Tissue and Cell 48 (2016) 18–24

Contents lists available at ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

Comparative analysis of the morphology and histochemistry of the duodenum of the coypu (*Myocastor coypus bonariensis*) during its prenatal and postnatal development

María Florencia Tano de la Hoz[a,b,1], Verónica Eyheramendy[c,1], Antonio Eduardo Felipe[c], Alcira Ofelia Díaz[b,*]

[a] Nacional Council of Scientific and Technical Research (CONICET), Argentina
[b] Institute of Marine and Coastal Research, Nacional Council of Scientific and Technical Research – Nacional University of Mar del Plata, Funes 3250, Mar del Plata 7600, Buenos Aires, Argentina
[c] Department of Biological Sciences, Faculty of Veterinary Science, U.N.C.P.B.A., Tandil, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 12 August 2015
Accepted 8 December 2015
Available online 25 December 2015

Keywords:
Hystricognathi
Myocastor coypus bonariensis
Duodenum development
Histochemistry
Glycoconjugates
Comparative morphology

ABSTRACT

The objective of this study focused on the comparative morphological and histochemical analysis of the duodenum of fetuses, juveniles and adult coypu (*Myocastor coypus bonariensis*), the major socioeconomic wildlife resource of Argentina. Histological and histochemical procedures for in situ characterization of glycoconjugates (GCs) were used. This study evidenced that fetal mucins differ histochemically in many respects from their adult counterparts. Only in fetuses from 90 days-post coitus (dpc) glycogen-rich sites were observed throughout the duodenal epithelium. The goblet cells appeared from 105 dpc and their secretory content varied considerably after birth. Duodenal glands presented scanty neutral and sulphated GCs in the 30-day juveniles; in adults the proportion of these GCs increased, and carboxylated and sialylated GCs were also observed. The results obtained in this work may be used in future studies to evaluate the effects of diet and intestinal pathologies in the glycosylation pattern of GCs. Also, knowledge of the normal glycoprofile of the duodenum of *M. coypus bonariensis* during its ontogenetic development may constitute a basis for the study of this organ in other Hystricognathi rodents.

1. Introduction

The ontogenetic development of the mammalian intestine is characterized by an early phase of morphogenesis and cell proliferation, an intermediate stage of cell differentiation and a subsequent period of physiological maturation. As a result of these processes, the intestinal epithelium is formed by highly specialized cells that carry out digestive, absorptive, secretory, endocrine and immunological functions (Pácha, 2000; Crosnier et al., 2006).

Among the matured cells of the intestinal epithelium the enterocytes, the Paneth cells, the M cells, the enteroendocrine cells and the goblet cells stand out; the latter being principally dedicated to the function of secreting mucus. The secreted mucosubstances are mainly composed of water, high molecular weight glycoproteins (mucins) and other minor components such as electrolytes. Mucins are involved in several important functions including lubrication and protection of the mucosa, and their physicochemical properties are highly determined by the presence of a large number of O-linked and N-linked glycans (Liquori et al., 2012).

The duodenal mucosa, being the first portion of the small intestine, is continuously exposed to mechanical abrasion from the ingested food. At the same time, the release of hydrochloric acid and bile acids create a hostile environment for the underlining epithelium (Ermund et al., 2013). Unlike the rest of the intestinal tract, the duodenum responds to this threat by releasing mucins into the lumen not only from goblet cells but also from the duodenal glands. Thus, the mammalian proximal duodenum of higher species shows a complex cellular architecture evidenced by distinct secreting cell types (Collaco et al., 2013).

So far, researches on the distribution and synthesis of glycoconjugates (GCs) during the intestinal ontogenetic development are scarce. According to Deplancke and Gaskins (2001), the distribution of goblet cells and the glycosylation pattern of mucus vary spatially and temporally all along the gastrointestinal tract of several mammals. Therefore, it is expected that the glycosylation patterns will

* Corresponding author.
E-mail address: adiaz@mdp.edu.ar (A.O. Díaz).
[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.tice.2015.12.002
0040-8166/

vary during pre and postnatal intestine development in response to morphological changes and different physiological requirements.

The coypu, *Myocastor coypus* (Molina 1782), is a Hystricognathi rodent distributed in all continents except Antarctica and Australia. Currently, the subspecies *M. coypus bonariensis* is the major socio-economic wildlife resource of Argentina, so it has suggested its inclusion in international economic development programs, and in basic and applied sciences (National Research Council, 2000). Moreover, given what is known about breeding in captivity of *M. coypus bonariensis*, this species has been proposed as a new model for morphological studies of development (Felipe and Masson, 2008).

Up to the present time, few studies are available on the developmental biology of *M. coypus bonariensis* (Felipe et al., 1999, 2002; Felipe and Masson, 2008). Among such researches, those related to the ontogenetic development of the digestive system represent an important landmark because of their possible transference to experimental essays on diet. Therefore, it is necessary to conduct researches that contribute to widen the knowledge of matters related to the ontogenetic development of the digestive system of *M. coypus bonariensis*.

The general objective of this study focused on the comparative morphological analysis and the histochemical characterization of the duodenum of *M. coypus bonariensis*, during its prenatal and postnatal development. For these purposes, histological techniques to describe morphological changes during the fetal and postnatal periods were used. Moreover, histochemical methods were performed to evaluate the distribution variation of GCs and its possible physiological role.

