Differential lectin binding patterns in the oviductal ampulla of the horse during oestrus

S. Desantis,[1] G. Ventriglia,[1] D. Zubani,[1] A. Corriero,[1] M. Deflorio,[1] F. Acone,[2] G. Palmieri,[2] G. De Metrio [1]

[1]Department of Animal Health and Well-being, Faculty of Veterinary Medicine, University of Bari;
[2]Department of Animal Biology, Faculty of Veterinary Medicine, University of Sassari, Italy

We investigated the oligosaccharide sequence of glycoconjugates, mainly sialoglycoconjugates, in the horse oviductal ampulla during oestrus by means of lectin and pre-lectin methods such as the KOH-neuraminidase procedure to remove sialic acid residues and incubation with *N*-glycosidase F to cleave *N*-linked glycans. Ciliated cells displayed *N*-linked oligosaccharides throughout the cytoplasm. The cilia glycocalyx expressed both *N*- and *O*-linked (mucin-type) oligosaccharides, both showing a high variety of terminal sequences. In the most non-ciliated cells, the whole cytoplasm contained *N*-linked oligosaccharides with terminal αGal as well as mucin-type glycans with terminal Forssman pentasaccharides. In a few scattered non-ciliated cells, the whole cytoplasm displayed sialylated *N*-linked oligosaccharides with terminal Neu5Ac-GalNAc and O-linked glycans terminating with neutral and/or αGalNAc, Neu5Acα2,6Gal/GalNAc, Neu5AcGalβ1,3GalNAc. Supra-nuclear granules, probably Golgi zones, of non-ciliated cells showed mainly O-linked glycans rich in sialic acid residues. The luminal surface of non-ciliated cells showed *N*-linked oligosaccharides, containing terminal/internal αMan/αGlc, βGlcNAc and terminal αGal, as well as mucin-type oligosaccharides terminating with a large variety of either neutral saccharides or sialylated sequences. Apical protrusions containing O-linked oligosaccharides with terminal Forssman pentasaccharide, Neu5Ac-Galβ1,4GlcNAc, Neu5Ac-GalNAc were seen in non-ciliated cells scattered along the epithelium. These findings show the presence of sialoglycoconjugates in the oviductal ampulla epithelium of the mare and the existence of different lectin binding profiles between ciliated and non-ciliated (secretory) cells, as well as the presence of non-ciliated cell sub-types which might determine functional differences along the ampullary epithelium of mare oviduct.

Key words: histochemistry, lectins, sialic acid, glycoconjugates, oviduct, horse.

Correspondence: Professor Gregorio De Metrio,
Department of Animal Health and Well-being,
Faculty of Veterinary Medicine, University of Bari,
S.P. per Casamassima Km. 3, I-70010 Valenzano (BA), Italy
Tel: +39.080.5443907.
Fax: +39.080.5443908.
E-mail: g.demetrio@veterinaria.uniba.it

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The ampulla is the oviductal region where fertilization and early embryonic development take place. The epithelium of the ampulla, like that of the entire oviduct, is of the simple columnar type and consists of two types of cells: ciliated and non-ciliated (secretory) cells. The ciliated cells play a role in the transport of germinal cells, whereas non-ciliated cells are considered secretory cells involved in the forming production of the oviductal fluid together with a selective trasudate of serum (Leese, 1988).

During late follicular development and oestrus, the mammalian oviduct undergoes specific morphological, biochemical and physiological modifications which determine changes in oviductal fluid composition involved in the optimisation of the microenvironment for fertilization and early cleavage-stage embryonic development. The previous changes appear to be regulated by ovarian steroids, above all estrogens, which also modulate ampulla glycoprotein synthesis in several species including pigs (Buhi *et al.*, 1990), hamsters (Abe *et al.*, 1998), sheep (Buhi *et al.*, 1991; DeSouza and Murray, 1995), mice (Kapur and Jonson, 1988), and humans (Arias *et al.*, 1994). Secretory oviduct-specific glycoproteins are involved in an increase in bovine (King *et al.*, 1994) and hamster (Boatman and Magnoni, 1995) sperm capacitation, and in the number of capacitated spermatozoa attaching to the surface oocytes, as well as in a reduction of the incidence of polyspermy in pigs (Funahashi and Day, 1997). In addition, oviductal glycoproteins associate with the zona pellucida and perivitelline space of oviductal oocytes and embryos in primates (O'day-Bowman *et al.*, 1995), cows (Wegner and Killian, 1991; Boice *et al.*, 1992), sheep (Gandolfi *et al.*, 1991), pigs (Buhi *et al.*, 1993) and hamsters (Malette *et al.*, 1995; El-Mestrah and Kan, 2001) as well as promoting early ovine embryonic cleavage They also promote early embryonic cleavage in ovine and porcine reproduction (Gandolfi *et al.*, 1995; Hill *et*

Table 1. Lectins used, their sugar specificities and inhibitory sugars used in control experiments.

Lectin abbreviation	Source of lectin	Concentration (μg/mL)	Sugar specificity	Inhibitory sugar	Reference
SNA	*Sambucus nigra*	15	Neu5Acα2,6Gal/GalNAc	NeuNAc	Shibuya *et al.* 1987
MAH*	*Maackia amurensis*	10	Neu5acα2,3Galβ1,3(Neu5Acα2,6)GalNAc	NeuNAc	Brinkman-Van der Linden *et al.* 2002
PNA	*Arachis hypogea*	20	Terminal Galβ1,3GalNAc	Galactose	Lotan *et al.* 1975
RCA_{120}	*Ricinus communis*	25	Terminal Galβ1,4GlcNAc	Galactose	Baenziger & Fiete 1979
SBA	*Glycine max*	10	Terminal α/βGalNAc	GalNAc	Hammarström *et al.* 1977
DBA	*Dolichos biflorus*	15	Terminal FP>GalNAcα1,3GalNAc	GalNAc	Hammarström *et al.* 1977
HPA	*Helix pomatia*	15	Terminal αGalNAc	GalNAc	Roth 1984
Con A	*Canavalia ensiformis*	25	Terminal and internal αMan>αGlc	Mannose	Goldstein & Hayes 1978
WGA	*Triticum vulgaris*	20	Terminal and internal βGlcNAc>>NeuNAc	GlcNAc	Debray *et al.* 1981
GSA I-B_4	*Bandeiraea simplicifolia*	25	Terminal αGal	Galactose	Hayes & Goldstein 1974
UEAI	***Ulex europaeus***	25	Terminal L-Fucα1,2Galβ1,4GlcNAcβ	Fucose	Pereira *et al.* 1978

