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Detection of glycoconjugates in the *ductus epididymis* of the prepubertal and adult horse by lectin histochemistry

F. Parillo[1], G. Stradaioli[2], A. Verini Supplizi[3] and M. Monaci[2]

Istituti di [1]Anatomia degli Animali Domestici, [2]Ostetricia e Ginecologia e

[3]Produzioni Animali, Facoltà di Medicina Veterinaria, Università degli Studi di Perugia, Perugia, Italy

Summary. This paper describes an approach for studying the structure of glycoconjugates found in the principal cells lining the epididymal duct in adult and prepubertal horses, using ten different lectin horseradish conjugates: Con-A, LCA, WGA, GSA-II, SBA, PNA, RCA-I, DBA, UEA-I, and LTA. Saponification and sialidase procedures, followed by lectin binding, were employed to visualize the distribution and to reveal the sequence of sialoglycoconjugates in *ductus epididymis*. In the adult horse the results demonstrated variations in the content and distribution of glycosidic residues of glycoconjugates in different epididymal regions (*caput, corpus, cauda*) and *vas deferens*, suggesting that each epididymal segment has a specific function. In particular, staining of the Golgi-zone in the principal cells lining *corpus epididymis* was interpreted as evidence for synthesis and secretion of glycoconjugates and sialoglycoconjugates. In the prepubertal horse, only the glycocalyx of the epithelial cells lining the epididymal duct showed reactivity toward the different lectins used, suggesting hormonal regulation of the epididymis activity. Additionally, the heterogeneity of the lectin staining pattern of the adult horse epididymis reported in this investigation also suggests the existence of different functional segments along the epididymal duct.

Key words: Horse, *Ductus epididymis,* Glycoconjugates, Lectins

Introduction

Morphological studies have allowed the identification of distinct regions (*caput, corpus, cauda* and *vas deferens*) within the epididymal duct of the horse (Johnson et al., 1978; Arrighi et al., 1991, 1993). These regions show clear differences in their histological and ultrastructural characteristics, suggesting different functional significance. During its passage through these tracts, the fluid secreted in the seminiferous tubules is modified as a result of the absorptive and secretory activity of epithelial cells lining the epididymis and *efferent ductules* (Bedford, 1975; Hamilton, 1975). In addition, spermatozoa complete their maturation in different segments of the epididymis, where they are stored before ejaculation. However, by the time the sperm reaches the *cauda epididymis* it undergoes biochemical and structural modifications (Turner, 1991). Many of these changes are sustained by androgen-dependent processes of the *ductuli efferentes* and epididymal epithelium (Dacheux and Dacheux, 1989); such processes take place at puberty when the epididymis acquires structural and functional properties, in particular the ability to synthesize specific glycoproteins.

Studies carried out in the epididymis of the mouse (Lee and Damjanov, 1984; Arya and Vanha-Pertulla, 1986a; Burkett et al., 1987), rat (Arya and Vanha-Pertulla, 1984, 1986b), bull (Arya and Vanha-Pertulla, 1985a) and man (Arenas et al., 1996) by lectin histochemistry, have identified several glycoconjugates with sugar moieties which appeared to have been synthesized and secreted by epididymal principal cells. Some of these glycoproteins have been characterized in epididymal fluid of bulls (Acott and Hoskins, 1978; Brand et al., 1978; Acott et al., 1979), and rats and hamsters (Moore, 1980). Since a characterization of epididymal glycoconjugates has not been reported in the horse, the aim of this study was to investigate the structure of glycoconjugates of principal cells lining the epididymal duct in sexually immature and adult horses by means of lectin histochemistry.

Materials and methods

Tissue collection

Epididymides, divided into *caput, corpus* and *cauda* and the proximal *vasa deferentia* from 8 to 10-month-old (n=5) and from 2- to 6-year-old (n=5) coldblood horses of proved fertility were obtained from an

Offprint requests to: Dr. F. Parillo, Istituto di Anatomia degli Animali Domestici, Facoltà di Medicina Veterinaria, via San Costanzo 4, 06126 Perugia, Italy

Table 1. Lectins used and corresponding carbohydrate specificities.

SOURCE OF LECTIN	ACRONIM	CARBOHYDRATE SPECIFICITIES[a]	LECTIN CONCENTRATION
Arachis hypogaea	PNA	ß-D-Gal-(1→3)-D-GalNAc	40µg/ml
Griffonia simplicifolia	GSA-II	α and ß GlcNAc	50 µg/ml
Ulex europeaus	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	10 µg/ml
Canavalia ensiformis	Con-A	α-D-Man>α-D-Glc	20 µg/mll
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.

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Lectin-histochemistry

The characterization of the glycosidic residues of glycoconjugates was performed by procedures described by Schulte and Spicer (1985). Immediately after slaughter, tissue samples 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 dehydrated through a graded ethyl alcohol series, cleared with xylene, and embedded in paraffin wax. Sections were cut at 5 µm and treated with Lugol's iodine solution prior to staining, in order to remove mercuric precipitates, and were then immersed in 0.3% H_2O_2-methanol solution for 30 min at 20 °C to inhibit endogenous peroxidase activity. After PBS washes, the sections were incubated with a solution of ten different lectin-horseradish peroxidase (HRP) conjugates (Sigma Chemical Co., USA) (Table 1) in 0.1M PBS, pH 7.2, containing 0.1mM $CaCl_2$, $MgCl_2$ and $MnCl_2$ and were placed in a moist chamber for 1 h at room temperature. Excess unbound reagent was removed by washing in PBS and the reaction was then developed in 0.025% diamino-benzidine-0.015% hydrogen peroxide (DAB-H_2O_2) medium, pH 7.0, for 10 min at room temperature.