2. Materials and methods

2.1. Study animals

The mating program used in the present study was performed according to Felipe and Masson (2008). Briefly, fifteen virgin and sexually mature females of *M. coypus bonariensis*, mean weight 3.8 ± 0.4 kg and a mean age of 6.5 ± 0.7 months, and three males, mean weight 4.5 ± 0.6 kg and 7 months old, were used in this study. Females were allotted in roofed pens with walls built with cement boards and wire fence and cement floor with a 3% slope. Males were allotted in pens adjacent to those of the females. Animals were fed with 300 g/day of balanced food and water ad libitum. The mating program implied a daily colpocytological examination, by using standardized routine techniques (Felipe et al., 2001a). Samples were stained with Harris' hematoxylin and Shorr stain. Observations of vaginal smears were immediately performed.

The controlled mating method was applied; hence, once the oestrus was detected by colpocytology, the female was immediately moved to a male's pen. Copulation was determined by direct observation, and colpocytological sampling one hour after mating (Felipe et al., 2001b). Once the mating was verified, the mating time was recorded as time 0 in order to express in hour units the age of the collected specimens in days post-coitus (dpc).

Gestating females were sacrificed according to the methods established by the Animal Welfare Act (2012) Faculty of Veterinary Science of the Universidad Nacional del Centro de la Provincia de Buenos Aires. The technique to collect the entire genital tracts was a laparatomy by the middle line of the abdomen. The reproductive apparatus was laid on a tray ad hoc with a saline solution at 37 °C. Fetuses were removed in a predetermined order, from the utero-tubaric joint to the cervical end of each hemiuteri. With the procedures described above three fetuses of each etarian group were obtained: 75, 90, 105, 120 and 135 dpc. For the post-natal analysis samples of three newborns 30-days age and three adults 6-months age were used.

2.2. Sampling and histological analysis

The necropsy was carried out immediately after sacrifice by taking samples of duodenum. Samples were fixed in 10% neutral-buffered formalin, dehydrated in a graded series of ethanol, and then embedded in paraffin wax. Four micrometer-thick histological sections were cut by microtome and then stained using the routine techniques hematoxylin and eosin (H–E), Masson trichrome stain for morphology and Shikata's orcein for identifying elastic fibers and sulphated mucins.

Micrographs were taken with an Olympus microscope, CH 30 (Olympus, Tokyo, Japan).

2.3. Histochemical analysis

Sections of tissue were also subjected to histochemical procedures for GCs identification. Sections were stained with: (1) PAS (periodic acid–Schiff's reagent) to demonstrate periodate-reactive vicinal diols (McManus, 1948); (2) α-amylase digestion before PAS reaction for a control of glycogen presence (Pearse, 1985); (3) PA*S (selective periodic acid Schiff reaction): used as a specific reagent for the selective visualization of sialic acids in the PAS procedure (Volz et al., 1987); (4) PAPS (periodic acid oxidation-phenylhydrazine-Schiff). This method demonstrates the presence of GCs with sialic acids without O-acyl substitution and with O-acyl substitution at C7 (Reid and Park, 1990); (5) KOH/PA*S (saponification-selective periodic acid-Schiff reaction) to allow the characterization of total sialic acid (Culling et al., 1976); (6) PA/Bh/KOH/PAS (periodic acid-borohydride reduction-saponification-periodic acid Schiff reaction): to reveal sialic acid with O-acyl substitutions at C7, C8, or C9 (Reid et al., 1973); (7) KOH/PA*/Bh/PAS (saponification-selective periodic acid-borohydride reduction-periodic acid Schiff reaction) for neutral sugar characterization (Volz et al., 1987); (8) AB pH 2.5 (Alcian Blue 8GX pH 2.5) to demonstrate GCs with carboxyl groups and/or with O-sulfate esters (Bancroft and Gamble, 2008); (9) AB pH 1.0 (Alcian Blue 8GX pH 1.0) to demonstrate GCs with O-sulfate esters (Bancroft and Gamble, 2008); (10) AB pH 0.5 (Alcian Blue 8GX pH 0.5) to demonstrate highly sulphated GCs (Bancroft and Gamble, 2008); (11) AB pH 2.5/PAS (Alcian Blue 8GX pH 2.5/periodic acid-Schiff staining) for investigating the differentiation of acidic and neutral mucins (Mowry, 1963).

Evaluation of labeling intensities was assessed by two independent observers through the examination of two sections per sample of all the animals tested and was classified as follows: no labeling (0), weak labeling (1), moderate labeling (2), strong labeling (3).

3. Results

3.1. Morphological analysis

The duodenum of 75 and 90 dpc fetuses exhibited an undifferentiated simple cylindrical epithelium (Fig. 1A–C), while the mucosa of those greater than or equal to 105 dpc was covered by a simple cylindrical epithelium where goblet cells were identified (Fig. 1D, g, h). In 75 and 90 dpc fetuses the lamina propria was highly vascularized, with incipient crypts (Fig. 1C). Starting from 105 dpc, the duodenum presented well-developed intestinal glands (Fig. 1E). Only in adults, Paneth cells were identified in the base of the intestinal glands showing acidophilus apical granules (Fig. 2c). The lamina muscularis mucosae was not observed in 75 and 90 dpc fetuses; it became very thin and discontinuous from 105 dpc, and was more evident in adults (Fig. 2C). At 120 dpc, the tela submucosa showed isolated or paired duodenal glands (Fig. 1G). Adults and

