa (Fig. 2A).

Reticulum. Positive staining was observed at 53 days (CRL 9 cm, 35 % gestation) in lamina propria-submucosa, tunica muscularis, serosa and myenteric plexus. The immunostained surface was higher distributed in tunica muscularis than lamina propria-submucosa (Fig. 3A).

Omasum. SPY positivity was detected at 53 days (CRL 9 cm, 35% gestation), in the lamina propria and submucosa, tunica muscularis and myenteric plexus (Fig. 4A). At an advanced stage of prenatal development, small stained areas were observed in the connective tissue of omasal laminae.

Non-neuronal enolase (NNE)

Rumen. NNE immunoreactivity was observed at 64 days (CRL 13.5 cm, 43% gestation) in all strata of the ruminal wall, although the distribution pattern differed between strata (Fig. 2B).

Reticulum. Positive staining for NNE was detected at 64 days (CRL 13.5 cm, 43% gestation) in lamina propria-submucosa, tunica muscularis, serosa, myenteric plexuses and epithelium. Epithelium was significantly more intense during the later stages of prenatal development (Fig. 3B).

Omasum. NNE-immunoreactivity was observed at 64 days (CRL 13.5 cm, 43% gestation) in the lamina propria-submucosa, tunica muscularis, serosa, epithelium and myenteric plexuses. The epithelium was the most heavily-stained layer in all groups (Fig. 4B).

Glial fibrillary acidic protein (GFAP)

Rumen. GFAP-immunoreactivity was clearly observed at 68 days (CRL 15 cm, 45% gestation) in lamina propria-submucosa, tunica muscularis, serosa and myenteric plexuses. The most intense staining was observed in myenteric plexuses (Fig. 2C).

Reticulum. Positive staining for GFAP was detected from 64 days (CRL 13.5 cm, 43% gestation), in the lamina propria-submucosa, tunica muscularis, serosa and myenteric plexuses (Fig. 3C). Staining increased in myenteric plexus as prenatal development progressed.

Omasum. GFAP immunoreactivity was detected at 64 days (CRL 13.5 cm, 43% gestation) in the lamina propria-submucosa, tunica muscularis and myenteric

plexuses. The greatest staining intensity was observed in myenteric plexuses (Fig. 4C). GFAP-Ir was detected occasionally in the connective tissue of omasal laminae.

Vimentin (VIM)

Rumen. VIM immunoreactivity was detected at 39 days (CRL 4.4 cm, 25% gestation) in mesenchymal cells and serosa (Fig. 2D). At 53 days, immunoreactivity was restricted to the lamina propria-submucosa, tunica muscularis, serosa and myenteric plexuses and a decreased in staining intensity was observed in each strata.

Reticulum. Positive staining for VIM was observed at 39 days (CRL 4.4 cm, 25% gestation) in mesenchymal cells and serosa (Fig. 3D). In later stages of prenatal development, immunoreactivity was observed in the different tissue layers and in the myenteric plexus.

Omasum. VIM positive glial cells were observed at 39 days (CRL 4.4 cm, 25% gestation) in pluripotential blastemic tissue and serosa (Fig. 4D). By 53 days (CRL 9 cm, 35% gestation), a moderate immunoreactivity was observed in lamina propria-submucosa and serosa and a low in tunica muscularis. Myenteric plexus showed also a low inmmunorreactivity.

Neuropeptide Y (NPY)

Rumen. NPY positive staining was detected at 113 days (CRL 33 cm, 75% gestation). The immunorreactivity was distributed by lamina propia-submucosa and tunica muscularis. Myenteric plexus showed a moderate immunoreactivity (Fig. 2E).

Reticulum. NPY immunoreactivity was first observed at 75 days (CRL 17.5 cm, 50% gestation), in lamina propria-submucosa and tunica muscularis. A moderate

immunorreactivity was observed in myenteric plexus. Stained areas were observed in the connective tissue of crest ribs (Fig. 3E).

Omasum. NPY immunoreactivity was detected at 95 days (CRL 20 cm, 63% gestation) in all tissue layers of omasal wall, except in epithelium. There was no significant difference in staining intensity between groups IV and V. NPY staining was observed in the connective tissue of omasal laminae (Fig. 4E).

Vasoactive intestinal peptide (VIP)

Rumen. VIP immunoreactivity was detected at 113 days (CRL 33 cm, 75% gestation) in lamina propria-submucosa, tunica muscularis and myenteric plexus (Fig. 2F).

Reticulum. VIP positive staining was observed at 75 days (CRL 17.5 cm, 50% gestation) in lamina propria-submucosa, tunica muscularis, serosa and myenteric plexus. Significant differences were found between group IV and V of gestation. (Fig. 3F).

Omasum. VIP immunoreactivity was detected at 95 days (CRL 20 cm, 63% gestation) in lamina propia-submucosa, tunica muscularis, serosa and myenteric plexus. Small stained areas were observed in the connective tissue of omasal laminae (Fig. 4F).

Discussion

This study sought to chart the spatial and temporal expression of neuroendocrine cell, glial cell and peptidergic innervation markers in the goat forestomach.

SPY-positive neuroendocrine cells were observed at 53 days of prenatal development (35% gestation). The similar finding was noted in the goat rumen and omasum [12, 13]. Similar results are reported for other ruminant species; in deer, for example, neuroendocrine cells have been detected at 97 days (36% gestation) in the rumen and reticulum [8, 9], but at an earlier stage of 67 days (26% gestation) in the omasum [32]. In sheep, by contrast, neuroendocrine cells are not reported until 81 days (54% gestation) [10, 11, 33]. SPY immunoreactivity was observed predominantly in the tunica muscularis and myenteric plexuses. However, a less immunorreactivity was extent in the lamina propria-submucosa and serosa. Staining intensity increased significantly with gestational age, as also reported in sheep [3].

NNE-positive neuroendocrine cells were detected at a later stage than SPY-positive cells. The staining distribution and intensity were similar, except that NNE staining was apparent in the epithelium of all forestomach compartments. This finding seems to suggest that these neuroendocrine cells belong to the diffuse neuroendocrine system, which is located in the gastrointestinal epithelium [38]. Diffuse neuroendocrine system cells in the digestive tract are thought to arise from the same precursor cell as other epithelial cells [16]; these neuroendocrine cells originating in the embryonic neural crest migrate to the gastrointestinal epithelium in the course of prenatal development [28].

Glial cells were detected by GFAP staining at 68 days (45% gestation) in the rumen and at 64 days (43% gestation) in reticulum and omasum. This finding is

reported somewhat later in the rumen and reticulum of sheep and deer, at 112 days (75% gestation) and 142 days (50% gestation), respectively [10, 11]. However, in the omasum of these species glial cells have been observed at earlier stages [32, 33]. GFAP-positive staining was visible in the lamina propria-submucosa, tunica muscularis and serosa, and was particularly marked in myenteric plexuses. The presence of glial cells in myenteric plexuses and submucosa has also been reported in sheep [45] and cows [40]. In other species, including rats [27] and cats [19], GFAP-positive cells have been observed in ganglionate plexuses. Glial cells are similar in structure and function to the astrocytes of the central nervous system, and play a key role in controlling gastrointestinal function and protecting enteric neurons [1].

Glial cells were also detected by VIM staining. VIM positivity was observed in all forestomach compartments at an early age of gestation. This finding indicated that VIM is an earlier glial-cell marker than GFAP, according to the reported in the prenatal development of the sheep pineal gland [7] and in the forestomach of sheep and deer [10, 11, 33]. At 39 days (25% gestation), VIM immunoreactivity was detected in pluripotential blastemic tissue and serosa. In later states of gestation, this immunoreactivity was distributed by the different tissue layers, which were already differentiated. VIM positivity was observed in the same strata as GFAP staining.

The peptidergic innervation markers detected in the goat forestomach have also been reported in lambs [15]. Here, they were observed earlier in the reticulum and omasum but rather later in the rumen. Similar findings are reported for red deer, in which these markers are detected at 142 days (50% gestation) in the reticulum [9, 11] and omasum [32, 33], but not until perinatal stages in the rumen [8,10]. Positive staining for NPY and VIP displayed a similar distribution pattern; in both cases,

myenteric plexuses exhibited the greatest staining intensity. In lambs, positive staining in the myenteric plexuses is of low to moderate intensity for NPY but more intense for VIP [15]. By contrast, immunoreactivity is most marked in the smooth muscle layers of the reticular groove, reticulum and rumen of cattle [17] and in sheep omasum [45]. Findings for peptide distribution, both here and in earlier studies [17, 18], indicate that peptide-containing nerve fibers are mainly intrinsic in origin, deriving from the intramural ganglia of the forestomach wall.

In conclusion, glial cell, neuroendocrine cell and peptidergic innervation markers were detected during the prenatal development of the goat forestomach. Differentiation of these cell types took place concurrently with the differentiation of tissue strata. These findings point to a possible function of the fetal goat forestomach in preparation for its postnatal function. Compared to other ruminant species, these cell types were detected earlier than in sheep, but at around the same stage as in deer.

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References

1. **Abdo H, Derkinderen P, Gomes P, Chevalier J, Aubert P, Masson D, Galmiche JP, Venden Berfhe P, Neunlist M, Landeux B.** Enteric glial cells protect neurons from oxidative stress in part via reduce glutathione. Faseb J 2012, **24**, 1082-1094.
2. **Calingasan NY, Kitamura N, Yamada J, Oomori Y, Yamashita T.** Immunocytochemical study of the gastroenteropancreatic endocrine cells of the sheep. Acta Anat 1984, **118**, 171-180.
3. **Ceccarelli P, Pedini V, Gargiulo AM**. Enteroendocrine cells in sheep fetuses. Small Ruminat Res 1991, **6**, 85-93.
4. **Cooke HJ**. Neurobiology of the intestinal mucosa. Gastroenterology 1986, **90**, 1057-1081.
5. **Deveney CW, Way WL.** Regulatory peptides of the gut. In: Greenspan FS, Forsham PH (eds). Basic and Clinical Endocrinology. Asian edn. pp. 479-499, Singapure, 1983.
6. **Evans HE, Sack WO.** Prenatal development of domestic and laboratory mammals: Growth curves, external features and selected references. Anat Histol Embryol 1973, **2**, 11-45.
7. **Franco A, Regodon S, Masot AJ, Redondo E.** A combined immunohistochemical and electron microscopic study of the second cell type in the developing sheep pineal gland. J Pineal Res 1997, **22**, 130-136.
8. **Franco A, Masot AJ, Gómez L. Redondo E.** Morphometric and immunohistochemical study of the rumen of red deer during prenatal development. J Anat 2004a, **204**, 501-513.

9. **Franco A, Redondo E. Masot AJ.** Morphometric and immunohistochemical study of the reticulum of red deer during prenatal development. J Anat 2004b, **205**, 277-289.
10. **Franco A, Masot AJ. Redondo E.** Ontogenesis of the rumen: a comparative analysis of the merino sheep and Iberian red deer. Anim Sci J 2011, **82**, 107-116.
11. **Franco A, Masot AJ, García A. Redondo E.** Ontogenesis of the reticulum with special reference to neuroendocrine and glial cells: a comparative analysis of the Merino sheep and Iberian red deer. Anat Histol Embryol 2012, **41**, 362-373.
12. **Garcia A, Masot J, Franco A, Gazquez A. Redondo E.** Histomorphometric and immunohistochemical study of the goat rumen during prenatal development. Anat Rec 2011, **295**, 776-785.
13. **Garcia A, Masot J, Franco A, Gazquez A. Redondo E.** Histomorphometric and immunohistochemical study of the goat rumen during prenatal development. Histol Histophatol 2012 (on line)
14. **Gershon MD, Kirchgessner AL, Wade PR.** Functional anatomy of the enteric nervous system. In: Johnson LR (ed). Physiology of the Gastrointestinal Tract, 3rd ed. pp. 381-422, Raven Press, New York, 1994.
15. **Groenewald HB**. Neuropeptides in the myenteric ganglia and nerve fibres of the forestomach and abomasums of grey, white and black Karakul lambs. Onderstepoort J Vet 1994, **61**, 207-213.

16. **Gurdip S, Sidhu MD.** The endodermal origin of digestive and respiratory tract APUD cells. Histopathologic evidence and a review of the literature. Am J Pathol 1979, **96**, 5-20.
17. **Kitamura N, Yamada J, Yamashita T.** Immunohistochemical study on the distribution of neuron-specific enolase and peptide-containing nerves in the reticulorumen and the reticular groove of cattle. J Comp Neurol 1986, **248**, 223-234.
18. **Kitamura N, Yamada J, Yamamoto Y, Yamashita J**. Substance P-immunoreactive neurons of the bovine forestomach mucosa: their presumptive role in a sensory mechanism. Arch Histol Cytol 1993, **56**, 399-410.
19. **Kleinschmidt S, Nolte I, Hewicher-Trautwein M.** Structural and functional components of the feline enteric nervous system. Anat Histol Embryol 2011, **40**, 450-456.
20. **Lazarides E**. Intermediate filaments as mechanical integrators of cellular space. Nature, 1980, **283**, 249-256.
21. **Llewellyn-Smith IJ.** Neuropeptides and the microircuitry of the enteric nervous system. Experientia 1987, **43**, 813-821.
22. **Lombardi G.** Optimum management and quality pastures for sheep and goat in mountain areas. Options mediterraneennes, Series A, No. 67. 2005, 19-29.
23. **Ludwin SK, Kosek JC, Eng LF.** The topographical distribution of S-100 and GFA proteins in the adult rat brain: An immunohistochemical study using horseradish peroxidise-labelled antibodies. J Comp Neurol **1976**, 165,197-208.

24. **Mawe GW, Schemann M. Wood JD, Gehrson MD**. Immunocytochemical analysis of potential neurotransmitters present in the myenteric plexus and muscular layers of the corpus of the guinea pig stomach. Anat Rec 1989, **224**, 431-442.

25. **Münnich J, Gäbel G, Pfannkuche H.** Intrinsic ruminal iinervation in ruminants of different feeding types. J Anat 2008, **213**, 442-451.

26. **Osami N, Takashi K.** Immunohistochemical identification of supportive cell types in the enteric nervous system of the rat colon and rectum. Cell Tissue Res 1988, **251**, 523-529.

27. **Pearse AGE, Takor T**. Neuroendocrine embryology and the APUD concept. Clinical Endocrinology 1976, **5**, 229-244.

28. **Pfannkuche H, Schellhorn C, Schemann M, Gäbel G.** Intrinsic innervation patterns of the smooth muscle in the rumen and reticulum of lambs. J Anat 2004, **204**, 293-299.

29. **Pfannkuche H, Schemann M.** Ruminal muscle of sheep is innervated by non-polarized pathways of cholinergic and nitrergic myneteric neurones. Cell Tissue Res 2002, **309**, 347-354.

30. **Pfannkuche H, Schellhorn C, Schemann M, Gäbel G.** Reticular groove and reticulum are iinervated by myenteric neurons with different neurochemical codes. Anat Rec 2003a, 917-922.

31. **Redondo E., Franco A. and Masot A.J.** (2005). Morphometric and immunohistochemical study of the omasum of red deer during prenatal development. J. Anat. **206**, 543-555.

32. **Redondo E., Masot J., Garcia A. and Franco A.** (2011). Ontogenesis of the omasum: a comparative analysis of the Merino sheep and Iberian red deer. Histol. Histopathol. **26**, 1135-1144.

33. **Ruckebusch Y.** Gastrointestinal motor functions in ruminants. In: Schultz SG, Wood JD, Rauner BB (eds). The Gastrointestinal System. pp. 1225-1283, Oxoford University Press, New York, 1989.

34. **Schachner M, Hedley-Whyte ET, Hsu DW, Schoonmarker G, Bignami A.** Ultraestructural localisation of glial fibrillary acidic protein in mouse cerebellum by immunoperoxidase labelling. J Cell Biol 1977, **75**, 67-73.

35. **Scharrer B.** The neuropeptide saga. Amer Zool 1990, **30**, 887-895.

36. **Schummer A, Nickel R.** Lehrbuch der Anatomie der Haustiere (3. ed), Vol IIVerlag pp. 149–173. Paul Parey, Berlin. 1975.

37. **Seng Pan Q, Ping Fang Z, Jie Huang F.** Identification, lozalization and morphology of APUD cell in gastroenteropan creatic system of stomach containing teleosts. Word J Gastoenterol 2000, **6**, 842-847.

38. **Sivachelvan M.N., Ghali Ali M. and Chibuzo G.A.** (1996). Foetal age estimation in sheep and goats. Small Ruminant Res 1996, **19**, 69-76.

39. **Teixeira AF, Wedel T, Krammer HJ, Kühnel W.** Structural differences of the enteric nervous system in the cattle forestomach revealed by whole mount immunohistochemistry. Annal Anat 1998, **180**, 393-400.

40. **Titchen DA.** Nervous control of motility of the forestomach of ruminants. In: W Heidel, CF Code (eds.). Handbook of Physiology, section 6. Alimentary Canal, pp 2705-2724, American Physiological Society, Washington, 1968.

41. **Von Boyen GB, Steinkamp M, Reinshagen M, Schäfer H, Adler G, Kirsch J**. Proinflammatory cytokines increase glial fibrillary adicic protein expression in enteric glia. Gut 2004, **53**, 222-228.
42. **Wathuta EM.** The distribution of vasoactive intestinal polypeptide-like, substance P-like and bombesin-like immunoreactivity in the digestive system of the sheep. Exp Physiol 1986, **71**, 615-631.
43. **Wathuta EM, Harrison FA.** The ontogeny of vasoactive intestinal polypeptide-like and substance P-like immunoreactivity in the digestive tract of the sheep. Exp Physiol 1987, **72**, 119-128.
44. **Yamamoto Y, Kitamura N, Yamada J, Yamashita T.** Immunoohistochemical study of the distributions of the peptide and cathecholamine containing nerves in the omasum of the sheep. Acta Anat 1994, **149**, 104-110.

Legends for Figures

Fig. 1. Immunoreactive density of each antibody the different layer of rumen, reticulum and omasum of goat.

Fig. 2. Distribution of immunoreactivity in the goat rumen.

(A) Synaptophysin-positive staining in tunica muscularis and myenteric plexus (arrow) in a section of the rumen wall at 64 days (43% gestation). Bar: 25 μm

(B) Non-neuronal enolase immunoreactivity in epithelial layer (arrow), lamina propria-submucosa, tunica muscularis and myenteric plexus (arrow) at 64 days (43% gestation). Bar: 25 μm

(C) Glial fibrillary acidic protein staining at 64 days (43% gestation) in lamina propria-submucosa, tunica muscularis, serosa and myenteric plexus (arrows) and within ruminal papillae. Bar: 30 μm

(D) Vimentin immunostaining in the mesenchymal cells and serosa of the ruminal wall at 39 days (25% gestation). Bar: 20 μm

(E) Neuropeptide Y-positive staining in lamina propria-submucosa, tunica muscularis and myenteric plexus (arrows) at 113 days (75% gestation). Bar: 25 μm

(F) Vasoactive intestinal peptide staining at 113 days (75% gestation) in lamina propria-submucosa, and intense immunostaining in tunica muscularis and myenteric plexus (arrows). Bar: 25 μm

Fig. 3. Distribution of immunoreactivity in the goat reticulum

(A) Synaptophysin-positive staining in lamina propria-submucosa, tunica muscularis, serosa and myenteric plexus (arrow) at 64 days (43% gestation). Bar: 25μm

(B) Non-neuronal enolase immunoreactivity visible in epithelium (arrow), lamina propria-submucosa, tunica muscularis, serosa and myenteric plexus (arrow) at 69 days (46% gestation). Bar: 20 μm

(C) Glial fibrillary acidic protein staining in lamina propria-submucosa, tunica muscularis and myenteric plexus (arrow) at 64 days (43% gestation). Bar: 25μm

(D) Vimentin immunostaining in pluripotential blastemic tissue layer and serosa at 39 days (25% gestation). Bar: 25 μm

(E) Neuropeptide Y-positive staining in lamina propria-submucosa, tunica muscularis and myenteric plexus (arrow) at 75 days (50% gestation). Bar:25 μm

(F) Vasoactive intestinal peptide staining in lamina propria-submucosa, tunica muscularis and myenteric plexus (arrows) at 75 days (50% gestation). Bar: 30 μm

Fig. 4. Distribution of immunoreactivity in the goat omasum.

(A) Synaptophysin-positive staining in lamina propria-submucosa, tunica muscularis and myenteric plexus in the omasal wall at 75 days (**5**0% gestation). Bar:25 μm

(B) Non-neuronal enolase immunoreactivity in epithelial layer, lamina propria-submucosa and tunica muscularis at 64 days (75% gestation). Bar: 20 μm

(C) Glial fibrillary acidic protein staining within myenteric plexus (arrow) and in lamina propria-submucosa, tunica muscularis and serosa at 69 days (46% gestation). Bar: 25μm

(D) Vimentin immunostaining in pluripotential blastemic tissue layer and serosa at 39 days (25% gestation). Bar:20 μm

(E) Neuropeptide Y-positive staining in lamina propria-submucosa, tunica muscularis and myenteric plexus. Immunostaining surface visible in connective tissue of omasal laminae at 95 days (63% gestation). Bar: 25µm

(F) Vasoactive intestinal peptide staining in the connective tissue and tunica muscularis of omasal laminae at 113 days (75% gestation). Bar: 20 µm

Table 1.The total stained surface (μm^2) in the goat rumen in the different group of gestation

Table 2.The total stained surface (μm^2) in the goat reticulum in the different group of gestation.

Table 3.The total stained surface (μm^2) in the goat omasum in the different group of gestation.

Table 1.The total stained surface (μm^2) in the goat rumen in the different group of gestation

	Group I	Group II	Group III	Group IV	Group V
SPY			838.86 ± 68.39	1044.30 ± 33.01	1530.49 ± 62.48
NNE			657.39 ± 133.53	855.98 ± 123.40	1143.59 ± 42.76
GFAP			623.15 ± 23.72	780.65 ± 118.46	1047.72 ± 47.07
VIM		1160.71 ± 20.54	1040.87 ± 15.69	838.86 ± 56.57	708.75 ± 71.90
NPY					1037.45 ± 107.24
VIP					1157.28 ± 65.23

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: 35-50% 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). SYP: synaptophysin, GFAP: glial fibrillary acidic protein, VIM: vimentin, NPY: neuropeptide Y VIP: vasoactive intestinal peptide.

Table 2.The total stained surface (μm^2) in the goat reticulum in the different group of gestation.

	Group I	Group II	Group III	Group IV	Group V
SPY			633.43 ± 23.72	941.58 ± 89.74	1133.32 ± 68.39
NNE			749.84 ± 37.03	869.67 ± 41.51	1164.13 ± 38.88
GFAP			653.97 ± 42.76	1051.14 ± 216.52	1575± 119.93
VIM		1119.62 ± 53.37	1047.72 ± 54.35	794.35 ± 104.91	653.97 ± 33.02
NPY				1006.63 ± 20.54	1355.87 ± 97.98
VIP				855.98 ± 79.78	1307.94 ± 25.86

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: 35-50% 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). SYP: synaptophysin, GFAP: glial fibrillary acid protein, VIM: vimentin, NPY: neuropeptide Y VIP: vasoactive intestinal peptide.

Table 3.The total stained surface (μm^2) in the goat omasum in the different group of gestation.

	Group I	Group II	Group III	Group IV	Group V
SPY			664.24 ± 42.76	931.30 ± 58.40	1194.95 ± 33.01
NNE			698.48 ± 87.76	849.13 ± 11.86	1078.53 ± 27.17
GFAP			486.19 ± 48.54	986.09 ± 61.63	1592.12 ± 178.20
VIM		1092.23 ± 33.01	955.27 ± 57.19	722.44 ± 15.69	582.06 ± 68.39
NPY				808.04 ± 46.32	1393.54 ± 74.31
VIP				623.15 ± 41.51	999.78 ± 21.38

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: 35-50% 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). SYP: synaptophysin, GFAP: glial fibrillary acidic protein, VIM: vimentin, NPY: neuropeptide Y VIP: vasoactive intestinal peptide.

Figures

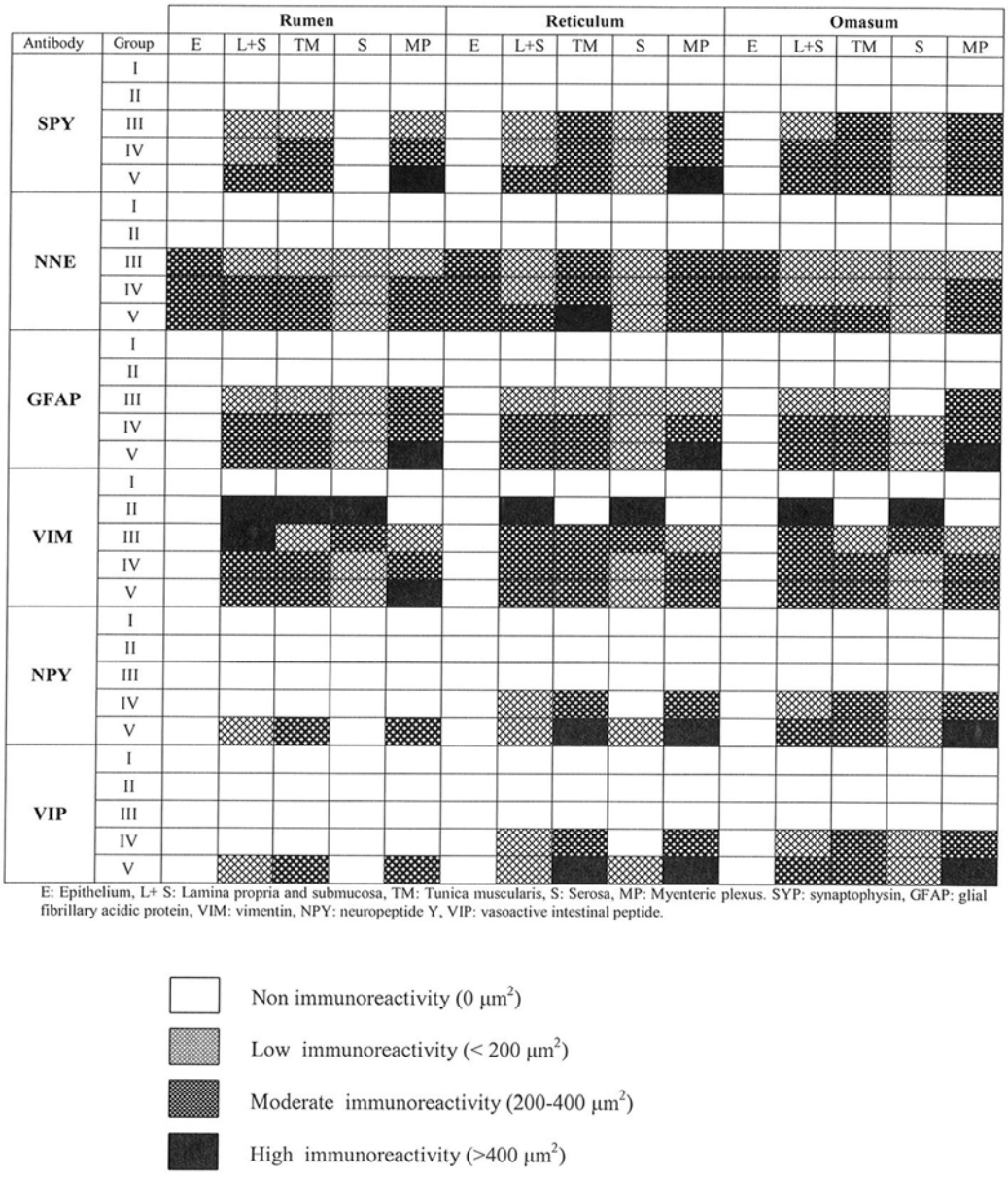

Figure 1

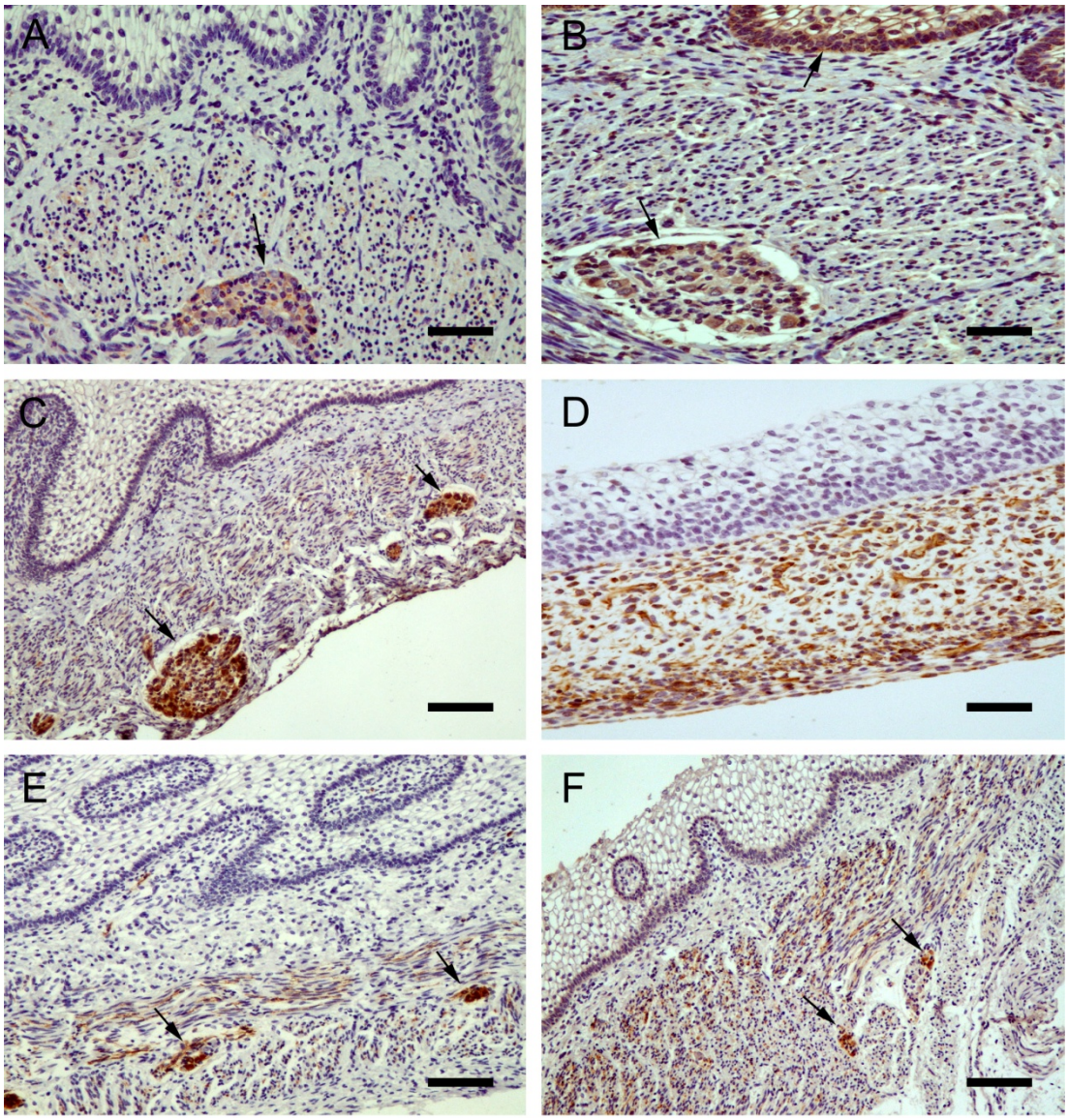

Figure 2

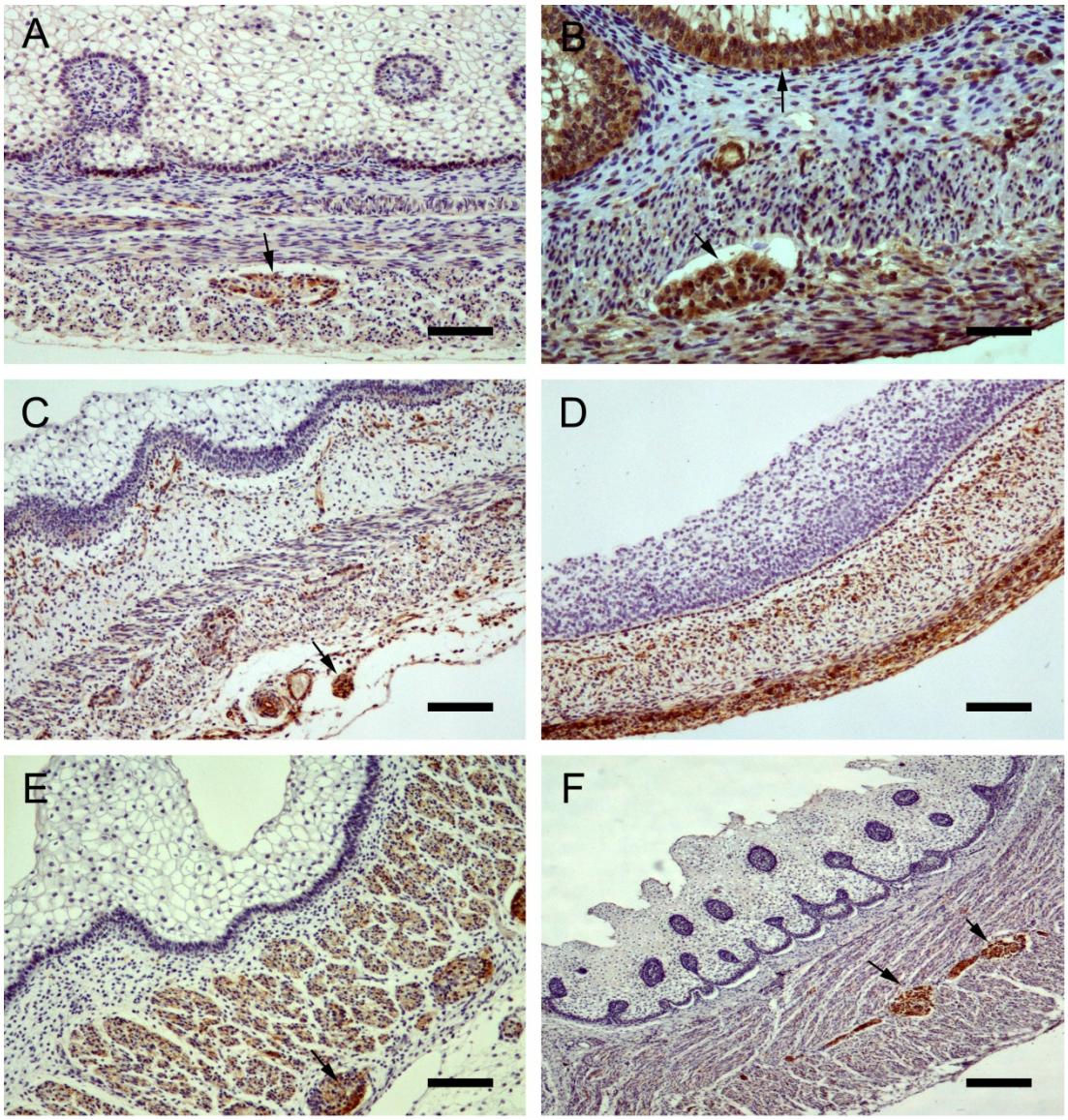

Figure 3

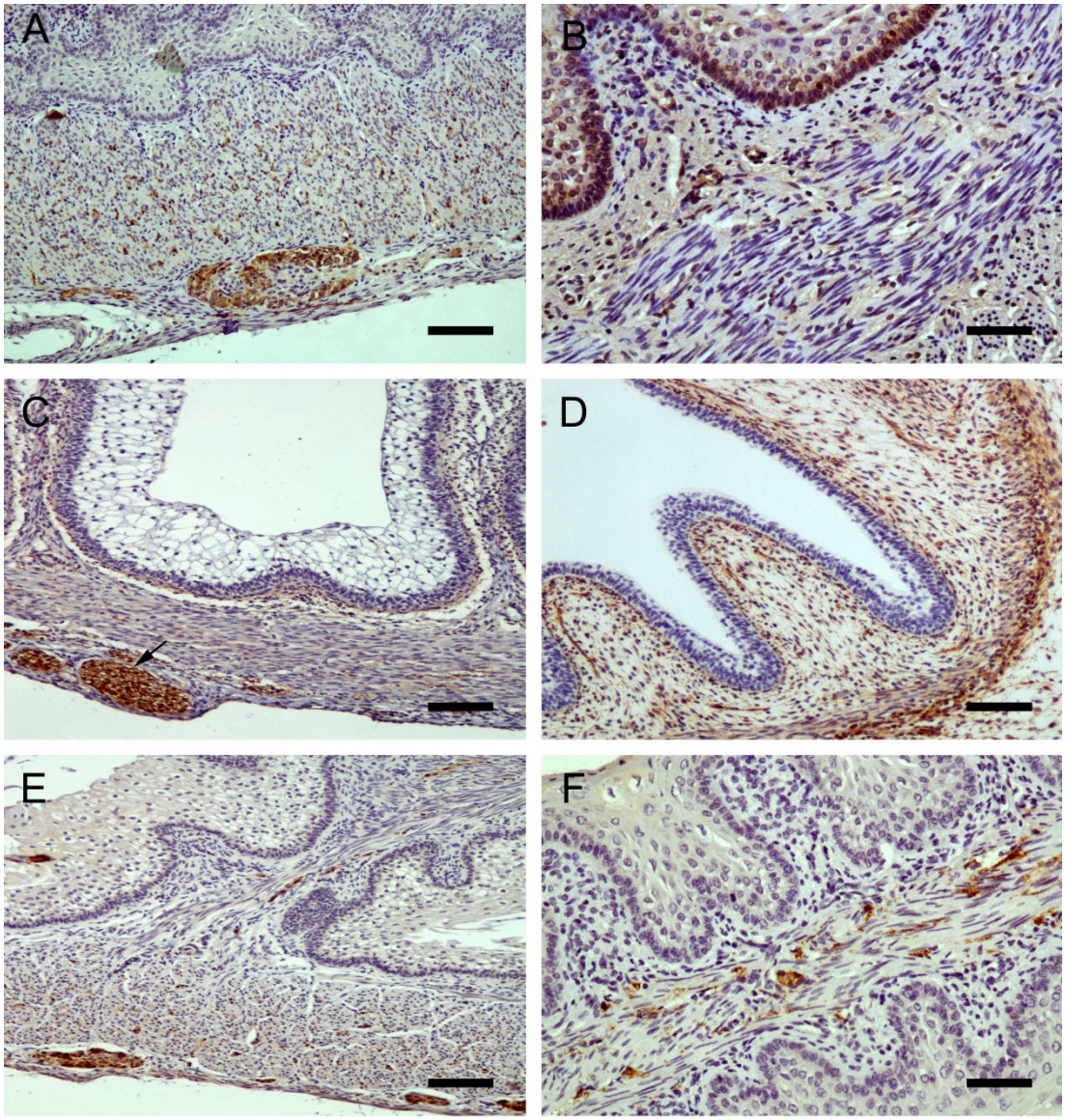

Figure 4

OTHER RESULTS OF STUDY IMMUNOHISTOCHEMICAL (NO PUBLISHED)

1. Immunohistochemical study of goat abomasum during prenatal development.

The total immunostained surface (μm^2) in abomasum for synaptophysin, non-neuroal enolase, glial fibrillary acidic protein, vimentin, neuropeptide Y, vasoactive intestinal peptide and gastrin is shown in Table 1. The staining intensity for each antibody in each stratum of the goat abomasums is summarized in Table 2. Staining distribution is illustrated in Figure 3.

