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Lectin-staining pattern in extratesticular *rete testis* and *ductuli efferentes* of prepubertal and adult horses

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Summary. This study was undertaken to determine the lectin affinity of the extratesticular *rete testis* and *ductuli efferentes* epithelial cells in adult and prepubertal horses, using ten different lectin horseradish peroxidase conjugates: Con-A, LCA, WGA, GSA-II, SBA, PNA, RCA-I, DBA, UEA-I, and LTA. In some cases, treatments with sialidase and KOH preceded the lectin staining. In sexually mature and immature horses the results showed the presence of different kinds of sialoglycoconjugates with the terminal sialic acid linked to D-GalNAc and ß-D-Gal residues in the *rete testis*. In the apical surface and cytoplasm of epithelial cells lining the *ductuli efferentes* of the adult horse, glycoconjugates with α-D-Man and/or α-D-Glc, GlcNAc, D-GalNAc and ß-D-Gal residues were evidenced, whereas in the prepubertal horse only the apical surface of the *ductuli efferentes* epithelial cells resulted reactive toward some lectins. The differences observed in the presence of glycoconjugates between adult and prepubertal horse *ductuli efferentes*, suggest a hormonal control of the function of these tracts of the post-testicular ducts.

Key words: Horse, *Ductuli efferentes*, *Rete testis*, Glycoconjugates, Lectins

Introduction

The horse *rete testis* can be divided into the septal, mediastinal and extratesticular portions (Amann et al., 1977). The last portion is the source of 11-18 *ductuli efferentes* (Hemeida et al., 1978). It has been well documented that testicular fluid transporting spermatozoa out of the testis undergoes many changes due to the absorptive and secretory functions of the epithelium lining the post-testicular ducts (Bedford, 1975; Hamilton, 1975; Cooper, 1990). In our previous investigations on the epithelial principal cells lining the ductus epididymis, these functions have been histochemically detected (Parillo et al., 1997).

Absorption of material (above all glycoproteins) from the lumen was the major role attributed to the *rete testis* (Morales and Hermo, 1983; Morales et al., 1984) and *ductuli efferentes* in mammals (Johnson et al., 1978; Aureli et al., 1984; Hermo and Morales, 1984; Arrighi et al., 1993, 1994; Ilio and Hess, 1994).

Studies carried out using lectin histochemistry on the epithelium lining the *ductuli efferentes* of mice (Burkett et al., 1987a,b) and humans (Arenas et al., 1996), have evidenced glycoconjugates with different sugar residues. It has been suggested that the reactions obtained in the principal cells of the *ductuli efferentes* are due to the staining of glycoconjugates endocytosed from the lumen and also to secretory material which coats spermatozoa as they pass from the testis to epididymis.

Considering the lack of histochemical evaluations of equine post-testicular ducts, this study was aimed to characterize the glycoconjugates present in the epithelial cells lining the extratesticular *rete testis* and *ductuli efferentes* in prepubertal and adult horses, using lectin-horseradish peroxidase conjugates, combined with potassium hydroxide treatment and sialidase digestion.

Materials and methods

Extratesticular *rete testis* and *ductuli efferentes* were taken from 2- to 6-year-old (n=5) coldblood horses of proven fertility and from 8- to 10-month-old (n=5) foals. The animals had been routinely slaughtered in an abbattoir. Specimens were fixed for 6 h at room temperature by immersion in a solution of 6% mercuric chloride in 1% sodium acetate containing 0.1% glutaraldehyde and were then routinely dehydrated through graded ethanols, cleared in xylene and subsequently embedded in paraffin (Schulte and Spicer, 1985).

Serial 5-μm sections were mounted on albumin-coated slides, deparaffinized and treated with Lugol's solution to remove mercury before all staining procedures. The sections were dipped in 0.3% H_2O_2/methanol for 30 min to inhibit endogenous peroxidase activity and, after washing with PBS, were incubated in a moist chamber for 1 h at room

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Table 1. Lectins used and corresponding carbohydrate binding.

SOURCE OF LECTIN	ACRONYM	NOMINAL SPECIFICITY[a]	LECTIN CONCENTRATION (μg/ml)
Arachis hypogaea	PNA	ß-D-GAL-(1→3)-D-GalNAc	40 μg/ml
Griffonia simplicifolia	GSA-II	α and ß GlcNAc	50 μg/ml
Ulex europaeus	UEA-I	α-L-Fuc	20 μg/ml
Lotus tetragonolobus	LTA	α-L-Fuc	20 μg/ml
Dolichos biflorus	DBA	α-D-GalNac	10 μg/ml
Glycine max	SBA	α-D-GalNac>ß-D-GalNAc	10 μg/ml
Triticum vulgare	WGA	GlcNAc>sialic acid	10 μg/ml
Canavalia ensiformis	Con-A	α-D-Man>α-D-Glc	20 μg/ml
Lens culinaris	LCA	α-D-Man>α-D-Glc	50 μg/ml
Ricinus communis	RCA-I	ß-D-Gal-(1→4)-D-GlcNAc	50 μg/ml