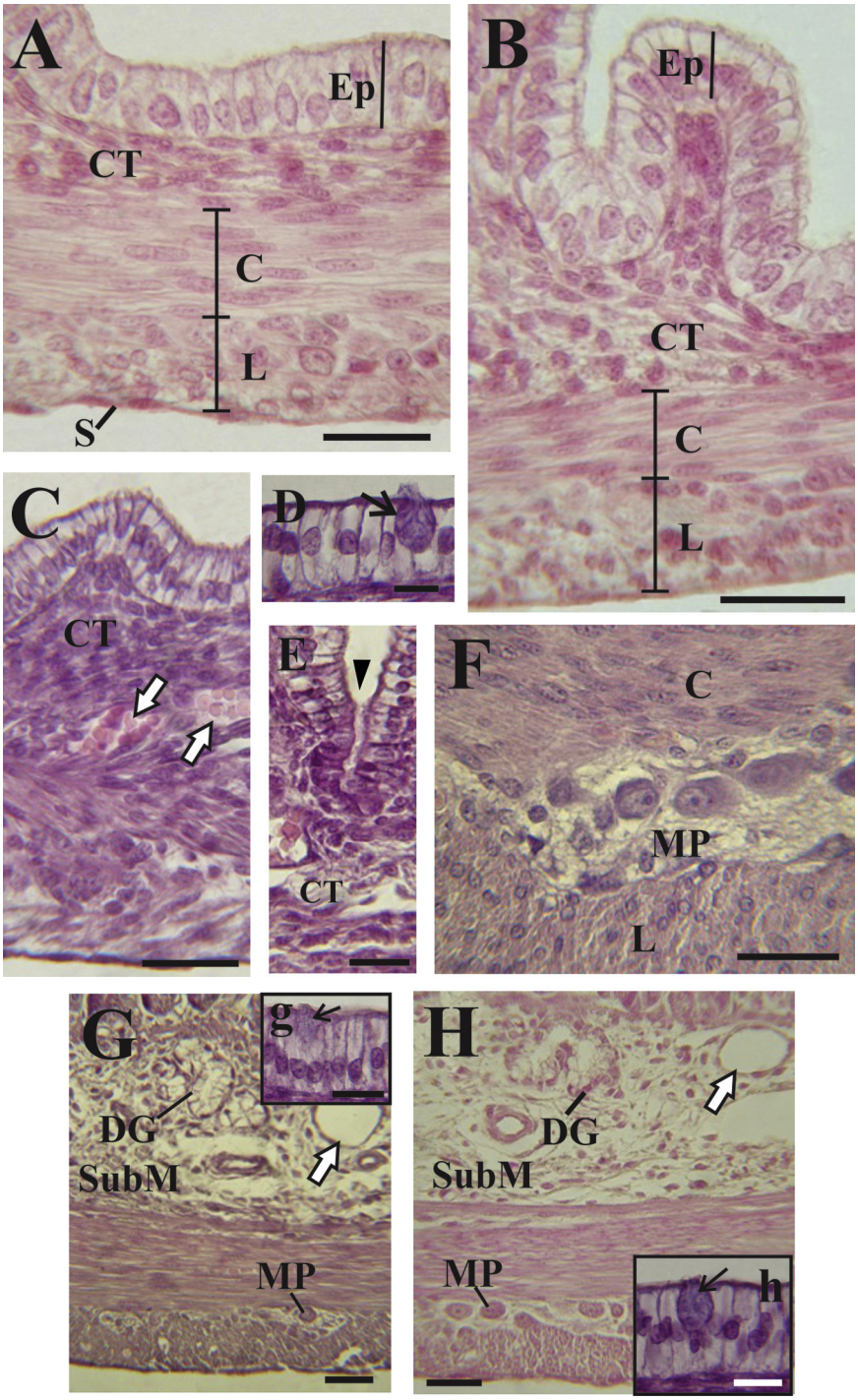

Fig. 1. Histological characteristics of the duodenum of *Myocastor coypus bonariensis* at different stages of its ontogenetic development. (A) Fetuses 75 dpc, H–E. (B, C) Fetuses 90 dpc, H–E. (D–F) Fetuses 105 dpc, H–E. (G, g) Fetuses 120 dpc, H–E. (H, h) Fetuses 135 dpc, H–E. Scale bars: 40 μm (A–C, E, G, H); 15 μm (D, g, h); 20 μm (F). C, circular layer of the tunica muscularis; CT, connective tissue; DG, duodenal glands; Ep, epithelial layer; L, longitudinal layer of the tunica muscularis; MP, myenteric plexus; S, tunica serosa; SubM, tela submucosa; →, goblet cell; ⇨, blood vessel; ►, intestinal gland.

juveniles had them located immediately below the lamina muscularis mucosae forming groups delimited by abundant collagenous and elastic fibers of unmodeled dense connective tissue (Fig. 2A, C–E). At 75 dpc, the tunica muscularis was formed by an inner circular layer and a developing outer longitudinal one (Fig. 1A). From 90 dpc, the tunica muscularis was composed of two layers of smooth muscle, among which the components of the Myenteric plexus were observed (Figs. 1F–H and 2F). The serosa was identified in all stages studied (Fig. 1A).

3.2. Histochemical characterization

The results of conventional histochemistry are summarized in Table 1.