Synaptophysin

SPY positive staining was detected at 64 days (CRL 13.5 cm, 43% gestation). The immunorreactivity was distributed by lamina propria and submucosa, tunica muscularis, serosa and myenteric plexus. A low immunorreactivity was detected in epithelial layer.

Non neuronal enolase

Positive staining for NNE was observed at 64 days (CRL 13.5 cm, 43% gestation) in the same region as SPY. The epithelial layer showed immunoreactivity higher than SPY.

Glial fibrillary acidic protein

GFAP immunoreactivity was detected at 64 days (CRL 13.5 cm, 43% gestation) in lamina propria and submucosa, tunica muscularis, serosa and myenteric plexus.

Vimentin

VIM positive staining was observed at 38 days (CRL 4.3 cm, 25% gestation) in mesenquimal cells and serosa. In later age gestational, the immunoreactivity was

distributed in the same as GFAP. The intervascular and perivascular connective tissue was also immunostaining.

Neuropeptide Y and vasoactive intestinal peptide

Positive staining for NPY and VIP was observed at 75 days (CRL 18 cm, 50% gestation) in lamina propria and submucosa, tunica muscularis and serosa. The myenteric plexus showed a high immunorreactivity.

Gastrin

Gastrin-immunoreactive cells were first detected at 76 days (CRL 18 cm, 50% gestation) in same epithelial cells. In the later gestational stages, the innmunoreactivity was observed in pyloric glands.

Table 5.The total stained surface (μm^2) in the goat abomasum in the different groups of gestation

	Group I	Group II	Group III	Group IV	Group V
SPY	-		838.86 ± 68.39	1044.30 ± 33.01	1530.49 ± 62.48
NNE	-		657.39 ± 133.53	855.98 ± 123.40	1143.59 ± 42.76
GFAP	-		623.15 ± 23.72	780.65 ± 118.46	1047.72 ± 47.07
VIM	-	1160.71 ± 20.54	1040.87 ± 15.69	838.86 ± 56.57	708.75 ± 71.90
NPY	-				1037.45 ± 107.24
VIP	-				1157.28 ± 65.23

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: 35-50% 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). SYP: synaptophysin, GFAP: glial fibrillary acidic protein, VIM: vimentin, NPY: neuropeptide Y VIP: vasoactive intestinal peptide.

Table 6. Immunoreactive density of each antibody the different layer of abomasum of goat

		Abomaso				
Antibody	Group	E	L+S	TM	S	MP
SPY	I					
	II					
	III					
	IV					
	V					
NNE	I					
	II					
	III					
	IV					
	V					
GFAP	I					
	II					
	III					
	IV					
	V					
VIM	I					
	II					
	III					
	IV					
	V					
NPY	I					
	II					
	III					
	IV					
	V					
VIP	I					
	II					
	III					
	IV					
	V					
GAS	I					
	II					
	III					
	IV					
	V					

E: Epithelium, L+ S: Lamina propria and submucosa, TM: Tunica muscularis, S: Serosa, MP: Myenteric plexus. SYP: synaptophysin, GFAP: glial fibrillary acidic protein, VIM: vimentin, NPY: neuropeptide Y, VIP: vasoactive intestinal peptide.

No inmunoreactivity

Low inmunoreactivity (<200µm^2)

Moderate inmunoreactivity (200-400µm^2)

High inmunoreactivity (>400µm^2)

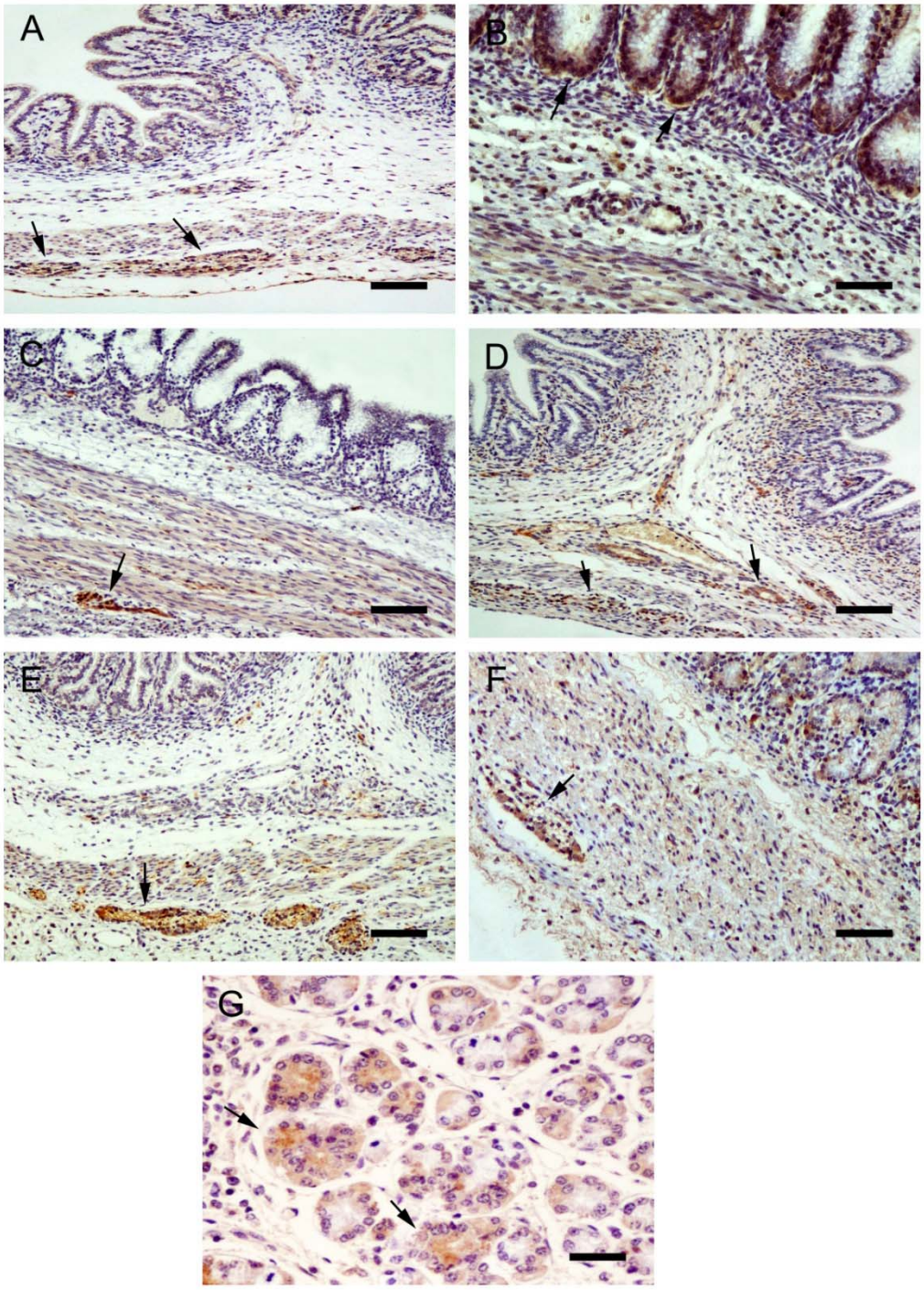

Imagen 20

Imagen 20. Distribution of immunoreactivity in the goat abomasum.

(A) Synaptophysin-positive staining in lamina propria-submucosa, tunica muscularis and myenteric plexus in the omasal wall at 64 days (**43**% gestation). Bar:25 µm

(B) Non-neuronal enolase immunoreactivity in epithelial layer, lamina propria-submucosa and tunica muscularis at 64 days (43% gestation). Bar: 20 µm

(C) Glial fibrillary acidic protein staining within myenteric plexus (arrow) and in lamina propria-submucosa, tunica muscularis and serosa at 64 days (43% gestation). Bar: 25µm

(D) Vimentin immunostaining in pluripotential blastemic tissue layer and serosa at 38 days (25% gestation). Bar:20 µm

(E) Neuropeptide Y-positive staining in lamina propria-submucosa, tunica muscularis and myenteric plexus. Immunostaining surface visible in connective tissue of omasal laminae at 75 days (50% gestation). Bar: 25µm

(F) Vasoactive intestinal peptide staining in the connective tissue and tunica muscularis of omasal laminae at 87 days (63% gestation). Bar: 20 µm

2. Double immunohistochemistry in gastric compartment of goat

The study of double-immunohistochemistry was performed by synaptophysin and glial fibrillary acidic protein in order to stain neuroendocrine and glial cells, together.

This study was conducted in one each of the gastric compartments in stages of prenatal developmental close to birth. The double immunostaining was observed in each compartment and located primarily in the myenteric plexus. The results obtened are shown in the Imagen.

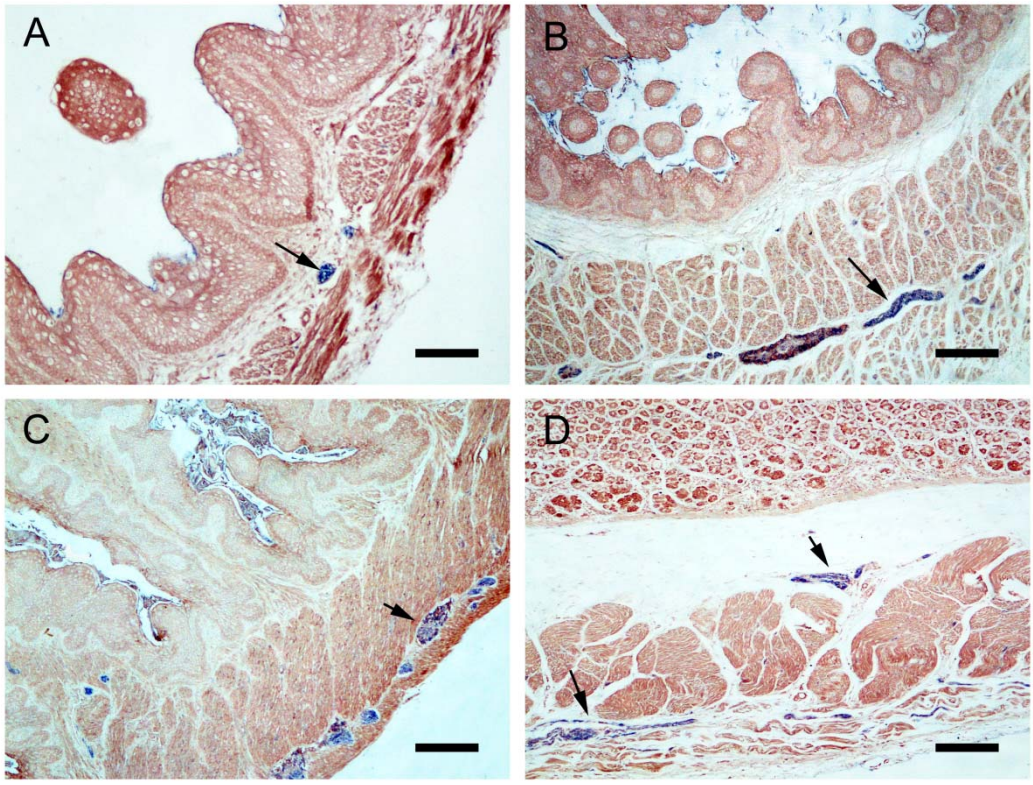

Imagen 21. Double immunostaining with synaptophysin and glial fibrillar acidic protein.

A. Ruminal wall at 113 days (75% gestation). Immunoreactivity in myenteric plexus (arrow).
B. Reticular wall at 113 days (75% gestation). Immustaining in myenteric plexus (arrow).
C. Omasal wall at 113 days (75% gestation). Immunoreactivity located in myenteric plexus (arrow).
D. Abomasal wall at 113 days (75% gestation). Immunoreactivity in submucosa plexus (up arrow) and myenteric plexus (under arrow).

Artículo 6

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Histomorphometric study of the goat stomach during prenatal development

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Running title: Histomorphometry in goat stomach

ABSTRACT

This study reports on the prenatal growth and development of the four compartments of the goat stomach. Data obtained by morphometric analysis of the gastric wall as a whole and of the individual component tissue layers was fitted to mathematical models and growth curves. A total of 140 embryos and fetuses were used, from the early stages of prenatal life until birth. The growth-rate of the gastric wall was slower than that of body length; rumen was the stomach compartment displaying slowest growth. In the three non-glandular compartments, the epithelial layer grew faster than the gastric wall itself, whilst the growth-rate of the abomasal epithelium declined in the early stages of development. A decline in growth-rate was also observed for the lamina propria and submucosa in rumen and reticulum from the early embryonic stages, whereas in omasum and abomasum these layers continued to grow as gestation progressed. The tunica muscularis displayed consistent growth in all compartments, growing faster than the gastric wall. Serosa thickness increased as gestation progressed, displaying a decline in growth-rate only in the omasum. In conclusion, the dynamics of gastric wall growth were governed by the growth-rate of each of the component tissue layers.

Keywords: *goat; histomorphometry; prenatal; development*

INTRODUCTION

The ruminant stomach is particularly remarkable for its ability to transform low-quality forage into products of great nutritional value (Lombardi 2005). In order to meet its functional needs, is has developed four separate compartments, each with its own morphological particularities: rumen, reticulum, omasum and abomasum. Each compartment is characterized by certain unique gross and histological features (Schummer & Nickel 1975) reflecting its morphological and functional adaption to the ingestion, processing and digestion of plant material.

The primitive digestive tract in ruminant animals is structurally and functionally similar to that of non-ruminant animals (Relling & Mattioli 2002). During prenatal life, however, the ruminant stomach undergoes certain morphological changes in order to meet functional demands in postnatal life. These morphological changes are linked to the growth and development of gastric viscera.

Growth and development are closely-linked and time-dependent biological processes, and can therefore be expressed mathematically by a model that is simply an equation representing the behavior of a system (France & Thornley 1984). Growth curves reflect the lifetime interrelationships between an individual's inherent impulse to grow and mature in all body parts and the environment in which these impulses are expressed. The term "growth curve" usually evokes a sigmoid curve depicting a sequence of measures of size over time (Fizthugh 1976). A dynamic model is the most appropriate, since the time factor accounts for the evolution of the events occurring in the course of prenatal development.

71 The choice of curve is dictated by three major factors: biological
72 interpretation of the parameters, goodness of fit to the actual data, and computational
73 difficulty, which varies with choice of function.

74 A common characteristic of growth models is that they each use two
75 biologically-relevant parameters. The first parameter establishes the position of the
76 individual (or group) in the general size space at a given reference age (Brinks et al.
77 1964, Brown et al. 1973, Taylor & Craig 1965). The second parameter is concerned
78 with growth rate relative to body size. Brody (1945) additionally uses a third
79 parameter to partition the growth curve into two stages, often referred to as the "self-
80 accelerating" and "self-inhibiting" stages, during which growth-rate velocity
81 increases or decreases, respectively.

82 Although morphometric analysis – clearly essential to any study of organic
83 growth over time – is a valuable tool for charting gastric development from early
84 embryonic stages until birth, surprisingly few morphometric studies have addressed
85 stomach growth in ruminants during prenatal life; even fewer have focussed on the
86 goat stomach, although there has been some research in sheep (Franco et al. 1993d).

87 The present paper reports on a histomorphometric analysis of the tissue layers
88 comprising the goat stomach wall. Data were fitted to mathematical models with a
89 view to obtaining formulas applicable to goat embryonic growth in general and
90 gastric growth in particular.

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MATERIAL AND METHODS

Animals

A total of 140 goat (*Capra hircus*) embryos and fetuses, ranging from the earliest 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). Goat embryos and fetuses were all obtained at a municipal slaughterhouse in Caceres (Spain), from routinely-slaughtered pregnant females; caesarean sections were performed in accordance with the provisions of Spanish Royal Decree 54/1995 on the protection of animals at slaughter. Gestational age was estimated using age-classification methods previously reported for sheep (Evans & Sack 1973) and goats (Sivachelvan et al. 1996).

Sampling and processing

Stomach compartments were extracted and small pieces of tissue were dissected. Tissue samples were fixed in 4% buffered formaldehyde for 24 hours, routinely processed and embedded in paraffin for histological examination. Sections 5µm thick were stained with Hematoxylin-Eosin (H-E).

Morphometric analysis

Eighteen specimens were selected from each group for histomorphometric analysis. Exact longitude in centimetres of the eighteen specimens in each group were as

follows, half male and half female : Group I: 6 x 3.5 cm CRL, 6 x 3.8 cm CRL and 6 x 4.3 cm CRL; Group II: 6 x 5 cm CRL, 6 x 6 cm CRL and 6 x 8 cm CRL; Group III, 6 x 13.5 cm CRL, 6 x 15 cm CRL, 6 x 17 cm CRL; Group IV, 6 x 19 cm CRL, 6 x 22 cm, 6 x 27.5 cm CRL; Group V, 6 x 33 cm CRL, 6 x 36 cm CRL and 6 x 42 cm CRL. Histological sections were viewed through a microscope (NIKON Eclipse 80i) equipped with a digital video camera (NIKON DXMI200F). Digital images were analyzed using the Nis-Element 2.30 software package. The variables studied were height of various tissue layers (epithelium, lamina propia and submucosa, tunica muscularis and serosa) and total wall thickness. In all selected specimens, one hundred measurements were made for each tissue layer.

Statistical analysis

Measurements were expressed in μm and are shown as mean ± SE. Data were subjected to factorial analysis of variance (ANOVA), with sex and group as factors. Where ANOVA revealed significant differences for the group factor ($p<0.05$), a post-hoc (Tukey) analysis was carried out to test for significant differences between means.

Tissue growth models were created using four mathematical models: linear ($y = a + bX$), quadratic ($y = a + bX + cX^2$), logarithmic ($y = a + bLOG\ (X)$), and exponential ($y = a\ exp(bX)$). Graphs for the best models were constructed by plotting averages of real growth values against embryo body length, together with the adjusted line of regression. The goodness of fit of each adjustment was measured using the linear coefficient of correlation, r. In all cases, embryo body length (crown-

rump length, in centimeters) was used as the independent variable; the thickness of each tissue layer served as dependent variable.

Allometry coefficients were calculated using the equation $y = aX^b$ of Huxley (1932) in which the independent variable was the thickness of the whole compartment wall and the dependent variable, the thickness of each tissue layer. The SPSS.19 statistical software package licensed to Extremadura University was used for this purpose.

RESUTLS

The thickness (absolute and relative values) of each tissue layer and total wall thickness are shown in Tables 1-5. Mathematical growth models adjusted for each tissue layer and for the gastric wall as a whole are shown in Figures 1-5.

Epithelial layer

The ruminal epithelium was significantly thicker in groups III, IV and V than in groups I and II, but no significant differences were also found between groups I and II. In the reticulum, the epithelium was significantly thicker in groups IV and V than in groups I, II and III. The epithelial layer of omasum was significantly thicker in group V than groups I, II, III and IV, without significant differences between groups I, II and III. Significant difference in thickness of the abomasal epithelium was observed between groups I and II with groups III, IV and V, and also between groups I and II but not between groups III, IV and V. No significant differences in epithelial thickness between the sexes were observed in any gastric compartments.

Epithelial growth fitted a polynomial model for all four stomach compartments. In rumen, reticulum and omasum, the growth-rate was slow from differentiation up to the 13.5-cm stage. Thereafter, a rapid growth was observed to the 30 cm stage in the rumen and omasum and to the 36 cm stage in the reticulum. However, active growth did not extend to the end of pregnancy in any of the non-glandular compartments; some inhibition of growth was observed in later stages in the rumen and reticulum, while the growth-rate stabilized in the omasum. In the abomasum, the epithelial growth-rate declined from the earliest stages of embryonic development to the 15-cm stage, stabilizing thenceforth until the end of gestation.

Lamina propria and submucosa

In the rumen, significant differences in the thickness of the lamina propria and submucosa were observed between group I and other groups. In the reticulum, these layers were significantly thicker in group V than in groups I, II, III and IV, with no significant difference between groups I, II and III. In the omasum, these tissue layers were significantly thicker in groups III, IV and V than in groups I and II, though no significant difference was detected between groups I and II. In the abomasum, both the lamina propria and the submucosa were significantly thicker in group V than in the other four groups. The thickness of the lamina propria and submucosa was not differed significantly between males and females.

Growth of these layers fitted a polynomial model for the rumen, reticulum and abomasum and a logarithmic model for the omasum.

The growth dynamics of the lamina propria and submucosa of the rumen and reticulum were characterized by an involutive phase which started in the early embryonic stages and persisted until the 20-cm and 15-cm stages in the rumen and reticulum, respectively. Involution was more marked in the rumen. Thereafter, active growth was observed in both compartments, lasting until birth. In the omasum and abomasum, the lamina propria and submucosa grew progressively from the early embryonic stages until birth.

Tunica muscularis

The tunica muscularis was not observed in the goat embryos and foetuses of group I. The tunica muscularis of rumen was significantly thicker in group V than in the other groups, but no significant differences were found between groups II, III and IV. In

212 reticulum, this layer was significantly thicker in groups IV and V than in groups II
213 and III, also significant differences were found between groups IV and V. In
214 omasum, significant differences were found between group V with groups II, III and
215 IV. The thickness of tunica muscularis in abomasum was significantly greater in
216 group IV and V than in the other groups, but no significant differences were
217 observed between groups II and III. Significant inter-sex differences in tunica
218 muscularis thickness were not recorded. However, tunica muscularis was thicker in
219 male than in female.

220 Tunica muscularis growth in the rumen fitted an exponential model. A slow
221 growth was recorded from the early embryonic stages to the 13.5-cm stage; thereafter
222 the growth-rate increased until the end of gestation. Growth of this layer in the
223 reticulum, omasum and abomasum fitted a polynomial model, progressive growth
224 being recorded from the earliest stages of gestation until birth; the growth-rate
225 increased from the 30-cm stage onwards.

226

227 **Serosa**

228 The ruminal serosa was significantly thicker in group V than in the other groups.
229 Significant differences were also observed between groups I, III and IV. In
230 reticulum, the serosa was also significantly thicker in groups III, IV and V than in the
231 other four groups, but no significant differences were found between groups I, II and
232 III. Significant differences were found in the thickness of the omasal serosa between
233 all groups. In the abomasum, the serosa was significantly less thick in groups I and II
234 than in groups III, IV and V. However, no significant differences were observed

between groups I and II. No significant inter-sex differences were observed in serosa thickness in any of the gastric compartments.

Serosal growth in the rumen fitted an exponential model. Growth was slow from the early embryonic stages until the 30-cm stage; thereafter, faster growth was observed until the end of the gestation period. Growth of the serosa in the reticulum and abomasum fitted a polynomial model. In the reticulum, slow growth was recorded from the start of pregnancy to the 17-cm stage; thenceforth, the growth-rate accelerated until the end of gestation. The abomasal serosa displayed rapid growth from the start of pregnancy to the 20-cm stage, thereafter leveling off and remaining stable until the end of gestation. The growth of serosa in omasum was adjusted to a logarithmic model. Its growth dynamics was characterized by an involution in thickness from the early stages of prenatal development until the end of the gestational period.

Total Wall

Total wall thickness in the rumen and reticulum was significantly lower in groups I, II and III than in groups IV and V. No significant differences were noted between the first three groups or between groups IV and V. In the omasum, significant differences were recorded between groups I and II with groups III, IV and V. Differences were also found between groups III, IV and V, but not between groups I and II. Total wall thickness in the abomasum was significantly greater in V than in groups I, II, III and IV; no significant differences were found between groups I, II and III. The wall thickness of all gastric compartments was no significantly different between males and females.

259 The growth-rate of the ruminal and reticular wall remained stable from early
260 embryonic stages to about 15 cm. The rapid-growth phase starting thereafter lasted
261 until the end of pregnancy in the rumen and to the 35-cm stage in the reticulum,
262 stabilizing in the latter to the end of gestation. The omasal and abomasal walls grew
263 progressively from the early embryonic stages until birth.
264

265 ## Allometric findings

266 Wall-thickness allometric coefficients, which correlate each gastric tissue layer with
267 body length, is shown in Table 6.
268 Results showed that the epithelial layer in all compartments grew faster than
269 gastric wall thickness, the fastest growth-rate being observed for the omasum. The
270 negative allometric coefficient in abomasum, indicated a dynamic involution of the
271 epithelial layer.
272 The lamina propria and submucosa in the rumen and reticulum grew more
273 slowly than the total gastric wall. Moreover, the negative coefficient confirmed some
274 inhibition of growth. In the omasum and abomasum, these layers grew faster than the
275 gastric wall in which they were immersed.
276 The tunica muscularis in all compartments grew around twice as fast as the total
277 gastric wall.
278 The allometric coefficient for the serosa in the rumen and particularly the
279 reticulum indicated a growth-rate faster than that of the total wall. In the omasum and
280 abomasum, growth-rates approached those of the gastric wall, tending towards
281 involution in the omasum.

The gastric wall displayed a slower growth-rate than body length; the slowest rate was recorded for the rumen, similar rates being observed for the other compartments.

DISCUSSION

The data obtained in this study revealed that the increase in epithelial thickness was directly proportional to the increase in embryo body length (crown-rump length) in non-glandular stomach compartments, as also reported in the rumen of buffalo by Osman and Berg (1981) and in red deer by Franco *et al*. (2004a). However, the abomasum decreased in thickness as gestation progressed; a finding also noted in sheep (Franco *et al.* 1993d) and in red deer (Masot *et al.* 2007).

A slow growth phase was observed in the epithelium of non-glandular stomach compartments in the early embryonic stages, coinciding with the transition from embryonic epithelium (Warner 1958) to stratified epithelium formed by the first two strata: basal and granulosum, as reported elsewhere in goats (Garcia *et al.* 2012, 2013). From 13.5 cm (64 days, 43% gestation) onwards, an active growth phase was observed, coinciding with an increase in the number of cells to form the stratum spinosum and the stratum corneum. This increased epithelial thickness may also be due to changes in the mucosa as a result of the formation of three structures corresponding to each compartment: rumen papillae have been observed at 9 cm (53 days, 35% gestation) in goats (Garcia *et al.* 2012), at 12.5 cm (61 days, 42% gestation) in sheep (Franco *et al.* 1992), and at 21 cm (142 days, 50% gestation) in deer (Franco *et al.* 2004a); reticular crests are reported at 11.5 cm (61 days, 41%

306 gestation) in goats (Ramkrishna & Tiwari 1979), at 9.6 cm (56 days, 38% gestation)
307 in sheep (Franco *et al.* 1993c), and at 21 cm (142 days, 50% gestation) in red deer
308 (Franco *et al.* 2004b); all four orders of omasal laminae have been noted at 4.3 cm
309 (38 days, 25% gestation), 7.7 cm (50 days, 33% gestation), 12 cm (59 days, 39%
310 gestation), and 13.5 cm (64 days, 43% gestation), respectively, in goats (Garcia *et al.*
311 2013) and also between 3.18 -7.4 cm in another goat study (Molinari & Jorquera
312 1988). However, epithelial growth inhibition was observed in the rumen and
313 reticulum in the final stages of gestation. This finding has also been noted in the
314 buffalo rumen by Osman and Berg (1981), where the number of epithelial layers was
315 reduced in perinatal stages due to the increased elevation of ruminal papillae into the
316 luminal surface. In the omasum, epithelial thickness remained constant with
317 increased fetal size, as described elsewhere in goats (Ramkrishna & Tiwari 1979). A
318 decrease in thickness or epithelial height was observed in the abomasum, due to the
319 transition from a pseudostratified epithelium to a simple coinciding with what
320 reported by previous studies (Age *et al.* 2007; Masot *et al.* 2007; Singh *et al.* 2007).
321 The growth of the lamina propria and submucosa was characterized by
322 regression in the early stages of prenatal development in the rumen and reticulum,
323 marking the differentiation of these two layers from the blastic tissue layer, as
324 reported in earlier studies (Franco *et al.* 1992, 1993a, b, c; Franco *et al.* 2004a,b;
325 Garcia *et al.* 2012, 2013). The differentiation of mesenchymal cells into fibroblast
326 cells and fibrillar elements could account for the progressive decrease in thickness of
327 these tissue layers in the early embryonic stages, as observed in the study of Kamel
328 et al. (1987) in the goat stomach. Two additional processes may also be involved in
329 the involution phase: firstly, active growth of the epithelium and tunica muscularis

330 could hinder the expansion of these strata (Osman & Berg 1981); secondly,
331 involvement of mesenchymal cells in the formation of the tunica muscularis, which
332 at this stage was also differentiated from pluripotent blastemic tissue according to
333 what describe in sheep (Franco *et al.* 1992, 1993a,b,c,), in deer (Franco *et al.* 2004a,
334 b) and in goat (Garcia *et al.* 2012, 2013). However, after the period of involution, an
335 active growth phase was observed in the rumen and reticulum, reflecting the
336 involvement of these layers in the formation of ruminal papillae (Vivo *et al.* 1990;
337 Franco *et al.* 1992; Franco *et al.* 2004a; Garcia *et al.* 2012) and reticular crests (Vivo
338 *et al.* 1990; Franco *et al.* 1993c; Franco *et al.* 2004b). By contrast, the lamina propria
339 and submucosa grew constantly from the start to the end of gestation. This
340 progressive growth may reflect their involvement in omasal laminae, reported in
341 goats from the early stages of pregnancy by Garcia *et al.* (2013). Moreover, the
342 increased thickness of these layers in the abomasum may be linked to the progressive
343 involution observed in the epithelial layer in that compartment, a finding not reported
344 in sheep by Franco *et al.* (1993d) and in cows by Vivo *et al.* (1990).

345 The growth of the tunica muscularis fitted an exponential model for the rumen,
346 as also noted in sheep by Franco *et al.* (1993d), while in the other compartments it
347 fitted a polynomial model. The fastest growth-rate for this muscle layer was recorded
348 in the abomasum, followed by the reticulum and finally the rumen and omasum,
349 which attained similar heights, as also reported in cattle by Vivo *et al.* (1990).
350 However, the growth-rate was slow until the 13.5-cm stage (64 days, 43% gestation),
351 by which point the two bundles making up the tunica muscularis were completely
352 configured (Garcia *et al.* 2012). Thereafter, growth-rates increased, possibly
353 reflecting the growth of the internal bundle, which is involved in the formation of the

354 muscular body of ruminal pillars (Panchamuki & Srivastava 1979; Franco *et al.*
355 1992; Franco *et al.* 2004a) and of the muscularis mucosae in reticulum (Franco *et al.*
356 1993c, Franco *et al.* 2004b), omasum (Franco *et al.* 1993a; Redondo *et al.* 2005) and
357 abomasum (Franco *et al.* 1993b; Masot *et al.* 2007).

358 Serosal growth varied depending on the compartment. A bottom-up growth was
359 observed in the rumen, reticulum and abomasum from the early stages of pregnancy.
360 These findings differ from those reported in cattle (Vivo *et al.* 1990) and sheep
361 (Franco *et al.* 1993d). By contrast, serosal involution in the omasum has been noted
362 in both species. This gradual reduction in the thickness of the serosa may result from
363 tissue differentiation of blast cells in the connective tissue of the subserosal, similar
364 to what describe in cow by Vivo *et al.* (1990).

365 Data for total wall thickness confirmed that growth-rates in the rumen and
366 reticulum remained stable until the 13.5-cm stage (64 days, 43% gestation),
367 accelerating thereafter. Similar growth phases are also reported in sheep by Franco *et*
368 *al.* (1993d). Moreover, the growth-rate of the rumen and reticulum coincided in this
369 study with that of the tunica muscularis. The omasal and abomasal walls displayed
370 consistent growth-rates from the start of pregnancy, coinciding with the growth
371 recorded by the lamina propria and submucosa in these compartments. Variations in
372 the dynamics of overall wall growth, also reported in sheep (Franco *et al.* 1993d), are
373 linked to specific characteristics of each of the tissue layers.