Enzymatic digestion

To detect the sugar residues penultimate to sialic acid, the terminal sialic acid residues were removed by incubating sections at 37 °C for 12 h in a 0.86 IU/ml solution of type V neuraminidase (sialidase) from *Clostridium perfringens* (Sigma Chemical Co., USA) in 0.1M sodium acetate buffer, pH 5.3.

The sections were then stained as above with SBA, PNA, DBA, RCA-I and WGA. Sialic acid residues with *O*-acetyl substituents at C_4 resisted enzymatic cleavage by sialidase but were rendered digestible with sialidase after removal of acetyl groups by saponification. Saponification 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

Omission of the respective peroxidase-conjugated lectins and/or addition of their appropriate blocking sugars (0.2-0.4 M) prevented the corresponding staining in control sections. As controls for the enzymatic digestion, sections were incubated in the respective enzyme-free buffer solution under the same conditions as in the digestion studies.

Results

In both the adult and prepubertal horse, the wall of the convoluted *ductus epididymis* is formed by a pseudostratified columnar epithelium that is highest in the *caput* and *corpus* with an ensuing gradual decrease in the *cauda* and *vas deferens*. Moreover, the epithelial cells lining the epididymal duct of the sexually immature horse are rather low in all regions and the intertubular connective tissue occupies about half of the surface area of the sections.

The binding patterns displayed by lectins in the principal cells lining the epididymal duct, in both adult and prepubertal horses, are summarized in tables 2 and 3. None of the negative control sections showed positive reactivity to the lectins employed (Fig. 1).

Adult horses

Caput epididymis. In the *caput epididymis* Con-A reacted weakly in the glycocalyx and cytoplasm of principal cells (Fig. 2). LCA-, WGA-, GSA-II-, and DBA-binding sites (Fig. 3) were strongly localized in the glycocalyx and moderately in the supranuclear cytoplasm of the epithelial principal cells. Pretreatment with sialidase did not modify WGA affinity. The glycocalyx showed a strong reaction with SBA, RCA-I and PNA. The binding of these three lectins in the

Table 2. Histologic and cytologic binding of lectins to the epididymal regions and *vas deferens* in adult horse.

LECTINS AND TREATMENTS	CAPUT EPIDIDYMIS		CORPUS EPIDIDYMIS			CAUDA EPIDIDYMIS		VAS DEFERENS	
	Glycocalyx	Cytoplasm	Glycocalyx	Cytoplasm	Golgi zone	Glycocalix	Cytoplasm	Glycocalyx	Cytoplasm
Con-A	-	+	++	+	-	++	++	++	++
LCA	+++	++	++	+	-	++	++	++	++
WGA/NEU-WGA[a]	+++	++	-	-	++	-	-	-	-
GSA-II	+++	++	-	-	-	-	-	-	-
SBA	+++	+	-	-	-	-	-	-	-
NEU-SBA[b]	+++	+	-	-	++	+++	++	-	-
KOH-NEU-SBA[c]	+++	++	+++	+	+++	+++	++	++	-
PNA	+++	-	-	-	++	+++	+++	+++	+++
NEU-PNA[d]	+++	-	-	-	++	+++	+++	+++	+++
KOH-NEU-PNA[e]	+++	++	+++	-	++	+++	+++	+++	+++
RCA-I	+++	+	-	-	-	-	-	-	-
NEU-RCA-I[f]	+++	+	-	-	+++	+	+	-	-
KOH-NEU-RCA-I[g]	+++	++	+++	-	+++	+	+	++	-
DBA	+++	++	-	-	-	++	-	-	-
NEU-DBA[h]	+++	++	-	-	++	++	-	-	-
KOH-NEU-DBA[i]	+++	++	-	-	++	++	-	++	-
UEA-I	++	++	-	-	++	++	-	-	-
LTA	++	++	-	-	-	++	-	-	-

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

Fig. 1. *Corpus epididymis* of adult horse. SBA-HRP with 0.2M D-GalNAc. Staining is completely inhibited. x 320

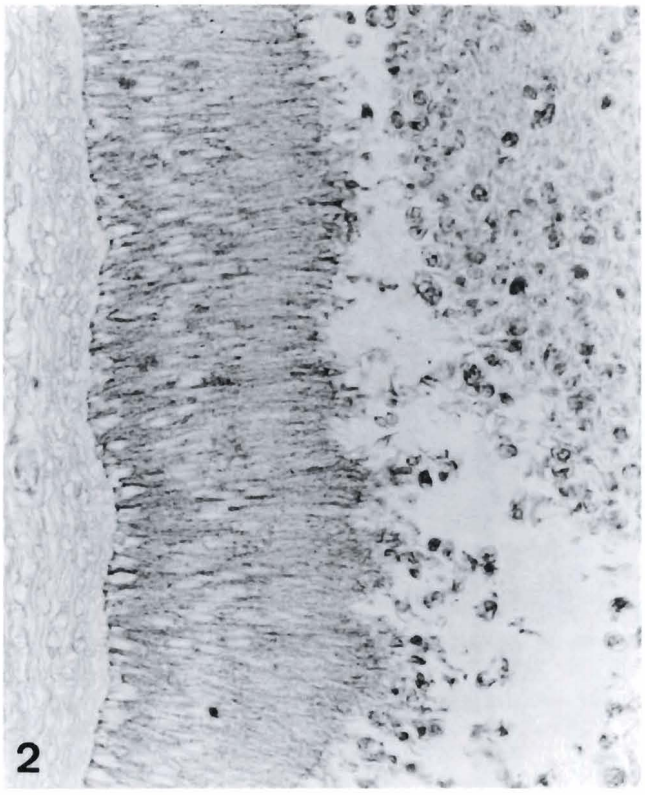


Fig. 2. *Caput epididymis* of adult horse. Con-A-HRP staining. The cytoplasm of epithelial principal cells shows a weak affinity for this lectin. x 320

supranuclear cytoplasm was variously affected by saponification prior to enzymatic degradation. In fact, this treatment induced a greater number of binding sites for SBA (Fig. 4) and RCA-I (Fig. 5). The same treatment promoted the positive reaction of PNA (Fig. 6). UEA-I and LTA (Fig. 7) moderately stained both glycocalyx and the supranuclear cytoplasm of the epithelial principal cells.