[a]: ß-D-Gal: ß-D-galactose; D-GalNAc: D-N-acetylgalactosamine; α-D-GalNAc: α-D-N-acetylgalactosamine; ß-D-GalNAc: ß-D-N-acetylgalactosamine; GlcNAc: N-acetylglucosamine; α-L-Fuc: α-L-fucose; α-D-Man: α-D-mannose; α-D-Glc: α-D-glucose

temperature, with a solution of horseradish peroxidase (HRP)-conjugated lectins (Sigma Chemical Co., St. Louis, MO) in 0.1M PBS, pH 7.2, containing 0.1mM $CaCl_2$, $MgCl_2$ and $MnCl_2$. The sections were rinsed briefly with PBS and the HRP was revealed by a diaminobenzidine-hydrogen peroxide (DAB-system) substrate medium. Plant lectins conjugated with HRP used in this research, along with their hapten sugars and their optimal concentration, are reported in Table 1.

Negative controls for the lectin labelling were run treating the sections as above with the addition of 0.2/0.4M hapten sugars to the HRP-conjugated lectin solutions.

An additional control was performed by dipping the sections in DAB system without lectins in order to evidence endogenous peroxidase activity in the tissue samples.

WGA, SBA, PNA, DBA and RCA-I stainings were also preceded by the incubation of adjacent sections in a solution containing 0.86 IU/ml of type V neuraminidase (sialidase) from *Clostridium perfringens* (Sigma Chemical Co., St. Louis, MO), in 0.1M sodium acetate buffer, pH 5.3, at 37 °C for 18h. Sialic acid residues with *O*-acetyl substituents at C-4 resisted sialidase but were cleaved after the removal of the acyl groups by saponification, which was performed by immersing the sections in a 1% solution of potassium hydroxide in 70% ethanol for 20 min at room temperature prior to staining (Schulte and Spicer, 1985). Controls for the enzymic digestion were provided by sections exposed to the buffer in which the enzyme was dissolved.

Results

In both the prepubertal and adult horses the extratesticular *rete testis* and the *ductuli efferentes* were lined by a cuboidal and pseudostratified columnar epithelium respectively; the transition between *rete testis* and *ductuli efferentes* was abrupt, evidenced by the epithelium changing from low cuboidal to columnar. The lectin binding profiles of epithelial cells lining the *rete testis* and *ductuli efferentes* in prepubertal and adult horses are reported in Tables 2 and 3.

Ductuli efferentes

The lectin binding pattern to the apical surface and microvilli of epithelial principal cells lining the *ductuli efferentes* were similar in both prepubertal and adult horses with the exception of the lectins specific for α-fucose. In fact, in the adult animals LTA- (Fig. 1) and UEA-I-binding sites were localized in the apical surface of principal cells, whereas in the immature horses UEA-I reacted as in the adults but LTA resulted negative.

In the prepubertal horses, the cytoplasm of principal cells lining the efferent ducts was always unreactive (Fig. 2); conversely, in the adults it showed numerous lectin binding sites (Table 3). In particular, granular material was stained moderately with Con-A (Fig. 3) and weakly with LCA (Fig. 4) and WGA. A diffuse reaction, with different intensity, was observed with SBA (Fig. 5)

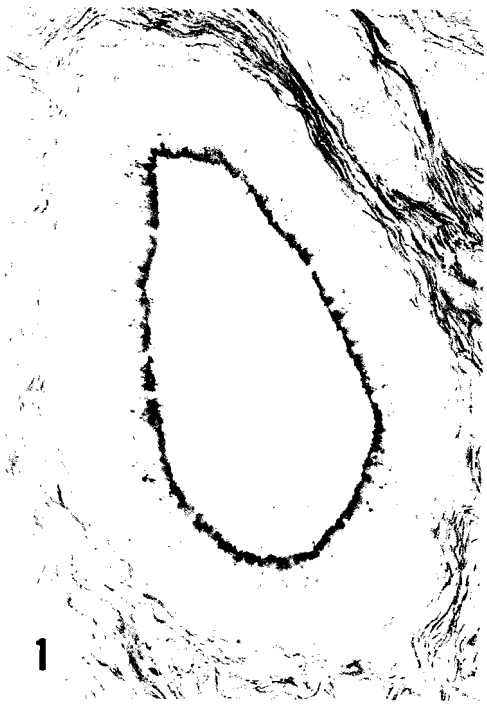

Fig. 1. Efferent ducts of the adult horse. LTA-HRP staining. Only the apical surface of epithelial cells appears moderately positive. x 280

and DBA (Fig. 6). Neither PNA nor RCA-I lectins showed a homogeneous reaction pattern in all the fields of the same section, where the majority of the *ductuli efferentes* showed moderate apical and weak cytoplasmic staining of epithelial cells (Fig. 7a), while in a minor number of *ductuli efferentes* only the apical surface resulted moderately reactive (Fig. 7b). Enzymic degradation with sialidase did not change the affinity of the cytoplasm principal cells towards WGA, SBA, DBA,

