J. Vet. Sci. (2003), 4(1), 21-28

JOURNAL OF
Veterinary
Science

Histochemical Detection of Glycoconjugates in the Male Reproductive System of the Horse

Tae-young Ha, Mee-jung Ahn, Yong-duk Lee, Jae-hyuk Yang, Hee-seok Kim and Tae-kyun Shin*

Department of Veterinary Medicine, Graduate School, Cheju National University, Jeju 690-756, Korea

Received March 5, 2003 / Accepted April 2, 2003

Abstract

Lectins are glycoproteins of plant and animal origin that have the ability to bind specific carbohydrate residues of cell glycoconjugates, particularly in terminal positions. In this study, the binding of lectins, *Dolichos biflorus* agglutinin (DBA), soybean agglutinin (SBA), *Bandeiraea simplicifolia* BS-1 (isolectin B4), *Triticum vulgaris* (WGA), *Arachis hypogaea* (PNA), and *Ulex europaeus* (UEA-I), was studied in the reproductive systems of male thoroughbred horses.

DBA was detected in the stereocilia of the caput and corpus epididymis, and in the vas deferens. It was weakly detected in connective tissue of the corpus epididymis. Strong SBA staining was seen in epithelial cells in the testis, stereocilia of the corpus and cauda epididymis, and in the vas deferens. There were intense positive reactions for isolectin B4 in interstitial cells in all tissue and serosa of the vas deferens. PNA staining was seen only in stereocilia in the caput and corpus epididymis, and in the vas deferens. Strong WGA staining was seen throughout the testis, except in Sertoli cells, stereocilia, and connective tissue. UEA-I was detected in secondary spermatids, stereocilia, and epithelial cells of the cauda epididymis.

These results show that degenerating cells in the testis, epididymal tubules, and vas deferens have differential affinities for lectins, and suggest that lectins play a role in the reproductive system of the horse. The heterogeneity of the lectin staining pattern in the reproductive tubules of adult horses suggests that the carbohydrate composition of each cell type is region specific.

Key words: lectin, horse, testis, reproductive tubules

*Corresponding author: Tae-Kyun Shin
Department of Veterinary Medicine, Cheju National University, Jeju 690-756, Korea
Tel: +82-64-754-3363; Fax: +82-64-756-3354
E-mail: shint@cheju.cheju.ac.kr

Introduction

Lectins are carbohydrate-binding proteins of nonimmune origin that are widely distributed in nature [9]. They are glycoproteins of both plant and animal origin that can bind to specific carbohydrate residues of cell glycoconjugates, particularly in terminal positions. All lectin molecules possess two or more carbohydrate-binding sites, a property that is essential for their ability to agglutinate cells and react with complex carbohydrates. Recently, sugar residues on the cell surface were shown to play an important role in cellular function, differentiation, and regeneration [3, 7], and histochemistry using lectins was demonstrated to be useful for detecting sugar residue expression in various tissues [4].

The male reproductive system is a tubular structure that is well suited for spermatozoa generation. Traditionally, male reproductive potential is based on the ability to deliver spermatozoa to the female genital tract. The function of the male reproductive system is sperm creation and maturation [8].

Many glycoproteins cover the epithelial lining of the male reproductive system. These macromolecules provide the specific intraluminal environment in which immature spermatozoa acquire progressive motility and fertilizing ability [6]. Accumulated evidence suggests that epididymal luminal proteins and glycoproteins bind to spermatozoa and may be responsible for altering the sperm plasma membrane, a vital component in early fertilization events [6].

Lectin binding studies in the male reproductive system have been carried out in dogs [5], cows [12], pigs [10], and horses [9]. These studies mainly examined a single organ, the testis. Little is known about patterns of lectin binding to glycoconjugates in the entire reproductive system of the male horse.

The aim of this study was to localize six lectins, *Dolichos biflorus* agglutinin (DBA), soybean agglutinin (SBA), *Bandeiraea simplicifolia* BS-1 (isolectin B4), *Triticum vulgaris* (WGA), *Arachis hypogaea* (PNA), and *Ulex europaeus* (UEA-I), in the reproductive tissues of male thoroughbred horses.

Materials and methods

Animals

Two two-year-old male thoroughbred horses were kindly supplied by Stud Farm of Korea Racing Association (Jeju), and the male reproductive organs were surgically removed under local anesthesia.