Corpus epididymis. The glycocalyx evidenced moderate staining with Con-A (Fig. 8) and LCA and became strongly reactive with SBA, PNA and RCA-I after KOH-sialidase sequence treatment.

The cytoplasm of the epithelial principal cells exhibited weak affinity for Con-A (Fig. 8), LCA and, after potassium hydroxide treatment prior to enzymatic degradation, for SBA. The same cells showed moderate WGA (Fig. 9) and UEA-I reactivity in the Golgi-zone. Neuraminidase degradation failed to modify WGA labeling. The Golgi-zone disclosed strong staining with RCA-I and moderate staining with DBA (Fig. 10) and SBA only following cleavage of sialic acid. In addition, saponification before enzymatic digestion imparted an increase in staining with SBA (Fig. 11), whereas it failed to engender RCA-I and DBA reactivity. The Golgi zone also reacted moderately with PNA (Fig. 12); KOH-sialidase sequence did not elicit new PNA binding-sites.

Cauda epididymis. The glycocalyx showed a reactivity which was weak with sialidase-RCA-I sequence, moderate with Con-A, LCA, DBA, UEA-I, LTA and strong with PNA and sialidase-SBA sequence. The cytoplasm of the principal cells resulted moderately stained with Con-A and LCA and strongly stained with PNA (Fig. 13) additionaly, following the enzymatic removal of sialic acid, it evidenced a moderate and weak affinity for SBA and RCA-I, respectively.

Vas deferens. In the *vas deferens*, the glycocalyx and the cytoplasm of principal cells displayed moderate staining with Con-A and LCA and strong staining with PNA. Pretreatment with KOH-sialidase sequence had the effect of imparting a moderate affinity for SBA, RCA-I and DBA on the glycocalyx of principal cells.

Immature horses

In immature horses, only the glycocalyx of the epithelium lining the *ductus epididymis* exhibited positive reactions of various intensities toward the lectins used (Table 3).

Con-A showed a reaction which was strong in the *caput epididymis* (Fig. 14) and moderate in the *corpus* and *cauda epididymis* and *vas deferens*.

LCA exhibited a moderate staining of the glycocalyx

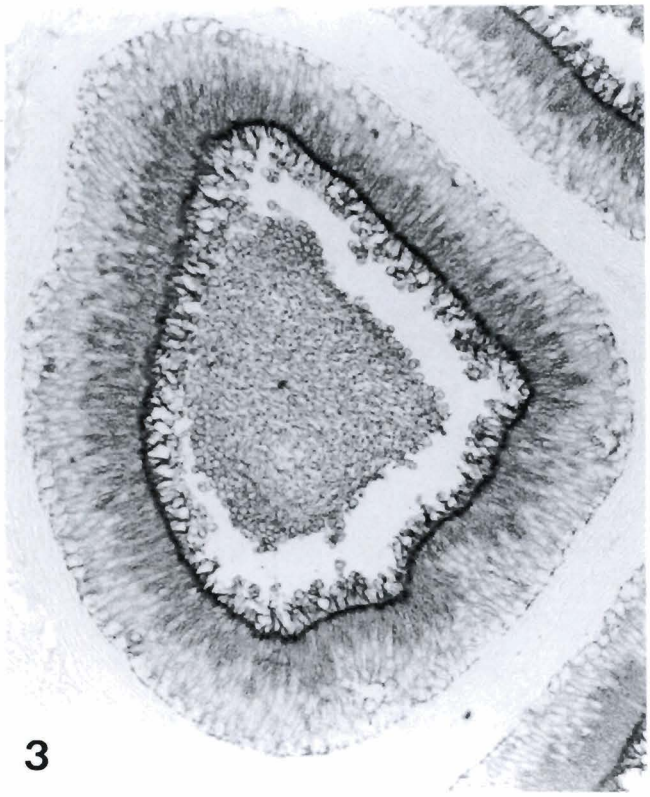


Fig. 3. *Caput epididymis* of adult horse. DBA-HRP staining. Binding sites are strongly localized in the glycocalyx and moderately in the supranuclear cytoplasm of the epithelial cells. x 200