3.3. Ninety and 105 dpc fetuses

The histochemical techniques revealed that the glucidic composition of surface and secretion GCs expressed in the duodenum was

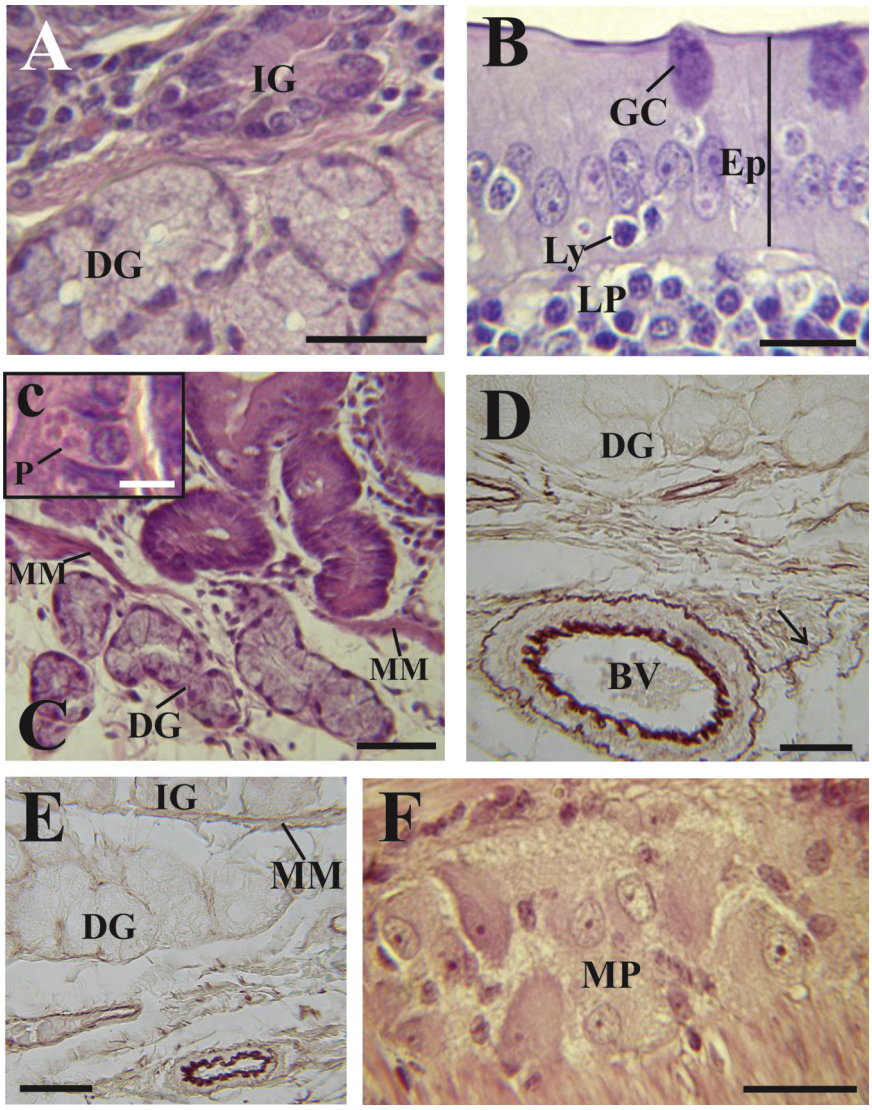

Fig. 2. Histological characterization of the duodenum of the coypu *Myocastor coypus bonariensis* during its postnatal development. (A) Juveniles 30 dpn, H–E. (B, C, c, F) Adults, H–E. (D, E) Adults, Shikata's orcein. Scale bars: 40 μm (A, C–E); 5 μm (B); 10 μm (c); 80 μm (F). BV, blood vessel; DG, duodenal glands; Ep, epithelial layer; GC, goblet cells; IG, intestinal gland; LP, lamina propria; Ly, lynphocyte; MM, lamina muscularis mucosae; MP, myenteric plexus; P, Paneth cell; SubM, tela submucosa; →, elastic fibers.

Table 1
Histochemical reactions of glycoconjugates in the duodenum of *Myocastor coypus bonariensis* during its ontogenetic development.

Procedures	Glycocalyx							Goblet cells					Brunner's glands			
	75	90	105	120	135	30	A	105	120	135	30	A	120	135	30	A
PAS	0	0	0	0	0	0	3	0	0	0	1	3	0	0	0	2
PA*S	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	2
PAPS	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2
KOH/PA*S	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	1
PA/Bh/KOH/PAS	0	0	0	1	1	1	2	1	1	1	2	3	0	0	0	2
KOH/PA*/Bh/PAS	0	0	0	1	1	1	3	1	1	1	2	3	0	0	1	3
AB pH 2.5	0	1	1	1	1	2	3	2	2	2	3	3	0	0	2	3
AB pH 1.0	0	1	1	1	1	2	3	1	2	2	2	3	0	0	1	2
AB pH 0.5	0	0	0	1	1	1	2	1	1	1	2	2	0	0	1	2
AB pH 2.5/PAS	0	1T	1T	1T	1T	2T	3P	2T	2T	2T	3T	3P-3M[a]	0	0	2T	2P

[a] Two goblet cell types were differentiated.
M, magenta; P, purple; T, turquoise. Staining intensity: 0, negative; 1, slightly positive; 2, moderate; 3, strong.

similar in both fetal stages. Only 90 dpc fetuses presented abundant PAS positive granules in the epithelial cells (Fig. 3A). After treatment with α-amylase, control sections were negative to the PAS reaction thus confirming the presence of large amounts of glycogen. Glycogen-rich sites were predominantly found in the enterocytes lining the villi.

At both fetal stages, the glycocalyx showed a slight positive reaction only with AB pH 2.5 and AB pH 1.0 techniques, demonstrating therefore the presence of scarce sulphated and carboxylated GCs.