Table 2. Lectin binding pattern to the epithelial cells of the *rete testis* and *ductuli efferentes* in prepubertal horse.

LECTINS AND TREATMENTS	*RETE TESTIS* Apical surface	*DUCTULI EFFERENTES* Apical surface and microvilli
Con-A	-	+++
LCA	-	+
WGA	-	++
NEU-WGA[a]	-	++
KOH-NEU-WGA[b]	-	++
GSA-II	-	-
SBA	-	+++
NEU-SBA[c]	++	+++
KOH-NEU-SBA[d]	++	+++
PNA	-	++
NEU-PNA[e]	+	++
KOH-NEU-PNA[f]	+	++
RCA-I	-	++
NEU-RCA-I[g]	+	++
KOH-NEU-RCA-I[h]	+	++
DBA	-	+++
NEU-DBA[i]	-	+++
KOH-NEU-DBA[l]	-	+++
UEA-I	-	++*
LTA	-	-

(+) and (-) indicate staining intensity on a subjective scale that attributes (-) to negative reaction and (+++) to strong reaction. [a,c,e,g,i]: NEU-WGA/SBA/PNA/RCA-I/DBA: neuraminidase treatment followed by WGA, SBA, PNA, RCA-I, DBA incubation, respectively. [b, d, f, h, l]: KOH-NEU-WGA/SBA/PNA/RCA-I/DBA: potassium hydroxide and neuraminidase digestion followed by WGA, SBA, PNA, RCA-I, DBA incubation, respectively. *: Microvilli were negative.

Table 3. Lectin binding pattern to the *rete testis* and *ductuli efferentes* epithelial cell in adult horse.

LECTINS AND TREATMENTS	*RETE TESTIS*	*DUCTULI EFFERENTES*	
	Apical surface	Apical surface and microvilli	Cytoplasm
Con-A	-	+++	++#
LCA	-	+	+#
WGA	+	++	+#
NEU-WGA[a]	+	++	+#
KOH-NEU-WGA[b]	+	++	+#
GSA-II	-	-	-
SBA	-	+++	+++
NEU-SBA[c]	+++	+++	+++
KOH-NEU-SBA[d]	+++	+++	+++
PNA	-	++	+
NEU-PNA[e]	+++	++	+
KOH-NEU-PNA[f]	+++	++	+
RCA-I	-	++	+
NEU-RCA-I[g]	+++	++	+
KOH-NEU-RCA-I[h]	+++	++	+
DBA	-	+++	+
NEU-DBA[i]	-	+++	+
KOH-NEU-DBA[l]	-	+++	+
UEA-I	-	++*	-
LTA	-	++*	-

(+) and (-) indicate staining intensity on a subjective scale that attributes (-) to negative reaction and (+++) to strong reaction. [a,c,e,g,i]: NEU-WGA/SBA/PNA/RCA-I/DBA: neuraminidase treatment followed by WGA, SBA, PNA, RCA-I, DBA incubation, respectively. [b, d, f, h, l]: KOH-NEU-WGA/SBA/PNA/RCA-I/DBA: potassium hydroxide and neuraminidase digestion followed by WGA, SBA, PNA, RCA-I, DBA incubation, respectively. #: Granular material. *: Microvilli were negative.

