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A morphological, morphometric and histochemical study of the oviduct in pregnant and non-pregnant females of the plains viscacha (*Lagostomus maximus*)

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Abstract

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The oviduct is a very thin organ with a very tortuous appearance. It is divided into three segments: the infundibulum, the ampulla and the isthmus. Particularly, the oviduct of the viscacha lacks the intramural portion described in other species. The mucosa shows longitudinal pleats. The free edge of the infundibulum ends as small fimbriae that are of variable length and do not completely cover the ovary. The proportion of ciliated and secretory epithelial cells varied both in relation to the segments of the oviduct analysed and to the physiological state (anoestrus, follicular phase, early pregnancy and late pregnancy). The glycocalix and the apical region of the superficial epithelial cells are PAS and alcian-blue positive. The muscular layers vary in thickness in different regions. Some lectins such as UEA-1 and DBA showed variations in the binding pattern during the different physiological stages analysed whereas RCA-1and WGA had a very stable pattern.

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Introduction

The mammalian oviduct is a tubular and flexuous organ necessary for the reception and transport of sperm, sperm capacitation, fertilisation of the oocyte and early embryo development (Yaníz *et al.* 2000; Welsch 2009). It is divided into different segments: the infundibulum, ampulla, isthmus and intramural portion. Each of these segments has structural adaptations to perform specific functions (Abe 1996; Banks 1996). It is lined by secretory cells and non-secretory ciliated cells wherein proportion varies throughout the sexual cycle depending on the concentration of steroid hormones (Anzaldúa *et al.* 2002; Desantis *et al.* 2011). The main function of ciliated cells is to transport gametes (Ferreira de Sant'Ana 2001). Secretions, especially glycoproteins produced by non-ciliated cells, play an important role during fertilisation and first days of development, as well as in sperm changes that occur before fertilisation (Buhi 2002).

The plains viscacha (*Lagostomus maximus*: Rodentia, *histrichognati*, Chinchillidae) (Woods 1998) is a natural inhabitant of different regions of Argentina. Some basic characteristics of the female reproductive organs and cycle were described decades ago by Weir (Weir 1971a). Among them, the vagina of this species has a medial longitudinal raphe, which is absent in other hystricomorph rodents. This structure extends some centimetres from the uterine neck and cranially divides the vagina into two cavities. The division of the cervix and vagina can be considered as an ancestral condition similar to what is observed in marsupials (Tyndale-Biscoe and Rodger 1978). From these pioneering studies it was shown that *L. maximus* had a long gestation period after which one to three pups were born. As the viscacha naturally polyovulates 200 to 800 oocytes, only 10 of them are implanted (Weir 1971b, 1974; Flamini *et al.* 2009).

The reproductive cycle of the viscacha varies during the year depending on the geographical region where animals are

living (Flamini *et al.* 2009). On the other hand, there have been several studies related to ovarian morphology and physiology showing that this organ is specifically adapted to polyovulation (Jensen *et al.* 2006, 2008; Gil *et al.* 2007; Flamini *et al.* 2009; Espinosa *et al.* 2011). The existence of a well-developed functional female prostate (Flamini *et al.* 2002) was also demonstrated.

Despite the reproductive particularities of *L. maximus* female and that several fundamental reproductive processes occur in the oviduct, there have been no detailed studies on the structural and histochemical characteristics of the organ in this species.

The goal of this study was to describe the oviduct of *L. maximus* histologically, morphometrically and histochemically at different periods of the reproductive cycle.

Materials and Methods

Animals and histochemical techniques

Thirty adult female viscachas weighing 4–5.5 kg were used. Animals were captured in the Estación de Cría de Animales Silvestres (ECAS), Ministry of Agricultural affairs, province of Buenos Aires, where they lived in the wild without control of reproduction. Captures were made with cage-traps, placed at noon and removed the next day morning.

Captures were carried out during March-April (probably at early pregnancy, oestrous or pro-oestrous), July–August (probably at late pregnancy and births) and December-January (probably non-pregnant anoestrous and late pregnancy), according to previous observations (Flamini *et al.* 2009). According to their ovarian morphological characteristics as previously described (Flamini *et al.* 2009) they were divided into four groups: (i) non-pregnant anoestrous females, (ii) females at follicular phase, (iii) females at early pregnancy, and (iv) females at late pregnancy.

Animals were anaesthetised with an i.m. single dose of ketamine hydrochloride (Ketanest, Laboratorio Scott Cassara) (50 mg/kg body weight) and then sacrificed by bleeding to white (Commision on life sciences national reserarch council 1996; Van Zutphen *et al.* 1999).

The uterine tubes were completely removed. To set their length they were dissected and stretched along their longitudinal axis. The oviduct was then fixed in 10% buffered formalin for 48 h. Subsequently, they were dehydrated, cleared and embedded in paraffin. Five micrometers thick slices were obtained. For conventional histological analysis, the following techniques were performed: haematoxylin–eosin, Masson trichrome, orcein and Gomori reticulin.

The Peryodic Acid Schiff (PAS) and the alcian blue at pH 2.5, 1 and 0.5 were applied as histochemical techniques. The reaction for these techniques was subjectively classified as (−) negative; (1) weak; (2) moderate and (3) strong. This classification was established according to previous reports (Freijo *et al.* 2009).