374 Morphometric data for males and females indicated no clear differences in
375 tissue-layer growth between sexes. However, tunica muscularis was thicker in males
376 than female in all stomach compartments. This difference may be attributed to the
377 anabolic effect of testosterone (Bhasin *et al.* 1997), which prompts an increase in

muscle fiber diameter, thus favoring an increase in overall muscle size coinciding with Griggs *et al.* (1989).

Allometric coefficients obtained for the epithelial layer and the tunica muscularis were similar to those reported for sheep (Franco *et al.* 1993d). However, the regression in serosal growth noted elsewhere was observed here only in rumen.

In conclusion, the mathematical models constructed using growth data for each of the stomach compartments closely reflected their prenatal development. This system could thus prove useful for determining fetal age based on the measured thickness of the wall as a whole and of each component tissue layer.

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401 **REFERENCES**

402 Age HM, Bhosale NS, Kadam SD, Kpadnis PJ. 2007. Histomorphological study of
403 abomasum in cattle. *Indian Journal Animal Research* **41**, 192-195.
404 Bhasin S, Storer TW, Berman N, Yarasheski KE, Clevenger B, Phillips J, Paul Lee
405 W, Bunnell TJ, Casaburi R. 1997. Testosterone replacement increases fat-free
406 mass and muscle size in hypogonadal men. *Journal of Clinical Endocrinology &*
407 *Metabolism* **82**, 407-413.
408 Brinks JS, Clark RT, Kieffer NM, Urick JJ. 1964. Estimates of genetic,
409 environmental and phenotypic parameters in range Hereford females. *Journal of*
410 *Animal Science* **23**, 711.
411 Brody S. 1945. Bioenergetics and growth. Reinhold Publ. Co., New York.
412 Brown JE, Brown CJ, Butts WT. 1973. Evaluating relationships among immature
413 measures of size, shape and performance of beef bulls. 1. Principal components
414 as measures of size and shape in young Hereford and Angus bulls. *Journal of*
415 *Animal Science* **36**, 1010.
416 Evans HE, Sack WO. 1973. Prenatal development of domestic and laboratory
417 mammals: Growth curves, external features and selected references. *Anatomia,*
418 *Histologia, Embryologia* **2**, 11-45.
419 Fizthugh HA. 1976. Analysis of growth curves and strategies for altering their shape.
420 *Journal of Animal Science* **42**, 1036-1051.
421 France V, Thornley I. 1984. Mathematicals models in agriculture. Butterworths,
422 London, U.K.

423 Franco A, Regodon S, Robina A, Redondo E. 1992. Histomorphometric analysis of
424 the rumen of the sheep during development. *American Journal Veterinary*
425 *Research* **53**, 1209-1217.
426 Franco A, Robina A, Regodon S, Vivo JM, Masot AJ, Redondo E. 1993a.
427 Histomorphometric analysis of the omasum of sheep during development.
428 *American Journal Veterinary Research* **54**, 1221-1229.
429 Franco A, Robina A, Guillen MT, Mayoral AI, Redondo E. 1993b.
430 Histomorphometric analysis of the abomasum of sheep during development.
431 *Anatomischer Anzeiger* **175**, 119-125.
432 Franco A, Robina A, Regodon S, Vivo JM, Masot AJ, Redondo E. 1993c.
433 Histomorphometric analysis of the reticulum of the sheep during development.
434 *Histology and Histopathology* **8**, 547-556.
435 Franco A, Rodriguez PL, Mayoral AI, Guillen MT, Robina A. 1993d. Modelos
436 matemáticos aplicados al crecimiento gástrico ovino durante la vida intrauterina.
437 *Anatomia, Histologia, Embryologia* **22**, 160-178.
438 Franco A, Masot AJ, Aguado MC, Gómez L. Redondo E. 2004a. Morphometric and
439 immunohistochemical study of the rumen of red deer during prenatal
440 development. *Journal of Anatomy* **204**, 501-513.
441 Franco A, Redondo E. Masot AJ. 2004b. Morphometric and immunohistochemical
442 study of the reticulum of red deer during prenatal development. *Journal of*
443 *Anatomy* **205**, 277-289.
444 Garcia A, Masot J, Franco A, Gazquez A. Redondo E. 2012. Histomorphometric and
445 immunohistochemical study of the goat rumen during prenatal development. *The*
446 *Anatomical Record* **295**, 776-785.

447 Garcia A, Masot J, Franco A, Gazquez A. Redondo E. 2013. Histomorphometric and
448 immunohistochemical study of the goat omasum during prenatal development.
449 *Histology and Histopathology* **28**, 737-748.
450 Griggs RC, Kingston W, Jozefowicz RF, Herr BE, Forbes G, Halliday D. 1989.
451 Effect of testosterone on muscle mass and muscle protein synthesis. *Journal of*
452 *Applied Physiology* **66**, 498-503.
453 Huxley US. 1932. Problems of relative growth. Methuen, Pub. Co., U.K, London.
454 Kamel G, Hassan A, Ali A, Moustafa M. 1987. Histological studies on the prenatal
455 development of goat stomach. *Assiut Veterinary Medical Journal* **19**, 23-30.
456 Lombardi G. 2005. Optimum management and quality pastures for sheep and goat in
457 mountain areas. *Options mediterraneennes* **67**, 19-29.
458 Masot AJ, Franco A, Redondo E. 2007. Morphometric and immunohistochemical
459 study of the abomasum of red deer during prenatal development. *Journal of*
460 *Anatomy* **211**, 376-386.
461 Molinari E, Jorquera B. 1988. Intrauterine development stages of the gastric
462 compartments of the goat (*Capra hircus*). *Anatomia, Histologia, Embryologia*
463 **17**, 121-137.
464 Osman AH, Berg R. 1981. Studies on the histogenesis of the tunica mucosa of the
465 stomach of the Egyptian Water buffalo (*Bos Bubalis*) 1. Histogenesis of the
466 ruminal mucosa. *Anatomischer Anzeiger* **149**, 232-240.
467 Panchamuki BG, Srivastava HC. 1979. Histogenesis of the rumen of the buffalo
468 (*Bubalus bubalis*) stomach. *Anatomia, Histologia, Embryologia* **8**, 97-105.
469 Ramkrishna V, Tiwari GP. 1979. Histological and histochemical observations on the
470 forestomach of goat during pre-natal life. *Cells Tissues Organs* **103**, 292-300.

471 Redondo E, Franco A, Masot AJ. 2005. Morphometric and immunohistochemical
472 study of the omasum of red deer during prenatal development. *Journal of*
473 *Anatomy* **206**, 543-555.
474 Relling AE, Mattioli GA. 2002. Fisiologia Digestive y Metabolica de los Rumiantes,
475 EDUL ed. Universidad Nacional de La Plata, Buenos Aires.
476 Schummer A, Nickel R. 1975. Lehrbuch der Anatomie der Haustiere 3th ed., Vol II
477 Verlag pp. 149–173. Paul Parey, Berlin.
478 Singh O, Roy KS, Kumar A, Bawa BS. 2007. Histomorphological studies on prenatal
479 development of abomasums in buffalo. *Indian Journal of Animal Science* **77**,
480 727-729.
481 Sivachelvan MN, Ghali Ali M, Chibuzo GA. 1996. Foetal age estimation in sheep
482 and goat. *Small Ruminant Research* **19**, 69-76.
483 Taylor SC, Craig J. 1965. Genetic correlation during growth of twin cattle. Animal
484 Production **7**, 83.
485 Vivo JM, Robina A, Regodon S, Guillen MT, Franco A, Mayoral AI. 1990.
486 Histogenetic evolution of bovine gastric compartments during prenatal period.
487 *Histology and Histopathology* **5**, 461-476.
488 Wardrop JD. 1961. Some preliminary observations on the histological development
489 on the forestomach of the lamb. I. Histological changes due to age in the period
490 from 46 days of foetal life to 77 days of postnatal life. *Journal of Agricultural*
491 *Science* **57**, 335-341.
492 Warner ED. 1958. The organogenesis and early histogenesis of the bovine stomach.
493 *American Journal of Anatomy* **102**, 33-63.
494

FIGURE LEGENDS

Figure 1 Mathematical models of prenatal growth of the epithelial layer: y = thickness of each tissue layer (micrometers); x = crown-rump length (centimeters).

(A) Mathematical model of epithelium in rumen. $y = -3.25+81.21x-1.33x^2$; $r=0.94$

(B) Mathematical model of epithelium in reticulum. $y = -108.23+93.22x-1.02x^2$; $r=0.96$

(C) Mathematical model of epithelium in omasum. $y = 44.85+18.61x-0.13x^2$; $r=0.96$

(D) Mathematical model of epithelium in abomasum. $y = 559.74-39.96x+0.71x^2$; $r=0.94$

Figure 2 Mathematical models of prenatal growth of lamina propria and submucosa: y = thickness of each tissue layer (micrometers); x = crown-rump length (centimeters).

(A) Mathematical model of lamina propria and submucosa in rumen. $y = 776.34-43.88x+0.99x^2$; $r=0.88$

(B) Mathematical model of lamina propria and submucosa in reticulum. $y = 499.25+13.27x+0.13x^2$; $r=0.88$

(C) Mathematical model of lamina propria and submucosa in omasum. $y = -1529.47+4040.63log\ (x)$; $r=0.98$

(D) Mathematical model of lamina propria and submucosa in abomasums. $y = 521.64-21.22x+1.95x^2$; $r=0.99$

Figure 3 Mathematical models of prenatal growth of tunica muscularis: y = thickness of each tissue layer (micrometers); x = crown-rump length (centimeters).

519 (A) Mathematical model of tunica muscularis in rumen. $y = 113.38\ exp^{(0.0565x)}$;

520 $r=0.96$

521 (B) Mathematical model of tunica muscularis in reticulum. $y = -$

522 $69.49+33.11x+0.28x^2; r=0.99$

523 (C) Mathematical model of tunica muscularis in omasum. $y = 137.97+9.28x+0.60x^2;$

524 $r=0.98$

525 (D) Mathematical model of tunica muscularis in abomasum. $y = -$

526 $124.27+33.75x+0.63x^2; r=0.97$

527

528 **Figure 4** Mathematical models of prenatal growth of serosa: y = thickness of each

529 tissue layer (micrometers); x = crown-rump length (centimeters).

530 (A) Mathematical model of serosa in rumen. $y = 67.46\ exp^{(0.04x)}$; $r=0.96$

531 (B) Mathematical model of serosa in reticulum. $y = 19.29+10.81x+0.0005x^2; r=0.98$

532 (C) Mathematical model of serosa in omasum. $y = 477.49+200.07log\ (x); r=0.96$

533 (D) Mathematical model of serosa in abomasums. $y = 23.06+14.26x-0.23x^2; r=0.95$

534

535 **Figure 5** Mathematical models of prenatal growth of parietal wall: y = thickness of

536 each tissue layer (micrometers); x = crown-rump length (centimeters).

537 (A) Mathematical model of ruminal wall. $y = 931.97+42.55x+0.36x^2; r=0.94$

538 (B) Mathematical model of reticular wall. $y = 344.27+152.11x-0.85x^2; r=0.97$

539 (C) Mathematical model of omasum wall. $y = 726.13+254.46x-1.98x^2; r=0.99$

540 (D) Mathematical model of abomasal wall. $y = 987.4-11.57x+3.01x^2; r=0.99$

Table 1. Thickness of epithelial layer in goat stomach during prenatal development (µm).

		Rumen		Reticulum		Omasum		Abomasum	
		Absolute	Relative (%)	Absolute	Relative (%)	Absolute	Relative (%)	Absolute	Relative (%)
Group (***)	*I*	$374.35^{a} \pm 21.85$	31.49	$332.73^{a} \pm 3.65$	28.67	$155.12^{a} \pm 2.17$	9.02	$471.21^{c} \pm 12.57$	43.07
	II	$408.74^{a} \pm 29.32$	32.67	$455.45^{a} \pm 13.13$	39.03	$171.35^{a} \pm 4.71$	8.10	$277.94^{b} \pm 44.79$	28.98
	III	$714.29^{b} \pm 85.98$	56.44	$777.11^{a} \pm 81.89$	37.76	$237.21^{a} \pm 21.31$	5.66	$75.16^{a} \pm 2.87$	5.69
	IV	$1401.17^{d} \pm 109.36$	57	$1603.61^{b} \pm 130.08$	43.20	$597.98^{b} \pm 93.51$	10.49	$70.03^{a} \pm 1.53$	3.10
	V	$1074.54^{c} \pm 60.08$	37.07	$1826.01^{b} \pm 183.32$	39.02	$910.12^{c} \pm 15.76$	12.56	$59.80^{a} \pm 3.63$	1.23
Sex (n.s)	*M*	777.42 ± 103.73	42.44	1012.40 ± 178.18	37.63	406.99 ± 83.89	8.76	190.10 ± 45.40	16.37
	F	811.82 ± 122.08	43.43	985.56 ± 170.13	37.45	421.72 ± 82.44	9.57	191.55 ± 44.76	16.46

***: significant differences ($p < 0.05$); n.s.: no significant differences; letters $^{(a, b, c, d)}$: group means significantly different.

Table 2. Thickness of lamina propria and submucosa in goat stomach during prenatal development (µm).

		Rumen		Reticulum		Omasum		Abomasum	
		Absolute	Relative (%)	Absolute	Relative (%)	Absolute	Relative (%)	Absolute	Relative (%)
Group (***)	*I*	*697^{c} ± 52.92	58.56	*691.83^{ab} ± 8.22	28.67	*1093.77^{a} ± 32.68	63.45	*508.31^{a} ± 20.49	46.22
	II	450.05^{ab} ± 30.82	36.65	453.60^{a} ± 16.73	39.03	1425.04^{a} ± 101.73	66.03	409.71^{a} ± 37.57	40.45
	III	291.02^{a} ± 30.69	24.18	649.04^{a} ± 68.33	37.76	3137.34^{b} ± 75.13	74.62	700.97^{ab} ± 8.28	52.71
	IV	340.04^{ab} ± 31.91	13.68	908.26^{b} ± 48.59	43.20	4019.70^{c} ± 74.34	72.28	1027.72^{b} ± 105.12	43.90
	V	488.53^{b} ± 57.48	16.38	1189.70^{c} ± 96.13	39.02	4866.55^{d} ± 211.94	66.65	2450.98^{c} ± 218.19	50.34
Sex (n.s.)	*M*	446.46 ± 42.75	29.36	754.07 ± 63.65	35.27	3006.66 ± 402.49	69.47	1057.86 ± 225.91	46.98
	F	469.19 ± 48	30.43	802.90 ± 85.19	36.84	2810.29 ± 386.72	67.75	981.21 ± 192.20	46.48

*: corresponded to means value of pluripotential blastemic tissue layer; (***): significant differences ($p < 0.05$); n.s.: no significant differences;
letters $^{(a, b, c, d)}$: group means significantly different.

Table 3. Thickness of tunica muscularis in goat stomach during prenatal development (µm).

		Rumen		Reticulum		Omasum		Abomasum	
		Absolute	Relative (%)	Absolute	Relative (%)	Absolute	Relative (%)	Absolute	Relative (%)
Group (***)	*I*	pbt	-	pbt	-	pbt	-	pbt	-
	II	$150.96^{a} \pm 14.47$	12.05	$157.95^{a} \pm 30.43$	13.21	$184.23^{a} \pm 28.24$	8.41	$194.79^{a} \pm 46.61$	18.60
	III	$294.41^{a} \pm 29.82$	23.69	$472.52^{a} \pm 57.34$	22.71	$496.75^{ab} \pm 22.51$	11.78	$386.28^{a} \pm 61.28$	27.90
	IV	$474.44^{a} \pm 50.36$	19.03	$882.62^{b} \pm 67.94$	23.83	$653.42^{b} \pm 45.47$	11.65	$954.21^{b} \pm 67.68$	41.25
	V	$884.04^{b} \pm 161.25$	29.35	$1509.43^{c} \pm 124.03$	31.81	$1288.65^{c} \pm 144.05$	17.42	$2089.11^{c} \pm 73.09$	43.59
Sex (n.s)	*M*	453.44 ± 104.68	20.59	773.10 ± 162.75	23.39	677.47 ± 137.06	12.38	934.99 ± 232.31	33.03
	F	448.48 ± 97.62	21.47	738.15 ± 159.37	22.38	634.55 ± 126.61	12.25	877.21 ± 220.19	32.64

pbt: pluripotential blastimic tissue; ***: significant differences ($p < 0.05$); n.s.: no significant differences; letters $^{(a, b, c)}$: group means significantly different.

Table 4. Thickness of serosa in goat stomach during prenatal development (µm).

		Rumen		Reticulum		Omasum		Abomasum	
		Absolute	Relative (%)	Absolute	Relative (%)	Absolute	Relative (%)	Absolute	Relative (%)
Group (***)	*I*	$65.28^{a} \pm 5.17$	5.55	$72.99^{a} \pm 0.68$	6.29	$383.63^{e} \pm 4.21$	22.37	$68.49^{a} \pm 7.29$	6.21
	II	$98.93^{ab} \pm 6.67$	7.95	$86.59^{a} \pm 2$	7.43	$287.83^{d} \pm 5.23$	13.59	$114.84^{a} \pm 8.16$	11.40
	III	$137.74^{bc} \pm 7.63$	11.27	$146.17^{b} \pm 17.26$	7.04	$238.72^{c} \pm 6.98$	5.71	$180.19^{b} \pm 8.86$	13.42
	IV	$171.76^{c} \pm 2.80$	7	$296.44^{c} \pm 8.63$	8.12	$209.38^{b} \pm 4.11$	3.78	$263.33^{c} \pm 21.53$	11.34
	V	$289.64^{d} \pm 33.33$	9.76	$418.55^{d} \pm 14.27$	8.87	$169.16^{a} \pm 4.25$	2.35	$229.95^{bc} \pm 3.54$	4.82
Sex (n.s)	*M*	154.49 ± 18.61	8.71	204.91 ± 36.15	7.55	255.73 ± 20.23	9.34	168.71 ± 20.53	9.13
	F	150.84 ± 26.16	7.91	203.38 ± 36.26	7.54	259.73 ± 19.71	9.78	174.01 ± 20.24	9.75

***: significant differences ($p < 0.05$); n.s.: no significant differences; letters $^{(a, b, c, d, e)}$: group means significantly different.

Table 5. Thickness of parietal wall in goat stomach during prenatal development (µm).

		Rumen	Reticulum	Omasum	Abomasum
Group (***)	*I*	1188.22^{a} ± 27.84	1160.14^{a} ± 2.92	1723.14^{a} ± 47.91	1097.77^{a} ± 18.54
	II	1245.86^{a} ± 50.24	1167.76^{ab} ± 37.03	2154.14^{a} ± 111.53	1003.28^{a} ± 48.61
	III	1252.54^{a} ± 79.32	2062.07^{b} ± 222.05	4204.68^{b} ± 101.14	1346.15^{a} ± 75.45
	IV	2478.03^{b} ± 111.74	3694.93^{c} ± 248.68	5580.93^{c} ± 204.01	2323.95^{b} ± 184.18
	V	2937.83^{c} ± 129.35	4729.53^{d} ±1 44.01	7310.54^{d} ± 352.24	4828.86^{c} ± 238.68
Sex	*M*	1810.29 ± 205.18	2568.86 ± 387.88	4299.87 ± 586.14	2177.64 ± 412.79
(n.s)	*F*	1830.70 ± 203.78	2556.91 ± 397.89	4089.89 ± 554.87	2062.36 ± 369.80

***: significant differences ($p < 0.05$); n.s.: no significant differences; letters $^{(a, b, c, d)}$: group means significantly different.

Table 6. Coefficient of allometry in each tissue layer of goat stomach during prenatal development.

	Rumen	Reticulum	Omasum	Abomasum
E/W	1.15	1.12	1.17	-1.04
Lp + Sb/W	-0.09	-0.51	1.05	1.07
TM/W	1.93	1.94	1.80	1.95
S/W	1.11	1.16	-0.49	0.63
W/C-R	0.40	0.68	0.67	0.63

E: epithelium; Lp+Sb: Lamina propria and Submucosa; TM: tunica muscularis; S: serosa; W: wall; C-R: crown-rump (cm)

Figures

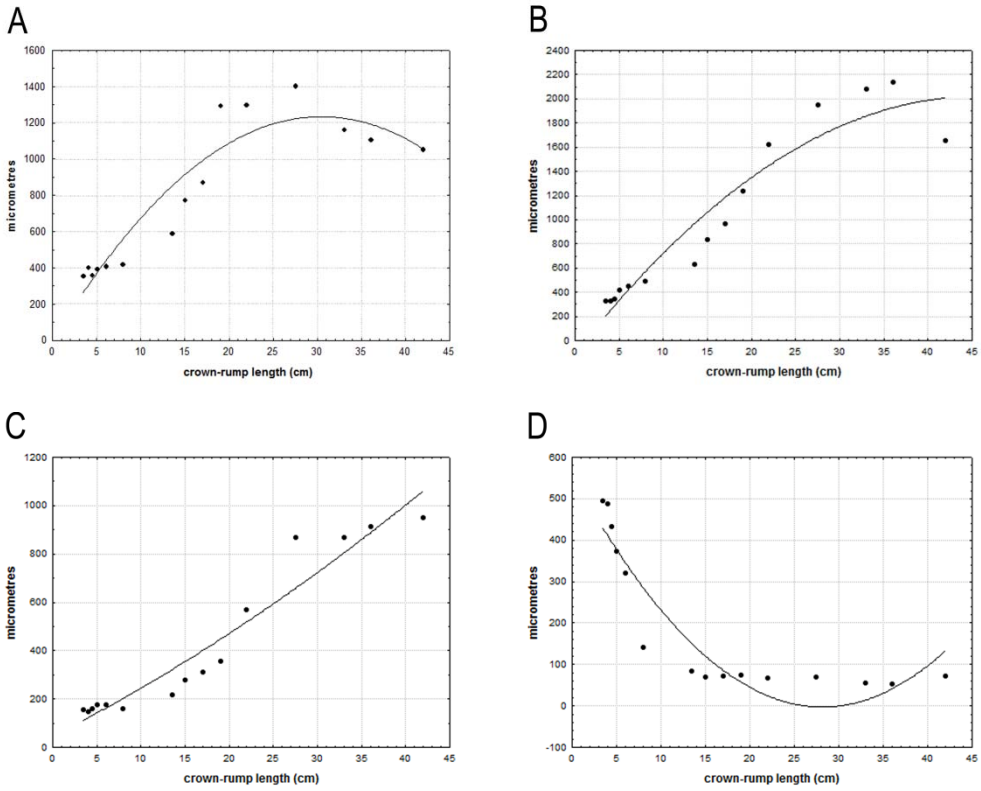

Figure 1

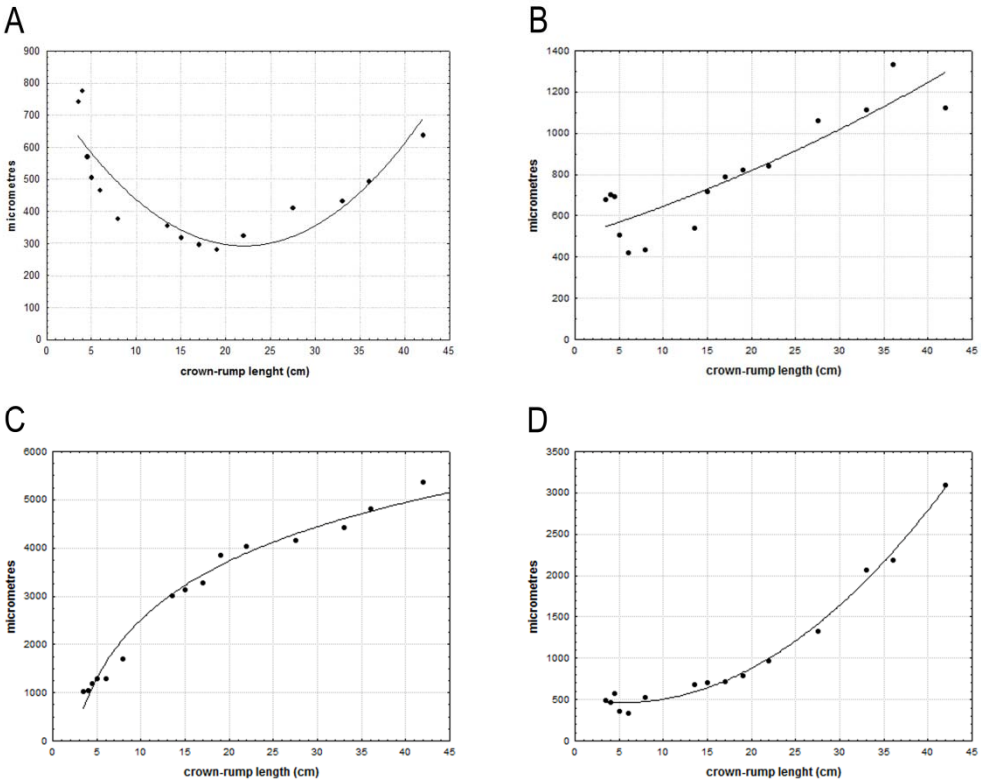
A
micrometres
crown-rump lenght (cm)
B
micrometres
crown-rump length (cm)
C
micrometres
crown-rump length (cm)
D
micrometres
crown-rump lenght (cm)

Figure 2

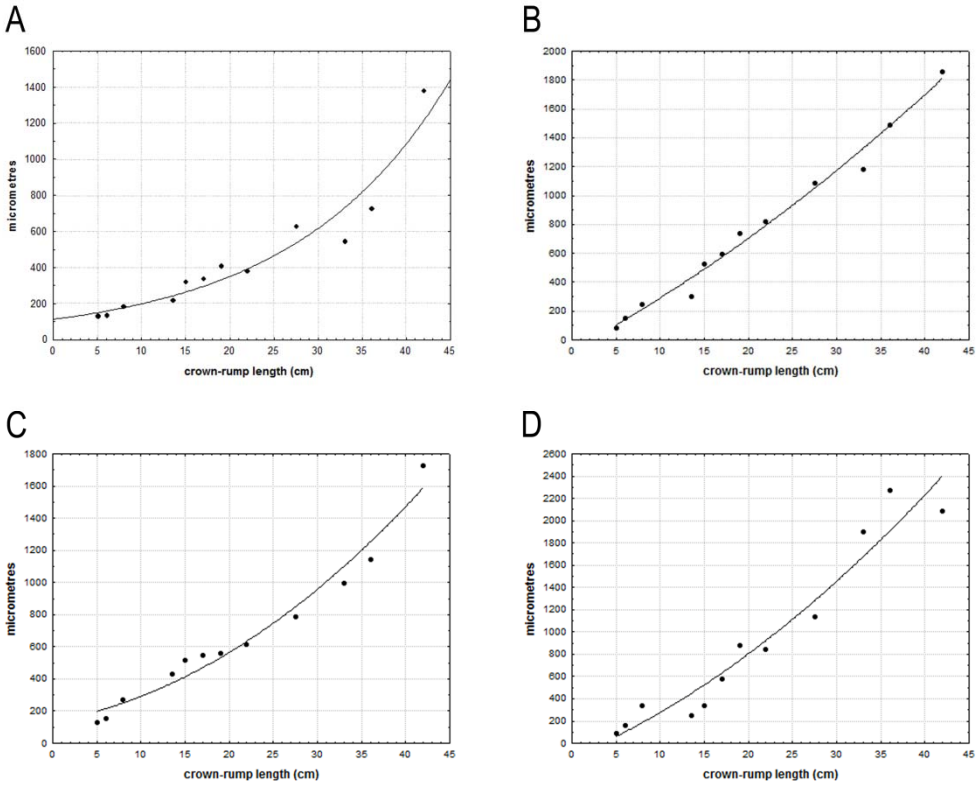
A
micrometres
crown-rump length (cm)
B
micrometres
crown-rump length (cm)
C
micrometres
crown-rump length (cm)
D
micrometres
crown-rump length (cm)

Figure 3

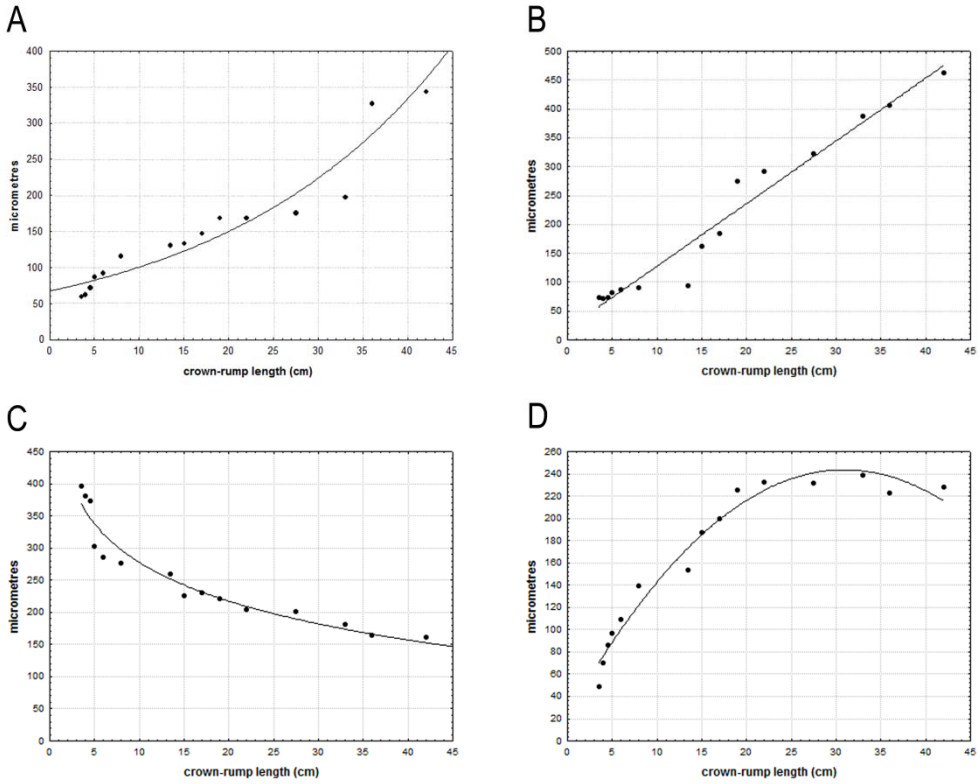
A
micrometres
crown-rump length (cm)
B
micrometres
crown-rump length (cm)
C
micrometres
crown-rump length (cm)
D
micrometres
crown-rump length (cm)

Figure 4

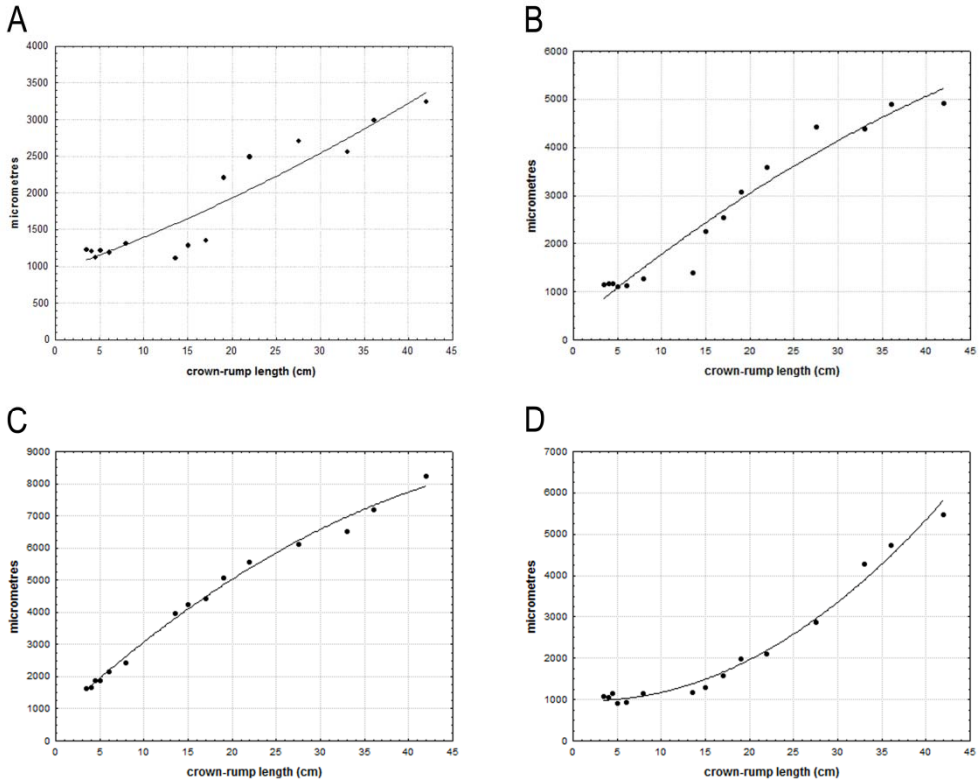
A
micrometres
crown-rump length (cm)
B
micrometres
crown-rump length (cm)
C
micrometres
crown-rump length (cm)
D
micrometres
crown-rump length (cm)

Figure 5

OTHER GRAPHICS ABOUT HISTOMORPHOMETRIC STUDY

(not published)

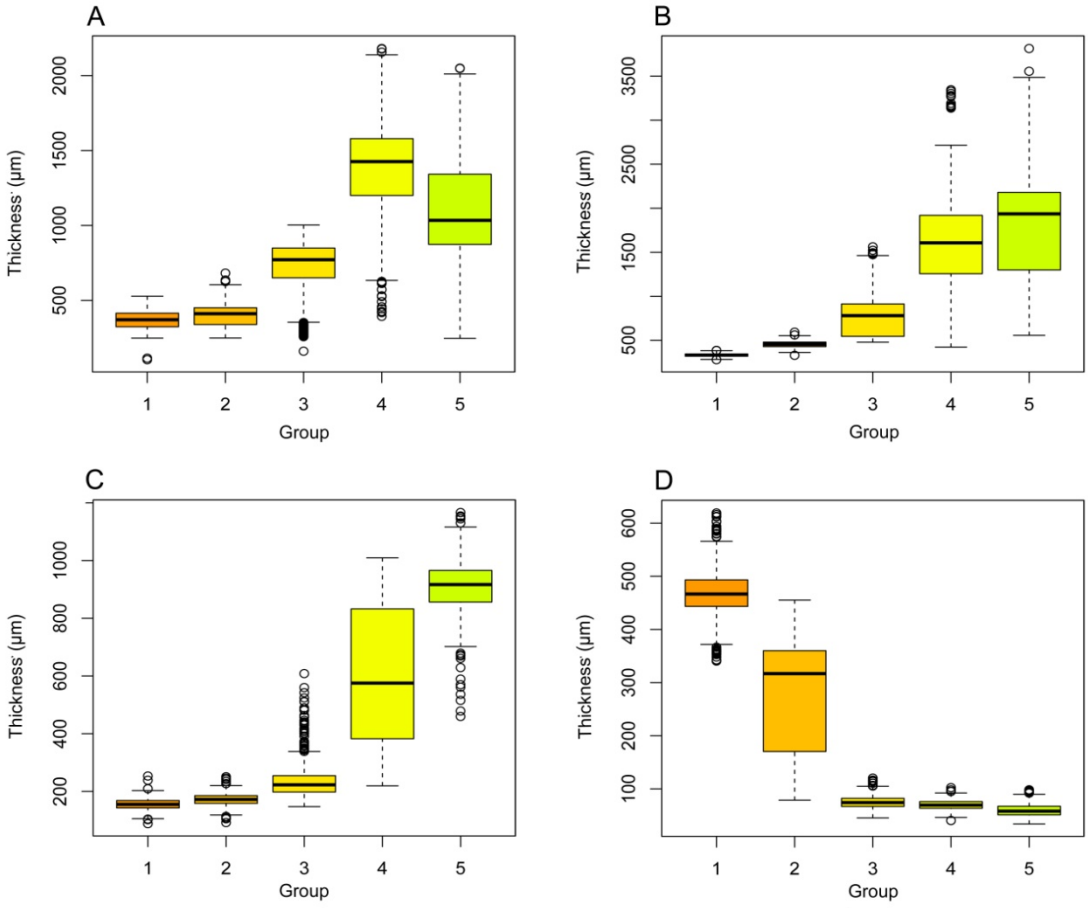

Imagen 22. Mean values of thickness of the epithelial layer in five groups of gestation.