*Fuc, Fucose; Gal, galactose; GalNAc, N-acetylgalactosamine; Glc, glucose; GlcNAc, N-acetylglucosamine; FP, Forssman pentasaccharide GalNAcα1,3GalNAcα1,3Galβ1,4Galβ1,4GlcNAc; Man, mannose; NeuNAc, N-acetyl neuraminic (sialic) acid. *We assumed MAH to be "MAL-II" on the basis of the general proporties for "MAL-II" reported by Vector Laboratories and according to Brinkman-Van der Linden et al. (2002).*

al., 1996; Kouba *et al.*, 2000). The presence of glycoconjugates in the ampullar oviduct epithelium during oestrus has been investigated successfully by means of lectin histochemistry in a variety of mammals such as humans (Schulte *et al.*, 1985; Wu *et al.*, 1993; Kiss *et al.*, 1998), hamsters (Kan *et al.*, 1990; El-Mestrah and Kan, 1999), pigs (Raychoudhury *et al.*, 1993; Walter and Bavdek, 1997), rabbits (Menghi *et al.*, 1995), and horses (Ball *et al.*, 1997). Among the carbohydrates that constitute the oligosaccharide chains in glycoproteins, sialic acids are known to be a large family of nine-carbon carboxylated sugars that usually occupy the terminal position of oligosaccharide chains in a variety of glycoconjugates (Schauer, 1985). Sialic acids and to act as ligands in recognition phenomena (Varki, 1997), *in vitro* sperm capacitation (Banerjee and Chowdhry, 1994; Focarelli *et al.*, 1995), maintaining sperm viability (Satoh *et al.*, 1995) and sperm-egg interaction (Geng *et al.* 1997). Oligosaccharide chains of glycoproteins have been classified into two families: *N*- and O-linked oligosaccharides. The first group is characterized by a reducing terminal *N*-acetylglucosamine (GlcNAc) bound *N*-glycosidically to asparagine. The O-linked (mucin-type) oligosaccharides contain a reducing terminal *N*-acetylgalactosamine (GalNAc) linked O-glycosidically to serine or threonine. In the present study, lectin histochemistry was applied in combination with enzymatic and chemical treatment to investigate the oligosaccharide sequences of carbohydrate moieties, mainly sialoglycoconjugates, in the oviductal ampulla of mares. Since the *in vitro* production of equine embryos has not been very successful with respect to other mammals (Squires *et al.*, 2003), we dealt with the sialoglycoconjugates in the horse ampullary oviduct epithelium of the horse oviduct during oestrus, when an increased rate of oviduct-specific glycoprotein synthesis occurs in numerous mammals (Buhi, 2002). Our investigation, by adding new data to the results recently obtained on the glucidic constituents of the oviductal isthmus (Desantis *et al.*, 2004), might help to clarify the role of carbohydrates to add to the data from a previous histochemical study on the isthmus (Desantis *et al.*, 2004) on the oviductal charbohydrate constituents which could play a role in mare reproduction.

Materials and methods

Tissue preparation

Oviducts from three oestrus mares (with a follicle >35 mm) were obtained from a local slaughterhouse. Immediately after collection, on the basis of general appearance, the ampulla was separated from the isthmus and fixed in Bouin's fluid for 12 h at room temperature (RT). Following fixation, the tissues were washed and dehydrated in an ethanol series, cleared in xylene, and embedded in paraffin wax. Sections, 4 μm thick, were cut and, after dewaxing with xylene and hydration in an ethanol series of descending concentrations, were stained with Mayer's Haematoxylin hematoxylin and Eosin eosin (to study the general morphology) or by one of the following histochemical methods.

Lectin histochemistry

The lectins used are listed in Table 1. The lectins PNA, DBA, RCA_{120}, SBA, WGA, HPA, Con A, GSA I-B4, and UEA I were HRP-conjugated. They were obtained from Sigma-Aldrich Co. (St. Louis, MO,

USA). SNA, and MAH were biotinylated lectins and were purchased from Vector Laboratories Inc. (Burlingame, CA, USA). Lectin staining was performed as previously described by Labate *et al.* (1997). De-waxed and re-hydrated tissue sections were immersed in 3% H_2O_2 for 10 min to suppress the endogenous peroxidase activity. After a rinse in 0.05 M Tris-HCl buffered saline (TBS), pH 7.4, the sections were incubated in a lectin solution at appropriate dilutions (Table 1) for 1 h at room temperature (RT). They were then rinsed 3 times in TBS, and the peroxidase activity of the bound lectins was visualized by incubation in a solution containing 0.05% 3,3′-diaminobenzidine (DAB) and 0.003% H_2O_2 in 0.05 M TBS (pH 7.6) for 10 min at RT. Finally, the sections were dehydrated and mounted. Tissue sections incubated in biotinylated lectins (SNA and MAH) were rinsed 3 times with 0.05 M phosphate-buffered saline (PBS) and were incubated in streptavidin/peroxidase complex (Vector Lab. Inc.) for 30 min at RT. After washing in PBS, peroxidase was developed in a DAB-H_2O_2 solution as above. Controls for lectin staining included: (1) substitution of the substrate medium with buffer without lectin; (2) incubation with each lectin in the presence of its hapten sugar (0.2-0.5 M in Tris buffer).