Lectin histochemistry was performed using a specific battery of seven biotinylated lectins (Table 1). Sections were mounted on slides coated with HistoGrip (Zimed Laboratories, San Francisco, CA, USA), dewaxed with xylene and immersed in absolute alcohol. Samples were then incubated with 0.3% hydrogen peroxide (H_2O_2) (100 volumes) in methanol for 30 min at room temperature to inhibit endogenous peroxidase activity. Samples were then rehydrated, washed in phosphate buffered saline (PBS) and incubated with 0.1% bovine serum albumin in humid chamber during 30 min to block any non-specific binding. Seven slices from each sample were then incubated with each of the seven listed biotinylated lectins during 2 h at 37 °C. Slides were washed three folds in PBS and then incubated with the avidin-biotin-peroxidase complex (ABC; Vector Laboratories, Burlingame, CA, USA). The horseradish peroxidase was left to react with the tissue during 4–10 min in a Tris-HCl (0.05 M, pH 7,6) buffer solution containing 0.02% diaminobenzidine (DAB - Biogenex, San Ramon, CA, USA) and 0.05% H_2O_2. Slices were then counterstained with Mayer haematoxylin. Time reaction depended on the lectin tested. The histochemical reaction of all slices incubated with the same lectin were stopped at the same time. Lectin reactivity was classified as (−) negative; (1) weak; (2) moderate and (3) strong. This criteria was established in previous reports (Freijo *et al.* 2009).

Image analysis

Images of all of the stained slides observed using 10× and 40× objectives were acquired using a digital colour video camera (Olympus DP71, Tokyo, Japan) attached to a microscope (Olympus BX50, Tokyo, Japan) and processed with a digital image analysis program (ImagePro Plus v6.3, Media Cybernetics, MA, USA). Morphometric analysis focused on the major and minor axes of epithelial cell regardless of cilia.

Table 1 Lectins used for the lectin histochemical analysis of the epithelium, showing their acronyms and affinities

Lectin	Acronym	Affinity
Group I		Glc/Man
Concanavalia ensiformis	Con A	β-D-Man; α-D-Glc
Group II		GlcNAc
Triticum vulgaris	WGA	β-D-GlcNAc; NeuNAc
Group III		GalNAc-/Gal
Dolichos biflorus	DBA	α-D-GalNAc
Glycine maximus	SBA	α-D-GalNAc; β-D-GalNAc
Ricinus communis	RCA-I	β-Gal
Arachis hypogaea	PNA	β-D-Gal (β1-3)> D-Gal Nac
Group IV		L-Fuc
Ulex europaeus	UEA-I	L-Fuc

Glc, glucose; Man, manose; Gal, galactose; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine; NeuNAc, N-acetylneuraminic acid (scialic acid); L-Fuc, L-fucose.

The recorded values obtained from 25 images of each slice were exported to a spreadsheet for statistical analysis.

Statistical analysis

Mean ± SEM were calculated from the morphometric data. ANOVA tests were used to establish differences between groups. The Bonferroni test was used as a posthoc test. Significant differences were considered those with a value of $P < 0.05$.

Results

Macroscopy

The oviduct of the viscacha was whitish, thin, tubular and very tortuous. It was very close to the ovary and joined it on almost all its length by a thin ligament, the mesosalpinx, except in the most cranial region of the ovary where they spread. The average length of the oviduct was 4.90 ± 0.17 cm. It presented many folds all along its length that gradually became thin from the fimbriated end to the end connected with the uterus.

Microscopically, three segments could be distinguished by their diameter: the infundibulum, the ampulla and the isthmus. In the cranial end of the ovary, the infundibulum opened through a hole: the *ostium abdominale*. The free edge of the infundibulum ended as small fimbriae that were of variable length and did not completely cover the ovary.

The infundibulum followed into the ampulla. This was a short portion, somewhat enlarged, of 0.23 ± 0.03 cm in diameter, showing 2–4 large folds. The ampulla was connected with the isthmus that was the narrowest and longest portion of the organ. Its diameter was 0.11 ± 0.08 cm (Fig. 1). It showed 8–12 short and close folds. The isthmus opens into the uterine horns through an orifice (uterine horn hole).

Microscopy

General structure. The wall of the oviduct had three tunicae: the mucosa, the muscular and the serous. The mucosa had a complex structure. It showed folds that branched to different degrees in all areas of the organ, resulting in primary, secondary and tertiary folds. The lumen of the organ was very irregular due to those folds.

The mucosa was formed by a simple columnar epithelium and chorion tissue. In the epithelium two types of cells with different characteristics could be found: ciliated and secretory cells. Both cell types were arranged in an alternate manner along the mucosa.

Ciliated cells had a cylindrical shape with rounded or oval nucleus. The nucleus was located in most cells toward the apical area. Its chromatin was widespread in fine granules. Morphometric studies showed that there were no differences neither in height nor in width of all ciliated cells in all the four physiological stages and for all the anatomical segments analysed. Height of these cells ranged from 13 to 21 μm whereas their width ranged from 6 to 10 μm.