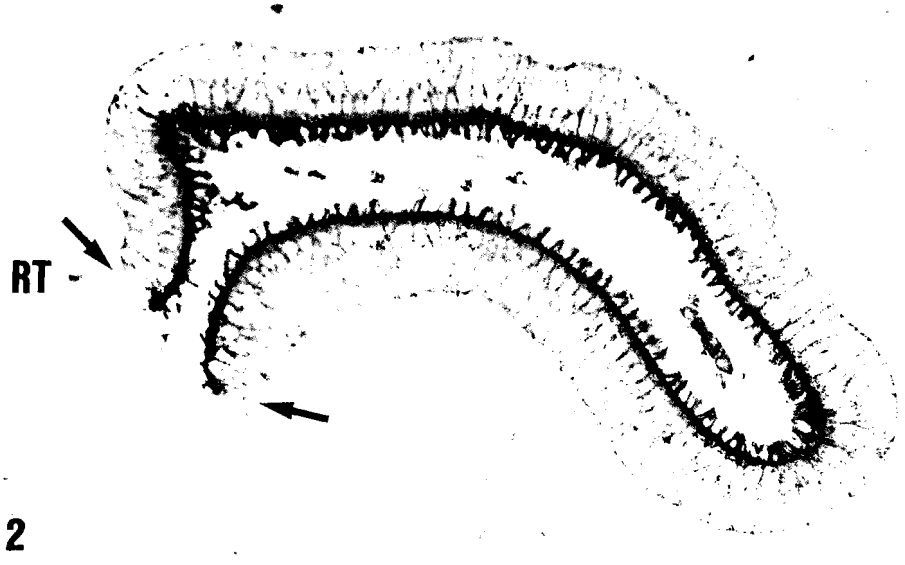

Fig. 2. Extratesticular *rete testis* and *ductuli efferentes* of the prepubertal horse. Con-A-HRP staining. The *rete testis* (RT) is unreactive, whereas the apical surface of the epithelium lining the *ductuli efferentes* evidences a strong reaction. x 330

PNA and RCA-I, even after potassium hydroxide treatment.

Rete testis

The reactivity of the *rete testis* epithelial cells resulted very similar in both the prepubertal and adult horses. However, WGA reactive sites were observed only in the adult animals; PNA (Fig. 8), SBA, DBA and RCA-I became positive only after cleavage of the terminal sialic acid with sialidase in both the adult and prepubertal horses. Deacetylation with KOH did not elicit new binding sites.

Staining control

None of control staining procedures disclosed appreciable reactivity at any of the sites described in the

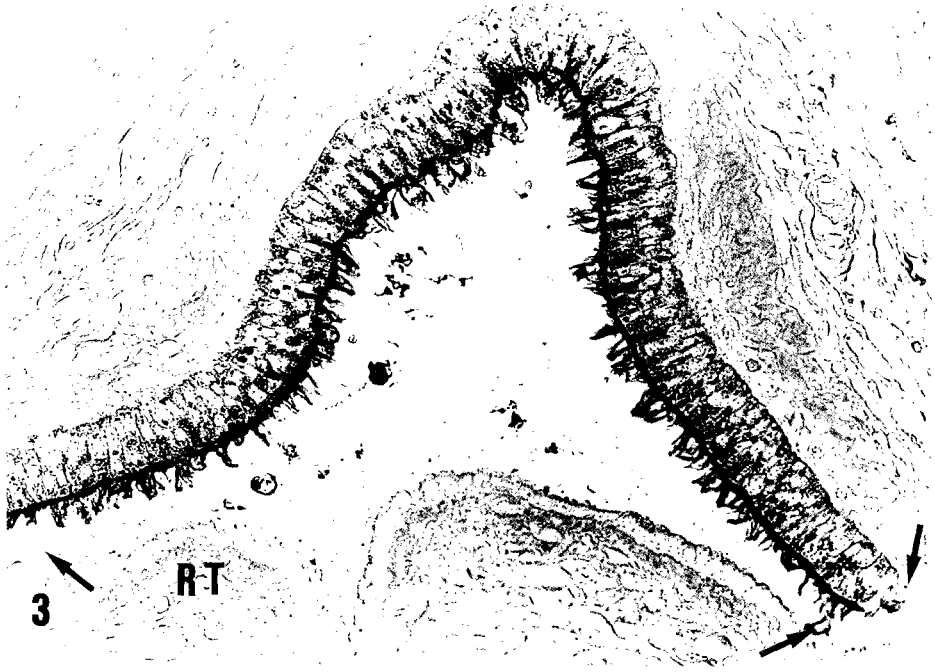

Fig. 3. Extratesticular *rete testis* and efferent ducts of the adult horse. Con-A-HRP staining. The *rete testis* (RT) results unreactive, whereas the apical surface of the epithelium lining the *ductuli efferentes* is strongly stained and the cytoplasm of the same cells presents a moderately stained material. Note the abrupt epithelial transitions (arrows, see also Figs. 2, 3, 7). x 290