A. Rumen
B. Reticulum
C. Omasum
D. Abomasum

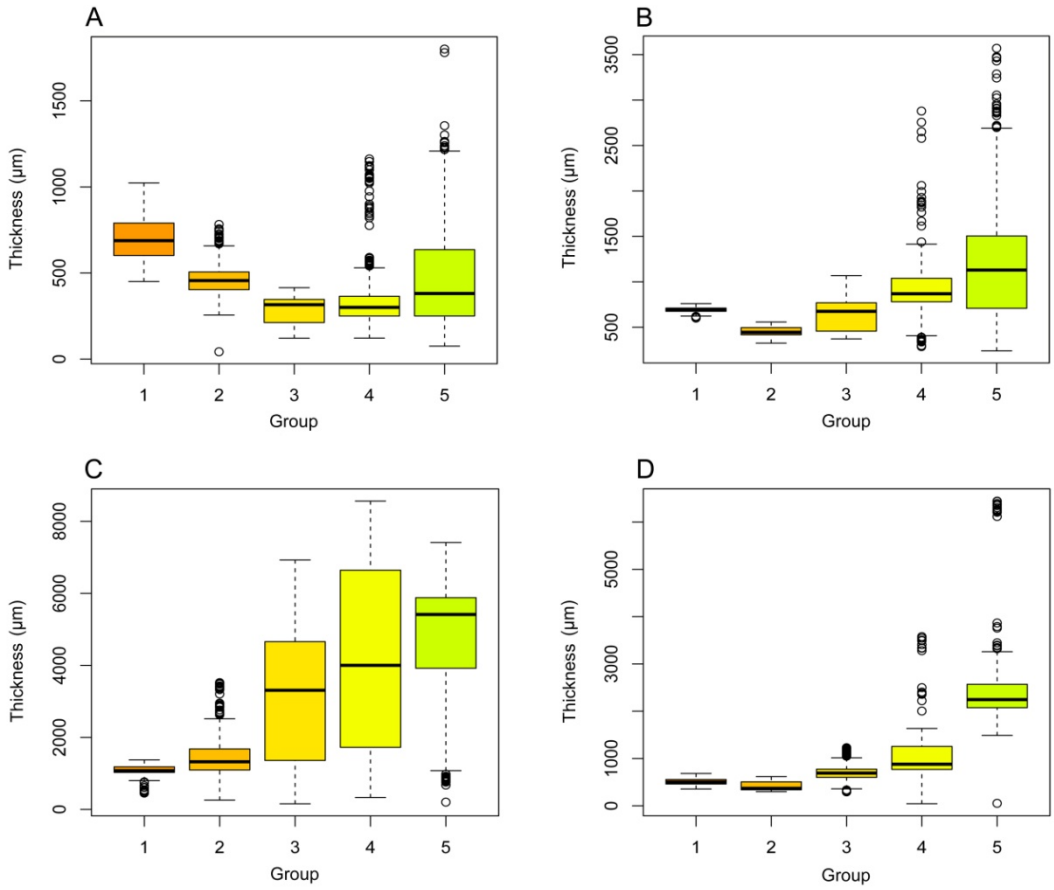

Imagen 23. Mean values of thickness of the lamina propria and submucosa in five groups of gestation.

A. Rumen
B. Reticulum
C. Omasum
D. Abomasum

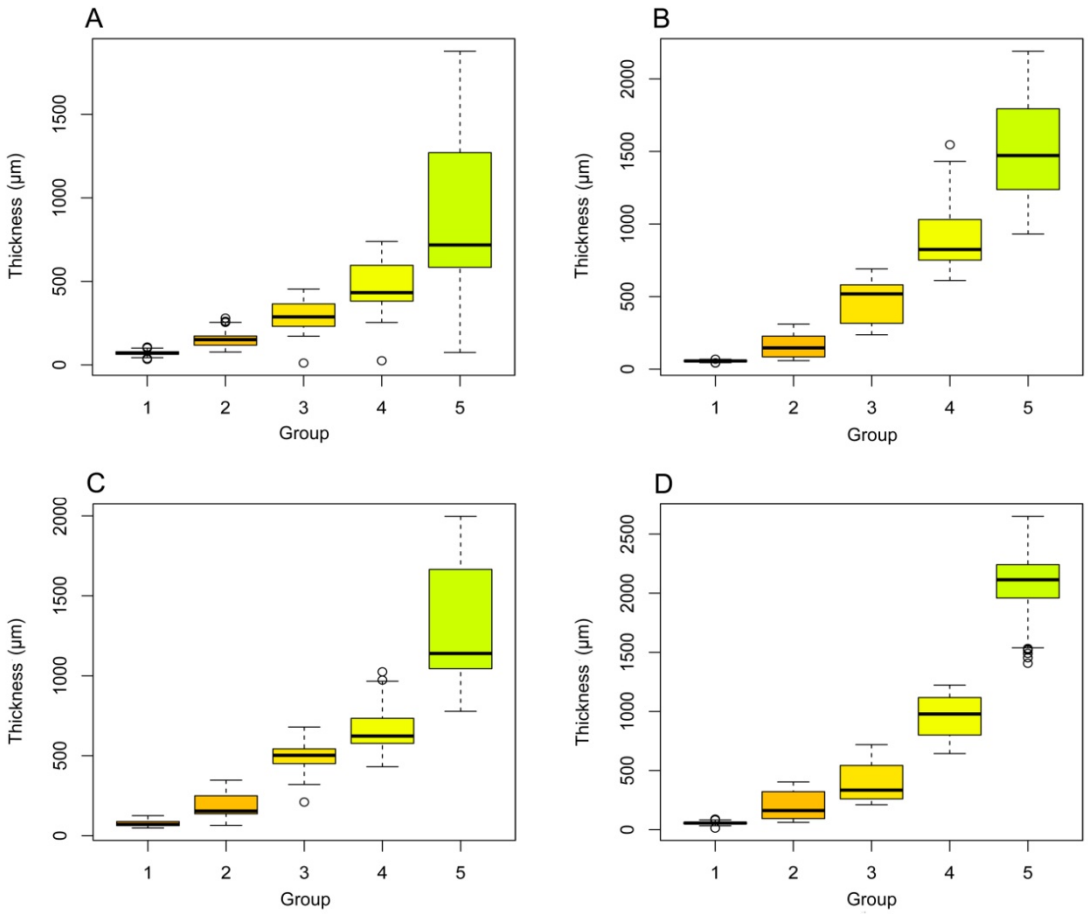

Imagen 24. Mean values of thickness of the tunica muscularis in five groups of gestation.

A. Rumen
B. Reticulum
C. Omasum
D. Abomasum

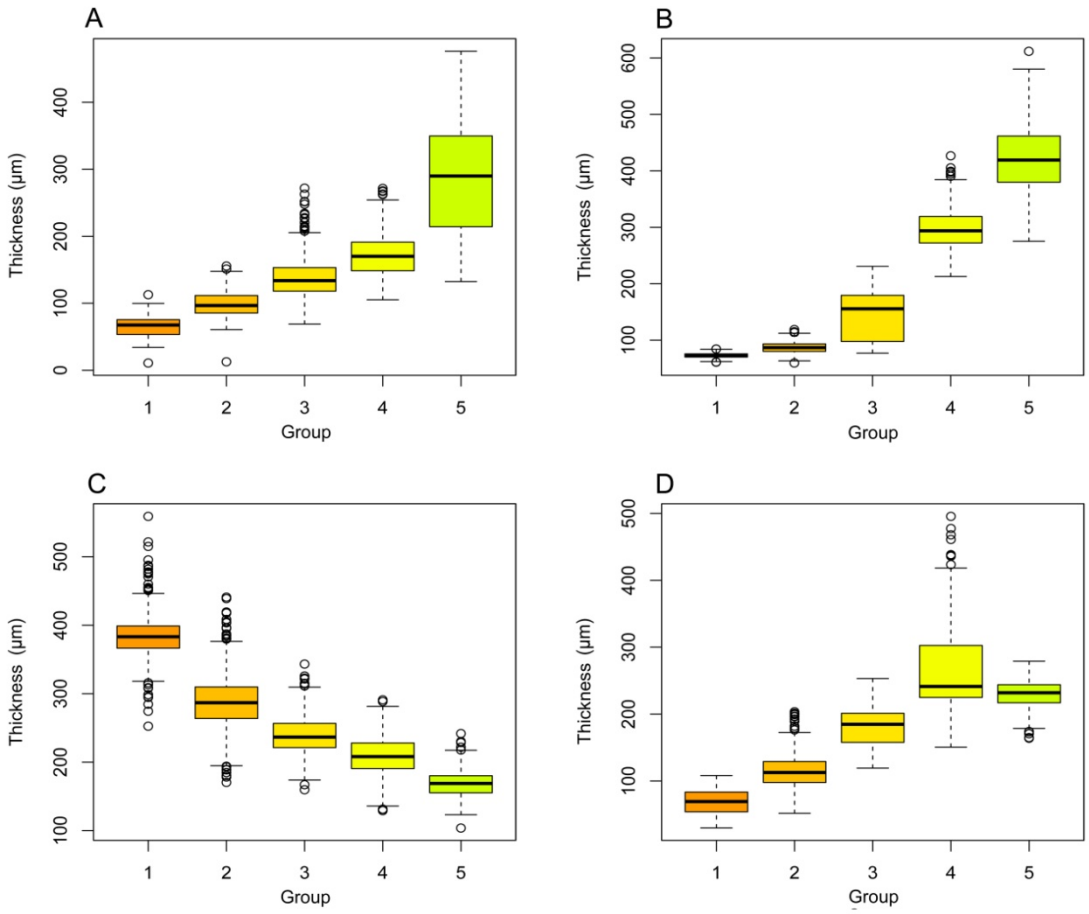

Imagen 25. Mean values of thickness of the serosa in five groups of gestation.

A. Rumen
B. Reticulum
C. Omasum
D. Abomasum

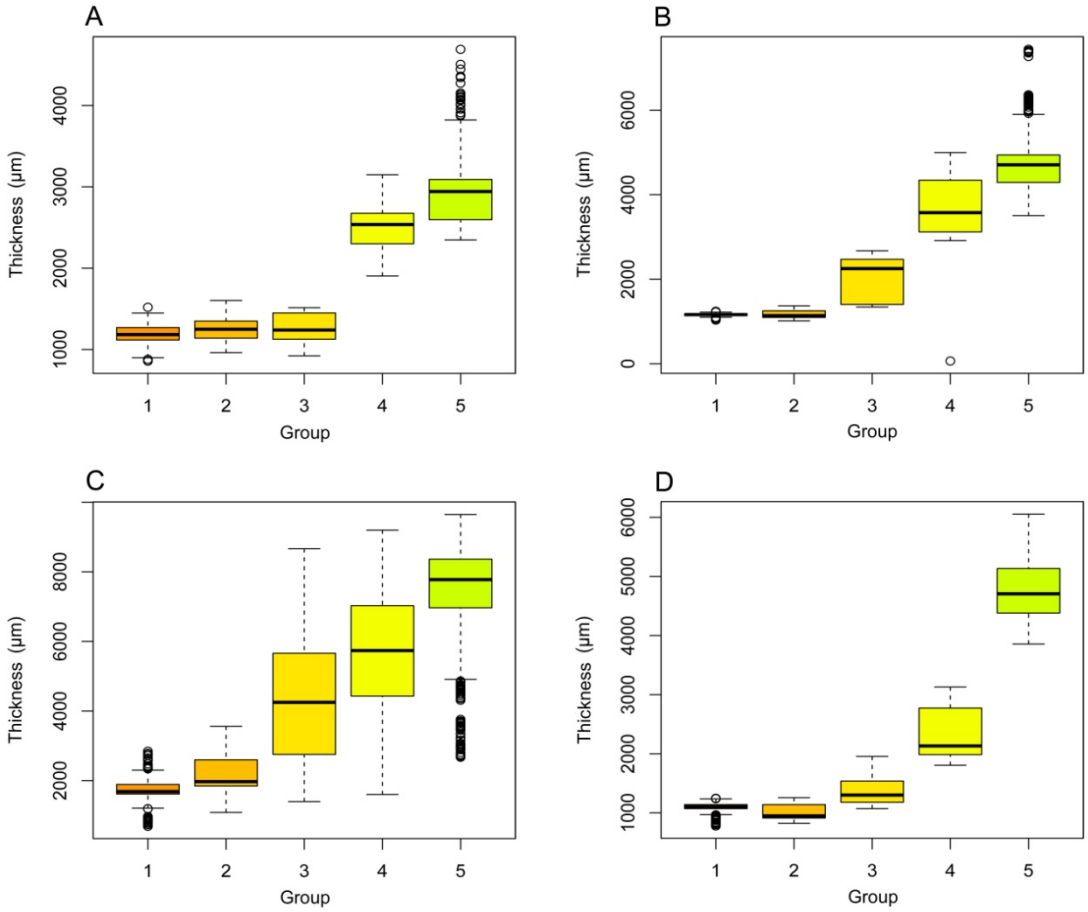

Imagen 26. Mean values of thickness of the total wall in five groups of gestation.

A. Rumen
B. Reticulum
C. Omasum
D. Abomasum

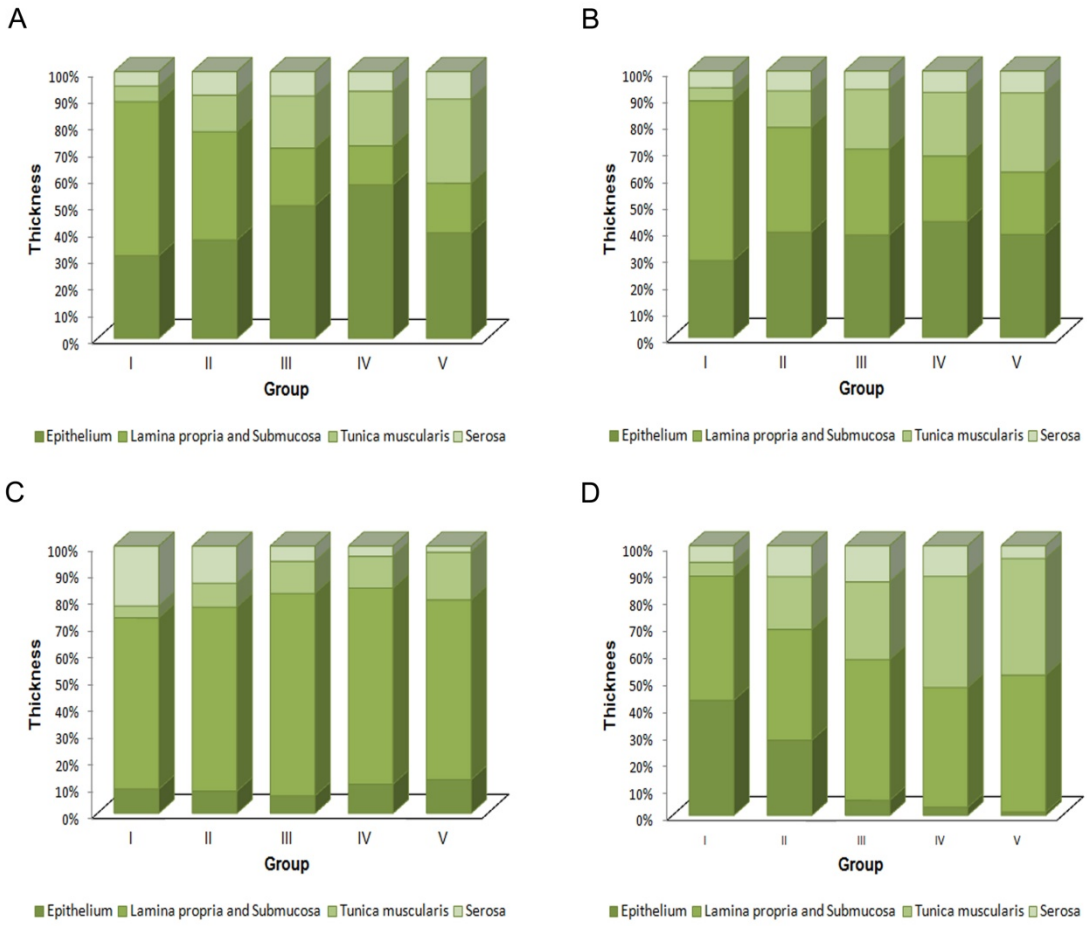

Imagen 27. Thickness (%) of tissue layers with respect to total wall in five groups of gestation.

A. Rumen
B. Reticulum
C. Omasum
D. Abomasum

7. CONCLUSIONES

CONCLUSIONS

1. La diferenciación histológica del rumen, retículo, omaso y abomaso como compartimentos individuales a partir del tubo gástrico primitivo fue observada a los 35 días (3 cm CRL, 23% de gestación). Su pared estaba constituida por tres capas: una interna o lámina epitelial, una media de tejido blástico pluripotencial y una externa o serosa.

2. El rumen, retículo y omaso se configuraron como compartimentos no glandulares y mostraron una mucosa tapizada por un epitelio estratificado plano queratinizado. La histogénesis secuenciada de la lámina epitelial de estos compartimentos fue acompañada de la diferenciación de estructuras específicas en su superficie (papilas ruminales, crestas reticulares y láminas omasales), representando una adaptación morfológica prenatal a la funcionalidad postnatal definitiva.

3. El abomaso, se conformó como compartimento glandular con la diferenciación de un epitelio simple cilíndrico y la aparición de estructuras glandulares en su mucosa a los 84 días (20 cm CRL, 55% de gestación). Las células productoras de gastrina fueron inmunodetectadas por primera vez en el epitelio a los 75 días (17,5 cm CRL, 50%) y localizadas posteriormente en las glándulas pilóricas.

4. La lámina propia y submucosa se diferenciaron a partir de la capa de tejido blástico pluripotencial a los 50 días (7,7 cm CRL, 33% gestación). Submucosa y lámina propia, en mayor proporción esa última, participaron activamente en la conformación del esqueleto de las papilas ruminales, crestas reticulares y láminas omasales.

5. La túnica muscular, diferenciada también a partir de la capa media de tejido blástico pluripotencial, estaba conformada por dos fascículos de fibras musculares lisas: uno interno circular y otro externo longitudinal. Esta capa tomó parte en la formación de los pilares ruminales y actuó de soporte

mecánico esquelético de la mucosa mediante derivaciones de su fascículo interno en la configuración de la muscular de la mucosa.

6. Las células neuroendocrinas positivas a sinaptofisina fueron observadas en lámina propia y submucosa, túnica muscular, plexos mientéricos y serosa, en todos los compartimentos gástricos, excepto en rumen, donde no fueron detectadas en serosa. Estas mismas células fueron inmunomarcadas con enolasa no neuronal en las mismas localizaciones y adicionalmente en la lámina epitelial.

7. Las células gliales fueron detectadas mediante vimentina en edades fetales más tempranas que la proteína ácida glial fibrilar. La immunorreactividad para ambos marcadores se distribuyó en las mismas localizaciones, siendo los plexos mientéricos las regiones de mayor intensidad de la inmunorreacción.

8. Los marcadores de inervación peptidérgica, neuropeptido Y y péptido intestinal vasoactivo fueron inmunodetectados en edades de gestación más tempranas en retículo y abomaso, seguidamente en el omaso y finalmente en el rumen. La inmunorreactividad para ambos marcadores fue localizada en lamina propia y submucosa, túnica muscular, plexos mientéricos y serosa, en todos los compartimentos, excepto en rumen, donde no fueron detectados en serosa.

9. La pared gástrica mostró un ritmo de crecimiento más lento que el de la longitud corporal, siendo el rumen el que más lento crecía. Además, la dinámica de crecimiento de la pared estaba supeditada a las características propias de crecimiento, bien de aumento o de involución, de cada uno de los estratos tisulares en los distintos compartimentos. La túnica muscular fue el único estrato tisular que creció más rápidamente que la propia pared parietal de todos los compartimentos.

1. The histological differentiation of rumen, reticulum, omasum and abomasum as individual compartments from the primitive gastric tube was observed at 35 days (CRL 3 cm, 23% of gestation). Its wall was composed of three layers: an internal or epithelial layer, a middle of pluripotential blastemic tissue and an external layer or serosa.

2. Rumen, reticulum and omasum were configured as no glandular compartments and showed a mucosa covered by a squamous keratizated stratified epithelium. The histogenesis sequenced of their epithelial layer was accompanied by differentiation of specific structures on its surface (ruminal papillae, reticular crest and omasal laminae), which represented a prenatal morphological adaptation to the postnatal functionality.

3. The abomasum was formed as a glandular compartment by the differentiation of a simple cylindrical epithelium and the appearance of glandular structures at 84 days (CRL 20 cm, 55% gestation). The gastrin-producing cells were first immunodetected in epithelial cells at 75 days (CRL 17.5 cm, 50% gestation) and later in pyloric gastric.

4. The lamina propria and submucosa were differentiated from pluripotent blastic tissue layer. Both layers, lamina propria and submucosa, in lesser proportion this last one, participated in the formation of the skeleton of ruminal papillae, reticular crest and omasal laminae.

5. The tunica muscularis, differentiated from pluripotent blastic tissue, was composed of two bundles of smooth muscle fibers: an inner circular and an outer longitudinal. This layer took part in the formation of ruminal pillars and acted as a mechanical skeletal support of mucosa by means of derivations of its internal fascicle in the configuration of the muscularis mucosae.

6. Neuroendocrine cells were detected by synaptophysin in lamina propria and submucosa, tunica muscularis, myenteric plexus and serosa, in all the gastric compartments, except in the serosa rumen. These same cells were immunolabelled by non-neuronal enolase in the same locations and additionally in the epithelial layer.

7. Glial cells positive to vimentin were detected at earlier fetal ages than with glial fibrillar acidic protein. Immunoreactivity for both markers was distributed in the same locations, being myenteric plexus the regions with the highest intensity.

8. Peptidergic innervation markers, neuropeptide Y and vasoactive intestinal peptide were immunodetected at earlier fetal ages in reticulum and abomasum, followed in omasum and by final stages of gestation in rumen. Immunoreactivity for both markers was observed in lamina propria and submucosa, tunica muscularis, myenteric plexus and serosa, in all gastric compartments except in the serosa rumen.

9. The gastric wall showed a slower growth rate than the body length, being rumen the slowest compartment in growing. In addition, the dynamics of wall growth was related to the proper characteristics, either increasing or involution growth, of each one of the tissue layers in the different compartments. The tunica muscularis was the only tissue layer that grew faster than the parietal wall itself of all the compartments.

8. RESUMEN

La finalidad de este trabajo ha sido estudiar el estómago de la cabra durante la vida prenatal mediante análisis macroscópico, histomorfológico y estructurales con microscopía electrónica de barrido y análisis inmunohistoquímicos. Además, fueron realizados estudios histomorfométricos en la pared compartimental y en cada uno de los estratos tisulares constituyentes de dicha pared para posteriormente ajustar su crecimiento a modelos matemáticos.

Un total de 140 embriones y fetos caprinos, en distintas fases de desarrollo, desde las primeras etapas embrionarias hasta el nacimiento fueron utilizados para este trabajo de investigación. Los especímenes fueron clasificados en cinco grupos de gestación de acuerdo a su longitud corporal (cabeza-nacimiento de la cola) y edad de gestación: grupo I (crown-rump length[CRL] 1.5-4.3 cm, 13-38 días, 1-25% de gestación), grupo II (CRL 4.4-8 cm, 39-52 días, 25-35% de gestación), grupo III (CRL 9-17.5 cm, 53-75 días, 35-50% de gestación), grupo IV (CRL 18-32 cm, 76-112 días, 50-75% de gestación) y grupo V (CRL 33-42 cm, 113-150 días, 75-100% de gestación).

El rumen, retículo, omaso y abomaso aparecieron diferenciados histológicamente como compartimentos individuales del tubo gástrico primitivo a los 35 días (23% de gestación). Sus paredes estuvieron conformadas por tres capas: una interna o lámina epitelial, una media de tejido blástico pluripotencial y una externa o serosa.

La estratificación de la lámina epitelial en los cuatro estratos (basal, espinoso, granuloso y córneo) en los compartimentos no glandulares (rumen, retículo y omaso) fue acompañada por modificaciones en su estructura, con la aparición de los pilares y papilas ruminales, crestas reticulares y láminas omasales.

En el rumen, los pilares ruminales fueron observados por primera vez a los 46 días (30% gestación) y los esbozos de las papilas ruminales comenzaron a insinuarse a los 53 días (35% gestación). A los 76 días (50% gestación), dichas papilas aparecían completamente desarrolladas como evaginaciones del estrato

basal hacia la luz ruminal y arrastrando en su configuración la lámina propria y la submucosa.

En retículo, la primera diferenciación de las celdillas reticulares fue marcada por la aparición de las crestas reticulares primarias a los 59 días (38% gestación) como pequeñas evaginaciones del estrato basal del epitelio. A los 87 días (61% gestación) fueron observadas unas expansiones laterales en la superficie de las crestas reticulares primarias, que se correspondían con las papilas córneas. Las crestas reticulares secundarias fueron visibles por primera vez a los 101 días (67% gestación), nunca sin alcanzar la altura de las primarias. Para la formación de las celdas del retículo fue necesario la combinación de dos tipos de crecimiento de las crestas: un crecimiento longitudinal, desde la superficie epitelial y responsable del crecimiento en longitud de las crestas y, un crecimiento transversal para la fusión de unas crestas con otras y formar las celdillas.

Las láminas omasales de primer, segundo, tercer y cuarto orden fueron observadas a los 38 días (25%), 50 días (33%), 59 días (38%) y 64 días (43%), respectivamente. A los 70 días (47% gestación), fueron visibles las primeras papilas córneas en la superficie de las láminas omasales de primer orden. A partir de los 113 días (75% gestación), las papilas córneas estuvieron presentes en los cuatro órdenes laminares.

En abomaso, la lámina epitelial sufrió una transición de epitelio pseudoestratificado a epitelio simple cilíndrico, acompañada de la diferenciación de las fosas gástricas y las glándulas gástricas a los 75 días (50% gestación) y a los 84 días (55% gestación), respectivamente.

La capa media de tejido blástico pluripotencial constituida por células mesenquimales, mostró a la edad de 50 días (33% de gestación) signos de diferenciación hacia células fibroblásticas para conformar la lámina propia y submucosa. Ambas capas estuvieron constituidas por un tejido conjuntivo fibroso, siendo la lámina propia más celular que la submucosa. No existió una delimitación histológica entre ambas capas, debido a la ausencia completa de la muscular de la

mucosa en el rumen y parcialmente en el retículo. Sin embargo, existió una separación espacial (no de génesis) como consecuencia del arrastre de la lámina propia para participar en la formación de las papilas ruminales y crestas reticulares.

A partir del tejido blástico pluripotencial se produjo también la diferenciación de la túnica muscular, formada en las primeras fases embrionarias por una capa de mioblastos, para conformarse posteriormente por dos fascículos de fibras musculares lisos: un interno circular y otro externo longitudinal. A partir del fascículo interno de fibras musculares lisas se configuró la muscular de la mucosa, la cual apareció a los 101 días (67% gestación) en la porción superior de las crestas reticulares primarias; a los 64 días (43% gestación) en las láminas omasales y a los 64 días (43% gestación) en el abomaso.

Externamente, cada uno de los compartimentos gástricos se cubrió por una serosa, formada por una subserosa de tejido conjunto laxo y tapizado por un mesotelio.

El estudio inmunohistoquímico fue realizado para detectar la presencia y la distribución de células neuroendocrinas, células gliales, marcadores de inervación peptidérgicas en todos los compartimentos gástricos, así como la detección de células productoras de gastrina exclusivamente en el compartimento abomasal.

La inmunodetección de células neuroendocrinas mediante sinaptofisina fue observada en rumen, retículo y omaso a los 53 días (35% gestación) y en abomaso a los 64 días (43% de gestación). Inmunorreactividad La frente a sinaptofisina fue detectada en la lámina propia y submucosa, túnica muscular, serosa y plexos mientéricos, en todos los compartimentos gástricos, excepto en la serosa del rumen, donde no fue detectada inmunorreacción. Estas células fueron inmunodetectadas mediante enolasa no neuronal (NNE) a los 64 días (43% de gestación) en todos los compartimentos gástricos. La inmunorreactividad frente a NNE fue observada en las mismas localizaciones que para SPY y de forma adicional en la lámina epitelial.

Las células gliales positivas a GFAP fueron observadas a los 64 días (43% gestación) de gestación en el retículo, omaso y abomaso y a los 68 días (15 cm CRL, 45% gestación) en rumen. Fueron distribuidas por lámina propia y submucosa, túnica muscular, serosa y plexos mientéricos. La superficie inmunomarcada aumentó a medida que avanzaba la edad fetal y las localizaciones con mayor inmunorreactividad fueron los plexos mientéricos. Immunorreactividad para vimentina fue observa a los 39 días (25% gestación) en rumen, retículo, omaso y 38 días (25%) en abomaso en el tejido blástico pluripotencial y serosa de cada uno de los compartimentos. En edades fetales más tardías, esta inmunorreactividad fue distribuida en las mismas capas tisulares que para GFAP.

Los marcadores de inervación peptidérgicos, NPY y VIP fueron detectados en rumen a los 113 días (75% gestación) y 120 días (80% gestación) respectivamente, a los 95 días (63% gestación) en omaso y a los 75 días (50% gestación) en retículo y abomaso. Las áreas inmunorreactivas fueron distribuidas por lámina propia y submucosa, túnica muscular, serosa y plexos mientéricos.

Las células immunorreactivas frente a gastrina fueron detectadas por primera vez a los 76 días (50% de gestación), en algunas áreas de la lámina epitelial y posteriormente fueron detectadas en las glándulas pilóricas en edades de gestación más avanzada.

El estudio histomorfométrico de cada una de las capas constituyentes de la pared parietal, reveló que la lámina epitelial mostraba un ritmo de crecimiento activo desde las primeras etapas embrionarias y llegó a valores estables en el momento del nacimiento en los compartimentos no glandulares. Por el contrario, la lámina epitelial del abomaso inicia un proceso involutivo desde las primeras fases embrionarias hasta el nacimiento. La lámina propia y submucosa en rumen y retículo evidenciaron una involución del crecimiento desde etapas iniciales. Sin embargo, su participación en las papilas ruminales y crestas reticulares hizo que esta involución se transformara en una fase de crecimiento activo. En omaso y abomaso, la lámina propia y submucosa siguieron un crecimiento acelerado desde las primeras fases de la gestación. La túnica muscular mostró un ritmo de

crecimiento constante desde las primeras fases embrionarias. En todos los compartimentos gástricos esta capa tuvo un crecimiento más rápido que la propia pared parietal. La serosa aumentó su grosor a medida que avanzaba la gestación en el rumen, retículo y abomaso, mientras que en el omaso mostró una involución de su crecimiento. La dinámica de crecimiento de la pared parietal estuvo relacionada con las características propias de crecimiento, bien de aumento o de involución, de cada uno de los estratos tisulares en los distintos compartimentos.

9. SUMMARY

The aim of this work was to study the goat´s stomach during its prenatal life by macroscopic analysis, histomorphological and a structural analysis with scanning electronic microscopy and immunohistochemical analysis. Furthermore, histomorphometric studies were performed on the wall compartment and each of the tissue layers of said wall constituents to adjust their growth subsequently applied mathematical models.

A total of 140 goat embryos and fetuses at various stages of development from early embryonic stages to birth were used for this research study. The specimens were classified into five sequential groups according to their size (crown-rump length-CRL) and gestational age: group I (CRL 1.5-4.3 cm, 13-38 days gestation 1-25%), group II (CRL 4.4-8 cm, 39-52 days, 25-35% of gestation), group III (CRL 9-17.5 cm, 53-75 days, 35-50% of gestation), group IV (18-32 cm CRL, 76 -112 days, 50-75% of gestation) and group V (CRL 33-42 cm, 113-150 days gestation 75-100%).

The rumen, reticulum, omasum and abomasum appeared as individual compartments histologically differentiated from primitive gastric tube at 35 days (23% of gestation). Its walls were composed of three layers: an internal or epithelial layer, a middle layer or pluripotent blastic tissue and an external or serosa.

The stratification of the epithelial layer in the four strata (basal, spinosum, granulosum and corneum) in no-glandulars compartments (rumen, reticulum and omasum) was accompanied by changes in its structure, with the appearance of ruminal pillars and papillae, reticular ridges and omasal laminae.

Ruminal pillars were visible at 46 days (30% gestation). The rudimentary ruminal papillae started to become visible at 53 days (35% gestation). At 76 days (50% gestation), these ruminal papillae were more developed, appearing as small elevations of the basal area towards the ruminal lumen, involving the lamina propria and submucosa.

In reticulum, the histological differentiation of future reticular cells was marked by the appearance of primary reticular crests at 59 days (38% gestation). At 87 days (61% gestation), lateral expansions on the surface of primary reticular

crests were observed, corresponding to the corneum papillae. Secondary reticular crests were visible at 101 days (67% gestation), though never attaining the same height as primary crests. To form reticular cells, reticular crests displayed two growth patterns: a longitudinal growth from the stratum basale towards the epithelial surface in order to lengthen crests and a transversally growth to fuse adjacent crest.

Omasal laminae of first, second, third and fourth orders were observed at 38 days (25% gestation), 50 days (33%), 59 days (38%) and 64 days (43%) respectively. At 70 days (47% gestation), conical papillae started to arise from the surface of primary omasal laminae. From 113 days (75% gestation), conical papillae were present in the four orders of omasal laminae.

The histogenesis of the epithelial layer of abomasum suffered a transition from a pseudoestratified epithelium to simple cylindrical epithelium, accompanied by the differentiation of gastric pits and gastric glands at 75 days (50% gestation) and 84 days (55% gestation) respectively.

The middle layer of blastic pluripotent tissue composed of mesenchymal cells, showed signs of differentiation into fibroblast cells to form the lamina propria and submucosa at 50 days (33% gestation). Both layers were formed by a fibrous connective tissue, being the lamina propria more cellular than the submucosa. There was no histological separation between both layers due to the complete absence of the muscularis mucosae into the rumen and reticulum partially. However, there was a spatial separation (but no genesis) because of the participation of the lamina propria in the formation of rumen papillae and reticular crest.

From pluripotent blastic tissue and at also 50 days (33% gestation), it was also produced the differentiation of tunica muscular, formed by a myoblastic layer. As the gestation progress, this layer became formed by two fiber bundles of smooth muscle fibers, a circular internal bundle and a longitudinal external bundle. From the inner bundle, it was formed the muscularis mucosae which was observed in the

upper portion of reticular crest at 101 days (67% gestation), in omasal laminae at 64 days (43% gestation), and also in abomasum at 64 days (43% gestation).