Enzymatic and chemical treatments

Before staining with SNA, MAH, PNA, DBA, RCA_{120}, SBA, HPA,and WGA, and GSA I-B4 some sections were incubated, at 37°C for 16 h in 0.86 U/mg protein of Type V Clostridium perfringens sialidase (Sigma Chemicals Co., St. Louis, MO, USA) dissolved in 0.1 M sodium acetate buffer, pH 5.5, containing 10 mM $CaCl_2$. Prior to the neuraminidase treatment, the sections were subjected to a saponification technique to render the enzyme digestion effective, using 0.5% KOH in 70% ethanol for 15 min at RT (Reid *et al.* 1978).

Hydrolysis of *N*-linked oligosaccharides was carried out by enzymatic treatment with 10 U/mL of *N*-glycosidase F (Roche, Mannheim, Germany) diluted in 20 mM sodium phosphate buffer, pH 7.2, containing 10 mM EDTA and 0.5% Triton X-100, overnight at 37°C. After a brief whash, the sections were incubated with each of the eleven lectins used in this study. As controls of the enzyme digestion procedures (sialidase and *N*-glycosidase F), sections were incubated in specific enzyme-free buffer solutions under the same experimental conditions.

Results

General morphology

The ampulla segment of the mare oviduct is characterized by both a less-developed muscle layers and extensively branched mucosal folds compared to the isthmus. The epithelium lining the mucosa is columnar and consists of ciliated cells and non-ciliated (secretory) cells (Figure 1).

Lectin histochemistry

The lectin-binding pattern in the ampulla oviduct is summarized in Table 2.

MAH weakly stained the cilia and the supranuclear cytoplasm of the ciliated cells and moder-

Table 2. Summary of lectin binding to epithelium lining the ampullary oviduct of estrus mares.

LECTIN	Ciliated cells	Non-ciliated cells
MAH	+ ci/+ sn	++as/±g
N-Glycosidase F/ MAH	-	++as
KOH-s- MAH	-	-
N-Glycosidase F/KOH-s- MAH	-	-
SNA	++ci	++as/± g /± c**
N-Glycosidase F/SNA	++ci	++as/±g/ ±c**
KOH-s-SNA	-	-
N-Glycosidase F/KOH-s-SNA	-	-
PNA	±ci	±as
N-Glycosidase F/PNA	±ci	± as
KOH-s-PNA	+++ci*	+++as/+g/++ c**
N-Glycosidase F/KOH-s-PNA	+++ci*	+++as/+g/++ c**
RCA120	+ci	+as
N-Glycosidase F/ RCA120	-	+as
KOH-s- RCA120	++ci	++as/+ap**
N-Glycosidase F/KOH-s- RCA120	++ci	++as+ap**
SBA	++ci	++as/++ap**
N-Glycosidase F/SBA	++ci	++as/++ap**
KOH-s-SBA	+++ci	+++as/+++ap**/++c**
N-Glycosidase F/KOH-s-SBA	++ci	+++as/+++ap**
DBA	+++ci/±c*	++as/++g/++ap/+c
N-Glycosidase F/DBA	-/**	++as/++g/++ap/ +c
KOH-s-DBA	+++ci/±c*	++as/++g/++ap/+c
N-Glycosidase F/KOH-s-DBA	-	++as/++g/++ap/+c
HPA	+++ci	+++as/+++ ap**/++c**
N-Glycosidase F/HPA	+++ ci	+++as/+++ap**/++c**
KOH-s-HPA	+++ci	+++as/+++ ap**/++c**
N-Glycosidase F/KOH-s-HPA	+++ ci	+++as/+++ap**/++c**
Con A	++ci/±c	+as/±c
N-Glycosidase F/Con A	-	-
WGA	++ci	+as
N-Glycosidase F/WGA	-	-
KOH-s-WGA	++ci	+as
N-Glycosidase F/KOH-s-WGA	-	-
GSA I-B4	++ci/++c	+as/++c
N-Glycosidase F/ BSA I-B4	-	-
KOH-s-GSA I-B4	++ci/++c	+as/++c
N-Glycosidase F/KOH-s- GSA I-B4	-	-
UEA I	±ci	-
N-Glycosidase F/ UEA I	-	-

*ap, apical protrusion; as, apical (luminal) surface; c, whole cytoplasm; ci, cilia; g, supranuclear granules; s, sialidase; sn, supra-nuclear cytoplasm. *, variable reactivity along the folds; **, few and scattered reactive cells; -, negative reaction; ±, faintly visibile reaction; +,++,+++, weak, moderate, strong positive reactions.*