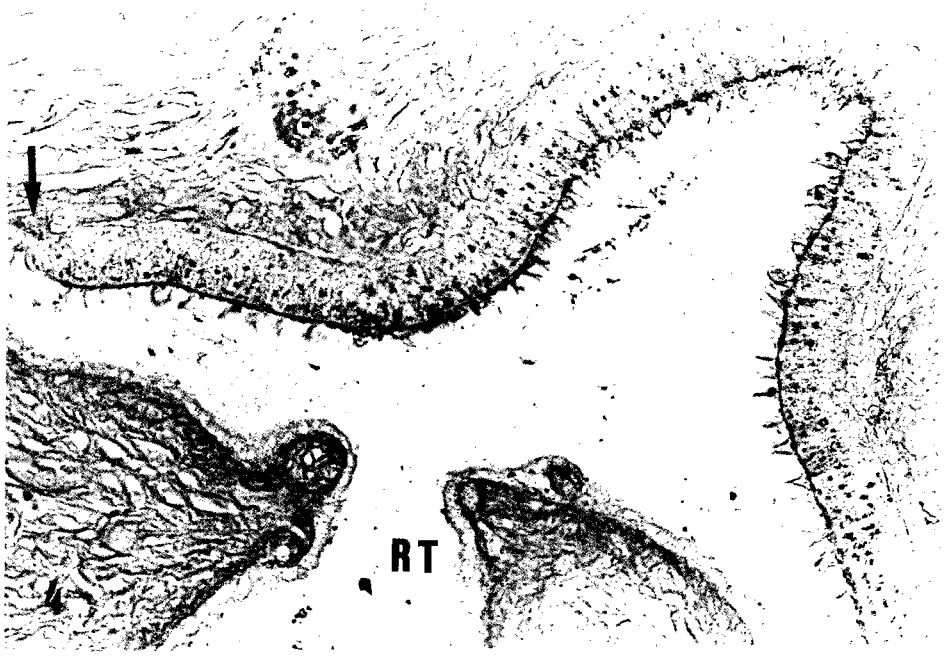

Fig. 4. Extratesticular *rete testis* and efferent ducts of the adult horse. LCA-HRP staining. The *rete testis* (RT) is unstained. The apical surface of the cells lining the *ductuli efferentes* is weakly stained and the cytoplasm of the same cells shows weakly stained granules. x 290

epithelial cells lining the *rete testis* and *ductuli efferentes* of immature and adult horses (Fig. 9).

Discussion

The lectin-staining pattern of equine *rete testis* and *ductuli efferentes* observed in this study showed some differences from those revealed in other species such as mice (Burkett et al., 1987a,b) and humans (Arenas et al., 1996).

Fig. 5. Efferent ducts of the adult horse. SBA-HRP staining. The apical surface and the cytoplasm of epithelial cells evidences a strong affinity for this lectin. x 330

In adult horses the apical surface of the *rete testis* epithelial cells reacted weakly with WGA and strongly with SBA, PNA and RCA-I after sialidase digestion, suggesting the presence of GlcNAc and of terminal sialic acid linked to D-GalNAc and ß-D-Gal residues, respectively. In prepubertal horses the pattern of lectin staining was similar to that observed in adults but WGA negativity related to the absence of GlcNAc was evidenced. The apparent discrepancy between WGA negativity and the presence of sialic acid linked to GalNAc and ß-Gal, revealed by sialidase digestion, could be due to the low level of sialic acid and to the scarce affinity of this lectin for such an acid (Monsigny et al., 1980).

In the adult and sexually immature horses, the apical surface and microvilli of the *ductuli efferentes* epithelial cells stained with almost all the lectins used, with the exception of UEA-I which stained only the apical surface, and GSA-II which resulted negative. In addition, LTA binding sites were revealed only on the apical surface of the epithelial cells of adult animals; in the prepubertal horses, the positivity of UEA-I and the negativity of LTA indicate that fucose is linked to D-Gal but not to GlcNAc.

D-Gal, α-D-GalNAc, α-Fuc and α-Glc residues were also observed in the apical surface and microvilli of mouse *ductuli efferentes* by Burkett et al. (1987a,b), whereas only GlcNAc residues were reported in human efferent ducts by Arenas and collegues (1996).

The positivity of the apical surface and microvilli in immature horses suggests that these glycoconjugates are structural and not related to endocytotic activity; additionally, the few differences observed between prepubertal and adult animals at these levels indicate small structural changes during sexual maturation. Some authors (Jeanloz and Codington, 1976; Schulte and

Fig. 6. Extratesticular *rete testis* and efferent ducts of the adult horse. DBA-HRP staining. The *rete testis* (RT) is unstained, whereas the *ductuli efferentes* shows a reaction which is strong in the apical surface and weak in the cytoplasm of epithelial cells. x 350

Spicer, 1992) suggest that glycoconjugates and terminal sialic acid play a role in several functions, such as protection of cells from dehydration, regulation of transcellular movement of metabolites and ions across the plasmalemma, and even hormone binding.

In the adult horses, the identification of Con-A-, LCA-, WGA-, SBA-, DBA-, PNA- and RCA-I- positive sites in the cytoplasm of the efferent duct epithelial cells, can be ascribed to the presence of glycoproteins having α-D-Man and/or α-D Glc, GlcNAc, D-GalNAc and ß-D-Gal residues.