At 105 dpc the goblet cells reacted positively with AB pH 2.5, AB pH 1.0 and AB pH 0.5, thus allowing the identification of GCs with sulphated and carboxylated groups (Fig. 3B). More specific

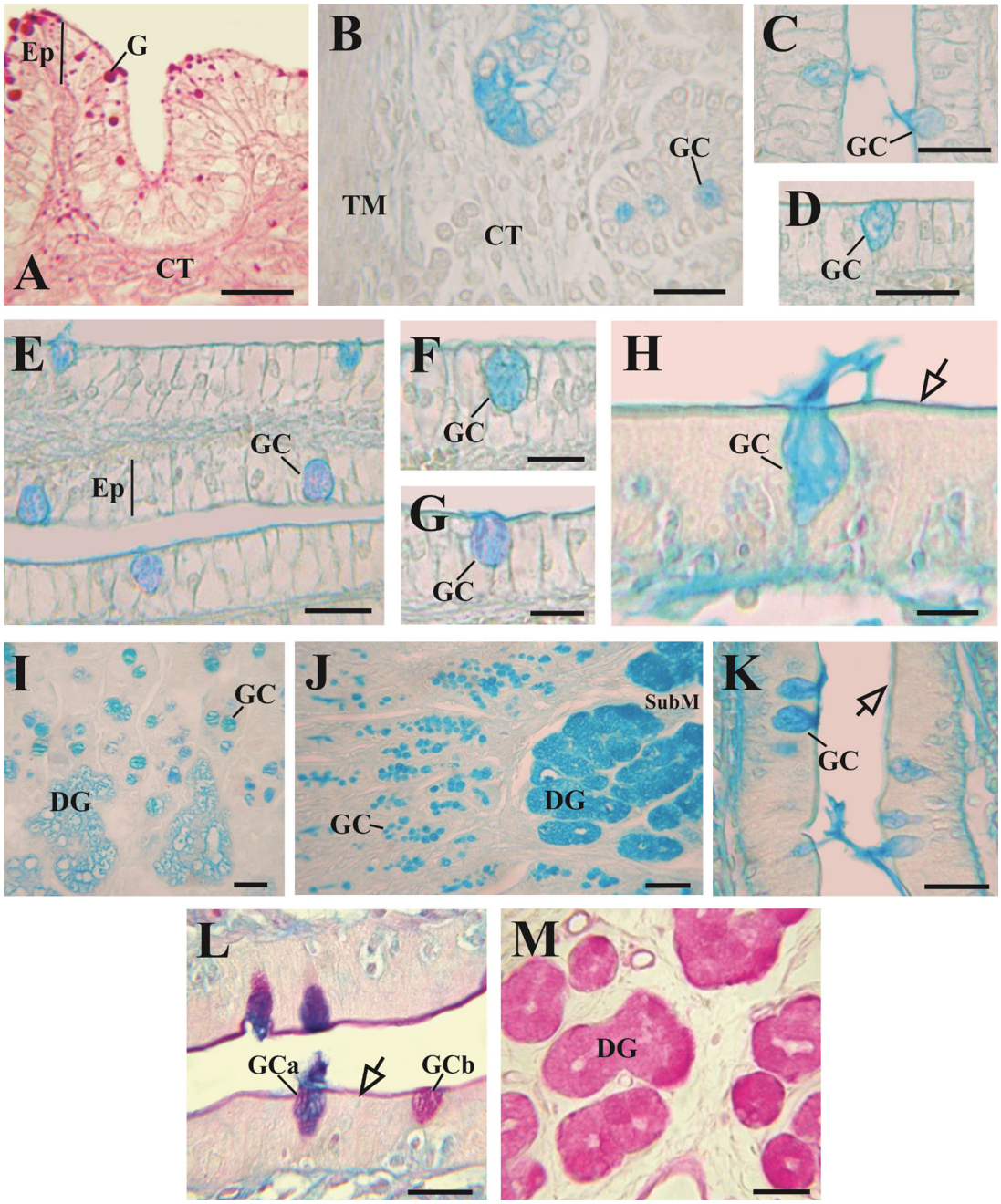

Fig. 3. Histological characteristics of the duodenum of *Myocastor coypus bonariensis* at different stages of its ontogenetic development. (A) Fetuses 90 dpc, PAS. (B) Fetuses 105 dpc, AB pH 2.5/PAS. (C) Fetuses 120 dpc, AB pH 2.5. (D) Fetuses 120 dpc, AB pH 1.0. (E) Fetuses 135 dpc, AB pH 2.5. (F) Fetuses 135 dpc, AB pH 1.0. (G) Fetuses 135 dpc, AB pH 2.5/PAS. (H) Juveniles 30 dpn, AB pH 2.5. (I) Juveniles 30 dpn, AB pH 2.5/PAS. (J) Adults, AB pH 2.5. (K) Adults, AB pH 1.0. (L) Adults, AB pH 2.5/PAS. (M) Adults, PA*S. Scale bars: 30 μm (A, C, D, E); 60 μm (B, J); 20 μm (F, G); 5 μm (H); 40 μm (I, M); 10 μm (K, L). CT, connective tissue; DG, duodenal glands; Ep, epithelial layer; G, glycogen granules; GC, goblet cells; GCa, goblet cells AB/PAS positive; GCb, goblet cells PAS positive; TM, tunica muscularis; SubM, tela submucosa; ⇾, glycocalyx.

techniques, like KOH/PA*/Bh/PAS and PA/Bh/KOH/PAS also showed a slightly positive labeling.

3.4. One hundred and twenty and 135 dpc fetuses

The duodenum of 120 and 135 dpc fetuses presented similar histochemical profiles. As in the former fetal stages, the glycocalyx evidenced scarce carboxylated and sulphated GCs (Fig. 3C and F). Moreover, a slight positive reaction with KOH/PA*/Bh/PAS and PA/Bh/KOH/PAS techniques was evidenced.

The goblet cells exhibited the same glycosylation pattern as the glycocalyx, although they presented a more intense reaction than with AB pH 2.5 and AB pH 1.0 techniques (Fig. 3C–G).