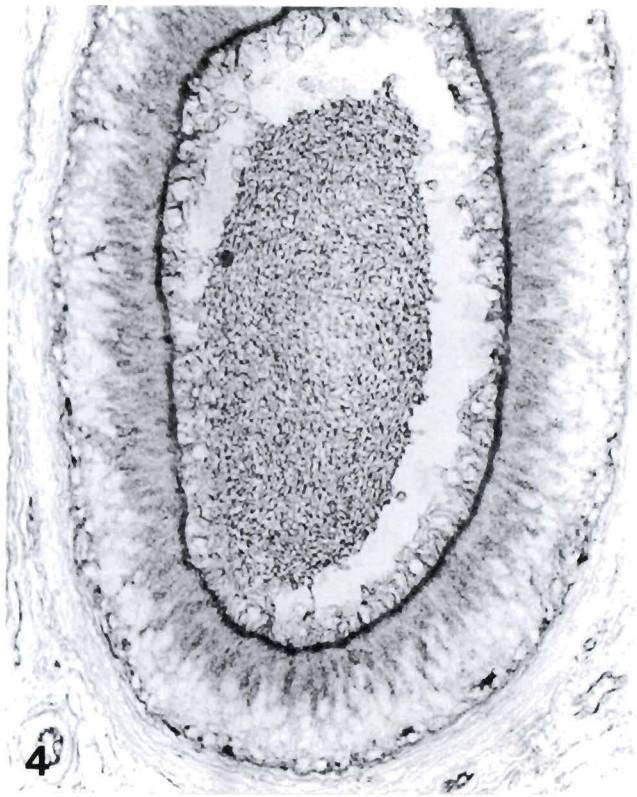


Fig. 4. *Caput epididymis* of adult horse. KOH-sialidase/SBA-HRP staining. A moderate increase in staining is observed in the supranuclear cytoplasm of the principal cells. x 300

Table 3. Binding of lectins to the glycocalyx of the epididymal regions and *vas deferens* in prepubertal horse.

LECTINS AND TREATMENTS	CAPUT EPIDIDYMIS	CORPUS EPIDIDYMIS	CAUDA EPIDIDYMIS	VAS DEFERENS
Con-A	+++	++	++	++
LCA	++	++	++	++
WGA/NEU/WGA[a]	++	-	-	-
GSA-II	+	-	-	-
SBA	+++	+++	-	-
NEU-SBA[b]	+++	+++	+++	+++
KOH-NEU-SBA[c]	+++	+++	+++	+++
PNA	+++	-	+++	+++
NEU-PNA[d]	+++	-	+++	+++
KOH-NEU-PNA[e]	+++	++	+++	+++
RCA-I	+++	++	-	-
NEU-RCA-I[f]	+++	++	-	-
KOH-NEU-RCA-I[g]	+++	++	-	-
DBA	+++	+++	+++	+++
NEU-DBA[h]	+++	+++	+++	+++
KOH-NEU-DBA[i]	+++	+++	+++	+++
UEA-I	++	-	-	-
LTA	-	-	-	-

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

in all epididymal regions and *vas deferens*.

WGA and GSA-II were moderately and weakly reactive respectively in the glycocalyx of the *caput epididymis*. Pretreatment with sialidase did not modify WGA affinity.

SBA strongly stained the glycocalyx of the *caput* and *corpus epididymis*; sialidase digestion failed to increase SBA staining in these two epididymal regions, whereas it promoted SBA positivity in the glycocalyx of *cauda epididymis* and *vas deferens*. Saponification before

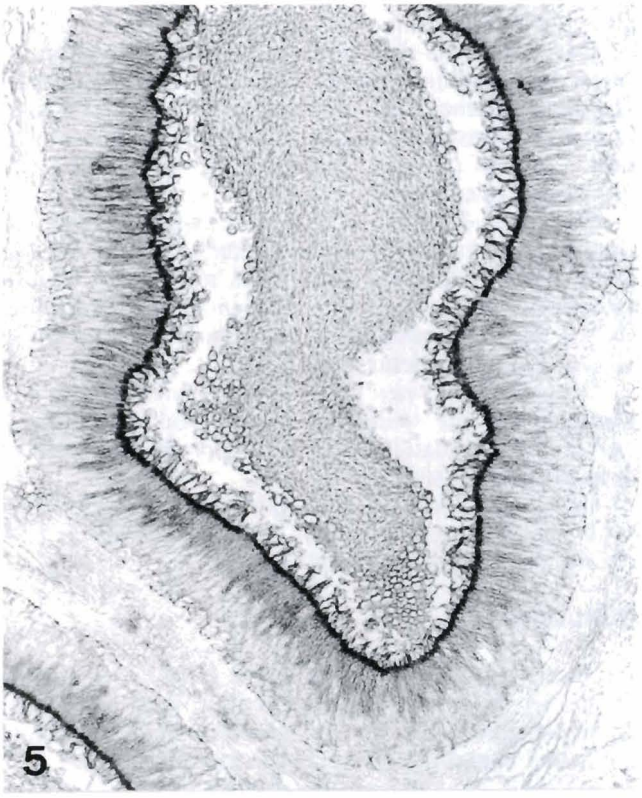


Fig. 5. *Caput epididymis* of adult horse. KOH-sialidase/RCA-I-HRP staining. Pretreatment with KOH-sialidase sequence promotes a moderate increase in staining in the supranuclear cytoplasm of the principal cells. x 200

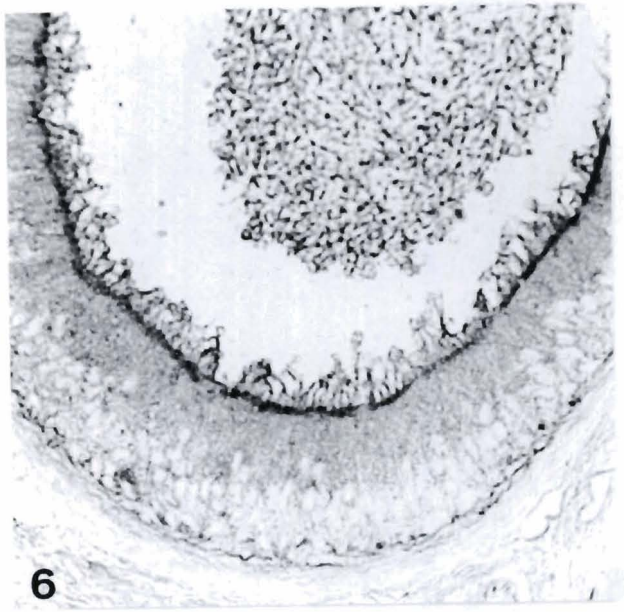


Fig. 6. *Caput epididymis* of adult horse. KOH-sialidase/PNA-HRP staining. New positive sites are visualized at the supranuclear cytoplasm of the principal cells. x 300