WGA lectin is reactive towards GlcNAc residues situated in the internal and terminal positions (Allen et al., 1973; Accilli et al., 1992), whereas GSA-II only

Fig. 7. Efferent ducts of the adult horse. PNA-HRP staining. **a.** Most of the tubules show a moderate reaction in the apical surface and a weak reaction in the cytoplasm. **b.** A minor number of tubules appear moderately reactive only in the apical surface. a, x 350; b, x 400

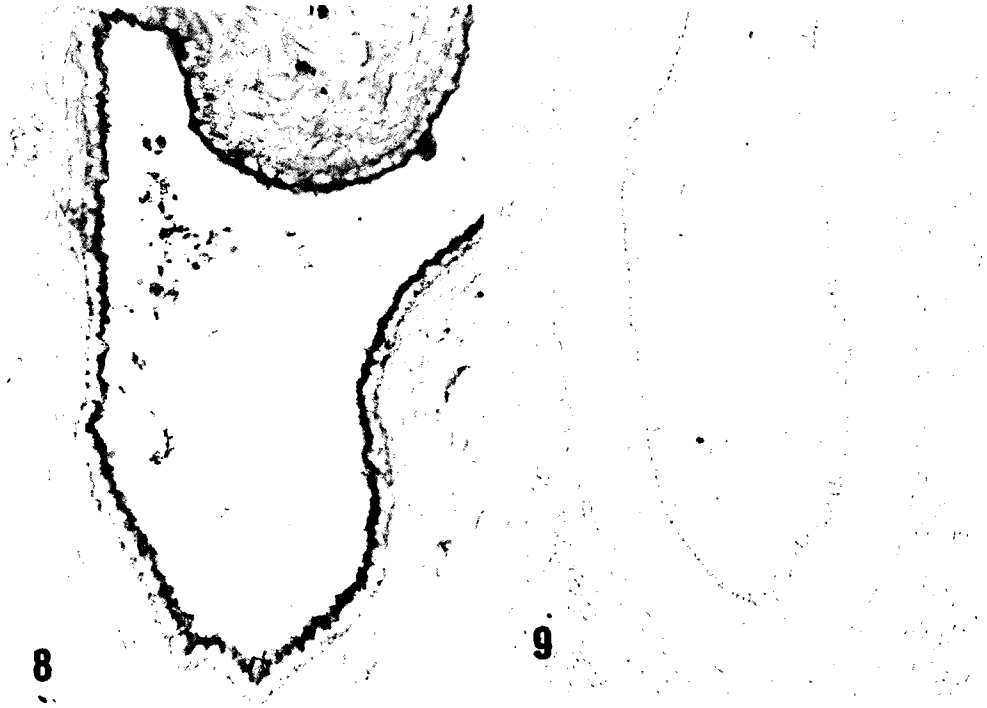

Fig. 8. Extratesticular *rete testis* of the adult horse. Sialidase/PNA-HRP staining. Following the removal of sialic acid the apical surface results strongly stained. x 290

Fig. 9. *Ductuli efferentes* of the adult horse. DBA-HRP with 0.2M D-GalNAc. The staining is completely inhibited. x 330

recognizes terminal GlcNAc. The lack of GSA-II staining may be correlated with the absence of terminal GlcNAc which, on the other hand, seems to occupy an internal position, as revealed by WGA positivity. In the case of the present work sialic acid did not compete with GlcNAc for WGA since sialidase treatment, even after saponification, did not influence WGA labeling in *ductuli efferentes* epithelial cells.

In the cytoplasm of the *ductuli efferentes* epithelial cells the presence of more binding-sites for SBA than for DBA indicated the presence of D-GalNAc in these cells, above all in the ß-anomeric form. Indeed, SBA and DBA are lectins with similar nominal specificity but DBA shows a clear preference for α-linked-D-GalNAc, in contrast to SBA which has no anomeric specificity.

PNA and RCA-I lectins were weakly reactive in the cytoplasm of the epithelial cells lining the *ductuli efferentes*; this can be ascribed to the presence of glycoproteins having the terminal disaccharides ß-D-Gal-(1-3)-D-GalNAc and ß-D-Gal-(1-4)-D-GlcNAc, respectively. Neuraminidase degradation, even after saponification, did not engender the affinity of SBA, DBA, PNA and RCA-I, indicating the absence or the presence of only low levels of the terminal sialic acid residues in the cytoplasm of epithelial cells lining the *ductuli efferentes*.