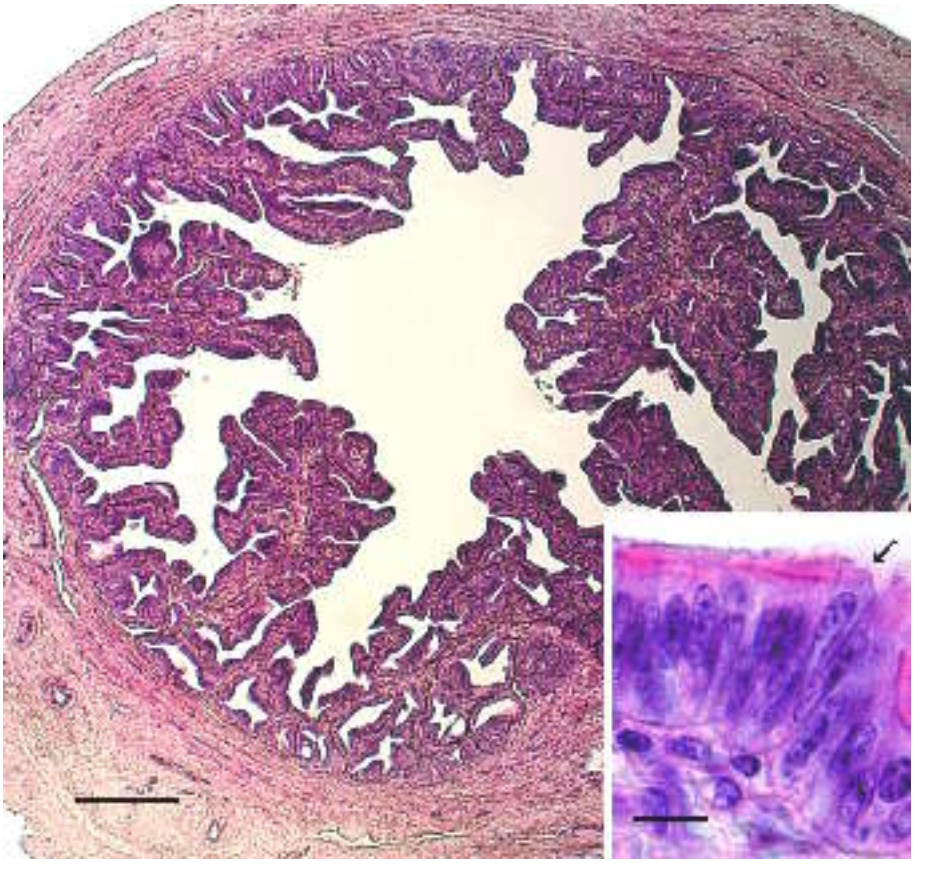

Figure 1. Cross-section of the horse oviductal ampulla. Inset: ciliated and non-ciliated cells. Mayer's hematoxylin-eosin staining. Bars indicate 280 μm in the low magnification picture and 14 Ìm in the inset. Arrow, non-ciliated cells.

ately marked the apical surface and faintly revealed supra-nuclear granules in the non-ciliated cells (Figure 2a,b). Removal of *N*-linked oligosaccharides by *N*-glycosidase F pre-treatment eliminated the staining in the ciliated cells as well as the affinity of supra-nuclear granules in the non-ciliated cells (Figure 2c). Saponification, followed by neuraminic acid cleavage (KOH-sialidase), abolished staining. SNA showed a moderate reaction for at the luminal surface of the epithelium and a faintly visible staining with at supra-nuclear granules of non-ciliated cells, as well as in the cytoplasm of few and scattered non-ciliated cells (Figure 3a ,b). The *N*-glycosidase F pre-treatment did not modify the SNA binding pattern. After KOH-sialidase treatment, no positive reaction was observed in either

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Figure 2. MAH staining. a. MAH-reactivity of ampulla observed at low magnification. b. Difference in the MAH-binding pattern between the ciliated cells and the non-ciliated cells. c. N-glycosidase F procedure retains MAH affinity only at luminal surface of non-ciliated cells.

Figure 3. SNA staining. a. Low magnification of ampulla stained with SNA. b. SNA reactivity at the cilia of ciliated cells and apical surface of non-ciliated cells. Occasional non-ciliated cells show a faintly visible reaction throughout the cytoplasm.

Figure 4. PNA staining. a, low and b, high magnification micrographs showing a weak reaction of the luminal surface of the epithelium lining the ampulla.

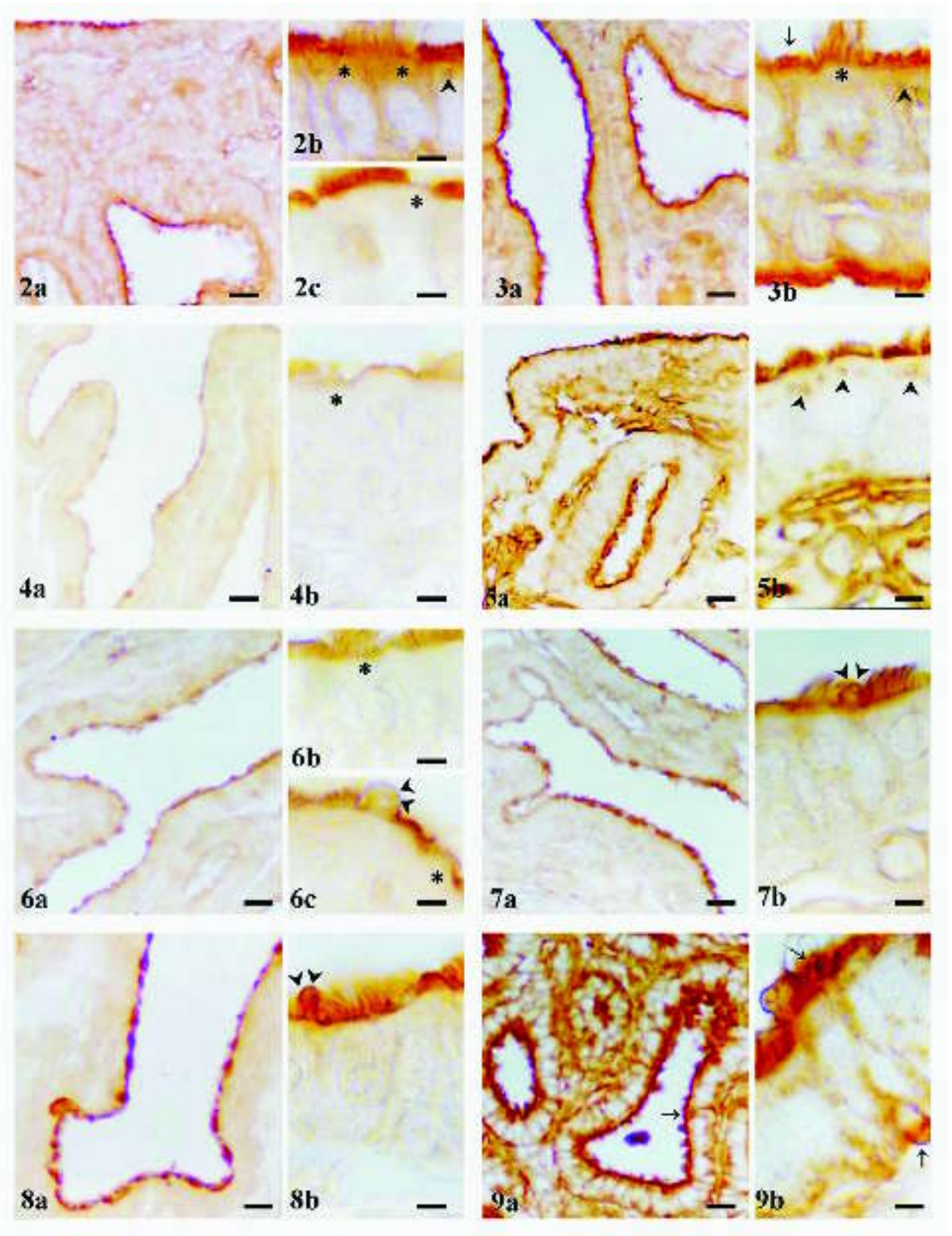
Figure 5. KOH-sialidase-PNA staining. a, low magnification micrograph showing strong PNA reactivity of luminal surface epithelium. b, KOH-sialidase pre-treatment reveals also supra-nuclear granules in non-ciliated cells.