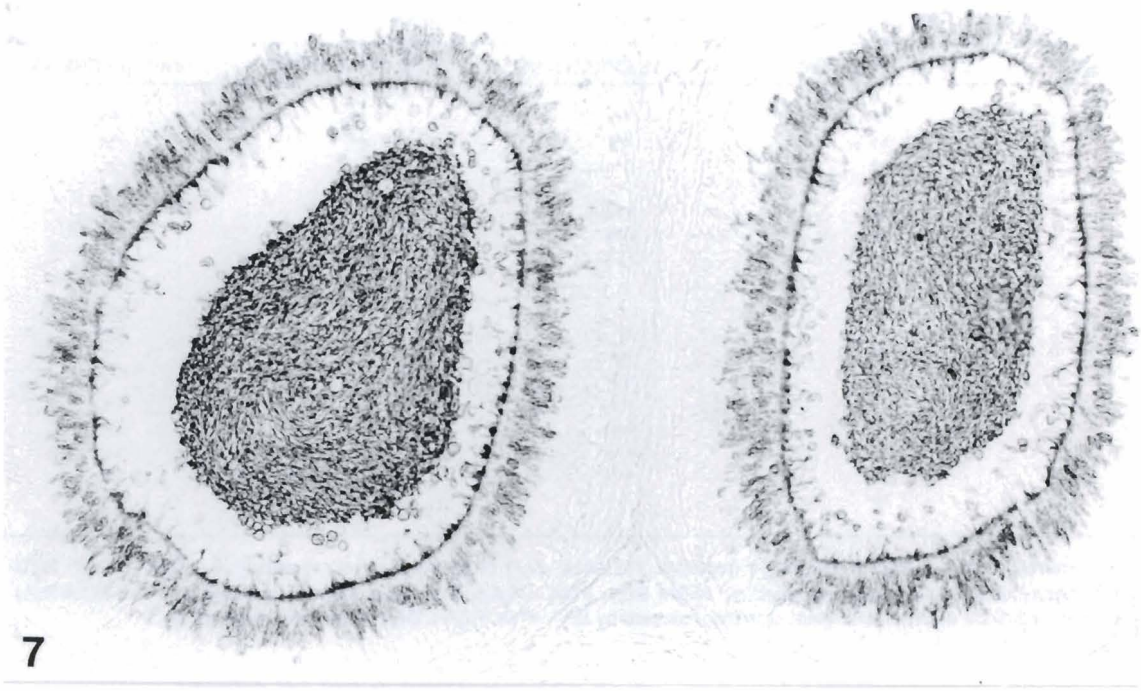


Fig. 7. *Caput epididymis* of adult horse. LTA-HRP staining. Reactive sites are moderately localized in both the glycocalyx and supranuclear cytoplasm of the epithelial principal cells. x 250

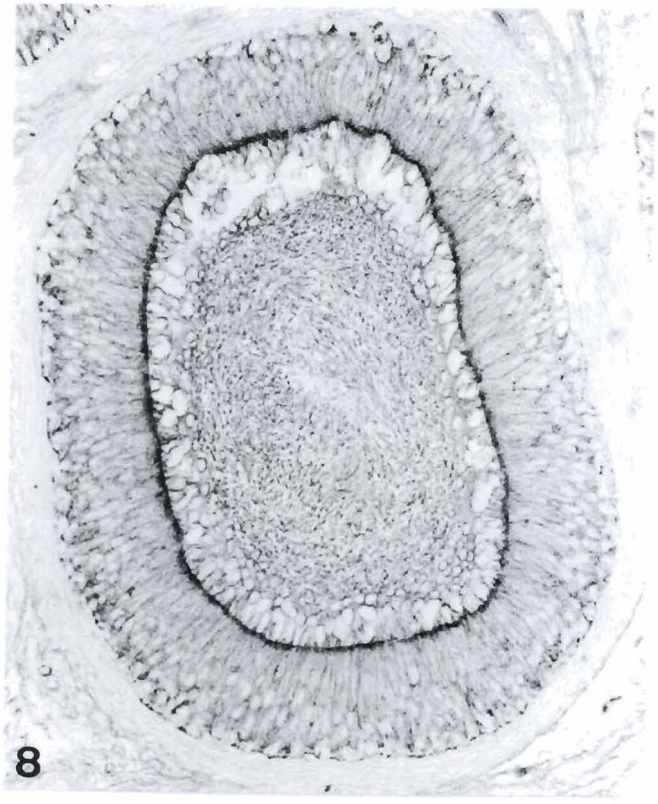


Fig. 8. *Corpus epididymis* of adult horse. Con-A-HRP staining. The glycocalyx and the cytoplasm of principal cells exhibit moderate and weak reaction respectively. x 250

enzymatic treatment did not enhanced SBA affinity in all epididymal regions or in *vas deferens*.

PNA showed a strong reaction in the glycocalyx of *caput* and *cauda epididymis* and in *vas deferens*. KOH-sialidase sequence did not elicit new PNA binding sites in these regions of the *ductus epididymis* whereas it was necessary to promote a moderate staining in the glycocalyx of epididymal *corpus*.