Secreting cells were cylindrical, but unlike ciliated cells, the nucleus was located towards its basal area. In the apical portion of the cytoplasm small granules were observed, which were not stained with haematoxylin and eosin, but were positive to PAS and to alcian-blue at different pH (Table 2). Morphometric studies showed that there were no differences

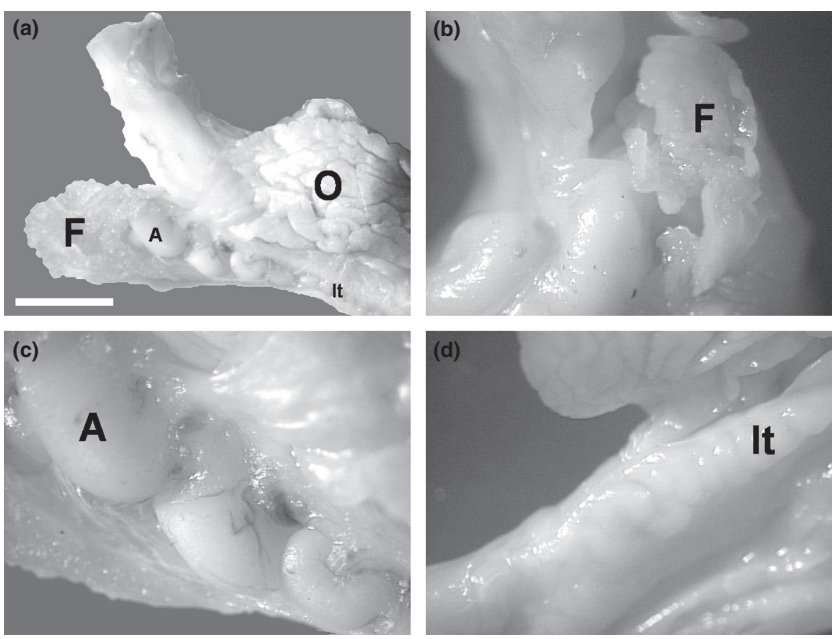

Fig. 1—Macroscopical view of the oviduct. (**a**) The oviduct and its relation with the ovary (O); (**b**) Detail of the fimbriae; (**c**) Detail of the ampulla; (**d**) Detail of the isthmus. Abbreviations: F = fimbriae; A = ampulla; It = Isthmus. Bar for **a** = 0.5 cm; Bar for (**b**–**d**) = 0.15 cm.

Table 2 Histochemical reaction of the secretory cells of the oviduct in the female viscacha. Reactivity to histochemical techniques: (3) strong, (2) moderate, (1) weak and (–) negative

	Sector		
Histochemical Technique	Infundibulum and fimbria	Ampulla	Isthmus
PAS	3	3	3
Alcian Blue pH 2.5	3	3	3
Alcian Blue pH 1.0	3	3	3
Alcian Blue pH 0.5	2	2	2

neither in height nor in width of all secreting cells except for their height in the ampulla where those observed at late pregnancy were significantly higher (18 μm) than those of anoestrus and early pregnant females (12 μm). The same differences were observed when comparing these cells with those in the fimbriae and infundibulum (14 μm). The width of these cells in all cases ranged from 5 to 8 μm.

The mucosal tunic lacked glands. The chorion was composed of loose connective tissue. The collagen fibres were arranged in thin beams, as shown by Masson trichrome technique (Fig. 2a). Reticular fibres ran longitudinally to the axis of the folds and were very abundant near the epithelium. Reticular fibres were also observed around small vessels present in the core of primary and secondary folds, especially in the region of the fimbriae (Fig. 2b–2b1). Elastic fibres were relatively scarce (Fig. 2c). In the connective tissue near the epithelium of the fimbriae blood cells were observed, particularly lymphocytes. As the oviduct was reduced in diameter the complexity of its folds decreased. The muscular tunica consisted of smooth muscle fibres. The thickness of the layer increased towards the uterine end. The serous tunica was represented by loose connective tissue with abundant intercellular substance and a simple squamous epithelium. Fig. 3 shows the different characteristics of the three segments of the oviduct.

Fimbriae and infundibulum: Fimbriae were thin structures that extended as fingers. They consisted of a core of tissue, the chorion, lined on both sides by a surface epithelium. Generally, connective tissue is very loose and is composed of thin collagen fibres arranged in a wavy bands form (Fig. 3a). The elastic fibres were sparse in the fimbriae and were found in higher quantities in the connective tissue that binded the fimbriae together. Reticular fibres followed the longitudinal axis of the folds. The chorion had many blood vessels of small diameter.

Both fimbriae and infundibulum presented a simple epithelium where secretory cells alternated with ciliated cells. In anoestrus females secreting cells predominated. Many of these cells had nuclear protrusions at the apical area. Secreting cells were proportionally more abundant in fimbriae and infundibulum of females in anoestrus than in pregnant. In females, in follicular phase, ciliated cells predominated over secreting