Each of the gastric compartments was covered externally by a serosa formed by a subserosa of loose connective tissue which was covered by a mesothelium.

Immunohistochemistry study was performed to detect the presence and distribution of neuroendocrine cells, glial cells, peptidergic innervations markers in all the compartments and the detection of gastrin-producing cells exclusively in abomasum.

Neuroendocrine cells were first detected by synaptophysin (SPY) at 53 days (35% gestation) in rumen, reticulum and omasum and at 64 days (43% gestation) in abomasum. Immunoreactivity-SPY was observed in lamina propria and submucosa, tunica muscularis, serosa and myenteric plexus in all the gastric compartments, except in the serosa rumen. These cells were immudetected by non-neuronal enolase (NNE) at 64 days (43% gestation) in all the gastric compartments. Immunoreactivity-NNE was distributed in the same locations as synaptophysin and in additional way in the epithelial layer.

Glial cells immunoreactive to GFAP were observed at 64 days (CRL 13.5 cm, 43% gestation) in the reticulum, omasum and abomasum and at 68 days (CRL 13.5 cm, 43% gestation) in rumen. Immunoreactivity-GFAP was distributed in lamina propria and submucosa, tunica muscularis, serosa and myenteric plexus. VIM-positive cells was detected at 39 days (CRL 4.4 cm, 25% gestation) in rumen, reticulum, omasum and in abomasum at 38 days (25% gestation) in the blastic pluripotent tissue and serosa of each one of the compartments. In the late gestational ages, this immunoreactivity was distributed in the same tissue layers as GFAP.

The peptidergic innervation markers NPY and VIP were first detected in rumen at 113 days (75% gestation) and 120 days (80% gestation) respectively; in omasum at 95 days and in reticulum and abomasum at 75 days (50% gestation).

Immunolabeling surface was located in lamina propria and submucosa, tunica muscularis, serosa and myenteric plexus.

Gastrin-immunoreactive cells first appeared in abomasums at 76 days (50% gestation) in some areas of the epithelium and appearing in pyloric glands at a later stage.

The histomorphometric study revealed the epithelial layer showed an active growth from early embryonic stages and reached stable values at the time of birth in the non-glandular compartments. By contrast, the epithelial layer of the abomasum started a regression from early embryonic stages until birth. The lamina propria and submucosa in rumen and reticulum showed a regression of growth from early stages. However, their participation in the rumen papillae and reticular crests made this involution lattice be transformed into a phase of active growth. In omasum and abomasum, both the lamina propria and submucosa continued an accelerated growth from the early stages of pregnancy. The tunica muscularis layer showed a steady growth since the early embryonic stages. In all gastric compartments, this layer grew than the parietal wall itself. The serosa thickness was increased as gestation progressed in rumen, reticulum and abomasum, while omasum showed a regression of its growth. The dynamics of parietal wall growth was related to the proper characteristics either of increasing or involution growth, of each one of the tissue layers in the different compartments.

10. BIBLIOGRAFÍA

Aage H.M., Bhosale N.S., Kadam S.D. and Kpadnis P.J. (2007). Histomorphological study of abomasum in cattle. *Indian. J. Anim. Res*., **41**: 192-195.

Abdalah O.Y., Shahin K.A. and Latif M.G.A. (1982). Allometric growth patterns of the alimentary tract in water buffalo and friesian cross-bred cattle. *Indian. J. Anim. Sci.*, **52**(7): 506-510.

Age H.M., Bhosle N.S. and Kapadnis P.J. (2008). Microscopic study of abomasum in sheep. *Indian. J. Anim. Res*., **42**: 134-135.

Agungpriyono S., McDonald A.A., Leus K.Y.G., Kitamura N., Andyane I.K.M., Goodall G.P., Hondo E. and Yamada J. (2000). Immunohistochemical study on the distribution of endocrine cells in the gastrointestinal tract of the babirusa, *Babyrousa babyrussa* (Suidae). *Anat. Histol. Embryol*., **29**: 173-178.

Agungpriyono S., Yamada J., Kitamura N., Nisa C., Sigit K. and Yamamoto Y. (1995). Morphology of the dorsal lingual papillae in the lesser mouse deer (Tragulus javanicus) with special reference to the internal surface. *J. Anat.,* **187**(3): 635.

Alison B.C. (1989). The distribution and antogeny of gastrin/CCK-somatostatine and neurotensin inmunoreactive cells in the gastrointestinal tract of the chicken. *Histol. Histopathol*., **4**: 55-62.

Al-Saffar F.J. (2012). Effect of different diets on number and distribution of gastrin cells in the mucosa of goat´s abomasum. *Asian. J. Anim. Sci*., **6**: 110-121.

Amaral C.M.C., Sugohara A., Resende K.T., Machado M.R.F. and Cruz C. (2005). Performance and ruminal morphologic characteristics of Saanen kids fed ground, pelleted or extruded total ration. *Small. Ruminat. Res.,* **58**: 47-54.

Amasaki H. and Daygo M. (1987). Prenatal development of sub epithelial vasculature related to appearance of ruminal papillae in the bovine rumen. *Anat. Anz*., **164**(2): 139-147.

Amasaki H. and Daygo M. (1988). Morphogenesis of the epithelium and the lamina propria of the rumen in bovine foetuses and neonates. *Anat. Histol. Embryol.,* **17**(1): 1-6.

Amer H.A. (2008). Determination of first pregnancy and foetal measurements in Egyptian Baladi goats (*Capra hircus*). *Veterinaria Italiana,* **44**(2): 429-437.

Anderson N., Hansky J. and Titchen D.A. (1975). Hipergastrinaemmia during parasitic gastritis in sheep. *J. Phisiol*., **256**: 51-52.

Arias J.L., Cabrera R. and Valencia A. (1978). Observations the histological development of the bovine rumen papillae. Morphological changes due to age. *Anat. Histol. Embriol*., **7**(2): 140-151.

Asari M., Ashige H., Wahui S., Fakaya K. and Kano Y. (1985). Histological development of bovine abomasum. *Anat. Anz*., **159**: 1-11.

Asari M., Fukaya K., Yamamoto M., Eguchi Y. and Kano Y. (1981). Developmental changes in the inner surface structure of the bovine abomasum. *Jpn. J. Vet. Sci.,* **43**(2): 211-220.

Avila C.G., Harding R., Young J.R. and Robinson P.M. (1989). The rule of gastrin in the development of the gastrointestinal tract in fetal sheep. *J. Exp. Phisiol.,* **74**: 169-180.

Balaskas C. and Gabella G. (1998). Glial fibrillary acidic protein (GFAP) immunoreactivity in enteric ganglia of the chick embryo. *Brain. Res.,* **804**: 275-283.

Balemba O.B., Mbassa G.K., Semugunka W.D., Assey R.J., Kahwa C., Hay-Schmidt K.B. and Dantzer V. (1999). The topography, architecture and structure of the enteric nervous system in the jejunum and ileum of cattle. *J. Anat.,* **195**: 1-9.

Bateman N. (1954). The measurement of milk production of mice through pre-weaning growth of suckling young. *Physiol. Zool.,* **27**: 163.

Beharka A.A., Nagaraja T.G., Morril J.L., Kennedy G.A. and Klemm R.D. (1998). Effects of form of the diet on anatomical, microbial, and fermentative development of the rumen of neonatal calves. *J. Dairy. Sci.,* **81**: 1946-1955.

Boyazoglu J., Hatziminaoglou I. and Morand-Fehr P. (2005). The role of the goat in society: past, present and perspectivas for the future. *Small. Ruminat. Res*., **60**: 13-23.

Brinks J.S., Clark R.T., Kieffer N.M. and Urick J.J. (1964). Estimates of genetic environmental and phenotypic parameters in range Hereford females. *J. Anim. Sci.*, **23**(3): 711-716.

Brody S. (1945). Bioenergetics and growth. Reinhold Publ. Co., New York.

Brookes S.J. (2001). Retrograde tracing of enteric neuronal pathways. *Neurogastroenterol. Motil.,* **13**: 1-18.

Brown J.E., Brown C.T. and Butts W.T. (1973). Evaluating relationships among immature measures of size, shape and performance of beef bulls. I. Principal components as measures of size and shape in young Hereford and Angus bulls. *J. Anim. Sci*., **36(**6): 1010-1020.

Brownlee A. (1956). The development of the rumen papillae in cattle fed on different diets. *Brit. Vet. J*., **112**(9): 369-375.

Bunnet N.M. and Harrison F.A. (1979). Endocrine cells in the alimentary tract of the sheep. *Ann. Rech. Vet*., **10**(2-3): 197-199.

Calingansan N.Y., Kitamura N., Yamda J., Oomori Y. and Yamashita Y. (1984). Inmunocytochemical study of the GEP endocrine cells of the sheep. *Act. Anat.,* **118**: 171-180.

Causton D.R. and Venus J.C. (1981). The biometric of plan growth. Arnold. Pub. Co., London, U.K.

Cecarrelli P., Pedini V. and Gargiulo A.M. (1995). Serotonin-containing cells in the horse gastrointestinal tract. *Anat. Histol. Embryol*., **24**: 97-99.

Chandrasekar V., Narayan P., Lalitha P.S. and Vijayaragavan C. (1993). Comparative gross anatomy of the omasum of sheep and goats. *Small. Ruminat. Res*., **9**: 377-388.

Church D.G., Jessup G.L. and Bogart R. (1962). Stomach development in the suckling lamb. *Am. J. Vet. Res.,* **23**: 220-225.

Clauss M., Hofmann R.R., Streich W.J., Fickel J. and Hummel J. (2010). Convergence in the macroscopic anatomy of the reticulum in wild ruminant species of different feeding types and a new resulting hypothesis on reticular function. *J. Zool.,* **281**: 26-38.

Cooke H.J. (1986). Neurobiology of the intestinal mucosa. *Gastroenterology*, **90**, 1057-1081.

Dall´Aglio C., Gargiulo A.M., Pedini V. and Ceccarelli P. (1999). Presence and distribution of neuroendocrine cells in the gastroenteropacnreatic endocrine system of fallow deer foetuses. *Anat. Histol. Embryol*., **28**: 331-336.

Del Rio Ortega S. (1973). Desarrollo prenatal del estómago de la oveja. Tesis Doctoral, Facultad de Veterinaria, Zaragoza, Spain.

Dickinson A.G. (1960). Some genetic implications of maternal effects. An hypothesis of mammalian growth*. J. Agric. Sci.,* **54**(3): 378-390.

Dickinson A.G., Hancock J.L., Hovell G.J.R. Taylor C.S. and Wiener G. (1962). The size of lambs at birth. A study involving egg transfer. *Anim. Prod*., **4**: 64-79.

Doize F., Vaillancourt D., Carabin H. and Belanger D. (1997). Determination of gestational age in sheep and goats using transrectal ultrasonographic measurement of placetomes. *Theriogenology,* **48**(3): 449-460.

Donald H.P. and Purser A.F. (1956). Competition in utero between twin lambs. *J. Agr. Sci.,* **48**: 245-249.

Dyce K.M., Sack W.O. and Wensing C.J.B. (2002). The abdomen of ruminants. In: Textbook of Veterinary Anatomy. WB Saunders Co. (3rd ed). Philadelphia, pp 666-690.

Eckstein P., McKeown T. and Record R.G. (1955). Variation in placental weight according to liter size in the guinea-pig. *J. Endocrinol.,* **12**(2): 108.

Edelman R. (1889). Vergleichend anatomische und physiologische untersuchungen über eine besondere Region der Magenschleimhaut (Cardiadrüsenregion) bei den Säugetieren. *Ctsch. Zschr. Thiermed*., **15**: 165-214.

El-Gendy, S.A.A. and Derbalah, A. (2010). Macroscopic and microscopic anatomy of the omasum of the Baladi goat. *J. Biol. Sci.,* **10**: 596-607.

Ellenberger W. (1884). Handbuch der Vergleichender Histologie und Physiologie der Haussaugtiere. Parey, Berlin.

Ellenberger W. and Baum H. (1912). Handbuch der Vergeichender Anatomie der Haustiere, (13th. ed.). Berlin.

Evans H.E. and Sack W.O. (1973). Prenatal development of domestic and laboratory mammals: Growth curves, external features and selected references. *Anat. Histol. Embryol.*, **2**: 11-45.

Fath El-Bab M.R., Schwart R. and Ali A.M.A. (1983). Micromorphological studies on the stomach of sheep during prenatal life. *Anat. Histol. Embryol*., **12**: 139-53.

Fizthugh H.A. (1976). Analysis of growth curves and strategies for altering their shape. *J. Anim. Sci.,* **42**: 1036-1051.

Fowden A.L. (2003). The insulin-like growth factors and feto-placental growth. *Placenta*, **24**(8): 803-812.

Franco A., Vivo J.M., Guillén M.T., Regodón S. and Robina A. (1989). Evolución parietal del retículo ovino de raza merina desde los 68 días de gestación hasta el nacimiento. *Histol. Med.,* **5**: 57-58.

Franco A., Regodón S., Robina A. and Redondo E. (1992). Histomorphometric analysis of the rumen of the sheep during development. *Am. J. Vet. Res*., **53**: 1209-1217.

Franco A., Regodón S., Gázquez A., Masot A.J. and Redondo E. (1993a). Ontogeny and distribution of gastrin cells in the gastrointestinal tract of the sheep. *Eur. J. Histochem*., **37**: 83-90.

Franco A., Robina A., Guillén M.T., Mayoral A.I. and Redondo E. (1993b). Histomorphometric analysis of the abomasum of sheep during development. *Anat. Anz*., **175**: 119-125.

Franco A., Robina A., Regodón S., Vivo J.M., Masot A.J. and Redondo E. (1993c). Histomorphometric analysis of the reticulum of the sheep during development. *Histol. Histopathol*., **8**: 547-556.

Franco A., Robina A., Regodón S., Vivo J.M., Masot A.J. and Redondo E. (1993d). Histomorphometric analysis of the omasum of sheep during development. *Am. J. Vet. Res*., **54**: 1221-1229.

Franco A., Rodríguez P.L., Mayoral A.I., Guillén M.T. and Robina A. (1993e). Modelos matemáticos aplicados al crecimiento gástrico ovino durante la vida intrauterina. *Anat. Histol. Embryol*., **22**(2): 160-178.

Franco A., Masot A.J., Gómez L. and Redondo E. (2004a). Morphometric and immunohistochemical study of the rumen of red deer during prenatal development. *J. Anat*., **204**: 501-513.

Franco A., Redondo E. and Masot A.J. (2004b). Morphometric and immunohistochemical study of the reticulum of red deer during prenatal development. *J. Anat*., **205**: 277-289.

Franco A., Masot A.J. and Redondo E. (2011). Ontogenesis of the rumen: a comparative analysis of the merino sheep and Iberian red deer. *Anim. Sci. J*., **82**: 107-116.

Franco A., Masot A.J., García A. and Redondo E. (2012). Ontogenesis of the reticulum with special reference to neuroendocrine and glial cells: a comparative analysis of the Merino sheep and Iberian red deer. *Anat. Histol. Embryol*., **41**: 362-373.

Franke W.W., Schmid E., Winter S., Osborn M. and Weber K. (1979). Widespread occurrence of intermediate filaments of the vimentin type in cultured cells from diverse vertebrates. *Exp. Cell. Res*., **123**: 25-46.

Fukaya K., Amasaki H., Ashary M., Eguchi Y. and Kato Y. (1979). Morphometric studies on the ruminants stomach of bovine fetuses and neonates based on observation of the casts produced by introduction of resin via the oesophagus. *Bull. Azabu. Vet. Coll*., **4**(2): 259-269.

Furness J.B. and Costa M. (1987). The Enteric Nervous System. Edinburgh: Churchill Livingstone.

Gabella G. (1971). Glial cells in the myenteric plexus. *Z. Naturforsch*., **266**: 244-245.

García A., Masot J., Franco A., Gázquez A. and Redondo E. (2012). Histomorphometric and immunohistochemical study of the goat rumen during prenatal development. *Anat. Rec*., **295**: 776-785.

García A., Masot J., Franco A., Gázquez A. and Redondo E. (2013). Histomorphometric and immunohistochemical study of the goat omasum during prenatal development. *Histol. Histopathol*., **28**: 737-748.

Garden I.C. and Scott A. (1971). The surface structure of the ovine omasum as seen with the scanning electron microscope (Mammalian, bovidae). *Z. Morph. Tiere*., **70**(3): 229-237.

Gázquez A. (1988). La necropsia de los animales domésticos. Interamericana. McGraw-Hill, Madrid.

Geboes K. and Collins S. (1998). Structural abnormalities of the nervous system in Crohn´s disease and ulcerative colitis. *Neurogastroenterol. Motil.,* **10**: 189-202.

Gihad E.A., El-Bedawy T.M. and Mehrez A.Z. (1980). Fiber digestibility by goats and sheep. *J. Dairy. Sci*., **63**: 1701-1706.

Gjesdal F. (1969). Age determination of bovine foetuses. *Acta. Vet. Scand.,* **10**: 197-218.

Goedloed J.F. (1972). The embryonic and postnatal growth of rat and mouse. *Cells Tissues Organs*, **82**(3): 305-336.

Gootwine E. (2005). Variability in the rate of decrine in bith weight as litter size increases in sheep. *Anim. Sci.,* **81**(3): 393-398.

Graham C. and Simmons N.L. (2004). Functional organization of the bovine rumen epithelium. *Am. J. Physiol. Regul. Integr. Comp. Physiol*., **288**: 173-181.

Green W.W. (1946). Comparative growth of the sheep and bovine animal during prenatal life. *Am. J. Vet. Res*., **7**: 395-402.

Gregory R.A. and Tracy H.J. (1964). The constitution and properties of two gastrins extracted from hog antral mucosa. *Gut.,* **5**: 103-117.

Groenewald H.B. (1994). Neuropeptides in the myenteric ganglia and nerve fibres of the forestomach and abomasums of grey, white and black karakul lambs. *Onderstepoort. J. Vet. Res*., **61**: 207-213.

Guilloteau P., Le Meuth-Metzinger V., Marisset J. and Zabielski R. (2006). Gastrin, cholecystokinin and gastrointestinal tract functions in mammals. *Nutr. Res. Rev*., **19**: 254-283.

Habel R.E. (1973). Anatomical and histological nomenclature of the ruminant stomach, in physiology of digestion of the ruminant. In: The Ruminant Stomach, Hofmann R.R. (ed.). East African literature Bureau, Nairobi, Kampala.

Habel R.E. (1975). Ruminant digestive system. In: The Anatomy of the Domestic Animals. S. Sisson and J. D. Grossman (5th ed.). W.B. Saunders Co., Philadelphia.

Hafez E.S.E. (1963). Symposium on Growth: Physio-genetics of prenatal and postnatal growth. *J. Anim. Sci*., **22**(3): 779-791.

Harfoot C.G. (1978). Anatomy, physiology and microbiology of the ruminant digestive tract. *Progress in lipid research*, **17**(1): 1-19.

Hentges J.F., Moore J.E. and Palmer A.Z. (1966). Replacement value of dried citrus meal for corn meal in beef-cattle diets. Agricultural Experiment Stations, Institute of Food and Agricultural Science. University of Florida, pp. 708.

Hofmann R.R. (1973). The Ruminant Stomach. Stomach structure and feeding habits of East African game ruminants. East African Literature Bureau, Nairobi.

Hofmann R.R. (1989). Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. *Oecologia*, **78**(4): 443-457.

Hofmann R. and Schnorr B. (1982). Die funktionelle Morphologie des wiederkaeuer Magens, Ferdinand Enke Verlag, Stuttgart.

Hunter G.L. (1956). The maternal influence on size in sheep. *J. Agric. Sci.,* **48**: 36-64.

Hurd R.G. (1977). Vegetative plant growth analysis in controlled environments. *Ann. Bot.*, **41**(4): 779-787.

Huxley U.S. (1932). Problems of relative growth. Methuen, Pub. Co., London. U.K.

Ito H., Yamada J., Kitamura N. and Yamashita T. (1985). Histological and inmunohistochemical studies of the Segi´s cap, a large aggregation of endocrine cells on the intestinal villi of porcine fetuses and neonatos. *Arch. Histol. Jap*., **48**(4): 399-409.

Jain R.N. and Samuelson L.C. (2006). Differentiation of the gastric mucosa. II. Role of gastrin in gastric epithelial cell proliferation and maturation. *Am. J. Physiol-Gastr. L*., **291**: 726-765.

Jessen K.R. and Mirsky R. (1983). Astrocyte-like glia in the peripheral nervous system: an immunohistochemical study of enteric glia*. J. Neurosci*., **3**: 2206-2218.

Jessen K.R., Polak J.M., Noorden S.V., Bloom S.R. and Burnstock G. (1980). Peptide-containing neurones connect the two ganglionated plexuses of the enteric nervous system. *Nature.,* **283**: 391-393.

Jotbert D. and Hammound J. (1958). A cross-breeding experiment with cattle with special reference to the maternal effect in South Devon-Dexter crosses. *J. Agri. Sci.*, **51**(3): 325-341.

Kamel G., Hassal A.H.S., Ali A.M.A., and Moustafa A.M.A. (1987). Histological studies on the prenatal development of goats stomachs. *Assiut. Vet. Med. J*., **19**(37): 23-30.

Kamler J. (2001). Morphological variability of forestomach mucosal membrane in red deer, fallow deer, roe deer and mouflon. *Small. Ruminat. Res.,* **41**: 101-107.

Kano Y., Fukaya K., Asari M. and Eguchi Y. (1981). Studies on the development of the fetal and neonatal bovine stomach. *Anat. Histol. Embryol.,* **10**: 264-274.

Karent A.M., Fattouh E.S.M. and Abu-Zeid S.S. (2009). Estimation of gestational age in Egyptian native goats by ultrasonographic fetometry. *Anim. Reprod. Sci.,* **114**: 167-174.

Karl H. (1915). Die Entwicklung des Magens beim Schafe. *Morfph. Jahrb*., **49**: 311-352.

King J.W.B. and Donald H.P. (1955). A study of variation in twin cattle. III. Growth. *J. Dairy. Res.,* **22**: 1.

Kitamura N., Tanimoto A., Hondo E., Andren A., Cottiell D.F., Sasaki M. and Yamada J. (2001). Immunohistochemical study of the ontogeny of prochymosin and pepsinogen-producing cells in the abomasum of sheep. *Anat. Histol. Embryol.,* **30**: 231-235.

Kitamura N., Yamada J., Calingasan N.Y. and Yamashita T. (1985). Histological and inmunocytochemical study of endocrine cells in the gastrointestinal tract of the cow and calf. *Am. J. Vet. Res*., **46**: 6.

Kitamura N., Yamada J. and Yamashita T. (1986). Immunohistochemical study on the distribution of neuron specific enolase, and peptide containing nerves in the reticulorumen and the reticular groove of cattle. *Comp. Neurol*., **248**: 223-234.

Kitamura N., Yamada J., Watanabe T. and Yamashita T. (1990). An immunohistochemical study on the distribution of endocrine cells in the gastrointestinal tract of the musk shrew, *Suncus murinus*. *Histol. Histopathol*., **5**: 83-88.

Kitamura N., Yamada J., Yamamoto Y. and Yamashita T. (1993). Substance P-immunoreactive neurons of the bovine forestomach mucosa: their presumptive role in a sensory mechanism. *Arch. Histol. Cytol*., **56**: 399-410.

Kitamura N., Yoshiki A., Sasaki M., Baltazar E.T., Hondo E., Yamamoto Y. and Agungpriyono S. (2003). Immunohistochemical evaluation of the muscularis mucosae in the ruminant forestomach. *Anat. Histol. Embryol*., **32**: 175-178.

Klein R.D., Kincaid R.L., Hodgson A.S., Harrison J.H., Hillers J.K. and Cronrath J.D. (1987). Dietary fiber and early weaning on growth and rumen development of calves. *J. Dairy. Sci.,* **70**: 2095-2104.

Konda Y., Kamimura H., Yokota H., Hayashi N., Sugano K. and Takeuchi T. (1999). Gatrin stimulates the growth of gastric pit with less-differentiated features. *Am. J. Physiol-Gastr. L*., **277**: 773-784.

Krahmer R. (1982). Anatomía de los animales domésticos. Acrabia S.A., Zaragoza.

Kressin M. and Sommer U. (1996). Differentiation of the surface mucous-cell line in the abomasum of the adult cow. *Anat. Histol. Embryol*., **25**: 177-189.

Kurnosov K.M., Danilova L.V. and Gurova N.I. (1960). The external appearance of the embryo and fetus of the cow. *Trudy Inst. Morfol. Zhivot.,* **29**: 103-109.

Lambert P.S. (1948). The development of the stomach in the ruminant. *Brit. Vet. J*., **102**: 302-310.

Larsson L-I. (1977). Ontogeny of peptide-producing nerves and endocrine cells of the gastro-duodeno-pancreatic region. *Histochemistry*, **54**: 133-142.

Larsson L-I. (2000). Developmental biology of gastrin and somatostatin cells in the antropyloric mucosa of the stomach. *Micros. Res. Techinq*., **48**:272-281.

Lee J.H., Huh C.K., Kim C.S. and Kwak S.D. (1994). Development of abomasum of fetuses and neonates in Korean native goats. *J. Vet. Res*., **34**: 219-227.

Lee Y., Lee O., Cho J., Shin H., Choi Y., Shim Y., Choi W., Shin H., Lee D., Lee G. and Shin S. (2005). Ultrasonic measurement of fetal parameters for estimation of gestational age in korean black goats. *J. Vet. Med. Sci.,* **67**(5): 497-502.

Lindh B., Hökfelt T., Elfvin L-G., Terenius L., Fahrenkrug J., Elde R. and Goldstein M. (1986). Topography of NPY, Somatostatin and VIP-immunoreactive, neuronal subpopulations in the guinea pig celiac superior mesenteric ganglion and their projection to the pylorus. *J. Neurosci*., **6**: 2371-2383.

Lombardi G. (2005). Optimum management and quality pastures for sheep and goat in mountain areas. *Options mediterraneennes*, 67, pp. 19-29.

Lubis D. and O´Shea J.D. (1978). Development of the omasum in sheep. *Acta. Anat*., **100**: 400-410.

Lucini C., de Girolamo P., Coppola L., Paino G. and Castaldo L. (1999). Postnatal development of intestinal endocrine cell populations in the water buffalo. *J. Anat*., **195**: 439-446.

Luginbuhl J.M. (1983). Comparative anatomy of the digestive tract in cattle sheep and goats. A review. Department of Animal Sciencie, North Carolina State University.

Lush J.L., Hetzer H.O. and Culbertson C.C. (1934). Factors affecting birth weight of swine. *Genetics,* **19**(4): 329.

Mannely R.B. (1952). Note on the ageing of the bovine embryo. *Vet. Rec.,* **64**: 509-511.

Martin P. (1933). Übersicht über einige neuere Forschungsergebnisse betr. Die Wiederkäuerma genentwicklung. *Münch. Thierärztl. Wschr*., **84**: 1-12.

Masot A.J., Franco A. and Redondo E. (2007a) Morphometric and immunohistochemical study of the abomasum of red deer during prenatal development. *J. Anat*., **211**: 376-386.

Masot A.J., Franco A. and Redondo E. (2007b). Comparative analysis of the forestomach mucosa in red deer during prenatal development. *Revue. Méd. Vét*., **7**: 397-409.

Mawe G.W., Schemann M., Wood J.D. and Gehrson M.D. (1989). Immunocytochemical analysis of potential neurotransmitters present in the myenteric plexus and muscular layers of the corpus of the guinea pig stomach. *Anat. Rec*., **224**, 431-442.

May M.D.S. (1964). The anatomy of the sheep (2nd ed.). Univ. of Queensland Press, Brisbane, Australia.

McGavin M.D. and Morril J.L. (1976). Scanning electron microscopy of ruminal papillae in calves feed various amounts and forms of roughage. *Am. J. Vet. Res.,* **37**(5): 497-507.

McGuigan J.E. and Harrison F.A. (1968). Gastrin mucosal intracellular localitation of gastrin by inmunofuorescence. *Gastroenterology*, **55**: 315-327.

Michel G. and Schwarze E. (1970). Compendio de anatomía veterinaria. Tomo VI: Embriología. Ed. Acribia. Zaragoza.

Molinari E. and Jorquera B. (1988). Intrauterine development stages of the gastric compartments of the goat (*Capra hircus*). *Anat. Histol. Embryol*., **17**: 121-137.

Münnich J., Gäbel G. and Pfannkuche H. (2008). Intrinsic ruminal innervations in ruminants of different feeding types. *J. Anat*., **213**: 442-451.

Murillo Ferrol N.L. (1973). Sistematización de las fases prenatales del desarrollo. *An. Des.,* **8**: 7-22.

Mutoh K. and Wakuri H. (1988). Early organogenesis of the caprine stomach. Nippon. *Juigaku. Zasshi.,* **51**: 474-484.

Nada O. and Kawana T. (1988). Immunohistochemical identification of supportive cell types in the enteric nervous system of the rat colon and rectum. *Cell. Tissue. Res.,* ***251***(3), 523-529.

Nagai R. Watanabe K., Wakatsuki A., Hamada F., Shinoshara K., Hayashi Y., Inamura R. and Fukaya T. (2008). Melatonin preserves fetal growth in rats by protecting against ischemical/reperfusion-induced oxidative/nitrosative mitochondrial damage in the placenta. *J. Pineal. Res.,* **45**(3): 271-276.

Neiva G.S.M., da Mota D.L., Batista A.M.V. and de Sousa-Rogrigues C.F. (2006). Mucous membrane of the rumen of ovines fed with spineless, Forrage cactus of Palm (Barbary Fig) (*Opuntia ficus indica* Mil): Hystochemical study by means of light microscopy. *Int. J. Morphol.,* **24**(4): 723-728.

Nichols C.W. (1944). The embryology of the calf: fetal growth weights, relative age and certain body measurements. *Am. J. Vet. Res.,* **5**: 135-141.

Nickel R., Schummer A., Seiferle E. and Sack W.O. (1973). The Viscera of the Domestric Mammals. Berlin. Verlage. Paul Parey, pp. 104-105.

Nickel R., Schummer A. and Seiferle E. (1979). The Viscera of Domestic Animals (2nd rev. ed.). Verlang Paul Parey. Spinger Verlag, Berlin.

Nockels C.F., Kintner L.D. and Pfander W.H. (1966). Influence of ration on morphology, histology and trace mineral content of sheep rumen papillae. *J. Dairy. Sci.,* **49**: 1069-1074.

Nwaogu C.I., Anya K.O. and Agada P.C. (2010). Estimation of foetal age using ultrasonic measurement of different foetal parameters in red Sokoto goats (*Capra hircus*). *Veterinarski arhiv.,* **80**(2): 225-233.

Nwaogu C.I. and Ezeasor N.D. (2008). Studies on the development of omasum in west African dwarf goats (*Capra hircus*). *Vet. Res. Commun*., **32**: 543-552.

Oppel A. (1896). Lehrbuch der Vergleichender Mikroskopischen Anatomie der Wirbeltiere 1. Der Magen. Fischer, Jena.

Ortega-Reyes L., Provenza F.D., Parker C.F. and Hatfield P.G. (1992). Drylot performance and ruminal papillae development of lambs exposed to a high concentrate diet while nursing. *Small. Ruminant. Res.,* **7**: 101-112.

Osman A.H.R. and Berg R. (1981a). Studies on the histogenesis of the tunica mucosa of the stomack of the Egyptian water buffalo (*Bos bubbalis*). I. Histogenesis of the ruminal mucosa. *Anat. Anz.***, 149**(3): 232-240.

Osman A.H.R. and Berg R. (1981b). Studies on the histogenesis of the tunica mucosa of the stomack of the Egyptian water buffalo (*Bos bubbalis*). II. Histogenesis of the reticular mucosa. *Anat. Anz, Jena*., **150**: 516-520.

Osman A.H.R. and Berg R. (1982a). Studies on the histogenesis of the stomach in the egiptyan water buffalo (*Bos bubbalis*). III. Histogenesis of the mucosa of the ventricular sulcus (Esophageal groove). *Anat. Anz. Jena*., **151**: 375-379.

Osman A.H.R. and Berg R. (1982b). Studies on the histogenesis of the tunica muscularis of the stomach in the egiptyan water buffalo (*Bos bubbalis*). IV. Histogenesis of the omasal mucosa. *Anat. Anz. Jena*., **151**: 467-471.

Panchamukhi B.G., Mudholkar D.R. and Srivastava H.C. (1975). Prenatal development of the buffalo (*Bubalus bubalis*) stomach 1. Organogenesis of the stomach. *Indian J. Anim. Sci*., **45**: 638-647.

Panchamukhi B.G. and Srivastava H.C. (1979). Histogenesis of the rumen of the buffalo (*Bubalus bubalis*). *Indian. J. Anim. Sci.,* **48**: 473-476.

Panchamukhi B.G. and Srivastava H.C. (1980). Histogenesis of the reticulum of the buffalo (*Bubalus bubalis*). Indian. *J. Anim. Sci*., **50**(12): 1064-1070.

Panchamukhi B.G. and Srivastava H.C. (1982). Histogenesis fo the reticular groove of the buffalo (*Bubalus bubalis*) stomach. *Am. J. Vet. Res*., **43**(2): 346-349.

Pearse A.G.E and Takor T. (1976). Neuroendocrine embryology and the APUD concept. *Clin. Endocrinol*., **5**: 229-244.

Pernkopf E. (1931). Die Entwicklung des Vorderdarmes, insbesondere des Magens der WiederKäuer. *Zschr. Anat. Entwickl. Ges*., **94**: 490-622.

Pfannkuche H. and Schemann M. (2002). Ruminal muscle of sheep is innervated by non-polarized pathways of cholinergic and nitrergic myenteric neurons. *Cell. Tissue. Res*., **309**: 347-354.

Pfannkuche H., Shcellhorn C., Schemann M. and Gäbel G. (2004). Intrinsic innervations patterns of the smooth muscle in the rumen and reticulum of lambs. *J. Anat*., **204**: 293-299.

Phillipson A.T. (1982). Ruminant Digestion. In: Physiology of Domestic Animals. M.J. Swenson (9th ed)., Comstock Publishing Associates, London, pp. 277.

Postma C. (1947). Determination of age in the bovine fetus. *Tijdschr Diergeneeskunde*, **72**: 463-531.

Ramkrishna V. and Tiwari G.P. (1979). Histological and histochemical observations on the forestomach of goat during pre-natal life. *Acta. Anat*., **103**: 292-300.

Rancourt M., Fois N., Lavin M.P., Tchakerian E. and Vallerand F. (2006). Mediterranean sheep and goat production: an uncertain future. *Small. Ruminat. Res*., **62**: 167-179.

Rawdon B.B. and Andrew A. (1981). An histocytochemical study of the distribution of pancreatic endocrine cells in chicks, with special reference to the

relationship between pancreatic polypeptide and somatostatin immunoreactive cells. *Immunochemical*., **59**: 189-197.

Redmer D.A., Wallace J.M. and Reynolds L.P. (2004). Effect of nutrient intake during pregnancy on fetal and placental growth and vascular development. *Domest. Anim. Endocrinol*., **27**(3): 199-217.

Redondo E., Regodón S., Masot A.J., Franco A. and Lignereux Y. (1997). The gastrin and CCK cells in the gastrointestinal tract of the sheep during development. A differential study. *Rev. Med. Vet*., **6**: 511-518.