With RCA-I the glycocalyx of epididymal *caput* and *corpus* reacted strongly and moderately, respectively. No RCA-I-positive sites were obtained in the *cauda epididymis* or *vas deferens*. Sequential KOH-sialidase RCA-I treatment resulted in an unmodified reaction in all regions of the *ductus epididymis*.

DBA-binding sites were strongly localized in the glycocalyx of the epididymal regions and *vas deferens;* enzymatic degradation, even after saponification, failed to increase DBA staining.

UEA-I-positive sites were moderately localized only in the glycocalyx of the *caput epididymis*, whereas LTA did not show appreciable binding.

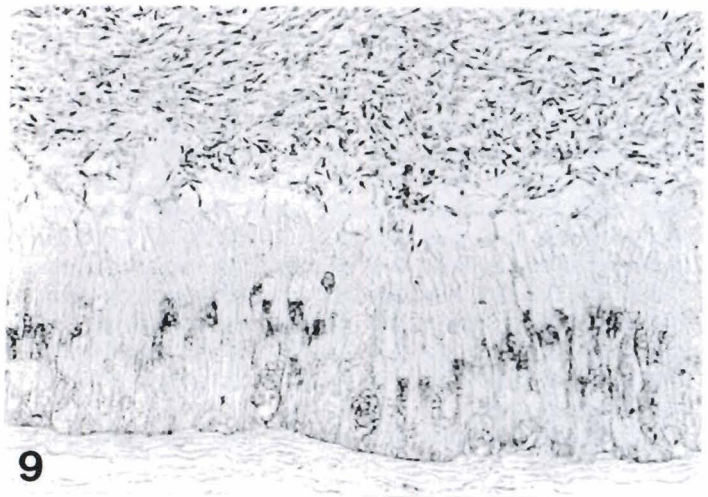
Discussion

In the present study we have used lectin histochemistry to analyze the chemical structure of glycoconjugates present in the epithelial principal cells lining the epididymal duct of prepubertal and adult horses.

In the *caput epididymis* of adult horse, the glycocalyx evidenced a positive reaction toward all the lectins used with the exception of Con-A. In the supranuclear cytoplasm of principal cells we visualized α-D-Man

and/or α-D-Glc, GlcNAc and α-L-Fuc residues. Moreover, positive binding was observed at this level for SBA, RCA-I, PNA and DBA. In particular the binding pattern of SBA and RCA-I was similar; both lectins being weakly reactive, indicating the presence of D-GalNAc and the dimer ß-D-Gal-(1-4)-D-GlcNAc in terminal position. In addition, these carbohydrates were also proved to be acceptor sugars for terminal sialic acid containing *O*-acyl substituents, as demonstrated by an increase in staining after KOH-sialidase-SBA and -RCA-I sequences. A moderate PNA positivity was evidenced only after saponification prior to neuraminidase degradation, indicating the presence of the terminal trimer sialic acid-ß-D-Gal-(1-3)-D-GalNAc. DBA showed moderate staining which did not increase after KOH-sialidase sequence. Reactivity, which was greater for DBA than for SBA, indicated the presence of terminal D-GalNAc above all in the α-anomeric form. Indeed, DBA and SBA have a very similar carbohydrate binding specificity, but DBA shows a clear preference for α-linked-D-GalNAc, in contrast to SBA which has no anomeric specificity for D-GalNAc, and also recognizes ß-D-Gal residues.

The epithelium lining the *corpus epididymis* evidenced different kinds of sialoglycoconjugates and α-D-Man and/or α-D-Glc residues in the glycocalyx. The



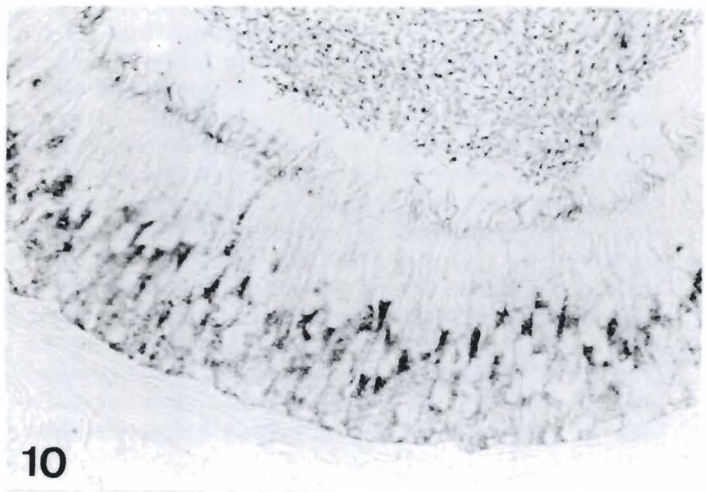
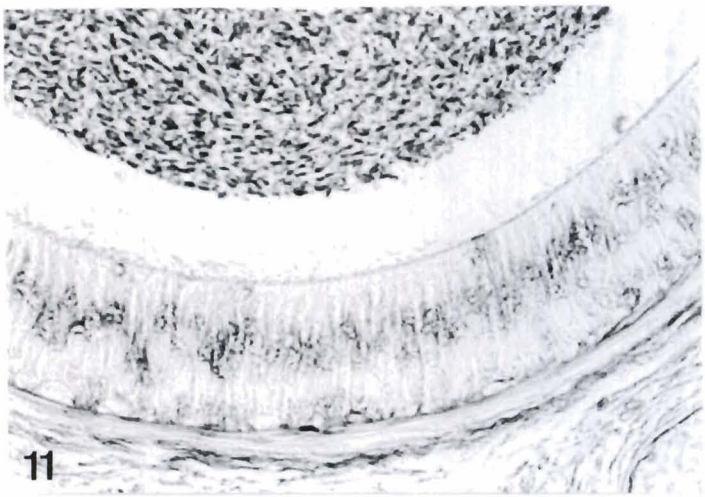