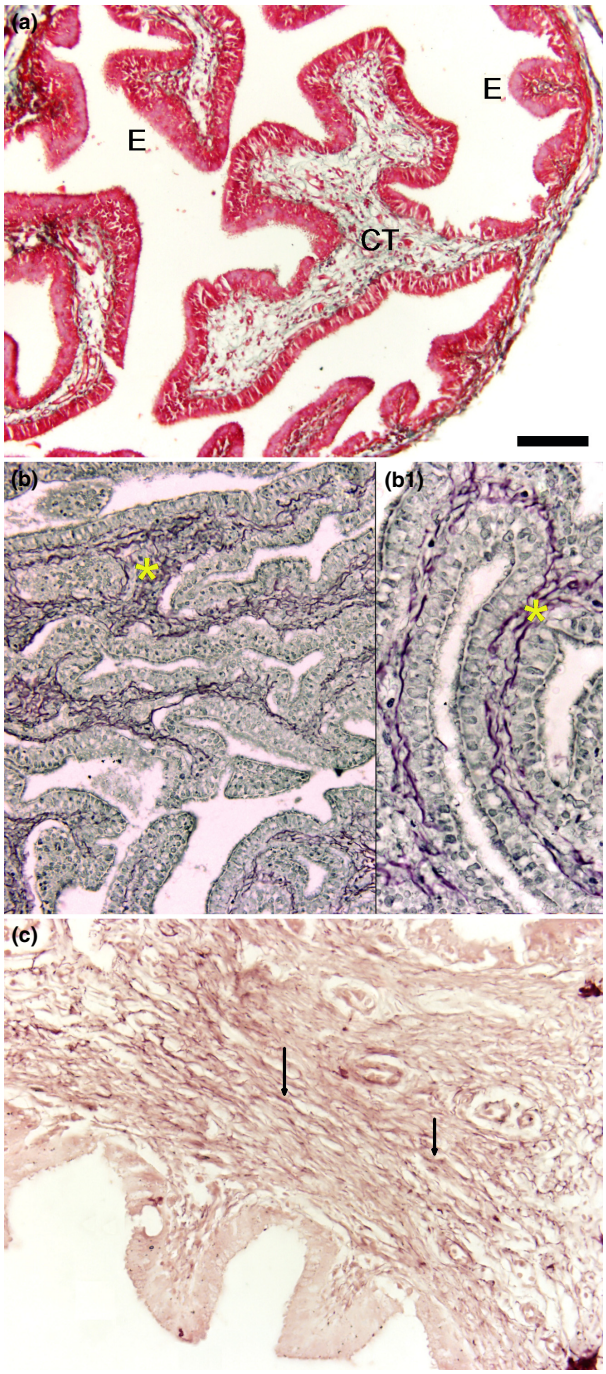

Fig. 2—Special histological staining techniques. (**a**) Staining of the ampulla of the oviduct with Masson trichromic technique. Red staining corresponds to epithelium. Green staining corresponds to connective tissue. Bar = 200 μm. CT = connective tissue. E = epithelium. (**b**) Staining of the ampulla of the oviduct with Gomori's reticulin technique. Asterisk indicates the presence of reticular fibres. Bar = 200 μm. (**b1**) is a magnification of (**b**) to show the reticular fibres in the inner portion of the primary folds. Bar = 50 μm. (**c**) Staining of the isthmus of the oviduct with orcein. Arrows are pointing to elastic fibres. Bar = 50 μm.

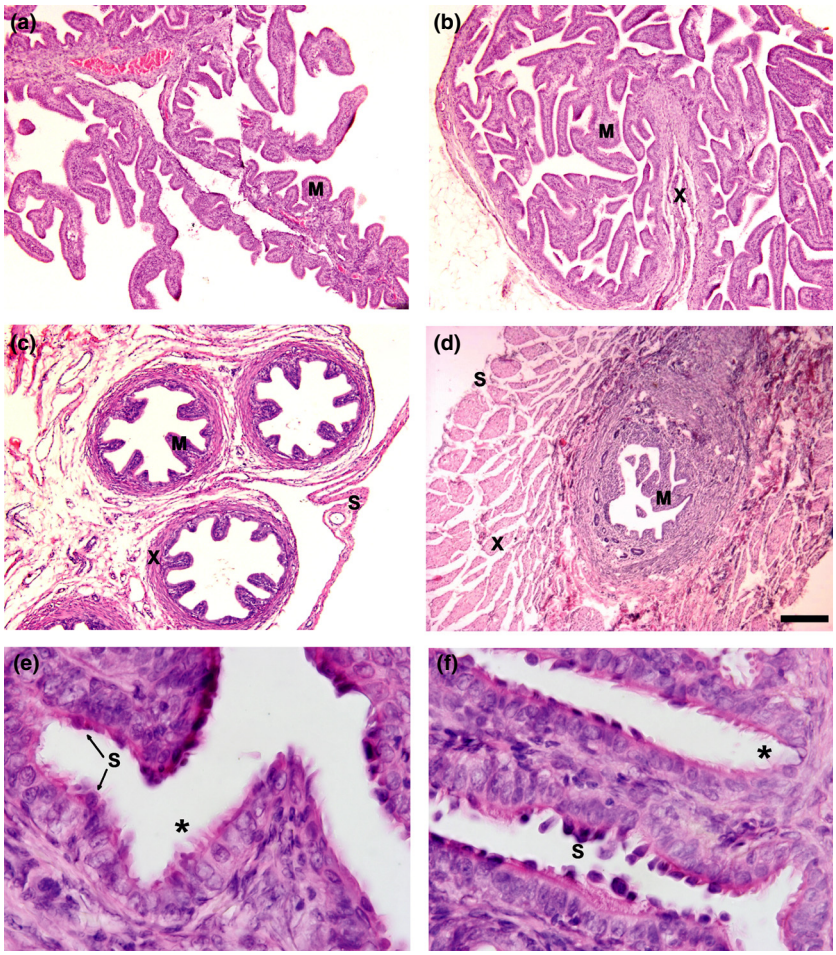

Fig. 3—Microscopical view of the oviduct. —**(a)**. Fimbriae; —**(b)**. Ampulla; —**(c)**. Isthmus; —**(d)**. Isthmus-uterus junction showing no net demarcation between both organs. —**(e,f)**. Higher magnification of the epithelium in the ampulla. * = Ciliated cells; S = Secretory cells. Haematoxylin-eosin staining. M = Tunica mucosa; X = Tunica muscular; S = Serous tunica. Bar for —**(a)**–**(d)** = 200 μm. Bar for —**(e,f)** = 20 μm.

cells both in the infundibulum and in the fimbriae. In pregnant females with multiple implantations and large foetuses, ciliated predominated over the secretory cells, both in the fimbriae and the infundibulum. Secreting cells had lower protrusions.