Redondo E., Franco A. and Masot A.J. (2005). Morphometric and immunohistochemical study of the omasum of red deer during prenatal development. *J. Anat*., **206**: 543-555.

Redondo E., Masot J., Garcia A. and Franco A. (2011). Ontogenesis of the omasum: a comparative analysis of the Merino sheep and Iberian red deer. *Histol. Histopathol.*, **26**: 1135-1144.

Regodón S., Franco A., Masot A.J. and Redondo E. (1996). Estudio ontogénico comparativo de la lámina epitelial de los compartimentos gástricos no glandulares en ovinos merinos. *Anat. Histol. Embryol*., **25**: 233-241.

Reid C.S.W. (1985). The progress of solid feed residues through the rumino-reticulum: the ins and outs of particles, in: Ruminants physiology: concepts and consequences. Baker, S.R., Gawthorne, J.M., Mackintosh, J.B., Purser, D.B. (eds). Perth: University of Western Australia Press, pp. 79-84.

Relling A.E. and Mattioli G.A. (2002). Fisiologia Digestive y Metabolica de los Rumiantes, EDUL ed. Universidad Nacional de La Plata, Buenos Aires.

Rompala R.E., Hoagland T.A. and Meister J.A. (1990). Modifications in growth and morfphology of ovine jejuna and ruminal epithelia as affected by inert dietary substances. *J. Anim. Sci.,* **68**: 2530-2535.

Roux M. (1990). Crecimiento, desarrollo y producción de carne. Curso de Nutrición Animal. C.I.H.E.A.M.I.A.M.Z., Zaragoza, España.

Ruckebusch Y. (1989). Gastrointestinal motor functions in ruminants. In: The Gastrointestinal System. Schultz S.G., Wood J.D., Rauner B.B. (eds). Oxoford University Press, New York, pp. 1225-1283.

Sander E.G., Warner R.G., Harrison H.N. and Loosli J.K. (1959). The stimulatory effet of sodium butyrate and sodium propionate on the development of rumen mucosa in the young calf. *J. Dairy. Sci*., **42**(9): 1600-1605.

Santiago-Moreno J., González-Bulnes A., Gómez-Brunet A., Toledano-Díaz A. and López-Sebastián A. (2005). Prediction of gestational age by transrectal ultrasonographic measurement in the mouflon (*Ovis gmelini musimon*). *J. Zoo. Wildlife. Med.,* **36**(3): 457-462.

Sbarbaty R. (1979). Quantitative aspects of the embryonic growth of the intestine and stomach. *J. Anat.,* **129**: 795-803.

Scala G., Corono M. and Maruccio L. (2011). Structural, Histochemical and Immunocytochemical study of the forestomach mucosa in domestic ruminants. *Anat. Histol. Embryol*., **40**: 47-54.

Scala G. and Maruccio L. (2012). Reticular Groove of the domestic ruminants: histochemical and immunocytochemical study. *Anat. Histol. Embryol*., **41**(6): 428-435.

Scharrer B. (1990). The neuropeptide saga. *Amer. Zool*., **30**, 887-895.

Schnorr B. and Vollmerhaus J. (1967). Fine structure of rumen epithelium of goat and bovine. *Zbl. Veter. Med.,* **14**: 789-818.

Schummer A. and Nickel R. (1979). The Viscera of the Domestic Animals. Verlag Paul Parey (2nd ed.), Berlin.

Schwartz V. (1977). Embriología animal comparada. Ed. Omega. Barcelona.

Schwarze E. and Schöder L. (1970). Conpendio de Anatomia Veterinaria. Tomo II. El Sistema Visceral. Editorial Acribia, Zaragoza.

Scott A. and Gardner I.C. (1973). Papillar form in the forestomach of the sheep. *J. Anat.*, **116**(2): 255-267.

Singh O. Roy K.S. Kumar A. and Bawa B.S. (2007). Histomorphological studies on prenatal development of abomasum in buffalo. Indian *J. Anim Sci*., **77**(8): 727-729.

Singh S., Sengar O.P.S. and Sing S.N. (1963). Prenatal development of Buffalo-*Bos Bubalis L. Agra, Univ. J. Res.,* **12**: 197-246.

Sisson S. (1974). Anatomía de los animales domésticos. Revolucionaria Inst (ed.) Cubano del Libro La habana Cuba.

Sivachelvan M.N., Ghali Ali M. and Chibuzo G.A. (1996). Foetal age estimation in sheep and goats. *Small. Ruminant. Res.,* **19**(1): 69-76.

Shulkes A. and Hardy K.J. (1982). Ontogeny of circulating gastrin and pancreatic polypeptide in the foetal sheep. *Acta Endocrinol*., **100**: 565-572.

Shulkes A., Chick P. and Hardy K.J. (1981). Development and regulation of gastrin and acid secretion in the chronically cannulated ovine fetus. *Gastroenterology*, **80**: 1284.

Soehartono R.H., Kitamura N., Yamagishi N., Taguchi K., Yamada J. and Yamada H. (2002). An immunohistochemical study of endocrine cells in the abomasum of vagotomized calf. *J. Vet. Med. Sci*., **64**: 11-15.

Sotillo J.L. and Vigil E. (1978). Producción animal. Bases fisiozootécnicas. Mijares, León.

Stallcup O.T., Kreider D.L. and Rakes J.M. (1990). Histological development and histochemical localization of enzymes in rumen and reticulum in bovine fetuses. *J. Anim. Sci.,* **68**: 1773-1789.

Stevens D.H. and Marshall A.B. (1970). Organization of the rumen epithelium. In: Phusiology and Digestion and Metabolism in the Ruminant. Phillipson A.T. (ed.). Newcastle upon Tynel. Oriel, pp. 80-100.

Stoss A. (1892). Vergleichende anatomische Untersuchungen über die entwicklung des verdauungskanales des wiederkäuer. *Dtsch. Zschr. Tiermed. Vergl. Path*., **16**: 96-124.

Suzuki M., Kaji K., Yamanaka M. and Ohtaishi N. (1996). Gestational age determination, variation of conception date and external fetal development of sika deer (*Cervus Nippon yesoensis Heude*, 1884) in eastern Hokkaido. *J. Vet. Med. Sci.*, **58**(6): 505-509.

Tamate H. (1956). The anatomical studies of the stomach of the goat. I. The post-natal development of the stomach with special reference to the weaning and prolonged suckling. Tohoku *J. Agr. Res.,* **7**: 209-229.

Tamate H., Kikuchi T., Onodera A. and Nagatani T. (1971). Scanning electron microscopic observation on the surface structure of the bovine rumen mucosa. *Arch. Histol. Jpn.,* **33**: 273-293.

Tamate H., McGilliard A.D., Jacobson N.L. and Getty R. (1962). The effect of various diets on the histological development of the stomach in the calf. *J. Dairy. Res.,* **45**: 408-420.

Taylor S.C. and Craig J. (1965). Genetic correlation during growth of twin cattle. *Anim. Prod.,* **7**(1): 83-102.

Teixeira A.F., Kühnel W., Vives P. and Wedel T. (2009). Functional morphology of unguiculiform papillae of the reticular groove in the ruminant stomach. *Ann. Anat*., **191**: 469-476.

Thomsen J.L. (1973). Body length, head circumference, and weight of bovine fetuses. Prediction of gestacional age. *J. Dairy Sci.,* **58**: 1370-1373.

Thornley J.H. and France J. (2007). Mathematical models in agriculture: quantitative methods for the plant, animal and ecological sciences. Cabi.

Timmermans J.P., Scheuermann D.W., Stach W. and Adriaensen D. and De Groodt-Lasseel M.H.A. (1990). Distinct distribution of CGFP, enkephalin, galanin, neuromedin U, neuropeptide Y, somatostatin, substance P, VIP and serotonin containing neurons in the two submucosal ganglionic neural networks of the porcine small intestine. *Cell. Tissue. Res.***, 260**: 367-379.

Tiwari G.P. and Jamdar M.N. (1979). Studies on gross and histological of development of the forestomachs of Indian water buffalo calf in early postnatal life with reference to normal feeding. Rumen. *Indian. J. Anim. Sci*., **57**: 335-340.

Tobe T., Chen S., Henmi K. and Fukuchi K. (1979). Distribution of gastrin in canine, cat, and human digestive organs. *Grant-in Aid, Sci. Res.,* **132**: 581-582.

Toner P., Carr K.E. and Wyburn G.M. (1971). The digestive system. London, Butterworth.

Vallejos E.R. and Colmenares A. (1972). Ultrastructure and functions of the rumen in adult bovines. *Acta cientifica venezolana*, **23**: 148-154.

Venge O. (1950). Studies of the maternal influence on the birth weight in rabbits. *Acta. Zool.,* **31**(1): 1-148.

Vivo J.M. (1987). El desarrollo del estómago del vacuno frisón: análisis desde el prisma morfológico y morfométrico. Tesis Doctoral. Universidad de Extremadura.

Vivo J.M., Robina A., Regodón S., Guillén M.T., Franco A. and Mayoral A.I. (1990). Histogenetic evolution of bovine gastric compartments during prenatal period. *Histol. Histopathol*., **5**: 461-476.

Von Bertalanffy L. (1957). Quantitative laws for metabolism and growth. *Quart. Res. of Biol.*, **32**(3): 217-231.

Von Boyen G.B., Steinkamp M., Reinshagen M., Schäfer H., Adler G. and Kirsch J. (2004). Proinflammatory cytokines increase glial fibrillary adicic protein expression in enteric glia. *Gut*., **53**, 222-228.

Wallace L.R. (1948). The growth of lambs before and after birth in relation to the level of nutrition. *J. Agri. Sci.,* **38**(4): 367-401.

Walton A. and Hammond J. (1938). The maternal effect on growth and conformation in the Shire horse-Shetland pony crosses. *Proc. R. Roy. Soc. Biol.,* **125**: 311-335.

Wardrop I.D. (1961a). Some preliminary observations on the histological development on the forestomach of the lamb. I. Histological changes due to age in the period from 46 days of foetal life to 77 days of postnatal life. *J. Agr. Sci.,* **57**: 335-341.

Wardrop I.D. (1961b). Some preliminary observations on the histological development of the fore-stomach of the lamb. II. The effects of diet on the histological development of the fore-stomach of the lamb during post-natal life. *J. Agr. Sci*., **57**(3): 343-346.

Warner ED. (1958). The organogenesis and early histogenesis of the bovine stomach. *Am. J. Anat*., **102**: 33-63.

Wathuta E.M. (1986). The distribution of vasoactive intestinal polypeptide-like, substance P-like and bombesin-like immunoreactivity in the digestive system of the sheep. *Q. J. Exp. Physiol.,* **71**: 615-631.

Wathuta E.M. and Harrison F.A. (1987). The ontogeny of vasoactive intestinal polypeptide-like and substance P-like immunoreactivity in the digestive tract of the sheep. *Q. J. Exp. Physiol*., **72**: 119-128.

Wu G., Bazer W., Wallace J.M. and Spencer T.E. (2006). Board-invited review: Intrauterine growth retardation: Implications for the animal sciences. *J. Anim. Sci.*, **84**(9): 2316-2337.

Xiao-guang L.I.U. (2010). Histogenesis of Mongolia sheep embryonic stomach. *Journal of Inner Mongolia University*, **4**: 9.

Yamamoto Y., Atoji Y. and Suzuki Y. (1995). Morphological study of the submucosal and mucosal plexuses of the sheep forestomach. *Ann. Anat*., **177**: 405-412.

Yamamoto Y., Atoji Y., Agungriyono S. and Suzuki Y. (1998). Morphological study of the forestomach of the Japanese Serow (*Capricornis crispus*). *Anat. Histol. Embryol.*, **27**(2): 73-81.

Yamamoto Y., Kitamura N., Yamada J. and Yamashita T. (1991). Muscular architecture in the omasal laminae of cattle and sheep. *Vet. Res. Commun*., **15**:249-256.

Yamamoto Y., Kitamura N., Yamada J.and Yamashita T. (1993). Three-dimensional architecture of the subepithelial connective tissue in the omasal laminae of sheep and cattle. *Acta Anat*., **146**(4): 238-243.

Yamamoto Y., Kitamura N., Yamada J., Andren A. and Yamashita T. (1994). Morphological study of the surface structure of the omasal lamiane in cattle, sheep and goats. *Anat. Histol. Embryol*., **232**: 166-176.

Yeates N.T.M. and Parer J.T. (1962). Effects of stress on reproduction. Reproduction in Farm Animals. E.S.E. Hafez (ed.) Lea & Febiger, Philadelphia, pp. 297-303.

Zimmerl U. (1900). Contributo alla Conoscenza della ontogenesi dello stomaco del rumianti. *Monitore Zool. Ital*., **11**: 13-29.

Zimmermann A. and Sal J. (1894). Beitrag zur Histologie des Pansens. Dtsch. Zschr. Tiermedizin, Leipzig.

Zitare I., Pilmane M. and Jemeljanovs A. (2013). Histomorphology of the digestive system of red deer (*Cervus elephus* L.) in Latvia. *J. Vet. Med. Anim. Health*., **5**(4): 99-106.

ANEXO

ANEXO 1. Listado de abreviaturas

CALB: calbidina

ChAT: Acetilcolintransferasa

CRL: crown-rump length (longitud desde la cabeza hasta la base de la cola)

CU: Catedrático de Universidad

ENK: Encefalina

FP: Formación profesional

GFAP: Proteína ácida fibrilar glial

GRP: Polipéptido liberador de gastrina

H-E: Hematoxilina-Eosina

IFI: Inmunofluorescencia indirecta

LENK: Leucina encefalina

mAc: Anticuerpo monoclonal

NNE: Enolasa no neuronal

NOS: Óxido nitico

NPY: Neuropéptido Y

NSE: Enolasa neuronal específica

pAc: Anticuerpo policlona

PAP: Técnica inmunohistoquímica de peroxidasa-antiperoxidasa

PBS: Tampón fosfato salino

RIA: Análisis radioinmunoensayo

SNED: Sistema Neuroendocrino Difuso

SP: Sustancia P

SOM: Somatostatina

TU: Profesor Titular de Univerisdad

VIM: Vimentina

VIP: Péptido intestinal vasoactivo

ANEXO 2. Comunicaciones a congresos

Redondo García, E., Masot Gómez-Landero, A.J., Franco Rubio, A., García González, A. *Histological findings of the rumen goat during prenatal development.* XV Congreso Nacional de la Sociedad Española de Histología e Ingeniería Tisular. Albacete 2009.

García González, A., Masot Gómez-Landero, A.J., Franco Rubio, A., Redondo García, E. *Histological findings of the reticulum goat during prenatal development.* XV Congreso Nacional de la Sociedad Española de Histología e Ingeniería Tisular. Albacete, 2009. (Accésit).

Redondo García, E., García González, A., Franco Rubio, A., Masot Gómez-Landero, A.J. *Histological findings of the omasum goat during prenatal development*. XV Congreso Nacional de la Sociedad Española de Histología e Ingeniería Tisular. Albacete 2009.

García González, A., Masot Gómez-Landero, A.J., Franco Rubio, A., Redondo García, E. *Histological findings of the abomasun goat during prenatal development*. XV Congreso Nacional de la Sociedad Española de Histología e Ingeniería Tisular. Albacete 2009.

Redondo, E., Franco, A., Gázquez, A., García, A., Masot A.J. *Ontogenesis of the rumen: A comparative analysis of the merino sheep and Iberian red deer*. TERMIS-EU Annual Meeting. Granada, 2011.

Redondo, E., Masot A.J., Gázquez A., García, A., Franco A. *Ontogenesis of the abomasum in the Iberian red deer*. TERMIS-EU Annual Meeting. Granada, 2011.

Masot, A.J., Franco, A., Gázquez A., García, A., Redondo E. *Ontogenesis of the omasum: a comparative analysis of the merino sheep and Iberian red deer*. TERMIS-EU Annual Meeting. Granada, 2011.

Franco, A., Masot, A.J., Gázquez, A., García, A., Redondo E. *Ontogenesis of the reticulum: a comparative analysis of the merino sheep and Iberian red deer.* TERMIS-EU Annual Meeting. Granada, 2011

Masot, A.J., García, A., Franco, A., Gázquez A., Redondo E. *Immunohistochemical study of the omasum of goat during prenatal development.* XXIX Congress of the European Association of Veterinary Anatomists. Bulgaria, 2012.

Masot, A.J., García, A., Franco, A., Gázquez A., Redondo E. *Immunohistochemical study of the reticulum of goat during prenatal development.* XXIX Congress of the European Association of Veterinary Anatomists. Bulgaria, 2012.

Masot, A.J., García, A., Franco, A., Gázquez A., Redondo E. *Immunohistochemical study of the rumen of goat during prenatal development.* XXIX Congress of the European Association of Veterinary Anatomists. Bulgaria, 2012.

HISTOLOGY AND HISTOPATHOLOGY

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SOCIEDAD ESPAÑOLA DE HISTOLOGÍA E INGENIERÍA TISULAR

XV CONGRESO DE LA SOCIEDAD ESPAÑOLA DE HISTOLOGÍA E INGENIERÍA TISULAR
3RD INTERNATIONAL CONGRESS OF HISTOLOGY AND TISSUE ENGINEERING

UNIVERSIDAD DE CASTILLA-LA MANCHA

HISTOLOGÍA COMPARADA / *COMPARATIVE HISTOLOGY*

26

HISTOLOGICAL FINDINGS OF THE RUMEN GOAT DURING PRENATAL DEVELOPMENT

Redondo García, E. (1); Masot Gomez-Landero, Antonio Javier (1); Franco Rubio, Antonio (1); Garcia Gonzalez, Angela (1)
Universidad De Extremadura (1)

Objectives
The aim of the present study was to sequence several histological phenomena that occur during the prenatal development of one of the gastric compartements, the rumen.

Material and methods
Histological and histochemical analyses were carried out on 50 embryos and fetuses of goat. The animals were divided for test purposes into five experimental groups: Group I (1-37 Days, 1-25% of gestation); Group II (38-52 Days, 25-35% of gestation); Group III (53-75 Days, 35-50% of gestation); Group IV (75-112 Days, 50-75% of gestation) and Group V (113-150 Days, 75-100% of gestation).

Results
The rumen of the primitive gastric tube was observed at approximately 33 days. At 37 days the rumen consisted of 3 layers: internal or mucosal, middle or muscular, and external or serosal layer. The stratification of the epithelial layer was accompanied by changes in its structure with the appearance of ruminal pillars and papillae.The outline of the ruminal papillae began to appear at 53 days of prenatal development as evaginations of the basal zone toward the ruminal lumen, pulling with it in its configuration the stratum basale, the lamina propria and the submucosa. At 59 days from the pluripotential blastemic tissue we witnessed the histodifferentiation of the primitive tunica muscularis composed of two layers of myoblasts with a defined arrangement. It was also from the pluripotential blastemic tissue, at 59 days, that the lamina propria and the submucosa were differentiated.The serosa showed continuity in growth as well as differentiation, already detected in the undifferentiated outline phase.

Conclusions
From the observations of our study, we can at similar stages of prenatal development than those detected in sheep; however, showed greater early in prenatal development that cow and red deer.

27

HISTOLOGICAL FINDINGS OF THE RETICULUM GOAT DURING PRENATAL DEVELOPMENT

García González, A. (1); Masot Gomez-Landero, Antonio Javier (1); Franco Rubio, Antonio (1); Redondo Garcia, Eloy (1).
Universidad De Extremadura (1)

Objectives
The purpose of this research work was to analyze the histological and histochemical goat reticulum during prenatal life.

Material and methods
Here we describe a histologic histochemical analysis of the stomach of 50 goat embryos and fetuses from 30 days of gestation until birth (150 days).

Results
Differentiation of the reticular compartment from the primitive gastric tube begins at 33 days, forming a three layered structure: epithelium, pluripotential blastemal tissue and serosa. The primitive reticular cells are initiated as small epithelial evaginations (primary ribs) at 64 days. At 74 days, lateral growths appear from the primary reticular ribs, forming the corneum papillae. The secondary reticular ribs form at 81 days as growths from the primary ribs. The uneven height of primary and secondary reticular ribs leads to the formation of cells of varying size. Growth of the reticular ribs involves the lamina propria but not the submucosa, so clear separation of these layers is maintained during histodifferentiation. Formation of the tunica muscularis from the pluripotential blastemal tissue begins at 59 days of intrauterine life, as two layers of longitudinally and circularly arranged myoblasts. Differentiation of the muscularis from the mucosa occurs at approximately 113 days, as longitudinal projections of the internal bundles of the tunica muscularis form the musculature of the primary ribs. The secretion of neutral and acid mucopolysaccharides by the reticu-

Conclusions
The reticulum of the goat during intrauterine life, showed a prenatal differentiation and development similar of other reported in the sheep. The capacity to secrete neutral mucopolysaccharides, after 67 days, meant a gradual adaptation of the mucosa to its protective function in postnatal life.

28

HISTOLOGICAL FINDINGS OF THE OMASUM GOAT DURING PRENATAL DEVELOPMENT

Redondo Garcia, Eloy (1); Garcia Gonzalez, Angela (1); Franco Rubio, Antonio (1); Masot Gomez-Landero, Antonio Javier (1).
Universidad De Extremadura (1)

Objectives
Research into the prenatal development of the stomach of ruminants has not been carried out to any great extent and in the species that concerns us here, there has been no published research to date. For this reason, the histological evaluation of the ontogenesis of the omasum in goat, the object of our study, is of an exclusive nature.

Material and methods
Histological and histochemical analyses were carried out on 50 embryos and fetuses of goat. The animals were divided for test purposes into five experimental groups: Group I (1-25% of gestation); Group II (25-35%); Group III (35-50%); Group IV (50-75%) and Group V (75-100% of gestation).

Results
At 33 embryonic days, the omasum wall was differentiated, and was composed of three layers: epithelial layer, pluripotential blastemic tissue and serosa. The stratification of the epithelial layer was accompanied by changes in its structure with the appearance of 4 laminae of different sizes whose sequence in order of appearance was: primary at 32 days; secondary at 38 days, tertiary at 50 days, and quaternary lamina at 59 days. At around mid-gestation, lateral evaginations were formed from the stratum basale of the primary and secondary smaller laminae. These were the primitive corneum papillae. From 113 days, the corneum papillae were present in all 4 sizes of laminae. The histodifferentiation of the lamina propia-submucosa, tunica muscularis and serosa showed patterns of behaviour similar to those referenced for the rumen and reticulum of goat.

Conclusions
The omasum of goat during prenatal life, above all from 67 days of gestation, was shown to be an active structure with full secretory capacity. Its histological development; its secretory capacity, detected by the presence of neutral mucopolysaccharides, was parallel to the development of the rumen and the reticulum.

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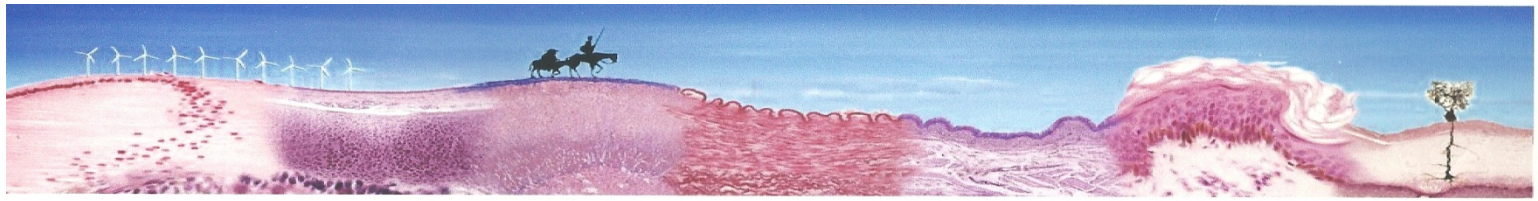
HISTOLOGICAL FINDINGS OF THE ABOMASUN GOAT DURING PRENATAL DEVELOPMENT

Garcia Gonzalez, Angela (1); Masot Gomez-Landero, Antonio Javier (1); Franco Rubio, Antonio (1); Redondo Garcia, Eloy (1).
Universidad De Extremadura (1)

Objectives
The objectives of this study are: 1°.- To explain sequentially the histology of the abomasum, from the differentiation of the primitive stomach to the gastric compartmentation in perinatal stages and 2°.- To describe the histochemical reaction towards the neutral and acidic mucopolysaccharides of the abomasal epithelial layer during prenatal development.

Material and methods
Histological and histochemical analyses were carried out on 50 embryos and fetuses of goat. The animals were divided for test purposes into five experimental groups: Group I (1-25% of gestation); Group II (25-35%); Group III (35-50%); Group IV (50-75%) and Group V (75-100% of gestation).

Results
In the organogenesis of the primitive gastric tube of red deer, the differentiation of the abomasum, took place at 33 days, forming a three-layered structure: epithelial layer (pseudostratified), pluripotential blastemic tissue and serosa. The abomasal wall displayed the primitive folds of the abomasum and by 38 days abomasal peak areas were observed on the fold surface. At 64 days the abomasal surface showed a single mucous cylindrical epithelium and gastric pits were observed in the spaces between abomasal areas. At the bottom of these pits the first outlines of glands could be observed. The histodifferentiation of the lamina propia-submucosa, tunica muscularis and serosa showed patterns of behaviour similar to those referenced for the forestomach of goat.



UNIVERSIDAD DE CASTILLA-LA MANCHA

XV Congreso Nacional de la Sociedad Española de Histología e Ingeniería Tisular

III International Congress of Histology and Tissue Engineering

Albacete del 8 al 11 de Julio de 2009

XV CONGRESO DE LA SOCIEDAD ESPAÑOLA DE HISTOLOGÍA E INGENIERÍA TISULAR
3rd INTERNATIONAL CONGRESS OF HISTOLOGY AND TISSUE ENGINEERING

ACCÉSIT

HONORARY MENTION

HISTOLOGÍA COMPARADA/ COMPARATIVE HISTOLOGY

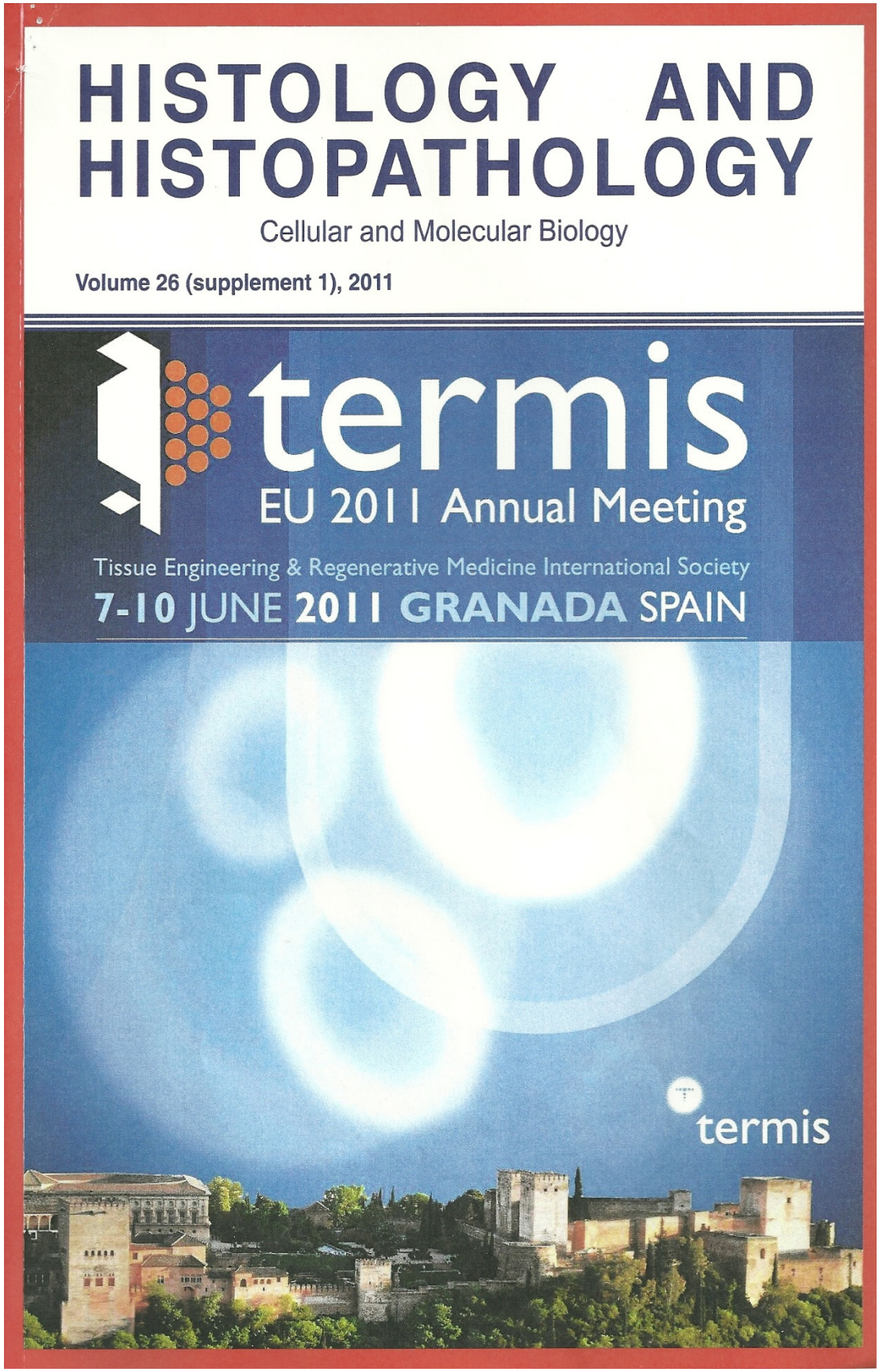
"HISTOLOGICAL FINDINGS OF THE RETICULUM GOAT DURING PRENATAL DEVELOPMENT"

García González, A.; Masot Gomez-Landero, Antonio Javier; Franco Rubio, Antonio; Redondo Garcia, Eloy.

Universidad De Extremadura

Prof. Dr. José Manuel Ruiz Gómez
President of Organization Committee

Prof. Dr. Vicente Crespo
President S.E.H.I.T.


HISTOLOGY AND HISTOPATHOLOGY
Cellular and Molecular Biology
Volume 26 (supplement 1), 2011
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termis

collagen IV probably reflects the inactive state of Leydig cell in photoinhibited testis.
Keywords. HSP-47, collagen IV, Leydig cells

(50.6.P12) HUNTER SCHREGER BANDS IN TEMPORARY DENTITION

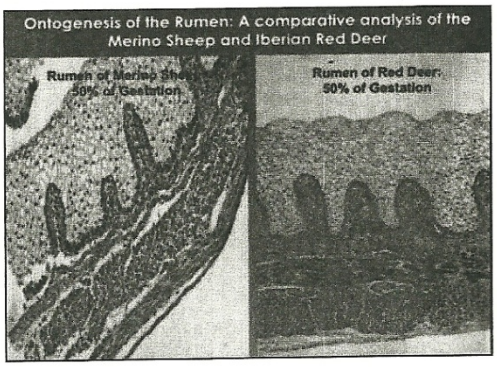
Durso G (1), Llompart G (1), Tanevitch A (1), Abal A (1), Martínez C (1), Llompart J (1), Carda C (2)
1. Asignatura Histología y Embriología, Facultad de Odontología, Universidad Nacional de La Plata, Argentina; 2. Departamento de Patología, Facultad de Medicina y Odontología, Universidad de Valencia, España

Introduction. The tooth enamel is a very hard but fragile tissue with two phylogenetic solutions to this problem, a continuous replacement of teeth and the specialization enamel microstructure. Prisms intercrosses as enamel with bands of Hunter Schreger (HSB) is an effective mechanism to increase resistance to fracture in mammals that employ high occlusal forces during the chewing. The purpose of this study was to determine the layout and microhardness of the bands in the temporary dentition.
Materials and methods. 5 anterior and 5 posterior teeth were included in epoxy resin, grinded with sandpaper of decreasing granulation, polished with powder aluminum oxide, and etched with 37% phosphoric acid during 3", cleaned both with flowing water and ultrasonically, metallized and observed under Scanning Electron Microscopy (SEM). Microhardness was determined by micro durometer Shimadzu with penetrators Vickers and loads of 25 grams for 5".
Results. Bands anterior teeth were observed in incisal zone as alternating layers groups of prisms in longitudinal and transverse section and in the medium and cervical third free face. In molars, in cervical and medial thirds of free faces, in the form of arcs from the connection to the dentin occupying two-thirds internal enamel thickness. The outer third of the enamel represents radial enamel with parallel prisms. Light bands to the SEM related to prisms in cross section and dark bands to prims in longitudinal section. Microhardness (arithmetic mean) was: light bands Hv25 = 227,5 Vk and dark bands HV25 = 174, 5 Vk.
Conclusions. As recorded in permanent teeth the HSB combine with radial enamel in the external portion. Microhardness variation between light and dark bands confirms the research that shows differences in wear and acid engraving. Location and layout relationship with functional areas and different crystalline orientation determines its physical properties.
Keywords. Bands, temporary, SEM, microhardness

(50.6.P13) ONTOGENESIS OF THE RUMEN: A COMPARATIVE ANALYSIS OF THE MERINO SHEEP AND IBERIAN RED DEER

Redondo E (1), Franco A (1), Gázquez A (1), García A (1), Masot AJ (1)
1. Histology. Fac. Vet. Med. University of Extremadura

Introduction. The aim of this study is to describe differences in the ontogenesis of the rumen in the sheep (domestic ruminant) and deer (wild ruminant).
Material and methods. A total of 50 embryos and fetuses of Merino sheep and 50 of 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.
Results. The appearance of the rumen from the primitive gastric tube was earlier in the sheep (22% gestation, 33 days) than in the deer (25% gestation, 66 days). In both cases it displayed a primitive epithelium of a stratified, cylindrical, non-ciliary type. At around 28% gestation in the sheep (42 days) and 26% (67 days) in the deer, the rumen was configured of three clearly-differentiated layers: internal or mucosal, middle or muscular and external or serosal. In both species the stratification of the epithelial layer was accompanied by modifications in its structure with the appearance of the ruminal pillars and papillae. The pillars appeared before the papillae and the appearance of both structures was always earlier in the deer (pillars: 70 days, 27% gestation; papillae: 97 days, 36% gestation) than in the sheep (pillars: 42 days, 28% gestation; papillae: 57 days, 38% gestation). The outlines of the ruminal papillae appeared as evaginations of the basal zone toward the ruminal lumen, dragging in their formation the basal membrane, the lamina propria and the submucosa.
Conclusions. The tegumentary mucosa of the rumen was without secretion capability in the first embryonic phases. From 67 days (26% gestation) the neutral mucopolysaccharides appeared in the deer and at 46 days (30% gestation) in the sheep. In both cases they continued to decrease until birth, this diminution being more pronounced in the deer. Finally, the presence of neuroendocrine and glial cells was detected in the deer at earlier stages than in the sheep.
Keywords. Ontogenesis; Rumen; Sheep; Red Deer

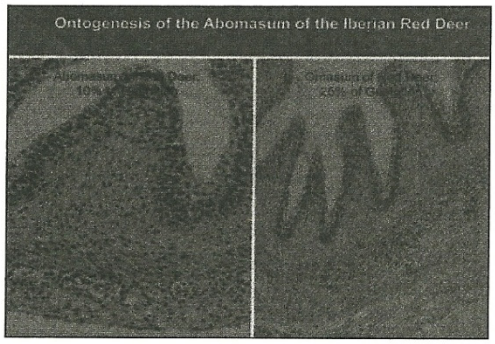


(50.6.P14) ONTOGENESIS OF THE ABOMASUM IN THE IBERIAN RED DEER

Redondo E (1), Masot AJ (1), Gázquez A (1), García A (1), Franco A (2)
1. Histology. Fac. Vet. Med. University of Extremadura; 2. Anatomy. Fac. Vet. Med. University of Extremadura