Sampling procedure

The testis, epididymal ducts and ductus deferens of each horse were fixed in 10% buffered formalin for 48 hrs to prepare for histological examination.

Histological examination

Specimens were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 ㎛, and stained with hematoxylin and eosin using routine histological techniques. All paraffin-embedded tissue sections stained for the lectin study were from normal horses.

Lectins used in this study

The lectins used in this study were *Bandeiraea simplicifolia* agglutinin (peroxidase-labeled isolectin B4, Sigma, St. Louis, MO), *Dolichos biflorus* agglutinin (peroxidase-labeled DBA, Sigma), glycine max agglutinin (peroxidase-labeled SBA, Sigma), *Triticum vulgaris* agglutinin (peroxidase-labeled WGA, Sigma), *Arachis hypogaea* agglutinin (peroxidase-labeled PNA, Sigma) and *Ulex europaeus* agglutinin I (peroxidase-labeled UEA-I, Sigma).

Histochemistry

Tissues were dehydrated by immersion in a graded ethanol series (70, 80, 90, 95 and 100%), cleared in xylene, embedded in paraffin wax, and sectioned at 5 ㎛ (m on a microtome. The sections were mounted on glass microscope slides, the wax was removed, and the sections were hydrated. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol for 30 min. After three washes with PBS, the sections were exposed to 10% normal horse serum, and then incubated with DBA-peroxidase (diluted 1:10), SBA-peroxidase (diluted 1:400), isolectin B4-peroxidase (diluted 1:50), WGA-peroxidase (diluted 1:20), PNA-peroxidase (diluted 1:10), or UEA-I-peroxidase (diluted 1:10) for 3 hrs at room temperature. Peroxidase was developed with diaminobenzidine (DAB)-hydrogen peroxide solution (0.001% 3,3′-diaminobenzidine and 0.01% hydrogen peroxide in 0.05 M Tris buffer). The sections were counterstained with hematoxylin before being mounted.

Results

Histological examination confirmed that all tissues, including testis, epididymal duct and ductus deferens, showed no pathological changes (Fig. 1).

In the testis, SBA (Fig. 2B), WGA (Fig. 2E), and UEA-1 (Fig. 2F) were specifically detected in the epithelial cells.

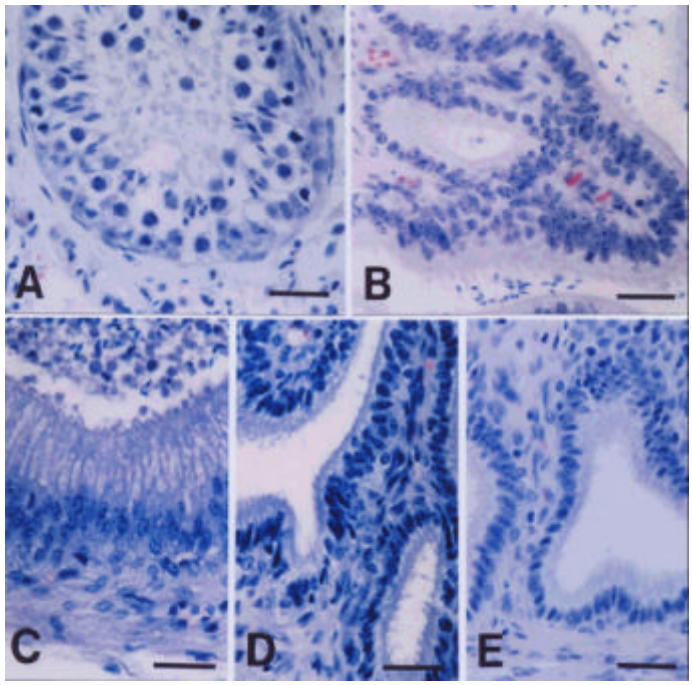

Fig. 1. Histological findings in the male reproductive system of thoroughbred horses. There was no inflammation in the testis (A), caput epididymis (B), corpus epididymis (C), cauda epididymis (D), or ductus deferens (E). Hematoxylin-eosin staining. Scale bar = 30 ㎛.