Ampulla: The ampulla had shorter folds but with the same level of complexity than that of the infundibulum (primary, secondary and tertiary folds) (Fig. 3b). It had the same layers shown in the infundibulum. The muscular layer was formed only by fibres arranged longitudinally, but was thicker than in the infundibulum.

In anoestrus females and those found in follicular phase, secretory and ciliated cells were approximately in the same proportion. In female carrying large foetuses, ciliated cells were more abundant. Secretory cells showed short protrusions. Both the apex of the cell and the protrusions were PAS and alcian-blue positive (Fig. 4a,b). In the epithelium infiltration of inflammatory cells, especially lymphocytes, was also observed.

In some females who had early pregnancy, secreting cells predominated over the ciliated cells, whereas in others the proportion of both cell types was similar.

Isthmus: The isthmus of the viscacha was the narrowest and longest sector of the oviduct. In the cross-sections it was shown that the lumen was uneven, but the folds were less complex than in other sectors. Only primary folds were found which were lower and thicker than in other sectors. The tunicae found area mucosa, muscular and serous (Fig. 3c).

The epithelium of the oviduct in this sector also had characteristics of secretory cells interspersed with ciliated columnar cells. In non-pregnant females in anoestrus the proportion of both cell types was similar, unlike non-pregnant females during the follicular phase in which secretory cells were found in greater quantity. In females with late pregnancy ciliated cells were seen in higher proportion, whereas in females with early pregnancy the proportion was variable, predominating ciliated cells in some females and secretory cells in others. In the isthmus of some females, round cells with pale cytoplasm and central nucleus were observed (Fig. 4c).

The muscular tunica was the one with greater thickness when compared with other segments and was arranged in two layers, an inner, where fibres were longitudinally arranged and an outer layer with thinner circular arrangement. In the latter, fibres were thinner than in the inner layer.

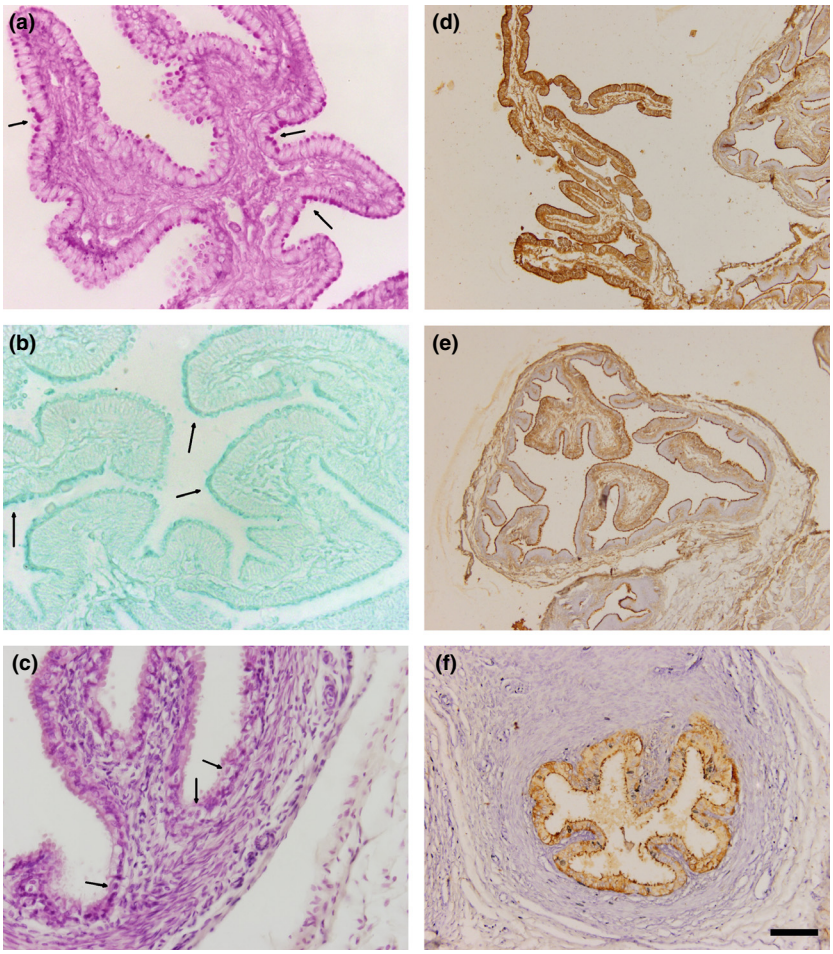

Fig. 4—Histochemical and lectin histochemical affinities of the epithelium. —**(a)**. Ampulla stained with PAS. Arrows point to secretory cells. —**(b)**. Ampulla stained with Alcian blue, pH 2.5. Arrows point to secretory cells. —**(c)**. Cells of the bottom of the folds (arrows). Haematoxylin-eosin staining. Bar for —**(a)**–**(c)** = 50 μm. —**(d)**. Fimbriae in follicular phase; —**(e)**. Ampulla in follicular phase; —**(f)**. Isthmus in early pregnancy. For —**(d,e)** staining is identifying L-Fuc with UEA-1. For —**(f)** staining is identifying β-galactose with RCA-1. Bar for —**(d**–**f)** = 100 μm.