Introduction. Histomorphometric and immunohistochemical analyses were carried out on 50 embryos and fetuses of red deer from the initial stages of prenatal life until birth.
Material and methods. The animals were divided for test purposes into five experimental groups: GROUP I (1.4 to 3.6, CRL Length cm; 30-60 Days, 1-25% of gestation); GROUP II (4.5 to 7.2, CRL Length cm; 67-90 Days, 25-35% of gestation) GROUP III (8 to 19, CRL Length cm; 97-135

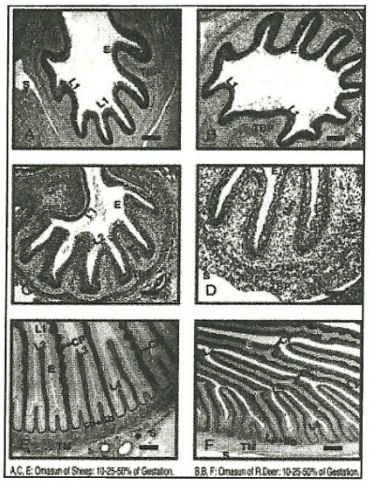
Days, 35-50% of gestation) GROUP IV (21 o 33, CRL Length cm; 142-191 Days, 50-70% of gestation) GROUP V (36 to 40, CRL Length cm; 205-235 Days, 75-100% of gestation).
Results. In the organogenesis of the primitive gastric tube of red deer, the differentiation of the abomasum, took place at 67 days, forming a three-layered structure: epithelial layer (pseudostratified), pluripotential blastemic tissue and serosa. The abomasal wall displayed the primitive folds of the abomasum and by 97 days villi were observed on the fold surface. At 135 days the abomasal surface is had by single mucous cylindrical epithelium and gastric pits were observed in the spaces between villi. At the bottom of these pits the first outlines of glands could be observed. The histodifferentiation of the lamina propia-submucosa, tunica muscularis and serosa showed patterns of behaviour similar to those referenced for the forestomach of red deer. The abomasum of red deer during prenatal life, above all from 67 days of gestation, was shown to be an active structure with full secretory capacity. Its histological development; its secretory capacity, detected by the presence of neutral mucopolysaccharides, and its neuroendocrine nature, detected by the presence of positive non-neuronal enolase cells and neuropeptides vasoactive intestinal peptide and neuropeptide Y, were parallel to the development of the rumen, reticulum and omasum. Gastrin immunoreactive cells first appeared in the abomasum at 142 days, and the number of positive cells increased during development. As in the case of the number of gastrin cells, plasma gastrin concentrations increased throughout prenatal life.
Conclusions . However, its prenatal development was later than that of the abomasum in sheep, goat and cow.
Keywords. Ontogenesis; Abomasum; Red Deer



(50.6.P15) ONTOGENESIS OF THE OMASUM: A COMPARATIVE ANALYSIS OF THE MERINO SHEEP AND IBERIAN RED DEER

Masot AJ (1), Franco A (2), Gázquez A (1), García A (1), Redondo E (1)
1. Histology. Fac. Vet. Med. University of Extremadura; 2. Anatomy. Fac. Vet. Med. University of Extremadura

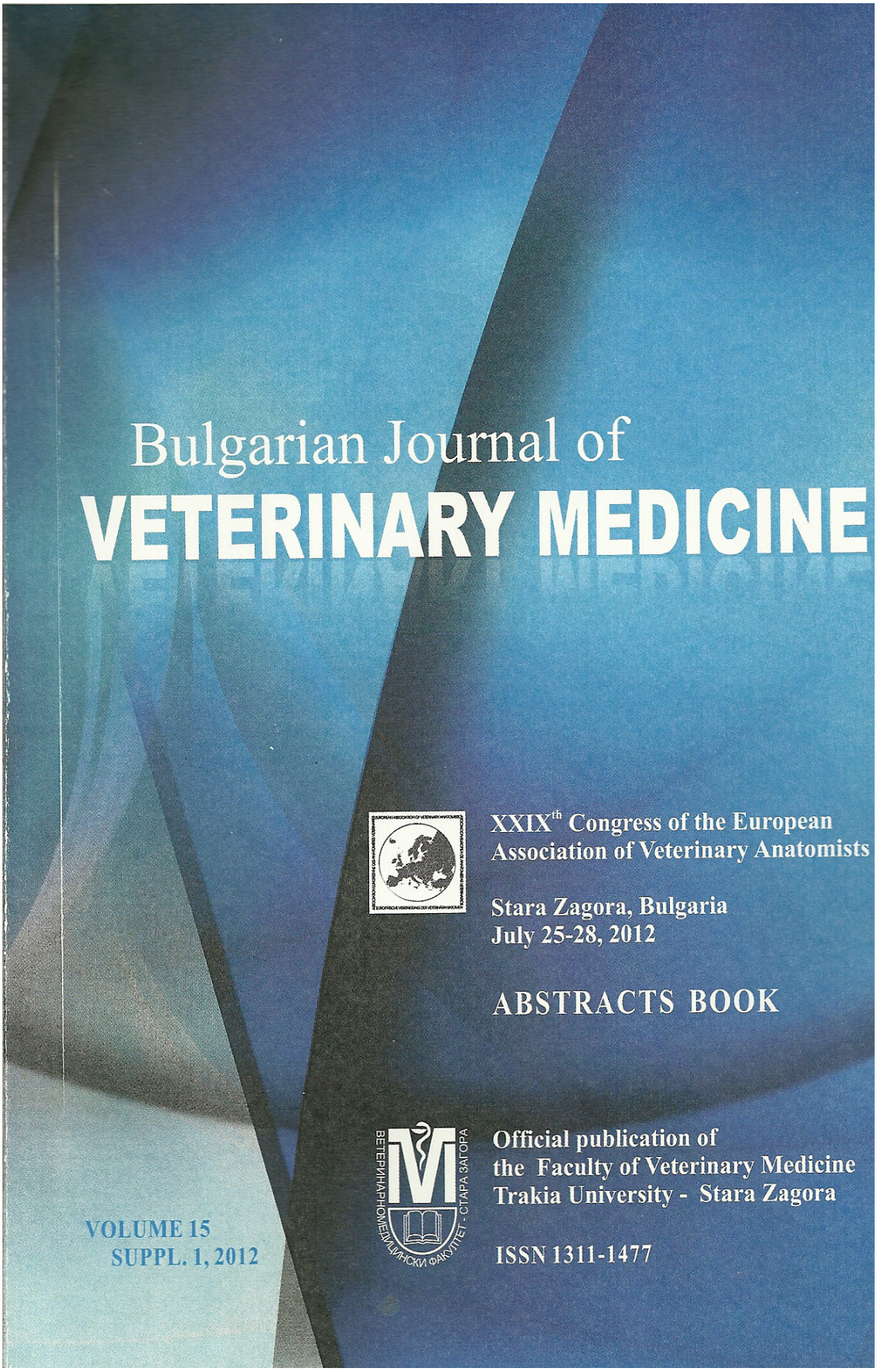
Introduction. The aim of this study is to describe differences in the ontogenesis of the omasum in sheep (domestic ruminant) and deer (wild ruminant).
Materials and methods. A total of 50 embryos and fetuses of Merino sheep and 50 Iberian deer were used, from the first stages of prenatal life until birth. 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.
Results. 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.
Conclusions. 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.
Keywords. Ontogenesis; Omasum; Sheep; Red Deer



(50.6.P16) ONTOGENESIS OF THE RETICULUM: A COMPARATIVE ANALYSIS OF THE MERINO SHEEP AND IBERIAN RED DEER

Franco A (1), Masot AJ (2), Gázquez A (2), García A (2), Redondo E (2)
1. Anatomy. Fac. Vet. Med. University of Extremadura; 2. Histology. Fac. Vet. Med. University of Extremadura

Introduction. The aim of this study is to describe differences in the ontogenesis of the reticulum in the sheep (domestic ruminant) and deer (wild ruminant).
Materials and methods. A total of 50 embryos and fetuses of Merino sheep and 50 of 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: phase I, gastric outline and compartmental individualization; phase II, primary parietal stratification; phase III, primitive epithelial stratification and appearance


Bulgarian Journal of
VETERINARY MEDICINE
XXIX th Congress of the European Association of Veterinary Anatomists
Stara Zagora, Bulgaria
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SMA-reactive cells particularly within the outer layer of the media detected in our study may correspond to vessel-wall-resident progenitor cells. Future studies are thus aimed on further IHC-characteristics in order to distinguish between putative progenitor cells committed towards the endothelial or the VSMC type.

P006 The branches variation of the right renal artery in a Wistar rat

Prof. Dr. İsmail Hakkı Nur[1]; Ass. Dr. Atilla Yoldaş[2] & Ass. Prof. Dr. Ayhan Düzler[3]

[1]Erciyes Universitesi Veteriner Fakultesi Anatomi, ABD, Kayseri, Türkiye (hnur_55@hotmail.com, hn63@erciyes.edu.tr); [2]Veteriner Kontrol ve Araştırma Enstitüsü, Adana, Türkiye (atillayoldas99@hotmail.com); [3]ERÜ, Veteriner Fakültesi, ABD, Kayseri/Türkiye (ayhanduzler@hotmail.com)

This study is the introduction of a case. A combination of 10 mg/kg xylazine (Rompun® enj; Bayer Turk Kimya San. CO. LTD Istanbul), and 100 mg/kg ketamine hydrochloride (Ketalar ® Eczacibasi Istanbul), was given by intraperitoneal route for the purpose of anaesthesia. Heparin was used in order to prevent clotting. Takilon mixture of 15 mL of liquid for corrosion cast of the renal artery (monomethylmetacrylate) was used. The Takilon mixture contained 9 g powdered (polymethylmethacrylate), and 2 mL colouring material obtained from board marker pen ink.

No anastomoses between branches of renal arteries have been reported by many sources. In a laboratory study, in a kidney weighing 0.835 g taken from a healthy rat with a body weight of 197.4 g, the right renal artery arose just before to the cranial mesenteric artery, continuing from the lateral side of the abdominal aorta, after it gave two fine branches to caudal and cranial poles of the kidney, respectively.

The right renal artery, just before entering the *hilus renalis* (Fig. 1/D) gave two main segmental branches – dorsal and ventral (Fig. 1/V). The right renal artery was about 0.75 mm in diameter, the dorsal branch was about 0.51 mm and the ventral branch was about 0.56 mm. The dorsal branch (D) is terminated to the dorsal surface of kidney. The ventral branch (V), immediately divided into cranial (*cr*) and caudal (*ca*) interlobar arteries. The cranial interlobar artery (*cr*) was divided immediately after entering the ventral hilus renalis into dorsal (Fig. 1a) and ventral (Fig. 1b) branches. It was also seen that these fine branches anastomosed to the arcuate arteries. The caudal interlobar artery (*ca*) without branching into the subdivision anastomosed to the arcuate arteries.

P007 Immunohistochemical study of the omasum of goat during prenatal development

A. J. Masot[1], A. García[1], A. J. Franco[2], A. Gázquez[1] & E. Redondo[1]

[1]Department of Veterinary Histology, [2]Department of Anatomy; Faculty of Veterinary Medicine, University of Extremadura, Cáceres, Spain

Introduction: The stomach of the domestic ruminants is a complex organ subdivided into four compartments: rumen, reticulum, omasum and abomasum. Each compartment has its own distinct morphological features and resulting physiological peculiarities, but all four are jointly involved in the digestive process. However, the ruminant is born with a digestive system that structurally and functionally resembles that of the non-ruminant and adaptive development requires the stomach to make fermentative digestion and rumination. Therefore, the aim of this study was to describe the morphological and immunohistochemical changes of the stomach, particularly the rumen of goats from the earliest stages of prenatal life until birth.

Methods: A total of 140 goat embryos and foetuses from the first prenatal stages at birth were sampled. Small pieces of omasum were fixed in 4% buffered formaldehyde. Immunohistochemical (peroxidase-diaminobenzidine) analysis was performed on tissue from the omasum to detect the neuroendocrine cells markers (synapthophysin, SPY), the glial cell markers (glial fibrillary acidic protein, GFAP and vimentin, VIM) and markers of peptidergic innervation as neuropeptide Y (NPY) and vasoactive intestinal peptide (VIP).

Results: Neuroendocrine cells were first detected by SYP staining at 52 days (35% gestation) in the lamina propria, submucosa, and tunica muscularis. Positive staining for GFAP was detected from 64 days (43% gestation) until birth in lamina propria, submucosa, tunica muscularis, the myenteric and the submucosal plexus. The same layers stained positive for VIM at 38 days (26% gestation). Positive staining for NPY and VIP was observed at 95 days (75% gestation) in both the lamina propria and the submucosa.

Conclusions: Compared with other ruminants, the prenatal development of the goat omasum was earlier than that of sheep and cattle, but similar to wild ruminants such as deer.

P008 Immunohistochemical study of the reticulum of goat during prenatal development

A. J. Masot [1], *A. García*[1], *A. J. Franco*[2], *A. Gázquez*[1] *& E. Redondo*[1]

[1]Department of Veterinary Histology, [2]Department of Anatomy; Faculty of Veterinary Medicine, University of Extremadura, Cáceres, Spain

Introduction: The stomach of the domestic ruminants is a complex organ subdivided into four compartments: rumen, reticulum, omasum and abomasum. Each compartment has its own distinct morphological features and resulting physiological peculiarities, but all four are jointly involved in the digestive process. However, the ruminant is born with a digestive system that structurally and functionally resembles that of non-ruminant and adaptive development requires the stomach to make fermentative digestion and rumination. Therefore, the aim of this study was to describe the morphological and immunohistochemical changes of the stomach, particularly the rumen of goats from the earliest stages of prenatal life until birth.

Methods: A total of 140 goat embryos and foetuses from the first prenatal stages at birth were sampled. Small pieces of reticulum were fixed in 4% buffered formaldehyde. Immunohistochemical (peroxidase-diaminobenzidine) analysis was performed on tissue from the omasum to detect the neuroendocrine cells markers (synapthophysin, SPY), the glial cell

markers (glial fibrillary acidic protein, GFAP and vimentin, VIM) and markers of peptidergic innervation as neuropeptide Y (NPY) and vasoactive intestinal peptide (VIP).

Results: Neuroendocrine cells were first detected by SYP staining at 64 days (43% gestation) in the lamina propria, submucosa and serosa. Positive staining for GFAP was detected from 64 days (43% gestation) until birth in lamina propria, submucosa, tunica muscularis, the mucosal and the myenteric plexus. The same layers stained positive for VIM at 39 days (26% gestation). Positive staining for NPY and VIP was observed at 113 days (75% gestation) in the lamina propria, submucosa and tunica muscularis.

Conclusions: Compared with other ruminants, the prenatal development of the goat reticulum was earlier than sheep and cattle, but similar to wild ruminants such as deer.

P009 Immunohistochemical study of the rumen of goat during prenatal development

A. J. Masot [1], *A. García*[1], *A. J. Franco*[2], *A. Gázquez*[1] *& E. Redondo*[1]

[1]Department of Veterinary Histology, [2]Department of Anatomy; Faculty of Veterinary Medicine, University of Extremadura, Cáceres, Spain

Introduction: The stomach of the domestic ruminants is a complex organ subdivided into four compartments: rumen, reticulum, omasum and abomasum. Each compartment has its own distinct morphological features and resulting physiological peculiarities, but all four are jointly involved in the digestive process. However, the ruminant is born with a digestive system that structurally and functionally resembles that of non-ruminant and adaptive development requires the stomach to make fermentative digestion and rumination. Therefore, the aim of this study was to describe the morphological and immunohistochemical changes of the stomach, particularly the rumen of goats from the earliest stages of prenatal life until birth.

Methods: A total of 140 goat embryos and foetuses from the first prenatal stages at birth were sampled. Small pieces of rumen were fixed in 4% buffered formaldehyde. Immunohistochemical (peroxidase-diaminobenzidine) analysis was performed on tissue from the omasum to detect the neuroendocrine cells markers (synapthophysin, SPY), the glial cell markers (glial fibrillary acidic protein, GFAP and vimentin, VIM) and markers of peptidergic innervation as neuropeptide Y (NPY) and vasoactive intestinal peptide (VIP).

Results: Neuroendocrine cells were first detected by SYP staining at 53 days (35% gestation) in the lamina propria, submucosa, tunica muscularis, the myenteric plexus, the intervascular and perivascular connective tissue. Positive staining for GFAP was detected from 68 days (45% gestation) until birth in lamina propria, submucosa, tunica muscularis and serosa. The same layers stained positive for VIM at 39 days (26% gestation). Positive staining for NPY and VIP was observed at 113 days (75% gestation) and 120 days (80% gestation), respectively, in both the lamina propria and the submucosa.

Conclusions: Compared with other ruminants, the prenatal development of the goat rumen was earlier than that of sheep and cattle, but similar to wild ruminants such as deer

HISTOLOGICAL FINDINGS OF THE RUMEN GOAT DURING PRENATAL DEVELOPMENT

Redondo[1] E. , Masot[1] A.J., Franco[2] A., García[1] A.
Histología[1] y Anatomía[2] Patológica. Dpto. de Medicina Animal. Fac. Vet. UEX

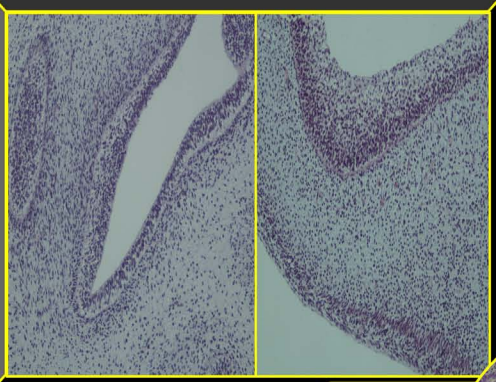
OBJETIVES AND METHODOLOGY

The aim of the present study was to sequence several histological phenomena that occur during the prenatal development of one of the gastric compartements, the rumen. Histological and histochemical analyses were carried out on 50 embryos and fetuses of goat. The animals were divided for test purposes into five experimental groups: Group I (1-37 Days, 1-25% of gestation); Group II (38-52 Days, 25-35% of gestation); Group III (53-75 Days, 35-50% of gestation); Group IV (75-112 Days, 50-75% of gestation) and Group V (113-150 Days, 75-100% of gestation).

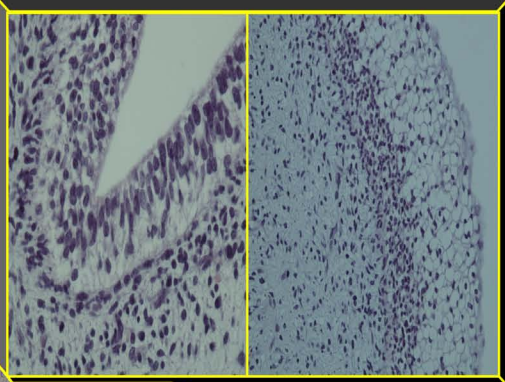
RESULTS

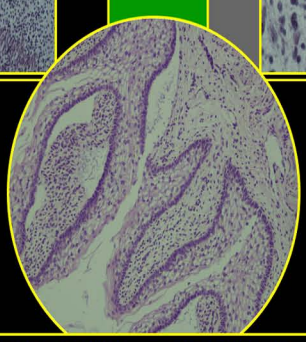
The The rumen of the primitive gastric tube was observed at approximately 33 days. At 37 days the rumen consisted of 3 layers: internal or mucosal, middle or muscular, and external or serosal layer. The stratification of the epithelial layer was accompanied by changes in its structure with the appearance of ruminal pillars and papillae.The outline of the ruminal papillae began to appear at 53 days of prenatal development as evaginations of the basal zone toward the ruminal lumen, pulling with it in its configuration the stratum basale, the lamina propria and the submucosa. At 59 days from the pluripotential blastemic tissue we witnessed the histodifferentiation of the primitive tunica muscularis composed of two layers of myoblasts with a defined arrangement. It was also from the pluripotential blastemic tissue, at 59 days, that the lamina propria and the submucosa were differentiated.The serosa showed continuity in growth as well as differentiation, already detected in the undifferentiated outline phase.

Group I (1-37 Days, 1-25% of gestation)



Group II (38-52 Days, 25-35% of gestation)



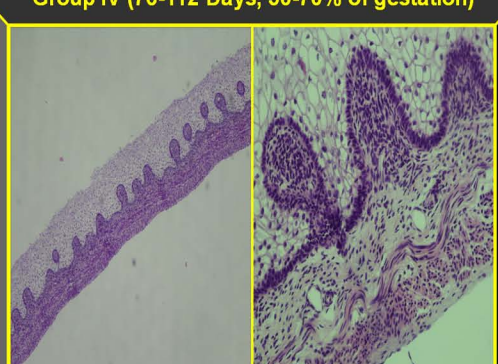


Group V (113-150 Days, 75-100% of gestation

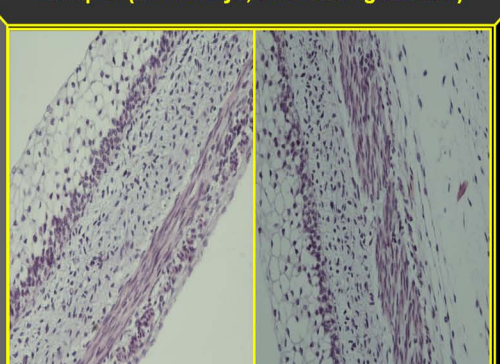
CONCLUSIONS

From the observations of our study, we can deduce that the the goat rumen was evident at similar stages of prenatal development than those detected in sheep; however, showed greater early in prenatal development that cow and red deer.

Group IV (76-112 Days, 50-70% of gestation)



Group III (53-75 Days, 35-50% of gestation)



HISTOLOGICAL FINDINGS OF THE RETICULUM GOAT DURING PRENATAL DEVELOPMENT

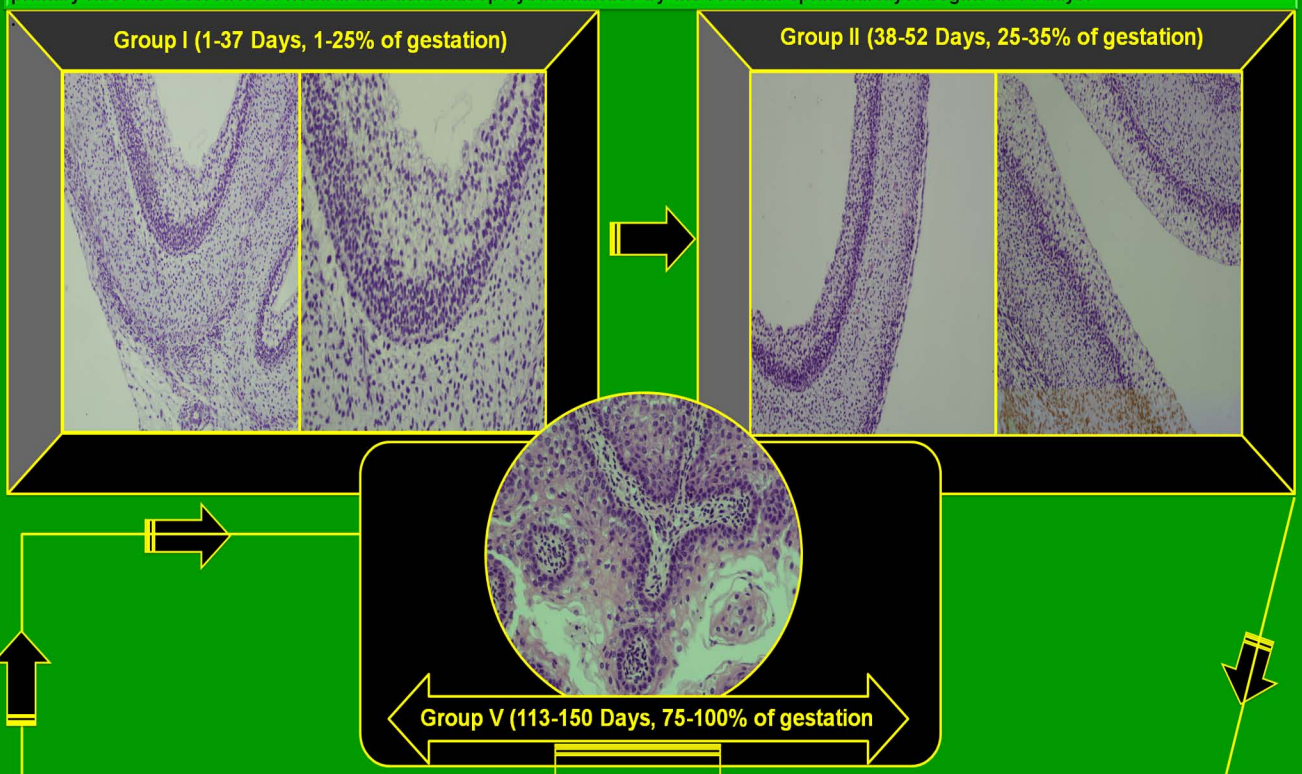
Redondo[1] E. , Masot[1] A.J., Franco[2] A., García[1] A.
Histología[1] y Anatomía[2] Patológica. Dpto. de Medicina Animal. Fac. Vet. UEX

OBJETIVES AND METHODOLOGY

The aim of the present study was to sequence several histological phenomena that occur during the prenatal development of one of the gastric compartements, the reticulum. Histological and histochemical analyses were carried out on 50 embryos and fetuses of goat. The animals were divided for test purposes into five experimental groups: Group I (1-37 Days, 1-25% of gestation); Group II (38-52 Days, 25-35% of gestation); Group III (53-75 Days, 35-50% of gestation); Group IV (75-112 Days, 50-75% of gestation) and Group V (113-150 Days, 75-100% of gestation).

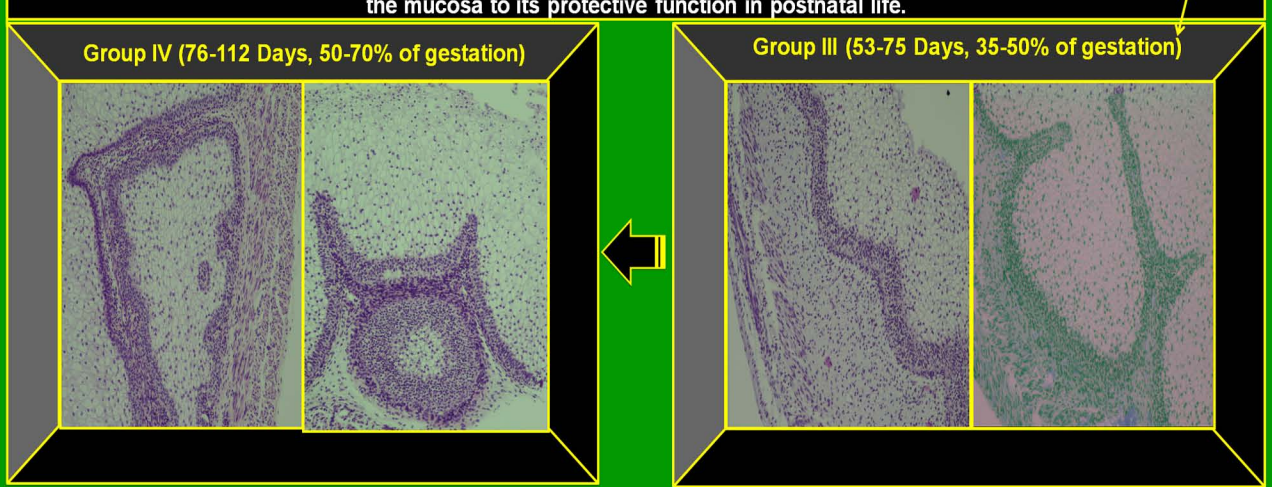
RESULTS

Differentiation of the reticular compartment from the primitive gastric tube begins at 33 days, forming a three layered structure: epithelium, pluripotential blastemal tissue and serosa. The primitive reticular cells are initiated as small epithelial evaginations (primary ribs) at 64 days. At 74 days, lateral growths appear from the primary reticular ribs, forming the corneum papillae. The secondary reticular ribs form at 81 days as growths from the primary ribs. The uneven height of primary and secondary reticular ribs leads to the formation of cells of varying size. Growth of the reticular ribs involves the lamina propria but not the submucosa, so clear separation of these layers is maintained during histodifferentiation. Formation of the tunica muscularis from the pluripotential blastemal tissue begins at 59 days of intrauterine life, as two layers of longitudinally and circularly arranged myoblasts. Differentiation of the muscularis from the mucosa occurs at approximately 113 days, as longitudinal projections of the internal bundles of the tunica muscularis form the musculature of the primary ribs. The secretion of neutral and acid mucopolysaccharides by the reticular epithelial layer begins at 64 days.



CONCLUSIONS

The reticulum of the goat during intrauterine life, showed a prenatal differentiation and development similar of other reported in the sheep. The capacity to secrete neutral mucopolysaccharides, after 67 days, meant a gradual adaptation of the mucosa to its protective function in postnatal life.



HISTOLOGICAL FINDINGS OF THE OMASUM GOAT DURING PRENATAL DEVELOPMENT

García[1] A., Masot[1] A.J., Franco[2] A., Redondo[1] E.
Histología[1] y Anatomía[2] Patológica. Dpto. de Medicina Animal. Fac. Vet. UEX

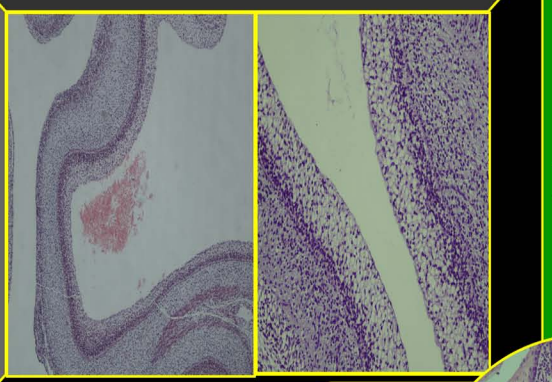
OBJETIVES AND METHODOLOGY

The aim of the present study was to sequence several histological phenomena that occur during the prenatal development of one of the gastric compartements, the omasum. Histological and histochemical analyses were carried out on 50 embryos and fetuses of goat. The animals were divided for test purposes into five experimental groups: Group I (1-37 Days, 1-25% of gestation); Group II (38-52 Days, 25-35% of gestation); Group III (53-75 Days, 35-50% of gestation); Group IV (75-112 Days, 50-75% of gestation) and Group V (113-150 Days, 75-100% of gestation).

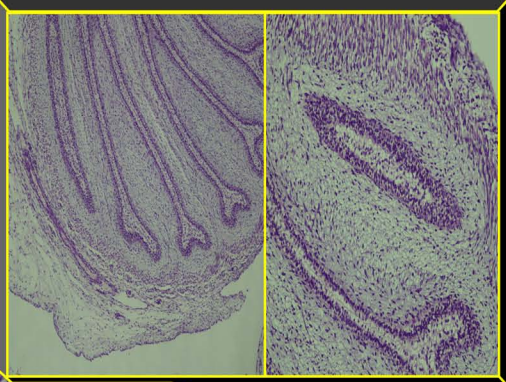
RESULTS

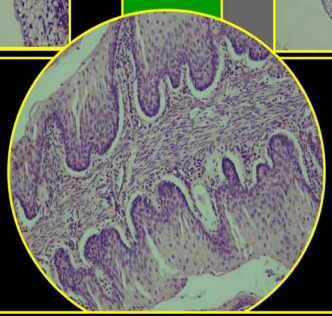
At 33 embryonic days, the omasum wall was differentiated, and was composed of three layers: epithelial layer, pluripotential blastemic tissue and serosa. The stratification of the epithelial layer was accompanied by changes in its structure with the appearance of 4 laminae of different sizes whose sequence in order of appearance was: primary at 32 days; secondary at 38 days, tertiary at 50 days, and quaternary lamina at 59 days. At around mid-gestation, lateral evaginations were formed from the stratum basale of the primary and secondary smaller laminae. These were the primitive corneum papillae. From 113 days, the corneum papillae were present in all 4 sizes of laminae. The histodifferentiation of the lamina propia-submucosa, tunica muscularis and serosa showed patterns of behaviour similar to those referenced for the rumen and reticulum of sheep and goat.

Group I (1-37 Days, 1-25% of gestation)



Group II (38-52 Days, 25-35% of gestation)



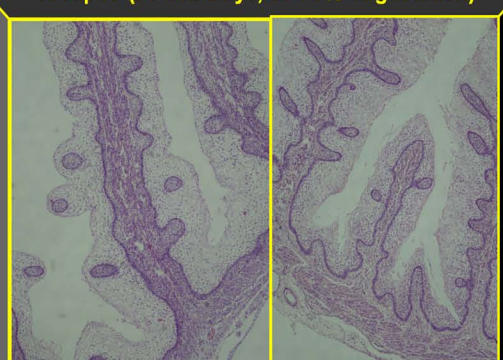


Group V (113-150 Days, 75-100% of gestation

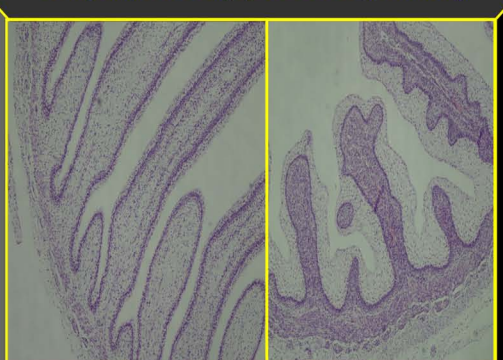
CONCLUSIONS

The omasum of goat during prenatal life, above all from 67 days of gestation, was shown to be an active structure with full secretory capacity. Its histological development; its secretory capacity, detected by the presence of neutral mucopolysaccharides, was parallel to the development of the rumen and the reticulum.

Group IV (76-112 Days, 50-70% of gestation)



Group III (53-75 Days, 35-50% of gestation)



HISTOLOGICAL FINDINGS OF THE ABOMASUM GOAT DURING PRENATAL DEVELOPMENT

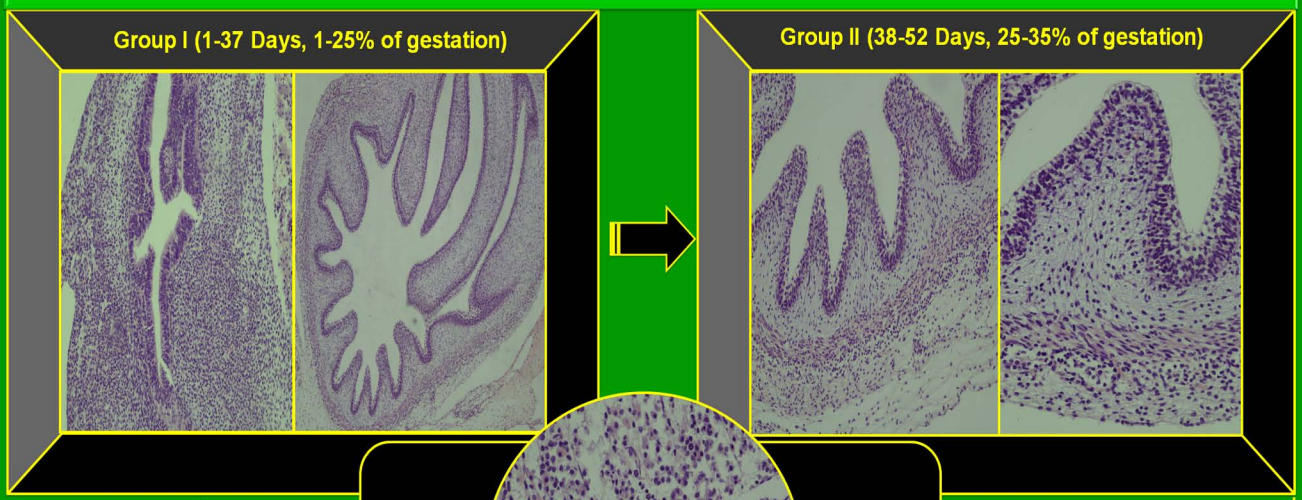
García[1] A., Masot[1] A.J., Franco[2] A., Redondo[1] E.
Histología[1] y Anatomía[2] Patológica. Dpto. de Medicina Animal. Fac. Vet. UEX

OBJETIVES AND METHODOLOGY

The aim of the present study was to sequence several histological phenomena that occur during the prenatal development of one of the gastric compartements, the abomasum. Histological and histochemical analyses were carried out on 50 embryos and fetuses of goat. The animals were divided for test purposes into five experimental groups: Group I (1-37 Days, 1-25% of gestation); Group II (38-52 Days, 25-35% of gestation); Group III (53-75 Days, 35-50% of gestation); Group IV (75-112 Days, 50-75% of gestation) and Group V (113-150 Days, 75-100% of gestation).