It is well established that absorption and degradation of luminal fluids are the major roles attributed to the *ductuli efferentes* in several mammalian species (Ilio and Hess, 1994; Stoffel and Friess, 1994). In fact, more than 90% of *rete testis* fluid is reabsorbed in the efferent ducts. Ultrastructural studies indicate that both ciliated and non ciliated epithelial cells of equine *ductuli efferentes* have a well developed endocytotic apparatus which is also capable of spermatozoa phagocytosis (Aureli et al., 1984; Arrighi et al., 1994). The homogeneous lectin-stained material observed in the cytoplasm of epithelial cells lining *ductuli efferentes* may represent endocytosis vescicles containing glycoproteins coming from the testicular fluid. The correspondence of the lectin staining pattern between the cytoplasm of epithelial cells and the luminal material present in both efferent ducts (Fig. 7a) and seminiferous tubules (unpublished data) supports this hypothesis.

Even if a secretory activity of these cells cannot be totally excluded, previous morphological investigations in domestic Equidae revealed a reduced synthetic apparatus (Aureli et al., 1984; Arrighi et al., 1994). Moreover, we did not observe any increase in lectin affinity of spermatozoa during their transit through the efferent ducts in contrast with the results obtained by Burkett et al. (1987a,b) in mouse where the principal cells of the *ductuli efferentes* secrete sperm-coating substances.

The absence of reactivity with PNA and RCA-I in some tracts of *ductuli efferentes* within the same section suggests that the ductuli are probably not synchronised in their activity. However, other studies, above all biochemical investigations, are needed to confirm this hypothesis and to establish why this phenomenon involves only these two lectins.

The granular material detected in the cytoplasm of the epithelial cells lining the *ductuli efferentes* and stained with Con-A, LCA and WGA might represent lipofuscin granules and residual bodies in general that were revealed to be present in large amounts and also intensely positive to the PA-TCH-SP reaction for polysaccharide complexes (Aureli et al., 1984). Negative control with DAB system and the inhibition of endogenous peroxidase activity prior to lectin incubation allow us to exclude aspecific staining due to peroxidase activity inside the lysosomes which was instead observed by Arenas et al. (1996) in human efferent ducts.

The greatest differences of lectin stainings between immature and sexually mature animals were observed in the *ductuli efferentes*. In the adult horse we evidenced the accumulation of large amounts of lectin-labelled material within the epithelial cells lining these ducts whereas in the prepubertal animals only the apical surface of the epithelium lining the *ductuli efferentes* showed reactivity toward some lectins. The cytoplasm staining in adults could be related to the development of the endocytotic apparatus for absorption and degradation of luminal fluids. These results may be explained by the fact that the histological and histochemical features of these ducts are greatly modified by sexual hormones (Turner, 1991; Ilio and Hess, 1994). However, it is still being discussed whether luminal androgens are only essential for the regulation of epithelial structure as determined by bilateral castration and ligation of the ductuli in the bull (Goyal and Hrudka, 1980) or for the mantainance of the reabsorbitive apparatus as observed by Gray et al. (1983) following ligation of the ductuli in the goat. It is also interesting to note that in mule, where spermatozoa production is lacking but the level of androgen is normal, the epithelium of *ductuli efferentes* is well developed, but there is a paucity of residual bodies and lipofuscinic granules (Arrighi et al., 1991).

In conclusion, the present findings indicate that lectins represent a suitable histochemical method for characterising glycoconjugates in the epithelial cells lining the *rete testis* and *ductuli efferentes* in adult and prepubertal horses. Current and previous (Parillo et al., 1997) data indicate that the distribution pattern of glycoconjugates in prepubertal and adult horse post-testicular duct epithelial cells could be under androgen control.

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References

Accili D., Menghi G., Bondi A.M. and Scocco P. (1992). Glycoconjugate composition of mammalian parotid glands elucidated in situ by lectins and glycosidases. Acta Histochem. 92, 196-206.

Allen A.K., Neuberger A. and Sharon N. (1973). The purification, compositon and specificity of wheat germ agglutinin. Biochem. J. 131, 155-159.

Amann R.P., Johnson L. and Pickett B.W. (1977). Connection between the seminiferous tubules and the *ductuli efferentes* in the stallion. Am. J. Vet. Res. 38, 1571-1579.

Arenas M.I., de Miguel M.P., Bethencourt F.R., Fraile B., Royuela M. and Paniagua R. (1996). Lectin histochemistry in the human epididymis. J. Reprod. Fertil. 106, 313-320.

Arrighi S., Romanello M.G. and Domeneghini C. (1991). Morphological examination of epididymal epithelium in the mule (*E. hinnus*) in comparison with parental species (*E. asinus* and *E. caballus*). Histol. Histopathol. 6, 325-337.

Arrighi S., Romanello M.G. and Domeneghini C. (1993). Ultrastructure of epididymal epithelium in Equus caballus. Ann. Anat. 175, 1-9.