Macroscopically, no evidence of a net demarcation at the junction of the oviduct to the uterine horns was seen. Serial sections obtained from the uterine horns until the appearance of the last segment of the oviduct, revealed that there was no intramural portion in *L. maximus*. Therefore, the isthmus continued to the uterine horns but there was a gradual transition to acquire the typical uterine structure (Fig. 3d). From the isthmus, the thickness of the mucosa increased and the lumen became larger. Cylindrical epithelial cells lowered their height towards the uterus. As the shape of the mucosa changes folds became shorter and less abundant and few small glands in the corium are seen.

The tunica muscular that surrounded the oviduct where it met the uterus composed of two layers: the inner one belonged to the isthmus whereas the outer continued with the outer muscle of the uterine horns. The outmost layer was a typical tunica serosa with loose connective tissue and mesothelium.

Lectin histochemistry of the epithelium of the oviduct

To determine the characteristics of cellular secretion of the oviduct, a histochemical study of lectin at each sector and at each physiological stage was carried out. Results are expressed in Table 3 and shown in Fig. 4d–f.

One of the outstanding features of the labelling with lectins was the negative reaction to *Dolichos biflorus* (DBA) lectin in all sectors of the oviduct in females in anoestrus stage. In contrast, in the same physiological state, the lectin *Concavalina ensiformis* (Con-A) showed a strong reactivity in the glycocalyx region in all sectors of the oviduct, whereas the apical region showed strong reaction in the fimbriae and infundibulum being weak in the other two sectors.

Labelling with the lectin *Ulex europaeus* (UEA-1) was also negative in the sector of the fimbriae and infundibulum, whereas the isthmus reacted differently when comparing pregnant females at early pregnancy with those at late pregnancy. Nevertheless, in the follicular phase, this lectin reacted quite strongly in the fimbriae (Fig. 4d) and ampulla (Fig. 4e).

In the area of the glycocalyx, lectins *Triticum vulgaris* (WGA) and *Ricinus communis* (RCA-1) (Fig. 4f) showed strong reactivity in all sectors and in the four analysed physiological states whereas in the apical and basal regions, the intensity of reaction to these lectins was variable.

In pregnant females at early pregnancy, the *Arachis hypogaea* (PNA) lectin reacted differently in the different sectors

Table 3 Lectin histochemical analysis of the epithelium at the fimbria, infundibulum, ampulla and isthmus in different reproductive stages. Reactivity to lectin: (3) strong, (2) moderate, (1) weak and (–) negative.

		Non pregnant anoestrus				Follicular phase				Early pregnancy				Late pregnancy			
Lectin	Region	F	I	A	T	F	I	A	T	F	I	A	T	F	I	A	T
WGA	*Glyc**	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3
	Apical	1	1	1	1	3	-	2	1	-	1	1	1	1	1	1	1
	Basal	-	-	-	-	3	-	-	-	-	-	-	2	1	1	-	-
CON-A	*Glyc*	3	3	3	3	3	3	3	3	-	-	-	3	3	3	3	3
	Apical	3	2	1	1	1	1	2	2	1	1	-	1	1	1	1	1
	Basal	2	2	-	-	1	-	1	1	-	-	-	-	1	1	1	1
DBA	*Glyc*	-	-	-	-	3	1	3	3	3	3	3	3	2	2	-	2
	Apical	-	-	-	-	2	1	2	2	1	1	1	2	1	1	-	2
	Basal	-	-	-	-	2	-	2	1	1	1	1	2	1	1	-	1
SBA	*Glyc*	3	3	2	2	2	-	2	3	2	3	3	3	3	2	2	3
	Apical	-	-	-	1	2	-	-	-	-	2	-	-	-	-	1	2
	Basal	-	-	-	-	-	-	-	-	-	-	1	-	1	1	1	2
PNA	*Glyc*	2	3	3	3	3	3	2	2	2	2	1	-	3	2	2	1
	Apical	-	-	1	2	-	1	1	1	-	1	-	-	-	1	-	1
	Basal	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
RCA-1	*Glyc*	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2	2
	Apical	1	1	1	1	2	-	1	-	-	1	-	-	-	-	-	1
	Basal	-	-	-	-	2	-	1	-	-	-	-	-	-	-	-	1
UEA-1	*Glyc*	1	1	3	3	2	1	3	3	-	-	-	3	1	-	2	2
	Apical	-	-	1	1	2	-	1	2	-	-	1	1	-	-	1	1
	Basal	-	-	-	-	2	-	1	-	-	-	-	2	-	-	-	1

F, Fimbriae; I, Infundibulum; A, Ampulla; T, Isthmus.
*glycocalyx.

with moderate affinity in the glycocalyx of the fimbriae and infundibulum, as they were weak in the ampulla and negative in the isthmus.