Figure 6. RCA120 staining. a, low and b, high magnification micrographs showing binding all over the luminal surface. c, N-Glycosidase F procedure abolishes the cilia reactivity.

Figure 7. KOH-sialidase- RCA120 staining. a, low and, b, high magnification micrographs showing evident increase of the RCA120-binding sites on the luminal surface epithelium. In b, apical protrusions of non-ciliated cells show a weak positive reaction.

Figure 8. SBA staining. a, low and b, high magnification micrographs showing binding-sites throughout the luminal surface epithelium. In b, a positive apical protrusion in a non-ciliated cell is evident.

Figure 9. KOH-sialidase-SBA. a, low and b, high magnification micrographs showing evidence of an increase of SBA reactivity on the luminal surface as well as appearance of binding sites in the cytoplasm of scattered non-ciliated cells.



Asterisk, ciliated cells; arrowhead, supra-nuclear granules; arrow, non-ciliated cells; double arrowhead, apical protrusions; bars indicate 35 μm in Figures 2a,3a,4a,5a,6a,7a,8a,9a,. Bars indicate 7 μm in Figures 2b,2c,3b,4b,5b,6b,6c7b,8b,9b.

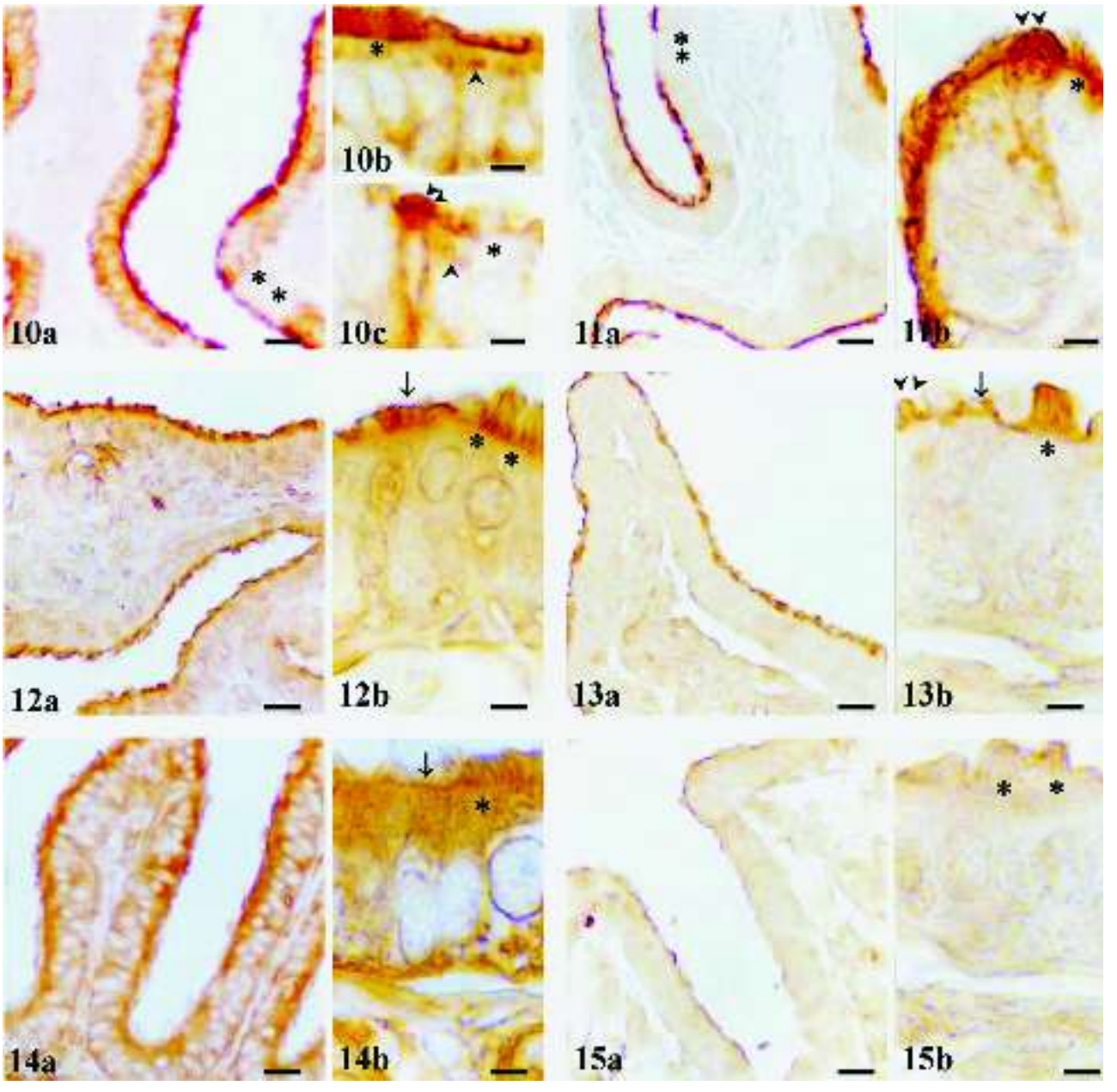


Figure 10. DBA staining. a, low magnification micrograph showing the epithelium positivity with scattered negative cytoplasm areas. b, high magnification micrograph evidencing supra-nuclear granules in non-ciliated cells. c, N-Glycosidase F procedure annulls the DBA staining in some ciliated cells.

Figure 11. HPA staining. a, low magnification micrograph showing the positive luminal surface epithelium interrupted by negative zones. b, positive apical protrusions in a scattered non-ciliated cell.

Figure 12. Con A staining. a, low and b, high magnification micrographs showing binding sites mainly localized overall on the luminal surface epithelium.

Figure 13. WGA staining. a, low and b, high magnification micrographs showing a different staining intensity between the luminal surface of ciliated and non-ciliated cells.

Figure 14. GSA I-B4 staining. a, low magnification micrograph showing the left mucosal fold epithelium with negative non-ciliated cells and the right mucosal fold epithelium with both positive cell types. b, positivity in both ciliated and non-ciliated cells.

Figure 15. UEA I staining. a, low and b, high magnification micrographs showing the presence of scarce binding sites only on the cilia.

ciliated or non-ciliated cells.

PNA gave a very weak reaction for the epithelial apical surface (Figure 4a, b). This staining was not affected by *N*-glycosidase F pre-treatment. After KOH-sialidase treatment, except for some scattered ciliated cells, the lectin revealed cryptic binding sites on the luminal surface of the epithelium, in the supra-nuclear granules of the majority of the non-ciliated cells (Figure 5a, b) and in the cytoplasm of a few non-ciliated cells. *N*-glycosidase F incubation followed by KOH-sialidase pre-treatment did not affected the PNA positivity.