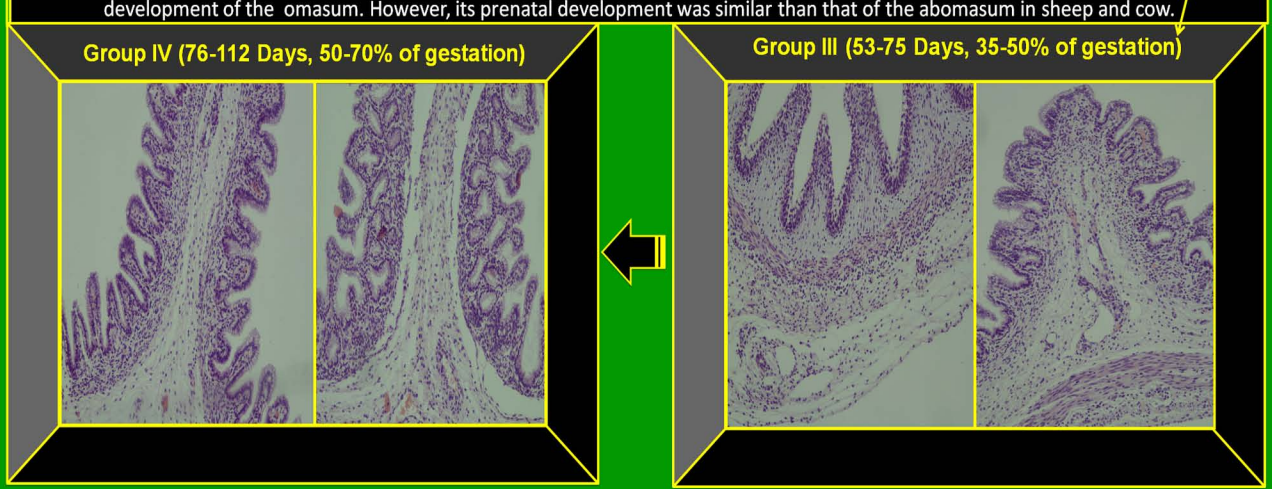
RESULTS

In the organogenesis of the primitive gastric tube of red deer, the differentiation of the abomasum, took place at 33 days, forming a three-layered structure: epithelial layer (pseudostratified), pluripotential blastemic tissue and serosa. The abomasal wall displayed the primitive folds of the abomasum and by 38 days abomasal peak areas were observed on the fold surface. At 64 days the abomasal surface showed a single mucous cylindrical epithelium and gastric pits were observed in the spaces between abomasal areas. At the bottom of these pits the first outlines of glands could be observed. The histodifferentiation of the lamina propia-submucosa, tunica muscularis and serosa showed patterns of behaviour similar to those referenced for the forestomach of sheep and goat.



CONCLUSIONS

The abomasum of red deer during prenatal life, above all from 67 days of gestation, was shown to be an active structure with full secretory capacity. Its histological development; its secretory capacity, detected by the presence of mucopolysaccharides, were parallel to the development of the omasum. However, its prenatal development was similar than that of the abomasum in sheep and cow.



ONTOGENESIS OF THE RUMEN: A COMPARATIVE ANALYSIS OF THE MERINO SHEEP AND IBERIAN RED DEER

[1]Franco A., [2]Masot A.J., [2]García A., [2]Redondo E.

Departaments of Veterinary [1]Anatomy and [2]Histology, Faculty of Veterinary Medicina, University of Extremadura, Cáceres, Spain

Introduction

The aim of this study is to describe differences in the ontogenesis of the rumen in the sheep (domestic ruminant) and deer (wild ruminant).

Materials and Methods

A total of 50 embryos and fetuses of Merino sheep and 50 of 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.

Results

The appearance of the rumen from the primitive gastric tube was earlier in the sheep (22% gestation, 33 days) than in the deer (25% gestation, 66 days). In both cases it displayed a primitive epithelium of a stratified, cylindrical, non-ciliary type. At around 28% gestation in the sheep (42 days) and 26% (67 days) in the deer, the rumen was configured of three clearly-differentiated layers: internal or mucosal, middle or muscular and external or serosal. In both species the stratification of the epithelial layer was accompanied by modifications in its structure with the appearance of the ruminal pillars and papillae. The pillars appeared before the papillae and the appearance of both structures was always earlier in the deer (pillars: 70 days, 27% gestation; papillae: 97 days, 36% gestation) than in the sheep (pillars: 42 days, 28% gestation; papillae: 57 days, 38% gestation). The outlines of the ruminal papillae appeared as evaginations of the basal zone toward the ruminal lumen, dragging in their formation the basal membrane, the lamina propria and the submucosa.

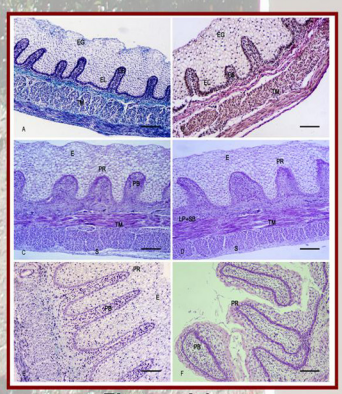


Figura nº 1

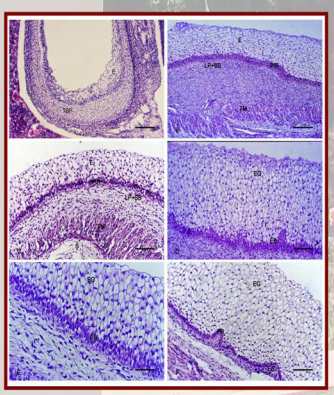


Figura nº 2

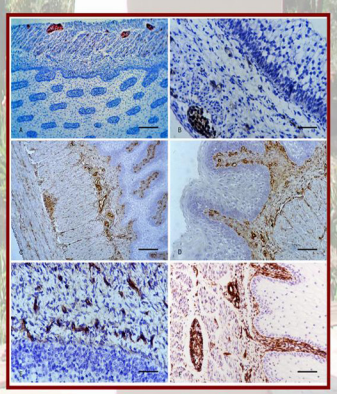


Figura nº 3

Conclusions

The tegumentary mucosa of the rumen was without secretion capability in the first embryonic phases. From 67 days (26% gestation) the neutral mucopolysaccharides appeared in the deer and at 46 days (30% gestation) in the sheep. In both cases they continued to decrease until birth, this diminution being more pronounced in the deer. Finally, the presence of neuroendocrine and glial cells was detected in the deer at earlier stages than in the sheep.

UNIÓN EUROPEA
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JUNTA DE EXTREMADURA
Vicepresidencia Segunda y Consejería de Economía, Comercio e Innovación

ONTOGENESIS OF THE RETICULUM: A COMPARATIVE ANALYSIS OF THE MERINO SHEEP AND IBERIAN RED DEER

[2]Masot A.J., [2]García A., [1]Franco A., [2]Redondo E.

Departaments of Veterinary [1]Anatomy and [2]Histology, Faculty of Veterinary Medicina, University of Extremadura, Cáceres, Spain

Introduction

The aim of this study is to describe differences in the ontogenesis of the reticulum in the sheep (domestic ruminant) and deer (wild ruminant).

Materials and Methods

A total of 50 embryos and fetuses of Merino sheep and 50 of 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: phase I, gastric outline and compartmental individualization; phase II, primary parietal stratification; phase III, primitive epithelial stratification and appearance of primary ribs; phase IV, definitive epithelial stratification and appearance of reticularis cells; phase V, parietal stabilisation and maturity of the mucosa.

Results

The appearance of the reticulum from the primitive gastric tube was earlier in the sheep (22% gestation, 33 days) than in the deer (25% gestation, 66 days). In both cases it displayed a primitive epithelium of a stratified, cylindrical, non-ciliary type. At around 48% gestation in the sheep (72 days) and 36% (97 days) in the deer, the reticulum was configured of 4 clearly-differentiated layers: mucosa (with epithelial layer and lamina propria), submucosa, tunica muscularis and serosa. In both species the stratification of the epithelial layer was accompanied by modifications in its structure with the appearance of the primitive reticular ribs. The primary ribs began to be formed first in the deer, at 117 days of prenatal life (40 % gestation) and later in the sheep (79 days, 53 % gestation). The differentiation of the corneum papillae in the primary ribs coincided with the appearance of secondary reticular rib. These structures began to be formed first in the deer, at 142 days of prenatal life (51 % gestation) and later in the sheep (83 days, 55 % gestation).

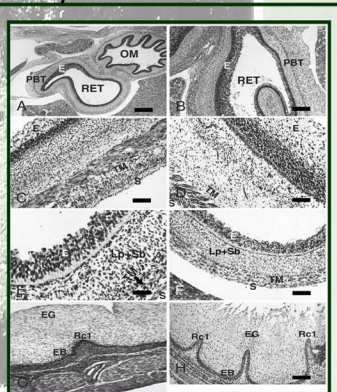


Figura nº 1

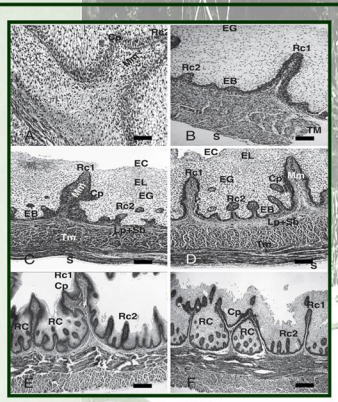
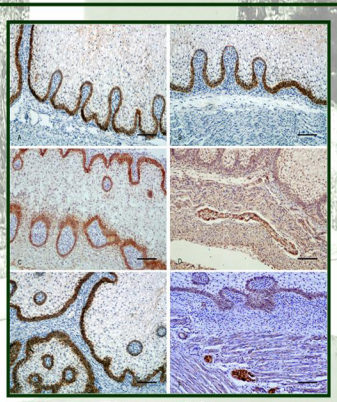


Figura nº 2



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Conclusions

The tegumentary mucosa of the reticulum was without secretion capability in the first embryonic phases. From 67 days (26% gestation) the neutral mucopolysaccharides appeared in the deer and at 46 days (30% gestation) in the sheep. In both cases they continued to decrease until birth, this diminution being more pronounced in the deer. Finally, the presence of neuroendocrine and glial cells was detected in the deer at earlier stages than in the sheep.

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ONTOGENESIS OF THE OMASO: A COMPARATIVE ANALYSIS OF THE MERINO SHEEP AND IBERIAN RED DEER

[2]García A., [1]Franco A., [2]Masot A.J., [2]Redondo E.

Departaments of Veterinary [1]Anatomy and [2]Histology, Faculty of Veterinary Medicina, University of Extremadura, Cáceres, Spain

Introduction

The aim of this study is to describe differences in the ontogenesis of the omasum in sheep (domestic ruminant) and deer (wild ruminant).

Materials and Methods

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.

Results

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.

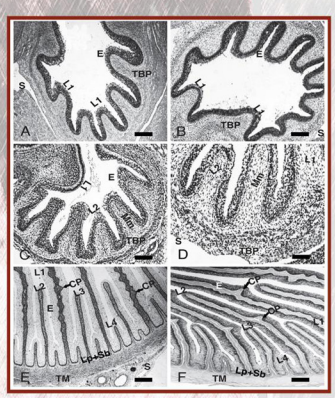
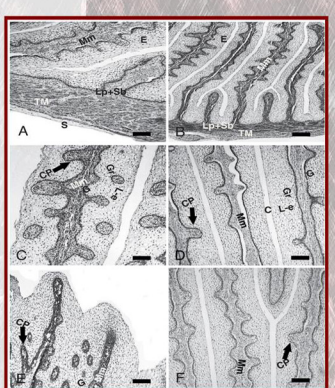


Figura nº 1



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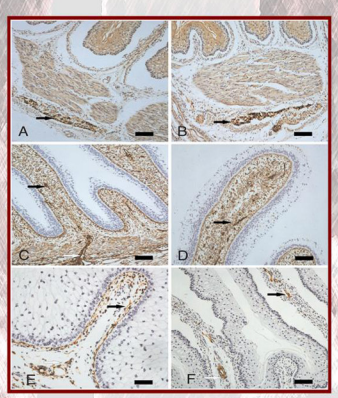

Figura nº 3

Conclusions

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.

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Vicepresidencia Segunda y Consejería de Economía, Comercio e Innovación

ONTOGENESIS OF THE ABOMASUM IN THE IBERIAN RED DEER

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Introduction

Histomorphometric and immunohistochemical analyses were carried out on 50 embryos and fetuses of red deer from the initial stages of prenatal life until birth.

Materials and Methods

The animals were divided for test purposes into five experimental groups: GROUP I (1.4 to 3.6, CRL Length cm; 30-60 Days, 1-25% of gestation); GROUP II (4.5 to 7.2, CRL Length cm; 67-90 Days, 25-35% of gestation) GROUP III (8 to 19, CRL Length cm; 97-135 Days, 35-50% of gestation) GROUP IV (21 o 33, CRL Length cm; 142-191 Days, 50-70% of gestation) GROUP V (36 to 40, CRL Length cm; 205-235 Days, 75-100% of gestation).

Results

In the organogenesis of the primitive gastric tube of red deer, the differentiation of the abomasum, took place at 67 days, forming a three-layered structure: epithelial layer (pseudostratified), pluripotential blastemic tissue and serosa. The abomasal wall displayed the primitive folds of the abomasum and by 97 days villi were observed on the fold surface. At 135 days the abomasal surface is had by single mucous cylindrical epithelium and gastric pits were observed in the spaces between villi. At the bottom of these pits the first outlines of glands could be observed. The histodifferentiation of the lamina propia-submucosa, tunica muscularis and serosa showed patterns of behaviour similar to those referenced for the forestomach of red deer. The abomasum of red deer during prenatal life, above all from 67 days of gestation, was shown to be an active structure with full secretory capacity. Its histological development; its secretory capacity, detected by the presence of neutral mucopolysaccharides, and its neuroendocrine nature, detected by the presence of positive non-neuronal enolase cells and neuropeptides vasoactive intestinal peptide and neuropeptide Y, were parallel to the development of the rumen, reticulum and omasum. Gastrin immunoreactive cells first appeared in the abomasum at 142 days, and the number of positive cells increased during development. As in the case of the number of gastrin cells, plasma gastrin concentrations increased throughout prenatal life.

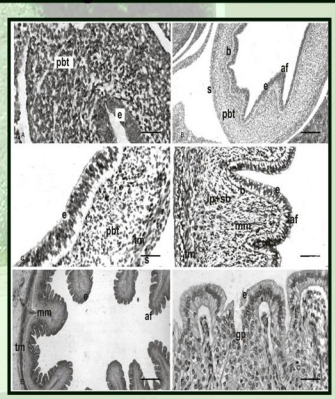
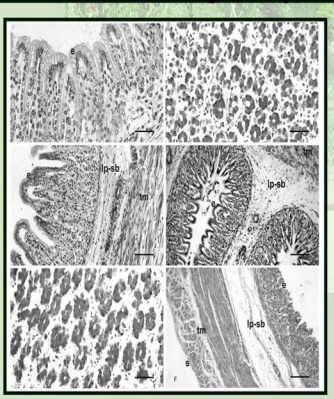


Figura nº 1



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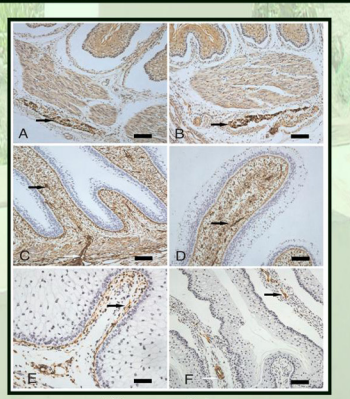

Figura nº 3

Conclusions

However, its prenatal development was later than that of the abomasum in sheep, goat and cow.

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IMMUNOHISTOCHEMICAL STUDY OF THE RUMEN OF GOAT DURING PRENATAL DEVELOPMENT

[1]Masot A.J., [1]Garcia A., [2]Franco A., [1]Gazquez A., [1]Redondo E.

Department of Veterinary [1]Histology and [2]Anatomy, Faculty of Veterinary Medicine, University of Extremadura, Caceres, Spain.

Introduction

Ruminants are animals with a high efficiency for the transformation of low-quality forage into high nutritional value due to the structure and function of their digestive tract **(Lombard, 2005).** Their stomach is divided into four compartments stomach: rumen, reticulum, omasum and abomasum. Each with different morphological characteristics.The first compartment stomach, el rumen, is the most important in physiological terms, in that it plays a key role in the breaksdown and subsequent micorbial digestion of celulose, an essential nutrient for ruminants **(Habel, 1973).** However, ruminant animals are born with a stomach that is structurally and functionally similar to non-ruminant animals and during prenatal life, the stomach of ruminant animals suffers some morphological changes to suit function in postnatal life. For this reason, the aim of this study was to detect neuroendocrine cells, glial cells, nerve cells and sympathetic and parasympathetic nerve fibers by immunohistochemical analysis in the rumen of goat from early embryonic stages until birth.

Materials and Methods

A total of 140 goat (*Capra hircus*) embryos and fetuses, ranging from the first prenatal stages to birth, were sampled. Gestionalage was estimated according to Evans and Sack (1973). Specimens were dividided into five 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, 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). Small pieces of rumen were fixed in 4% buffered formaldehyde. The UltraVision One HRP polymer (polymer conjugated to horseradish peroxidase) was performed on deparaffinized tissue from the omasum to detect the neuroendocrine cell marker with synaptophysin (SYP), the glial cell marker with glial fibrillary acidic protein (GFAP) and vimentin (VIM) and markers of peptidergic innervation as neuropetide Y (NPY) and vasoactive intestinal peptide (VIP).

	Group I (1.5-4.3 cm CRL, 26-38 days; 1-25% gestation)				Grupo II (4.4-8 cm CRL, 39-52 days; 25-35% gestation)				Grupo III (9-17.5 cm CRL, 53-75 days; 35-50% gestation)				Grupo IV (18-32 cm CRL, 76-112 days; 50-75% gestation)				Grupo V (33-47 cm CRL, 113-150 days; 75-100% gestation)			
	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
SYP	-	-	-	-	-	-	-	-	-	+	+	-	-	++	++	-	+	++	++	-
GFAP	-	-	-	-	-	-	-	-	-	-	-	-	-	++	++	++	-	++	++	++
VIM	-	-	-	-	+	+	+	+	+	+	+	-	+	++	++	++	-	+++	+++	+++
NPY	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	++	-	-	
VIP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	

- no reactivity; + low reactivity; ++, high reactivity; +++, strong reactivity. E= epithelium, LP-S= lamina propia-submucosa, TM= tunica muscularis, S= serosa.

Table 1. Immunohistochemical analysis of goat rumen during prenatal development

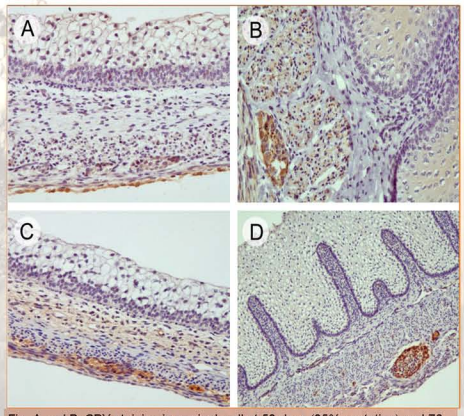

Fig. A and B. SPY-staining in ruminal wall at 53 days (35% gestation) and 76 days (50% gestation).

Fig. C and D. GFAP-positive cells in ruminal wall at 69 days (46% gestion) and 113 days (75% gestation).

Results

➢ Neuroendocrine cells were first detected by SYP staining at 53 days (35% gestation) in the lamina propria, submucosa, tunica muscularis, myenteric plexus and intervascular connective tissue **(Fig. A).** By 76 days (50% gestation) staining intensity was greater in these layers **(Fig. B).**

➢ Cells staining positive to GFAP were first observed at 68 days (45% gestation) in lamina propria, submucosa, tunica muscularis and serosa **(Fig. C).** These cells were more abundant at these localization by 113 days **(Fig. D).**

➢ VIM-positive glial cells were observed at 39 days (26% gestation) in lamina propria, submucosa, and tunica muscularis **(Fig. E).** By 101 days, VIM-positive cells were observed in myenteric plexus, lamina propia, submucosa, tunica muscularis and perivascular connective tissue **(Fig. F).**

➢ Sympathetic and parasympathetic nerve fibers and nerve bodies were detected with NPY and VIP at 113 days (75% gestation) and 120 days (80% gestation), respectively, in both the lamina propria and submucosa **(Fig. G and H).**

Discussion

➢ Immunohistochemical analysis of the goat rumen reveled SPY-positive neuroendocrine cells at ages similar to those described by Franco et al. (2011), in red deer (97 days, 36% gestation), in contrast to sheep, these cells were observed in later stages.

➢ Glial cells were detected in ealier stages by VIM than GFAP. According to Franco et al. (1997) in the prenatal development of the pineal gland, VIM was identified as an ealy glial cell marker.

➢ Sympathetic and parasympathetic nerve fibers adn nerve cells were observed during late stages of development. Similar results were noted for red deer by Franco et al. (2004).

➢ In conclusion, and compared with other ruminants the prenatal development of the goat rumen was ealier than sheep, but similar to wid ruminants such as deer.

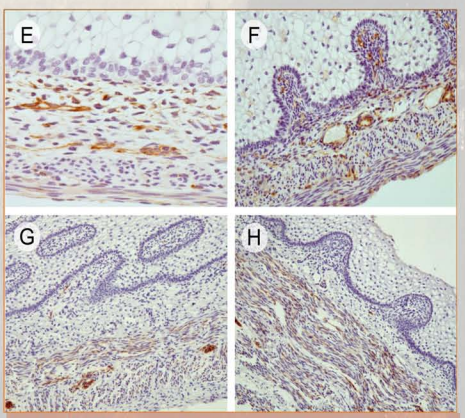

Fig. A and B. VIM-positive cells in ruminal wall at 38 days (25% gestation) and 101 days (67% gestation).

Fig. C and D. NPY-positive staining and VIP-positive staining in ruminal wall at 113 days (75% gestion) and 120 days (80% gestation), respectively.

Reference

Evans H.E. and Sack W.O. (1973). Prenatal development of domestic and laboratory mammals: Growth curves, external features and selected references. Anat. Histol. Embryol. 2, 11-45.

Franco A., Regodón S., Masot A.J. and Redondo E. (1997). A combined immunohistochemical and electron microscopic study of the second cell type in the developing sheep pineal gland. J. Pineal. Res. 22, 130-136.

Franco A., Masot A.J., Gómez L. and Redondo E. (2004a). Morphometric and immunohistochemical study of the rumen of red deer during prenatal development. J. Anat. 204, 501-513.

Franco A., Masot A.J. and Redondo E. (2011). Ontogenesis of the rumen: a comparative analysis of the merino sheep and Iberian red deer. Anim. Sci. J. 82, 107-116.

Habel R.E. (1973). Anatomical and histological nomenclature of the ruminants stomach, in physiological of digestion of the ruminant:. In: Hofmann, R.R. (Ed.), The Ruminant Stomach. East African Literature Bureau, Nairobi, Kampala.

Lombardi, G., 2005. Optimum management and quality pastures for sheep and goat in mountain areas. Options mediterraneennes, 67.

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IMMUNOHISTOCHEMICAL STUDY OF THE RETICULUM OF GOAT DURING PRENATAL DEVELOPMENT

[1]Masot A.J., [1]Garcia A., [2]Franco A., [1]Gazquez A., [1]Redondo E.

Department of Veterinary [1]Histology and [2]Anatomy, Faculty of Veterinary Medicine, University of Extremadura, Caceres, Spain.

Introduction

Ruminants are animals with a high efficiency for the transformation of low-quality forage into high nutritional value due to the structure and function of their digestive tract **(Lombard, 2005).** Their stomach is divided into four compartments stomach: rumen, reticulum, omasum and abomasum. Each with different morphological characteristics.The first compartment stomach, el rumen, is the most important in physiological terms, in that it plays a key role in the breaksdown and subsequent micorbial digestion of celulose, an essential nutrient for ruminants **(Habel, 1973).** However, ruminant animals are born with a stomach that is structurally and functionally similar to non-ruminant animals and during prenatal life, the stomach of ruminant animals suffers some morphological changes to suit function in postnatal life. For this reason, the aim of this study was to detect neuroendocrine cells, glial cells, nerve cells and sympathetic and parasympathetic nerve fibers by immunohistochemical analysis in the rumen of goat from early embryonic stages until birth.

Materials and Methods

A total of 140 goat (*Capra hircus*) embryos and fetuses, ranging from the first prenatal stages to birth, were sampled. Gestionalage was estimated according to Evans and Sack (1973). Specimens were dividided into five 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, 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). Small pieces of rumen were fixed in 4% buffered formaldehyde. The UltraVision One HRP polymer (polymer conjugated to horseradish peroxidase) was performed on deparaffinized tissue from the omasum to detect the neuroendocrine cell marker with synaptophysin (SYP), the glial cell marker with glial fibrillary acidic protein (GFAP) and vimentin (VIM) and markers of peptidergic innervation as neuropetide Y (NPY) and vasoactive intestinal peptide (VIP).

Table 1. Immunohistochemical analysis of goat rumen during prenatal development

Results

- Neuroendocrine cells were first detected by SYP staining at 53 days (35% gestation) in the lamina propria, submucosa, tunica muscularis and serosa **(Fig. A).** By 76 days (50% gestation) staining intensity was greater and these cells were observed in myenteric plexus **(Fig. B).**
- Cells staining positive to GFAP were first observed at 65 days (43% gestation) in lamina propria, submucosa, tunica muscularis, serosa and myenteric ans submucosal plexuses **(Fig. C).** These cells were more abundant at these localization by 113 days (75% gestation; **Fig. D).**
- VIM-positive glial cells were observed at 38 days (25% gestation) in lamina propria, submucosa, tunica muscularis and serosa **(Fig. E).** By 113 days (75% gestation), VIM-positive cells were observed in myenteric and submucosal plexus, lamina propia, submucosa, tunica muscularis and perivascular connective tissue **(Fig. F).**
- Peptidergic nerves were detected with NPY and VIP at 76 days (50% gestation) in both the lamina propria and submucosa, tunica muscularis, serosa and myenteric plexus **(Fig. G and H).**

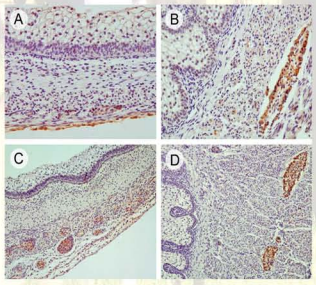

Fig. A and B. SPY-staining in ruminal wall at 53 days (35% gestation) and 76 days (50% gestation).

Fig. C and D. GFAP-positive cells in ruminal wall at 69 days (46% gestion) and 113 days (75% gestation).

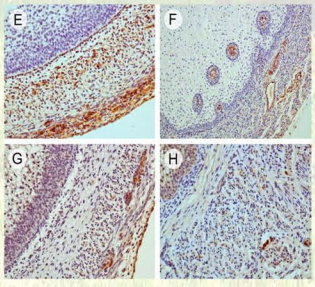

Fig. A and B. VIM-positive cells in ruminal wall at 38 days (25% gestation) and 101 days (67% gestation).

Fig. C and D. NPY-positive staining and VIP-positive staining in ruminal wall at 113 days (75% gestion) and 120 days (80% gestation), respectively.

Discussion

- Immunohistochemical analysis of the goat reticulum reveled SPY-positive neuroendocrine cells at 53 days (35% gestation) according to those described by Franco et al. (2004), in red deer (97 days, 36% gestation) and goat rumen by Garcia et al. (2012).
- Glial cells were detected in ealier stages by VIM than GFAP. Similar findings were described by Franco et al. (2012) in red deer and sheep. Moreover, the difference in glial-ceels labelling refects the fact taht VIM is an earlier marker, as noted by Franco et al. (1997), in a study of prenatal pineal gland development in sheep.
- Peptidergic nerves were observed at 76 days (50% gestation), according to those described in deer by Franco et al. (2004). However, Garcia et al. (2012) observed nerve fibers in the rumen goat deer at late stage of development (113 days, 75% gestation).
- In conclusion, and compared with other ruminants the prenatal development of the goat reticulum was ealier than sheep, but similar to wid ruminants such as deer.

Reference

Evans H.E. and Sack W.O. (1973). Prenatal development of domestic and laboratory mammals: Growth curves, external features and selected references. Anat. Histol. Embryol. 2, 11-45.

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IMMUNOHISTOCHEMICAL STUDY OF THE OMASUM OF GOAT DURING PRENATAL DEVELOPMENT

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Department of Veterinary [1]Histology and [2]Anatomy, Faculty of Veterinary Medicine, University of Extremadura, Caceres, Spain.

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	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, 35-50% 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	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	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	++	+	+

E: Epithelium, Lp + Sb: Lamina propia and submucosa, TM: Tunica muscularis, S: Serosa. -, non immunoreactivity; +, low immunoreactivity; ++, moderate immunoreactivity; +++, high immunoreactivity.

Table 1. Immunohistochemical analysis of goat omasum during prenatal development

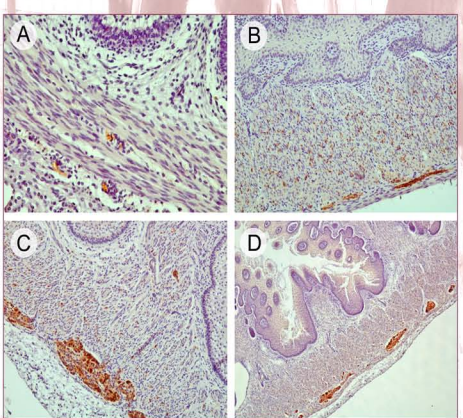

Fig. A and B. SPY-staining in ruminal wall at 53 days (35% gestation) and 76 days (50% gestation).

Fig. C and D. GFAP-positive cells in ruminal wall at 69 days (46% gestion) and 113 days (75% gestation).

Results

The results of immunohistochemical staining of the omasum are shown in Table 1.

➢Neuroendocrine cells were first detected by SYP staining at 53 days (35% gestation) in the lamina propria, submucosa, tunica muscularis, myenteric plexus **(Fig. A).** Positive-staining cells were more abundant at these sites by 64 days (43% gestation; **Fig. B).**

➢ Cells staining positive to GFAP were first observed at 64 days (43% gestation) in lamina propria, submucosa, tunica muscularis and myenteric and sbumucosal plexuses **(Fig. C).** These cells were more abundant at these localization by 113 days (75% gestation; **Fig. D).**

➢VIM-positive glial cells were observed at 38 days (25% gestation) in lamina propria, submucosa, tunica muscularis and serosa **(Fig. E).** By 101 days (67% gestation), VIM-positive cells were observed in myenteric plexus and scattered thorught the lamina propia and submucosa of omasal laminae **(Fig. F).**

➢Peptidergic nerves were detected by immunolabeling with NPY and VIP at 95 days (63% gestation) in both the lamina propria and submucosa, tunica muscularis and serosa **(Fig. G and H).**

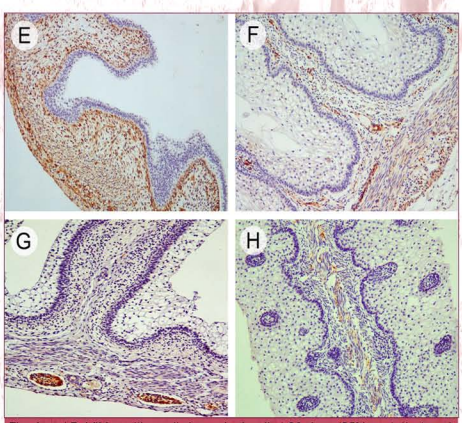

Fig. A and B. VIM-positive cells in ruminal wall at 38 days (25% gestation) and 101 days (67% gestation).

Fig. C and D. NPY-positive staining and VIP-positive staining in ruminal wall at 113 days (75% gestion) and 120 days (80% gestation), respectively.

Discussion

➢ Immunohistochemical analysis of the goat omasum reveled SPY-positive neuroendocrine cells at ages similar to those described by Redondo et al. (2005), in red deer (97 days, 36% gestation).

➢ Glial cells were detected in ealier stages by VIM than GFAP. A similar timelag in the immunohistochemical detection of glial cells has also been reported by Redondo et al. (2011) in a comparative study of the omasum in deer and sheep. Moreover, the difference in glial-ceels labelling refects the fact taht VIM is an earlier marker , as noted by Franco et al. (1997), in a study of prenatal pineal gland development in sheep.

➢Peptidergic nerves were observed during late stages of development. However, Redondo et al. (2005) observed nerve fibers in the omasum deer at en earlier stage of development (142 days, 51% gestation).

➢In conclusion, and compared with other ruminants the prenatal development of the goat omasum was ealier than sheep, but similar to wid ruminants such as deer.

References

Evans H.E. and Sack W.O. (1973). Prenatal development of domestic and laboratory mammals: Growth curves, external features and selected references. Anat. Histol. Embryol. 2, 11-45.

Franco A., Regodón S., Masot A.J. and Redondo E. (1997). A combined immunohistochemical and electron microscopic study of the second cell type in the developing sheep pineal gland. J. Pineal. Res. 22, 130-136.

Lombardi, G., 2005. Optimum management and quality pastures for sheep and goat in mountain areas. Options mediterraneennes, 67.

Phillipson A.T. (1982). Ruminant Digestion. In: M.J. Swenson Duke´s Physiology of Domestic Animals. 9th edn. Comstock Publishing Associates (ed). London. pp 277.

Redondo E., Franco A. and Masot A.J. (2005). Morphometric and immunohistochemical study of the omasum of red deer during prenatal development. J. Anat. 206, 543-555.

Redondo E., Masot J., Garcia A. and Franco A. (2011). Ontogenesis of the omasum: a comparative analysis of the Merino sheep and Iberian red deer. Histol. Histopathol. 26, 1135-1144.

Yamamoto Y., Kitamura N., Yamada J., Andren A. and Yamashita T. (1994). Morphological study of the surface structure of the omasal laminae in cattle, sheep and goats. Anat. Histol. Embryol. 232, 166-176.

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