Arrighi S., Romanello M.G. and Domeneghini C. (1994). Ultrastructure of epithelium that lines the *ductuli efferentes* in domestic equidae, with particular reference to spermatophagy. Acta Anat. 149, 174-184.

Aureli G., Arrighi S. and Romanello M.G. (1984). Ultrastructural and cytochemical study on the epithelium lining *ductuli efferentes* in *Equus asinus*. Basic Appl. Histochem. 28, 101-115.

Bedford J.M. (1975). Maturation, transport and fate of spermatozoa in the epididymis. In: Handbook of physiology. Sect. 7: Endocrinology Vol. V: Male reproductive system. Am. Physiol. Soc. Washington. pp 303-317.

Burkett B.N., Schulte B.A. and Spicer S.S. (1987a). Histochemical evaluation of glycoconjugates in the male reproductive tract with lectin-horseradish peroxidase conjugates: I. Staining of principal cells and spermatozoa in the mouse. Am. J. Anat. 178, 11-22.

Burkett B.N., Schulte B.A. and Spicer S.S. (1987b). Histochemical evaluation of glycoconjugates in the male reproductive tract with lectin-horseradish peroxidase conjugates: II. Staining of ciliated cells, basal cells, flask cells, and clear cells in the mouse. Am. J. Anat. 178, 23-29.

Cooper T.G. (1990). In defense of a function for the human epididymis. Fert. Ster. 54, 965-975.

Goyal H.O. and Hrudka F. (1980). The resorptive activity in the bull efferent ductules. A morphological and experimental study. Andrologia 12, 404-414.

Gray B.W., Brown B.G., Ganjam V.K. and Whitesides J.F. (1983). Effect of deprival of *rete testis* fluid on the morphology of *ductuli efferentes*. Biol. Reprod. 29, 525-534.

Hamilton D.W. (1975). Structure and function of the epithelium lining the *ductuli efferentes*, ductus epididymis and ductus deferens in the rat. In: Handbook of physiology. Sect. 7: Endocrinology. Vol.V: Male reproductive system. Am. Physiol. Soc. Washington. pp 259-301.

Hemeida N.A., Sach W.O. and McEntee K. (1978). *Ductuli efferentes* in the epididymis of boar, goat, ram, bull and stallion. Am. J. Vet. Res. 39, 1892-1900.

Hermo L. and Morales C. (1984). Endocytosis in nonciliated epithelial cells of the *ductuli efferentes* in the rat. Am. J. Anat. 171, 59-74.

Ilio K.Y. and Hess R.A. (1994). Structure and function of the *ductuli efferentes*: a review. Microsc. Res. Tech. 29, 432-467.

Jeanloz K.W. and Codington J.F. (1976). The biological role of sialic acid at the surface of the cell. In: Biological roles of sialic acid. Rosenberg A. and Schengrund C.L. (eds). Plenum Press. New York, London. pp 201-238.

Johnson L., Amann R.P. and Pickett B.W. (1978). Scanning electron microscopy of the epithelium and spermatozoa in the Equine excurrent duct system. Am. J. Vet. Res. 39, 1428-1434.

Monsigny M., Roche A.C., Sene C., Maget-Dana R. and Delmotte F. (1980). Sugar-lectin interactions: how does wheat-germ agglutinin bind sialoglycoconjugates? Eur. J. Biochem. 104, 147-153.

Morales C. and Hermo L. (1983). Demonstration of fluid-phase endocytosis in epithelial cells of the male reproductive system by means of horseradish peroxidase-colloidal gold complex. Cell Tissue Res. 230, 503-510.

Morales C. Hermo L. and Clermont Y. (1984). Endocytosis in epithelial cells lining the *rete testis* of the rat. Anat. Rec. 209, 185-195.

Parillo F., Stradaioli G., Verini Supplizi A. and Monaci M. (1997). Detection of glycoconjugates in the *ductus epididymis* of the prepubertal and adult horse by lectin histochemistry. Histol. Histopathol. 12, 691-700.

Schulte B.A. and Spicer S.S. (1985). Histochemical methods for characterizing secretory and cell surface sialoglycoconjugates. J. Histochem. Cytochem. 33, 427-438.

Schulte B.A. and Spicer S.S. (1992). Diversity of cell glycoconjugates shown histochemically: a perspective. J. Histochem. Cytochem. 40, 1-38.

Stoffel M.H. and Friess A.E. (1994). Morphological characteristics of Boar *ductuli efferentes* and epididymal duct. Microsc. Res. Tech. 29, 411-431.

Turner T.T. (1991). Spermatozoa are exposed to a complex microenvironments as they traverse the epididymis. Ann. NY Acad. Sci. 637, 364-383.

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