RCA_{120} weakly stained the luminal surface of the mucosal folds (Figure 6a, b). Removal of *N*-linked oligosaccharides eliminated the cilia positivity (figure 6c). After KOH-sialidase, cryptic RCA_{120} binding sites were observed on the luminal surface of the epithelium non-ciliated cells as well as in the apical protrusions of the rare non-ciliated cells (Figure7a, b). Removal of *N*-linked oligosaccharides combined with sialic acid cleavage did not modify this binding pattern.

SBA showed moderate staining of the mucosal luminal surface and of the apical protrusions of some scattered non-ciliated cells (Figure 8a, b). *N*-glycosidase F pre-treatment did not modify the binding pattern. KOH-sialidase caused an increase in SBA affinity in the above-mentioned structures as well as revealing positive cytoplasm in scattered non-ciliated cells (Figure 9a, b). Removal of *N*-linked oligosaccharides and neuraminic acid abolished the cytoplasm positivity.

DBA displayed a moderate reactivity for the luminal surface of the epithelium and a weak positivity of the cytoplasm in both the cell types (Figure 10a,b). Scattered areas with negative cytoplasm were observed along the epithelium (Figure 10a). Non-ciliated cells contained supra-nuclear positive granules (Figure 10b). Some non-ciliated cells exhibited moderate staining at their apical protrusions (Figure 10c). *N*-glycosidase F incubation annulled the binding sites in some ciliated cells (Figure 10c). KOH-sialidase procedure followed following or not by *N*-glycosidase F incubation did not reveal significant changes in the DBA binding pattern.

HPA gave strong but not continuous labelling of the luminal surface as well as a moderate reaction of the cytoplasm and a strong staining of apical protrusions in scattered non-ciliated cells (Figure 11a,b). *N*-glycosidase pre-treatment increased the negative epithelial areas. KOH-sialidase procedure following or not *N*-glycosidase F incubation did not reveal changes in the HPA staining.

Con A showed a faintly visible cytoplasmic reaction as well as a moderate and weak reaction of the cilia and luminal surface of the ciliated and non-ciliated cells, respectively (Figure 12a, b). Removal of *N*-linked oligosaccharides abolished the Con A positivity.

WGA moderately marked the cilia of ciliated cells and weakly marked the luminal surface of non-ciliated cells (Figures 13a, b). *N*-glycosidase F incubation abolished the WGA positivity. KOH-sialidase-WGA treatment as well as *N*-glycosidase F incubation followed by KOH-sialidase-WGA treatment did not modify the WGA binding pattern.

GSA I-B4 displayed moderate and weak staining of the cilia and luminal surface of non-ciliated cells, respectively, as well as moderate labelling with in the cytoplasm of both cell-types (Figures 14a, b). Negative non-ciliated cells were observed in some mucosal folds. Removal of *N*-linked oligosaccharides abolished the posititypositivity. KOH-sialidase procedure following or not *N*-glycosidase F incubation did not show changes in the GSA $I\text{-}B_4$ binding pattern.

UEA-I faintly reacted with the cilia of ciliated cells (Figure 15a, b). No labelling was observed after removal of *N*-linked oligosaccharides.