Fig. 9. *Corpus epididymis* of adult horse. WGA-HRP staining. The Golgi zone of the principal cells shows a moderate reaction. x 350

Fig. 10. *Corpus epididymis* of adult horse. Sialidase/DBA-HRP staining. Enzymatic removal of sialic acid discloses a moderate reaction at the Golgi zone of principal cells. x 250



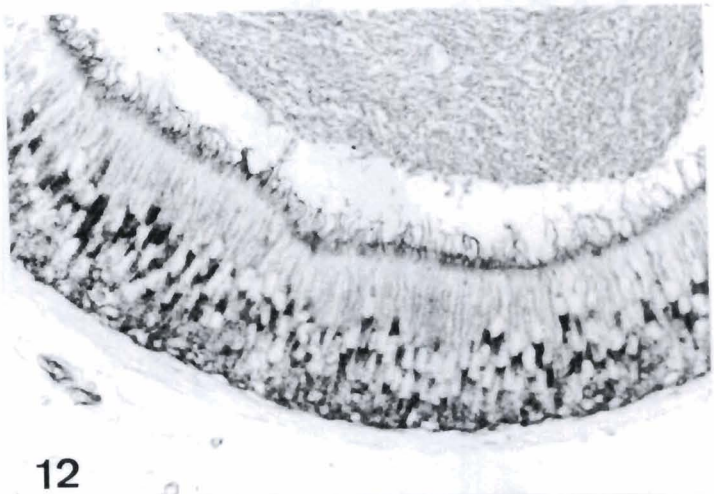


Fig. 11. *Corpus epididymis* of adult horse. KOH-sialidase/SBA-HRP staining. Saponification before enzymatic digestion promotes strong and weak staining in the Golgi zone and cytoplasm of principal cells respectively. x 300

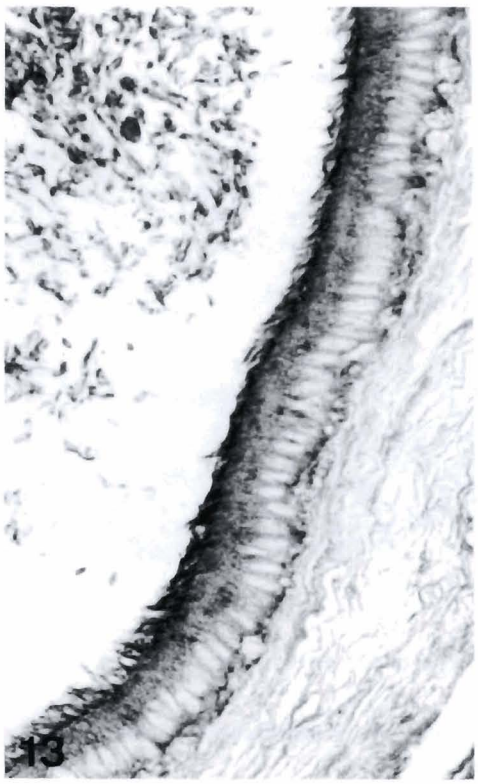
Fig. 12. *Corpus epididymis* of adult horse. Sialidase/PNA-HRP staining. The Golgi zone of principal cells is moderately stained after enzymatic digestion x 300

presence of the latter two sugars was also observed in the cytoplasm of principal cells. In the same cells of epididymal corpus the Golgi zone reacted moderately with WGA but not with GSA-II, indicating the presence of GlcNAc residues only in internal position. WGA can also bind sialic acid, although much less avidly than GlcNAc (Monsigny et al., 1980). However, since digestion with sialidase did not change the binding of WGA, it can be confirmed, in the case of the present study, that glucosamine is the only sugar bound to this lectin.

As α-L-Fuc was visualized in the Golgi area, we found that UEA-I and LTA showed non-comparable results, even though these two lectins have the same nominal specificity. In fact, LTA did not show any affinity, while UEA-I revealed binding-sites for α-fucose. These apparent differences in lectin behaviour could be due to the different type linkage which binds fucose to penultimate sugar. In particular, fucose visualized by LTA seems to be involved in the linkages with GlcNAc and/or D-Gal, while fucose recognized by UEA-I seems bound to D-Gal. The Golgi apparatus also synthesizes sialoglycoconjugates as demonstrated by positive SBA, RCA-I and DBA binding sites after sialidase digestion with or without prior saponification. In particular, digestion with sialidase promoted a notable RCA-I and a moderate DBA positivity, indicating the presence of two sialoglycoconjugates with the terminal sialic ß-D-Gal-(1-4)-D-GlcNAc and sialic acid-D-GalNAc, respectively. Saponification before sialidase digestion did not increase RCA-I and DBA reactivity. Indeed, KOH treatment intensified the staining of the sialidase-SBA sequence. This result suggests that terminal sialic acid with *O*-acetyl groups in the C-4 side chain acts as receptor sugar for penultimate ß-D-GalNAc. Indeed, KOH-sialidase treatment failed to increase the moderate positivity of PNA indicating the presence of the dimer ß-D-Gal-(1-3)-D-GalNAc in terminal position in the Golgi zone.