The duodenal glands reacted with none of the employed techniques.

3.5. Thirty dpn juveniles and adults

At both stages the glycocalyx exhibited carboxylated, sulphated and very sulphated GCs, neutral sugars and GCs with sialic acids

with O-acyl substitutions at C7, C8 and/or C9 (Fig. 3H, K). Only in the adult stage, the PAS technique strongly labeled the glycocalyx (Fig. 3L).

The goblet cells presented similar histochemical profiles in both etarian groups, although the reacting intensity was always higher in the adult than in the juvenile stage. These unicellular glands exhibited GCs with oxidizable vicinal diols, carboxylated, sulphated and very sulphated GCs, neutral sugars and GCs with or without O-acyl substitutions at C8 and/or C9 (Fig. 3H–K). Furthermore, the sequence AB pH 2.5/PAS allowed the identification of goblet cells with two different histochemical patterns only in the duodenum of adults. Goblet cells PAS positive as well as a higher proportion of AB/PAS positive were observed (Fig. 3L).

The duodenal glands presented a positive labeling with KOH/PA*/Bh/PAS, AB pH 2.5, 1.0 and 0.5 techniques, always having the adult a more intense histochemical reaction. The histochemical profile of these glands demonstrated the presence of neutral sugars, carboxylated, sulphated and very sulphated GCs in both etarian groups. In adults, duodenal glands reacted moderately and strongly to PAS, PA*S, PAPS, KOH/PA*S and PA/Bh/KOH/PAS techniques, which evidences that these glands secrete GCs with oxidizable vicinal diols and sialic acids with or without O-acyl substitutions at C7, C8 and/or C9 only at the adult stage (Fig. 3M).

4. Discussion

Several studies on rodents have demonstrated the existence of morphological changes in the gastrointestinal tract in response to food quality, dietary habits, energy requirements and ontogenetic development (Mastrodonato et al., 2014; Tano de la Hoz et al., 2014). Up to now, few are the researches on the ontogenetic development of the digestive system of Hystricognathi rodents. In the case of the viscacha (*Lagostomus maximus*), Tano de la Hoz et al. (2014) have reported the histological and histochemical changes in the duodenum during the pre and postnatal development. As observed in *L. maximus*, the duodenum of *M. coypus bonariensis* presented a more advanced pre-natal histogenesis than other rodents, showing a complete differentiation of tissue layers and adenogenesis as from 75 dpc. In *M. coypus bonariensis*, the crypts themselves developed early, beginning around prenatal day 105. In contrast, descriptive studies on the fetal development have demonstrated that in most rodents the crypts appear later, only after birth (Crosnier et al., 2006). Unlike rodents, early crypt development is characteristic of mammals with a long gestation period such as humans and sheep (Pácha, 2000). However, compared to other animals of the order Rodentia the hystricognathis show a longer gestation period. Likewise, in the suborder Hystricognathi, *M. coypus bonariensis* presents a mean gestation period of 132 days and has precocial offspring (Felipe and Masson, 2008).

Several mammal studies have indicated that the mucin glycosylation varies according to animal species, anatomical region, position along crypt-villus axis, pathological conditions, and dietary habits (Hedemann et al., 2007). However, little attention has been paid to the age-dependent changes in mucin secretion and composition. Likewise, there are few studies regarding the distribution of glycoconjugates in the intestine of mammalian fetuses (Felipe et al., 1999; Beyaz and Liman, 2009; Tano de la Hoz et al., 2014). Therefore, we aimed to analyze the carbohydrates composition in the duodenum of *M. coypus bonariensis* in different pre and postnatal stages. In the present study a temporal variation of the mucin subtypes during the ontogenetic development of the duodenum of *M. coypus bonariensis* was observed. Although duodenal glycoconjugate secretion was higher during postnatal development, mucins were already present in early fetal stages. In *M. coypus bonariensis*, carboxylated GCs predominated throughout fetal life. Mucin sulphation and carboxylation started in the glycocalix of the duodenal epithelium as early as 90 days of gestation, whereas highly sulphated GCs were evident after 105 days, together with the appearance of goblet cells. In a recent study, Beyaz and Liman (2009) reported similar finding in bovine fetuses. Previous studies suggested that the existence of acidic glycoproteins in the fetal and neonatal intestine might be important as a protective barrier since the acquired immune system is not completely functional in early developmental stages (Deplancke and Gaskins, 2001; Beyaz and Liman, 2009); this is consistent with researches indicating that acidic mucins hamper bacterial translocation (Robertson and Wright, 1997; Conour et al., 2002). Yet other studies suggested that the presence of highly sulphated mucins may be related with secretory products of immature goblet cells (Pácha, 2000). In the present study, traditional histochemical methods also demonstrated that the amount of glycogen present in the duodenal epithelium varies considerably during the ontogeny of *M. coypus bonariensis*. Abundant glycogen was found in the epithelia of 90 dpc fetuses and it gradually declined thereafter, having been scanty in 105 dpc fetuses and nonexistent in adults. Similar results were described by Beyaz and Liman (2009) and Tano de la Hoz et al. (2014) who observed an increase in glycogen accumulation during early fetal development in mammals and a gradual diminution toward birth. Previous studies have described a possible storing role of glycogen in cellular metabolism, presumably to provide energy reserves for use during early development and after birth (Beyaz and Liman, 2009). Moreover, it has been postulated that the glycogen stores could be used in more advanced stages of ontogeny for the synthesis of glycoconjugates (Tano de la Hoz et al., 2014).