Discussion

In mammals, the ampulla of the oviduct is the place where sperm-oocyte interaction and early embryonic development occur. In the present study, lectin histochemical characterization of oligosaccharides in the oviductal ampulla epithelium of oestrus mares shows the presence of sialoglycoconjugates, the existence of a different lectin binding profile between ciliated and non-ciliated (secretory) cells as well as differences in the lectin-binding pattern of non-cilated cells along the epithelium lining the mucosal folds.

The ciliated cells showed cytoplasmic reactivity, from the base to the apical region, with Con A and GSA $I\text{-}B_4$, and variable binding up to negative with DBA, α and tThe supra-nuclear zone of the ciliated cells was also labelled with MAH. These lectins recognize terminal/internal αMan/αGlc, terminal αGal, GalNAcα1,3GalNAcα1,3Galβ1,4Galβ1, 4GlcNAc (Forssman pentasaccharide), Neu5Acα2,

3Galβ1,3 (Neu5Acα2,6)GalNAc (Konami *et al.*, 1994), respectively. The abolition of these reactions after *N*-glycosidase F digestion suggests that these carbohydrates belong to *N*-linked oligosaccharides. The cilia surface reacted with all the lectins used, indicating a complex glycocalyx. The affinity for PNA, RCA_{120}, and SBA increased after sialidase digestion revealing the presence of terminal sialic acid on galactosyl penultimate residues identified with these lectins. WGA recognizes both GlcNAc and sialic acid residues (Debray *et al.,* 1981); but in this investigation it identified only found GlcNAc, since the KOH-sialidase procedure did not modify the labelling. *N*-glycosidase F digestion abolished the cilia reactivity for the lectins MAH, DBA, RCA_{120}, Con A, WGA, GS I-B4 and UEA I lectins. This Thus, compared with the cytoplasm, indicates that the cilia glycocalix proves to contains also specific *N*-linked oligosaccharides both *N*- and *O*-linked oligosaccharides. With respect to the cytoplasm, the cilia also express *N*-linked oligosaccharides with terminal Galβ1,4GlcNAc, terminal/internal βGlcNAc, and terminal αL-Fuc(1,2)βGal1, 4βGlcNAc residues (shown with RCA_{120}, WGA, and UEA I, respectively). The ineffective action of *N*-glycosidase F digestion on the SNA, PNA, KOH-sialidase-PNA, KOH-sialidase-RCA_{120}, SBA, KOH-sialidase-SBA, and HPA binding provides indirect evidence that the cilia glycocalyx contains also O-linked oligosaccharides with terminal Neu5Acα2, 6Gal/GalNAc, Galβ1,3GalNAc, Neu5Ac-Galβ1,3GalNAc, sialic acid-Galβ1,4GlcNAc, sialic acid-αGalNAc, and αGalNAc. These findings indicate that the ciliated cells of the horse ampullar oviduct contain sialylglycoconjugates as well as the previously demonstrated galactosyl glycoconjugates (Ball *et al.*, 1997). Sialylglycoproteins have also been also found in cilia of the ampulla oviduct of humans (Schulte *et al.*, 1985) and rabbits (Menghi *et al.*, 1995). Sialoglycoconjugates provide a negative charge to ciliated cells glycocalyx. The presence of a negatively charged glycocalyx of ciliated cells in the oviduct of rabbits (Norwood *et al.*, 1978) and humans (Kiss *et al.*, 1998) has been considered the basis of the electrostatic interaction between cilia and oocyte-cumulus cell complexes as well as for oocyte pickup and transport. In addition, sialic acid residues might keep the cilia separated from one other, maintain ciliary motility (Ito *et al.*, 1990), and provide the progression of capacitated spermatozoa (Hunter *et al.*, 1991).

The whole cytoplasm was reactive with DBA, Con A and GSA I-B_4 in the majority of non-ciliated cells. The application of *N*-glycosidase F abolished Con A and GSA I-B_4 affinity, not DBA affinity, thus indicating the presence of O-linked (mucin-type) glycans with terminal Forssman pentasaccharides and *N*-linked oligosaccharides with terminal/internal αMan/αGlc and internal terminal αGal residues. A few scattered non-ciliated cells showed the whole cytoplasm labelled with SNA, HPA, and, after KOH-sialidase treatment, also with PNA and SBA. *N*-glycosidase F treatment annulled the KOH-sialidase-SBA reaction suggesting that the cytoplasm of these few non-ciliated cells contains *N*-linked oligosaccharides terminating with sialic acid-αGalNAc and *O*-linked glycans ending with Neu5Acα2, 6Gal/GalNAc, Neu5Ac-Galβ1, 3GalNAc, and αGalNAc. The non-ciliated cells also showed supra-nuclear granules reactive to MAH, SNA, PNA after the KOH-sialidase procedure, and DBA. *N*-glycosidase F application only affected the MAH binding (the reaction was abolished). This indicates that these granules contain *N*-linked oligosaccharides with Neu5acα2,3Galβ1,3 (Neu5Acα2,6)GalNAc and mainly *O*-linked glycans with Neu5Acα2,6Gal/GalNAc, Neu5Ac-Galβ1, 3GalNAc, and Forssman pentasaccharide. As revealed by electron microscope studies (Schulte *et al.*, 1985; Abe 1996), the morphological basis of the supra-nuclear lectin affinity could be the rough endoplasmic reticulum (RER), the secretory granules and the Golgi zones. In particular, the affinity of supra-nuclear granules to MAH, SNA, KOH-sialidase-PNA, and DBA could be considered as histochemical evidence for the Golgi zones because the O-linked glycosylation and the sialylation occur in the Golgi apparatus (Schauer, 1985).