Principal cells lining epididymal *cauda* showed a moderate reaction with Con-A and LCA, both in the glycocalyx and cytoplasm, testifying to the presence of D-Man and/or α-D-Glc residues, whereas terminal α-L-Fuc was detected only at the glycocalyx level. SBA and RCA-I reactivities were elicited by sialidase, in both the glycocalyx and the cytoplasm, indicating the presence of the terminal dimer sialic acid-D-GalNAc and the terminal trimmer sialic-acid-ß-D-Gal-(1-4)-D-GlcNAc, whereas the binding affinity of PNA was not modified by enzymatic degradation, even after saponification, suggesting that the dimer ß-D-Gal-(1-3)-D-GalNAc appeared as a normally exposed sugar. Additionally, the penultimate ß-D-Gal could be linked to D-GalNAc rather than to GlcNAc, as PNA exhibited strong reaction while RCA-I had a weak reaction. DBA-binding sites were observed only in the glycocalyx, indicating the presence of the terminal D-GalNAc in the α-anomeric form, since SBA failed to react in the glycocalyx of epididymal *cauda*.

Epithelial principal cells in the *vas deferens*



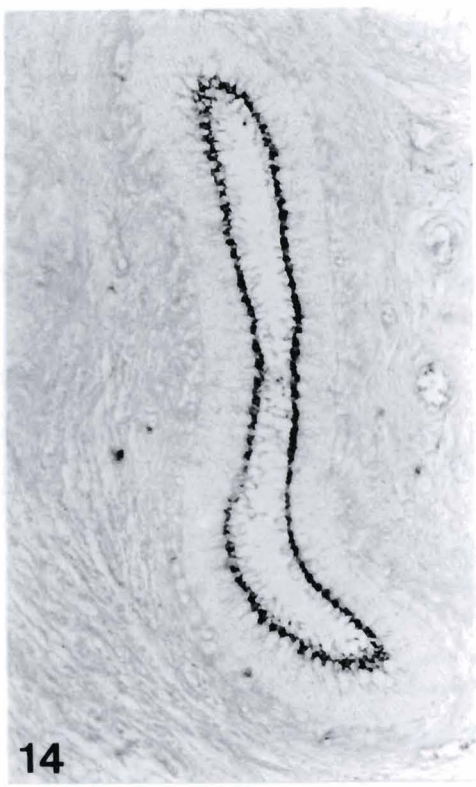


Fig. 13. *Cauda epididymis* of adult horse. PNA-HRP staining. The glycocalyx and the cytoplasm of principal cells result strongly positive. x 500

Fig. 14. *Caput epididymis* of prepubertal horse. Con-A-HRP staining. The glycocalyx evidences a strong reaction. x 300

evidenced α-D-Man and/or α-D-Glc residues in both the glycocalyx and cytoplasm, whereas the terminal sequences sialic acid-D-GalNAc and sialic acid-ß-D-Gal-(1-4)-D-GlcNAc were evidenced only in the glycocalyx, since sialidase digestion induced SBA, DBA and RCA-I positivity, only after KOH treatment. Conversely, PNA behaviour in the *vas deferens* was the same as that obtained in the epididymal *cauda*.

Glover and Nicander (1971) suggested that each epididymal region has a specific function. In fact, they consider the epididymis to be in three segments: an initial one (*caput*) involved in absorption; a middle one (*corpus*), in which sperm maturation occurs; and a terminal one (*cauda*), in which fertile spermatozoa are stored until ejaculation. Although the principal cells of the *ductus epididymis* are capable of both absorption and secretion, the histochemical results we obtained in the adult horse, evidenced a possible subdivision of these two functions. The *caput*, *cauda* and proximal *vas deferens* are probably the epididymal regions which were more likely to be involved in absorption than secretion since the Golgi zone seems unreactive. The lectin-stained material, observed in the cytoplasm of the principal cells lining the epithelium of the aforementioned epididymal regions, may be stained glycoproteins endocytosed from the lumen (Arya and Vanha-Pertulla, 1985a). Conversely, the Golgi apparatus was mainly visualized with some lectins in the epididymal *corpus*, suggesting that this area is the most active in the synthesis and secretion of intraluminal glycoproteins. These compounds may mix with the tubular sperm and contribute to the final maturation of spermatozoa.

These explanations are consistent with studies carried out in other mammals (Arya and Vanha-Pertulla, 1985a, 1986a,b; Burkett et al., 1987).

In all regions examined, the glycocalyx of epididymal principal cells is particularly rich in carbohydrate moieties, above all sialoglycoconjugates. Some authors (Jeanloz and Codington, 1976) suggest that terminal sialic acid residues play a role in several functions, including protection of cells from dehydration, transport of metabolites and ion across the plasmalemma and even hormone binding.

In the prepubertal horses, only the glycocalyx of the epithelium lining the *ductus epididymis* exhibited a positive reaction toward the lectins employed, whereas the cytoplasm and the Golgi zone of the cells resulted unreactive. These features may be explained by the fact that the epididymis is an androgen target organ and its metabolism is regulated by androgens which are necessary to normal secretory and absorptive epithelial functions (Turner, 1991).

In fact, in the rat the removal of androgens by castration determines an involution of epididymal epithelium (Sun and Flickinger, 1979) and also a suppression of lectin staining indicating a block of glycoprotein synthesis and/or absorption (Arya and Vanha-Pertulla, 1985b).

In conclusion, our studies show that a notable difference exists between the presence of glycoconjugates in adult and prepubertal horse epididymis epithelium, probably related to the immature structure of the organ. The heterogeneity of the lectin-binding pattern of the adult horse epididymis reported in the present work also suggests the existence of different functional segments along the epididymal duct. Further studies are needed to establish the relations between the activities of the epithelium lining the *ductus epididymis* and sperm maturation in the horse.

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