Greater variability in terms of reactivity and intensity was observed in females in follicular phase. In fimbriae, the lectins DBA, *Glycine maximus* (SBA) and PNA were those that were more heterogeneous in the intensity of staining.

Discussion

In our study we observed that the oviduct of the viscacha was long and like other mammalian females, presented an infundibulum, an ampulla and an isthmus near the proximal part of the uterus. However, the oviduct of the viscacha lacked an intra-uterine portion. Unlike what was observed in other rodents including hystricomorph such as guinea pigs, and non-hystricomorph such as mice and meriones, the oviduct had an intramural portion penetrating the uterine wall. (Breazile and Brown 1976; Almeida *et al.* 2001).

The oviduct in the viscacha was not as convoluted as it was observed in other hystricomorph such as coypus (Felipe *et al.* 1998) and myomorpha such as rats and mice (Griffith and Farris 1942; The staff of the Jackson Laboratory 1966).

The relationship established between the size of the fimbriae and the ovary during ovulation is essential to capture the oocyte and ensure its entry into the oviduct. This relationship also varies in different animal species. Mammalian females holding a well-developed ovarian bursa such as rats, mice, mink and bitches have small fimbriae, whereas in those females with a small ovarian bursa, the fimbriae are longer (Hafez 1970; Yaníz *et al.* 2000; Steinhauer *et al.* 2004).

The ovary of some mammals including the guinea pig (a hystricomorph rodent) is completely enclosed during ovulation, given the vast expansion of the fimbriae, whereas in the mouse and rat fimbriae cover, only a small area of the ovary (Hafez 1970) is enclosed. In the other hystricomorph such as South African porcupine (*Atherurus africanus*) females, fimbriae of the infundibulular portion are fully associated with ovarian surface (Mayor *et al.* 2003). In the viscacha, the oviduct is attached to the full extent of the edge of the ovary by a mesosalpinx. The shortness of fimbriae and the absence of ovarian bursa are characteristic of this species. Probably, the finger-like structures of the fimbriae spread at the time of ovulation to capture the largest possible number of oocytes released. From the observation that some oocytes remained stacked in the folds of the ovary, Weir suggested that they are trapped to be released at a later stage into the oviduct (Weir 1971b; Weir and Rowlands 1974). However, according to our own observation (Flamini *et al.* 2009) no oocytes were detected in the ovarian cords in all the serial sections of the ovary analysed. Even though the hypothesis proposed by Weir

could not be ruled out, it would imply that there should be a favourable environment in the spaces between cords for the survival of oocytes, a fact not proven yet.

Another hypothesis to explain that *L. maximus* fimbriae do not cover the ovarian surface and that the species lack an ovarian bursa is the large number of oocytes released in each cycle. The fact that many of them are lost in the peritoneal cavity does not represent a problem as many other oocytes could be fertilised and implanted without affecting the reproductive efficiency and jeopardizing the survival of the species. Although the viscacha presents some reproductive characteristics reminiscent of marsupials, they differ in that the latter have a developed ovarian bursa (Kress and Morson 2007). Regarding the other group of polyovulating eutherians such as elephant-shrews (Macroscelidea), Tripp (1971) refers to capsule as a structure that completely surrounds the ovary and that could be similar to the ovarian bursa of other species. It should be noted that the ovary of Macroscelidea lacks the cordonal structure of the viscacha and furthermore, their polyovulation produce fewer mature oocytes. Thus, the structure and size of the capsule could prevent the loss of reproductive cells into the abdominal cavity.

The simple epithelium of the oviduct is lined by ciliated and non-ciliated cells (secretory cells). We have found both cell types in the epithelium of the oviduct of the viscacha in all the analysed reproductive states. Both cells types were randomly arranged in a similar way as described in other species (Abe and Oikawa 1993; Priedkalns 1993). Nevertheless, unlike the porcupine species where no changes related to ovarian activity were described (Mayor *et al.* 2003), we observed that changes in the different physiological states of the viscacha were related to the height of the secretory cells. In the oviduct of the armadillo (*Chaetophractus villosus*), Codón and Casanave (2009) describe a third cell type characterised by cells with pale stained cytoplasm at the bottom of the folds. In the viscacha, we observed some cells that resembled those described in the armadillo. To our knowledge, no studies include these cells in the oviduct of rodents.

We found no studies that analysed the relationship between secretory and ciliated cells in rodents. Therefore, we could only compare our results with those of phylogenetically more distant mammals such as cows and sows. The appearance of the epithelium of the oviduct of *L. maximus* during pregnancy differed from that observed by Abe and Oikawa (1993) during the luteal phase in the cow. This might be due to predominant cells in the viscacha during pregnancy which was ciliated and not secretory as in that species. However, similar to what was described in cattle during the luteal phase, secretory cells of viscacha presented low projections resembling bulbs.

In our study, we compared the height of ciliated and secretory cells in different physiological states. We noted that the height of ciliated cells showed no significant differences between sectors and between physiological states considered, unlike what was observed for the sow (Ferreira de Sant'Ana 2001). As for the secretory cells, they are higher in the ampulla of pregnant females with late pregnancy, not presenting in this case significant differences when compared with females in follicular phase. This difference could be due to the oviduct epithelium which prepared itself for the next ovulation, adapting its morphology to this new phase of the cycle. This result was consistent with that observed by Ferreira de Sant'Ana (2001) for the sows in follicular phase.