Scattered epithelial non-ciliated cells showed apical protrusions staining with KOH-sialidase RCA_{120}, SBA (affinity increased after KOH-sialidase), DBA, and HPA. Since *N*-glycosidase F incubation did not modify the lectin -binding, it can be suggested that these apical protrusions express terminal sialic acid-Galβ1,4GlcNAc, αGalNAc, sialic acid-αGalNAc, and Forssman pentasaccharide in *O*-linked oligosaccharides. Although the apical protrusions have been interpreted as a feature of epithelial degeneration in cows (Abe and Oikawa, 1993) and of epithelial regeneration in pigs (Walter and Bavdek, 1997), in horses they could be considered as a feature of probable apocrine secretion because

we observed them duringoccurring in the oestrus phase. In mammals the glycoconjugates contained in ampulla secretory-glycoproteins play various roles such as constituting the glycocalyx of the luminal plasmamembrane (Schulte *et al.*,1985), binding to the sperm membrane and enhancing sperm capacitation and ability to fertilize (Boatman and Magnoni, 1995; Funahashi and Day, 1997), increasing spermatozoa viability (Satoh *et al.*, 1995), decreasing polyspermy (McCauley *et al.*, 2001; Kouba *et al.*, 2000). Furthermore, oviductal secretory glycoproteins can add to the zona pellucida and perivitelline space of oviductal oocytes (Gandolfi et al., 1991; Weger and Killian, 1991; Boice *et al.*, 1992; Buhi *et al.*, 1993; El-Mestrah and Kan, 2001), can promote early embryonic cleavage (Gandolfi, 1995; Hill *et al.*, 1996; Kouba *et al.*, 2000), and can remain associated with the embryo until implantation (Malette *et al.*, 1995; O'day-Bowman *et al.*, 1995). Regarding sialoglycoconjugates, an oestrogen-dependent oviductal mucin-type secretory sialoglycoprotein has been revealed in sheep (DeSouza and Murray,1995), and an oviduct-specific mucin-type sialo-glycoprotein important for the maintenance of sperm viability has been found in cattle (Satoh *et al.*, 1995). The secretion of sialylglycoconjugates into the lumen has been related to the stratification of mucoploysaccharide coat components around the blastocyst in the rabbit ampullar oviduct (Menghi *et al.*, 1995). A possible interaction of ampullar sialaglycoconjugates with the mucin-like (capsule) egg coat could occur in horse because fertilized ova are retained at the oviductal ampullary-isthmus junction for as long as 120 hours post-ovulation (Betteridge and Mitchell. 1974), and late morula- to early blastocyst-stage embryos are in transit from the ampulla to the uterus for up to 132 hours post-ovulation (Weber *et al.*, 1991).

The luminal surface of non-ciliated cells showed both *N*-and *O*-linked oligosaccharides. The *N*-linked glycans contain terminal and internal αMan/αGlc, βGlcNAc and terminal αGal. The O-linked oligosaccharides (shown by the ineffective action of *N*-glycosidase F treatment on PNA, RCA_{120}, SBA, DBA, HPA, MAH, SNA, KOH-sialidase-PNA, KOH-sialidase-RCA_{120}, and KOH-sialisade-SBA reactivity) terminate with neutral saccharides such as Galβ1,3GalNAc, Galβ1,4GlcNAc, Forssman pentasaccharide, and αGalNAc (PNA, RCA_{120}, DBA, SBA, HPA labelling) and with sialylated sequences such as Neu5acα2,3Galβ1,3(Neu5Acα2,6) GalNAc, Neu5Acα2,6Gal/GalNAc, Neu5Ac-Galβ1,3GalNAc, Neu5Ac-Galβ1,4GlcNAc, Neu 5Ac-GalNAc (MAH, SNA, KOH-sialidase-PNA, KOH-sialidase-$RCA_{120,}$ and KOH-sialidase-SBA reactivity). These findings confirm that the luminal surface glycocalyx of the non-ciliated ampullar cells contains glycoconjugates with terminal galactosydes (Ball *et al.*, 1997) and, in addition, provide evidence for the presence of sialic acid residues with in sialomucin-type glycans which are more widely expressed than *N*-linked oligosaccharides.

Histochemical evidence of sialoglycoconjugates on the ampulla luminal surface has been found in humans (Schulte *et al.*, 1985), rabbits (Menghi *et al.*, 1995), and monkeys (Jones *et al.*, 2001). At present, the role played by sialylglycoconjugates of the glycocalyx of the luminal surface of the epithelium lining the ampulla oviduct is not known. Mucin glycoproteins expressed at the apical surfaces of epithelia are generally believed to be involved in lubrication of epithelial surfaces and preventing tissue dehydration (Jentoff, 1990; Devine and McKenzie, 1992; Lagow *et al.*,1999), transport of metabolites and ions across the plasmalemma, and hormone-binding (Jeanloz and Codington, 1976; Arenas *et al.*, 1998).

In conclusion, the present histochemical analysis indicates differences in the lectin-binding pattern between CCs ciliated cells and NCs non-ciliated cells of the ampullar oviductal epithelium of the oestrus horse. Furthermore, the different lectin-binding profile observed among the non-ciliated cells could indicate the presence of non-ciliated cell subtypes as well as functional differences along the mucosal epithelium of the mare ampullar oviduct. The presence of galactosides and sialylgalactosides, particularly O-linked types, in non-ciliated cells could be consistent with the roles played by this oviductal region. Sialoglycoconjugates could inhibit intermolecular and intercellular interactions by virtue of their negative charge as well as by acting as the critical ligand recognized by a variety of sialic-acid binding lectins (reviewed by Varki *et al.*1997). Thus, sialylglycoconjugates may represent a crucial component of a ligand which is recognized by the endogenous lectin present on the spermatozoa as well as on the oocyte and/or embryo during flow in the ampulla of the oviduct.

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