The present study demonstrated that the glycosylation pattern of goblet cells and duodenal glands in the *M. coypus bonariensis* duodenum rapidly changes after birth. The duodenum of the adults showed the most complex glycosylation pattern, with goblet cells having two different histochemical profiles. One of the cell types was found in greater proportion and presented a mixed secretion of acid and neutral GCs, while the other was characterized by acidic GCs alone, like what had been observed in *L. maximus* (Tano de la Hoz et al., 2014). Therefore, it can be hypothesized that the glycosylation profiles found in the goblet cells of the duodenum of *M. coypus bonariensis* might be due to the presence of goblet cells in different secretory stages or to the existence of two cell subpopulations that produce and secrete different GCs. These findings correlate with the study of Boonzaier et al. (2013), which showed that mixed (acid and neutral) GCs were the predominant type of mucin in the large intestine of some mammalian species.

In addition, the PAS technique demonstrated a progressive enhancement of neutral components in goblet cells during the duodenal development of *M. coypus bonariensis*. In agreement with this, Deplancke and Gaskins (2001) have shown that the relation of neutral to acidic mucins usually increases after birth. Several studies have shown that the glycosylation pattern of goblet cells varies along the vertical crypt-villus axis (Galotta et al., 2009); however, in contrast to our study, no histochemical differences were observed between crypts and intestinal villi goblet cells.

The duodenal glands have been studied in different species; noticeable differences both in their distribution and in the secreted types of GCs have been determined (Krause, 2000; Verdiglione et al., 2002). Like in most mammals, the distribution of duodenal glands in *M. coypus bonariensis* was just limited to the tela submucosa (Verdiglione et al., 2002). However, in juveniles and adults, these glands were not located all along the thickness of the submucosa, but immediately below the lamina muscularis mucosae forming groups delimited by abundant collagenous and elastic fibers.

Some studies carried out in mammalian species have reported that duodenal glands principally produce neutral mucin, while acidic mucosubstances represent a smaller percentage of the total

glandular secretion (Krause, 2000). However, according to many studies the glycosylation pattern of these glands varies significantly among species (Ergün et al., 2010). In the present study, the histochemical techniques showed that the duodenal glands of *M. coypus bonariensis* produced both neutral and acidic (carboxylated and sulphated) GCs. The duodenal glands were characterized by an unusual glycopattern in some mammals (Verdiglione et al., 2002; Tano de la Hoz et al., 2014). In bovines, the neutral GCs were secreted by the whole gland, while the acidic mucins were produced only by the peripheral adenomeres of the glands (Verdiglione et al., 2002). A similar histochemical pattern was described for *L. maximus* (Tano de la Hoz et al., 2014); they demonstrated that the duodenal glands from the submucosa deep area showed a histochemical profile different from that of the glands from the superficial area and a larger amount of acid GCs. Nevertheless, in the present study it was observed that the duodenal glands of adults showed similar histochemical profiles, regardless of their location in the tela submucosa. The results of these studies suggest that the glycosylation pattern of the duodenal glands varies even between closely related species.

In conclusion, our data indicate that in *M. coypus bonariensis* the fetal mucins differ histochemically in many respects from their adult counterparts. In relation to the potential role of mucins, the high histochemical complexity observed in the duodenum of the adult *M. coypus bonariensis* was probably related to the multiple functions carried out by the mucus of the digestive tract, such as protection, lubrication and transport between the luminal content and the epithelial lining (Ergün et al., 2010). Further, the presence of acidic mucins in the duodenum of *M. coypus bonariensis* fetuses could be principally involved in the protection of the intestinal mucosa against potential pathogens (Pácha, 2000; Beyaz and Liman, 2009). The results obtained in this work may be used in future studies to evaluate the effects of diet and intestinal pathologies in the glycosylation pattern of GCs. Also, knowledge of the normal glycoprofile of the duodenum of *M. coypus bonariensis* during its ontogenetic development may constitute a basis for the study of this organ in other Hystricognathi rodents.

Acknowledgements

This research was partly supported by grants from Universidad Nacional de Mar del Plata (UNMdP, Argentina) and Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA, Argentina), Centro de Investigaciones Biológicas (CIB).

References

Animal Welfare Act, 2012. Facultad de Cs. Veterinarias, UNCPBA, Retrieved 12 June 2005, from: http://www.vet.unicen.edu.ar/html/facultad/Bienestar%20Animal.html.

Bancroft, J.D., Gamble, M., 2008. Theory and Practice of Histological Techniques. Churchill Livingstone Elsevier, Philadelphia, PA.

Beyaz, F., Liman, N., 2009. The prenatal development and histochemistry of the ileal mucins in the bovine fetuses. Anat. Histol. Embryol. 38, 436–442.

Boonzaier, J., Van der Merwe, E.L., Bennett, N.C., Kotze, S.H., 2013. Comparative gastrointestinal morphology of three small mammalian insectivores: *Acomys spinosissimus* (Rodentia), *Crocidura cyanea* (Eulipotyphla), and *Amblysomus hottentotus* (Afrosoricida). J. Morphol. 274, 615–626.

Collaco, A.M., Jakab, R.L., Hoekstra, N.E., Mitchell, K.A., Brooks, A., Ameen, N.A., 2013. Regulated traffic of anion transporters in mammalian Brunner's glands: a role for water and fluid transport. Am. J. Physiol. Gastrointest. Liver Physiol. 305 (3), 258–275.

Conour, J.E., Ganessunker, D., Tappenden, K.A., Donovan, S.M., Gaskins, H.R., 2002. Acidomucin goblet cell expansion induced by parenteral nutrition in the small intestine of piglets. Am. J. Physiol. Gastrointest. Liver Physiol. 283, 1185–1196.