Ovarian hormones play a fundamental role in the differentiation of oviductal epithelium and the appearance of proteins and glycoproteins related to their secretion (Buhi 2002).

Tubaric secretion is composed mainly of glycoproteins produced by secretory cells of the oviduct. These cells vary in structure and function also changes the chemical composition of the glycocalyx and its secretion. There are a variety of methods to identify and differentiate glycoconjugates (Spicer *et al.* 1981; Díaz *et al.* 2001). Histochemical techniques used to detect carbohydrates in the oviductal epithelium of the viscacha showed that the intensity of staining at different stages of sexual cycle only varied when staining with alcian blue at pH 0.5. This finding agrees with some observations made by Ferreira de Sant'Ana (2001) who applied some histochemical techniques to different portions of the oviduct of sows in the follicular and luteal phase. In the three portions of the oviduct of the viscacha (infundibulum, ampulla and isthmus) the epithelium was positive to PAS and alcian blue at different pH. Similar to what was observed in the sow, the intensity of staining in viscacha was lower in the epithelium of the isthmus. Due to the characteristics of the captured animals we could not detect changes during ovulation in relation to the reactivity with the PAS technique, as were described in the sow (Gregoraszczuk *et al.* 2000). However, as in the latter species, the highest reaction was seen in the glycocalyx and apical cytoplasm, as well as in the protrusions of the secretory cells (Gregoraszczuk *et al.* 2000; Ferreira de Sant'Ana 2001).

We did not find studies in literature related to the identification of carbohydrates using lectin hsitochemistry in the oviduct of any Hystricognathi. Therefore, we cannot compare the results obtained in the viscacha with closely related animals. In the oviduct cells of the viscacha a strong reaction to lectins WGA and RCA was observed in all studied sectors and physiological states, similar to that described in the hamster, a non-hystricomorph rodent (El-Mestrah and Kan 1999). However, in the latter species, the lectin UEA-1 and Con-A were negative in the ampulla at all physiological stages considered (El-Mestrah and Kan 1999).

By comparing our results with those previously found in other, not rodents mammals species, it can be observed that in both the viscacha and heifers (Cobo *et al.* 2004), the lectin RCA-1 intensively stains the glycocalyx. By contrast, in the supranuclear region the reaction was poor or absent in all sectors of the oviduct. However, differences in staining with other lectins were observed. Thus, the reactivity for the lectin WGA was very intense in the cell glycocalyx of the viscacha, whereas

in heifers the immunoreactivity was moderate. Contrary to what was determined in heifers of the same study, staining with the lectin Con-A was highly positive in the glycocalyx and moderate in the apical portion of the viscacha, whereas no reaction was observed in heifers.

Ball *et al.* (1997), conducted a study on the distribution of glycoconjugates in the oviduct of mares during oestrus, diestrum and pregnancy stages. Their results showed some differences to what was observed in the viscacha. In the latter species, labelling with DBA was negative in the anoestrous phase in all sectors of the oviduct, whereas in females in follicular phase and early pregnancy, the glycocalyx strongly reacted to this lectin. The most outstanding results in the mares showed that the DBA positively reacted in the epithelium of the isthmus in all stages of the oestrous cycle, which differed with our observations. Unlike to what occurred in the oviduct of the mare that had a negative reactivity to WGA, staining with this lectin was strong in all considered physiological states and in all sectors of the oviduct of the viscacha.

The presence of some glycoproteins in oviducts of sexually mature New Zealand rabbits was studied by Menghi *et al.* (1985) who stated that there were marked differences in the distribution pattern of some lectins in the ampulla and the isthmus of anoestrus females. In viscacha, the used lectins reacted very similar in both sectors of the oviduct of females in anoestrus phase, although the lectins PNA and WGA showed greater reactivity in the isthmus than the ampulla, which did not occur in the rabbit. These data suggest that in the viscacha some carbohydrates are synthesised in greater amounts in the isthmus.

In all sectors of the oviduct of the viscacha in anoestrous, when circulating estrogen levels were low, the staining with the lectin DBA was negative. Something similar occurs in the ampulla of the oviduct of postmenopausal women whose estrogen levels are decreased and the DBA does not have reactivity (Gheri *et al.* 2001). However, the lectins WGA and Con-A, which do not react on the oviduct of postmenopausal women, showed a positive reaction in anoestrous viscachas, especially in the glycocalyx.

The above presented results suggest that binding of lectins varies in different animal species. This would show that despite phylogenetic differences, galactose residues appear to be necessary for the functioning of the oviductal epithelium.

Lagostomus maximus has some outstanding features in their reproductive system and cycle such as high mortality in uterus, polyovulation and embryonic physiology. However, in the oviduct, we found no such differential specificities as found in the ovary (Flamini *et al.* 2009) and vagina (Flamini *et al.* 2011) that might be associated with a peculiar reproductive physiology. Nevertheless, it must be pointed out that the oviduct has particular anatomical features such as the short length of their fimbriae despite the lack of a recognizable ovarian bursa and the absence of an intra-uterine portion present in other related rodents such as guinea pigs (Breazile and Brown 1976).

As in other species, the morphological and histochemical characteristics of the oviductal epithelium vary with the physiological status of the female and the region analysed.

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