Culling, C.F.A., Reid, P.E., Dunn, W.L., 1976. A new histochemical method for the identification and visualization of both side-chain acylated and non-acylated sialic acids. J. Histochem. Cytochem. 24, 1225–1230.

Crosnier, C., Stamataki, D., Lewis, J., 2006. Organizing cell renewal in the intestine: stem cells, signals and combinatorial control. Nat. Rev. Genet. 7, 349–359.

Deplancke, B., Gaskins, H.R., 2001. Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. Am. J. Clin. Nutr. 73, 1131–1141.

Ergün, E., Ergün, L., Özen, A., Kürüm, A., Bayraktaroglu, A.G., 2010. Histomorphology of the Brunner's glands in the Angora rabbit. J. Anim. Vet. Adv. 9, 887–891.

Ermund, A., Schütte, A., Johansson, M.E.V., Gustafsson, J.K., Hansson, G.C., 2013. Studies of mucus in mouse stomach, small intestine, and colon I. Gastrointestinal mucus layers have different properties depending on location as well as over the Peyer's patches. Am. J. Physiol. Gastrointest. Liver. Physiol. 305, 341–347.

Felipe, A., Cabodevila, J., Callejas, S., 1999. Anatomicohistological characteristics of female genital tubular organs of the South American nutria (*Myocastor coypus*). Anat. Histol. Embryol. 27, 245–250.

Felipe, A., Cabodevila, J., Callejas, S., 2001a. Characterization of the estrous cycle of the *Myocastor coypus* (coypu) by means of exfoliative colpocytology. J. Neotrop. Mastozool. 8, 129–137.

Felipe, A., Teruel, J., Cabodevila, J., Callejas, S., 2001b. Controlled mating in *Myocastor coypus* (coypu). Scientifur 25, 99–104.

Felipe, A., Teruel, M., Cabodevila, J., Callejas, S., 2002. Pre-implantational timetable of embryonal development of *Myocastor coypus* (coypu). Reprod. Nutr. Dev. 42, 15–24.

Felipe, A.E., Masson, P.G., 2008. Observations on the fetal morphology in *Myocastor coypus bonariensis* (coypu) (Rodentia, Myocastoridae). Anat. Histol. Embryol. 37, 469–474.

Galotta, J.M., Márquez, S.G., Zanuzzi, C.N., Gimeno, E.J., Portiansky, E.L., Barbeito, C.G., 2009. Lectin binding pattern of intestinal goblet cells in horse, pig and rabbit. Anim. Biol. 1, 49–58.

Hedemann, M.S., Højsgaard, S., Jensen, B.B., 2007. Lectin histochemical characterization of the porcine small intestine around weaning. Res. Vet. Sci. 82, 257–262.

Krause, W.J., 2000. Brunner's glands: a structural, histochemical and pathological profile. Prog. Histochem. Cytochem. 35, 255–367.

Liquori, G.E., Mastrodonato, M., Mentino, D., Scillitani, G., Desantis, S., Portincasa, P., Ferri, D., 2012. *In situ* characterization of O-linked glycans of Muc2 in mouse colon. Acta Histochem. 114, 723–732.

Mastrodonato, M., Mentino, D., Portincasa, P., Calamita, G., Liquori, G.E., Ferri, D., 2014. High-fat diet alters the oligosaccharide chains of colon mucins in mice. Histochem. Cell. Biol. 142, 449–459.

McManus, J.F.A., 1948. Histological and histochemical uses of periodic acid. Stain Technol. 23, 99–108.

Mowry, R.W., 1963. The special value of methods that color both acidic and vicinal hydroxyl groups in the histochemical study of mucins with revised directions for the colloidal iron stain, the use of Alcian blue 8GX, and their combination with the periodic acid-Schiff reaction. Ann. N. Y. Acad. Sci. 106, 402–423.

National Research Council (USA), 2000. Coypu. In: Vietmeyer, N. (Ed.), Microlivestock: Little-Known Small Animals with a Promising Economic Future. Office of International Affairs (OIA), National Academy Press, Washington, DC, pp. 217–224.

Pácha, J., 2000. Development of intestinal transport function in mammals. Physiol. Rev. 80, 1633–1667.

Pearse, A.G.E., 1985. Histochemistry: Theoretical and Applied, vol. 2. Churchill Livingstone, Edinburgh.

Reid, P.E., Culling, C.F.A., Dunn, W.L., 1973. Saponification-induced increase in the periodic acid-Schiff reaction in the gastrointestinal tract. Mechanism and distribution of the reactive substance. J. Histochem. Cytochem. 21, 473–483.

Reid, P.E., Park, C.M., 1990. Carbohydrate histochemistry of epithelial glycoproteins. Prog. Histochem. Cytochem. 21, 1–170.

Robertson, A.M., Wright, D.P., 1997. Bacterial glycosulphatases and sulphomucin degradation. Can. J. Gastroenterol. 11, 361–366.

Tano de la Hoz, M.F., Flamini, M.A., Díaz, A.O., 2014. Histological and histochemical study of the duodenum of the plains viscacha (*Lagostomus maximus*) at different stages of its ontogenetic development. Acta Zool. 95, 21–35.

Verdiglione, R., Mammola, C.L., Filotto, U., 2002. Glycoconjugate histochemistry of bovine Brunner glands. Ann. Anat. 184, 61–69.

Volz, D., Reid, P.E., Park, C.M., Owen, D.A., Dunn, W.L., 1987. A new histochemical method for the selective periodate oxidation of total tissue sialic acids. Histochem. J. 19, 311–318.