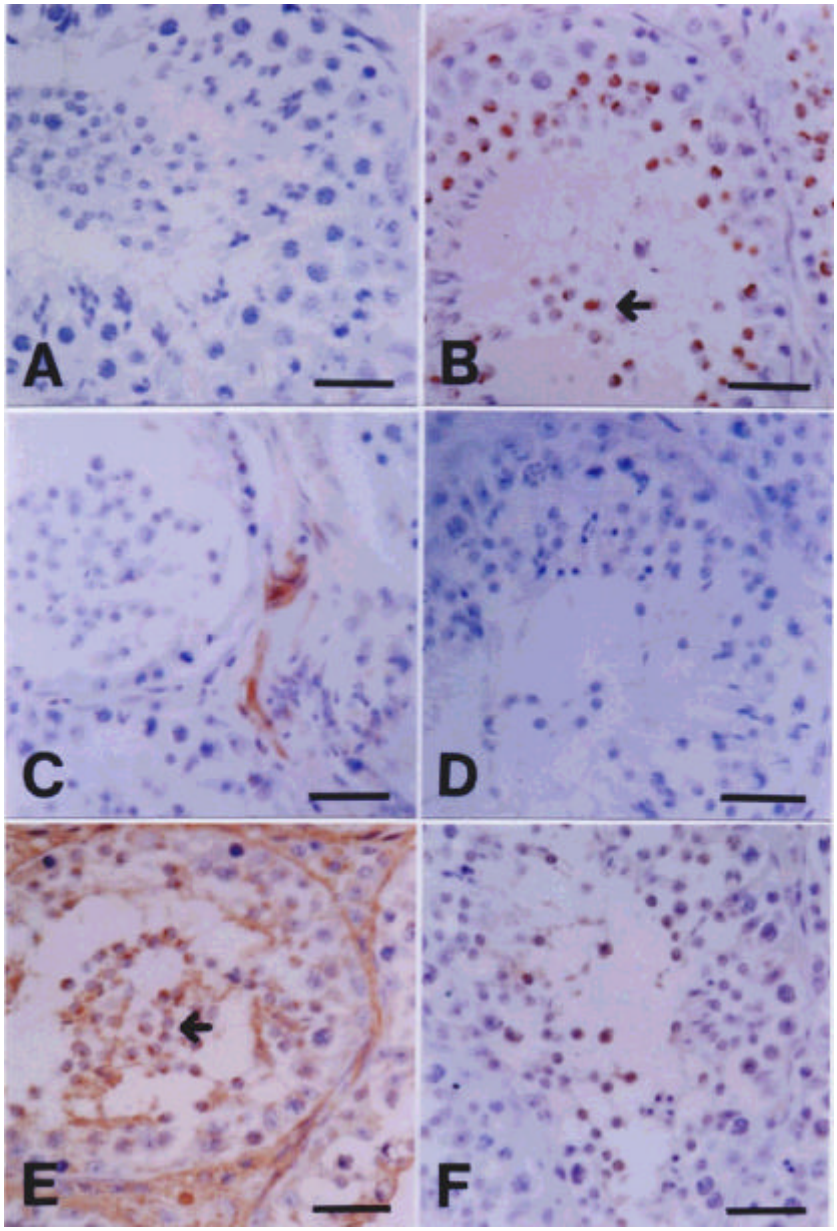

Fig. 2. Immunohistochemical detection of DBA (A), SBA (B), isolectin B4 (C), PNA (D), WGA (E), and UEA-I (F) in the testis of thoroughbred horses. SBA (B), WGA (E) and UEA-1 (F) were specifically detected in the epithelial cells. The secondary spermatids were largely positive for SBA (B), WGA (E), and UEA-I (F). SBA (B) and WGA (E) were also detected in the spermatocytes (arrow). Isolectin B4 (C) and WGA (E) were detected in interstitial cells, while DAB (A) and PNA (D) were localized in a few cells. Counterstaining was with hematoxylin. Scale bar = 30 μm.

Secondary spermatids were largely positive for SBA (Fig. 2B), WGA (Fig. 2E), and UEA-I (Fig. 2F). SBA (Fig. 2B) and WGA (Fig. 2E) were also detected in the spermatocytes. Isolectin B4 (Fig. 2C) and WGA (Fig. 2E) were detected in interstitial cells, while DAB (Fig. 2A) and PNA (Fig. 1D) were localized in very few cells.

In the epididymis, DBA, isolectin B4, PNA, WGA, and UEA-I were detected the caput epididymis (Fig. 3). DBA (Fig. 3A), SBA (Fig. 3B), PNA (Fig. 3D) and WGA (Fig. 3E) were strongly detected in the stereocilia of the caput epididymis. Isolectin B4 (Fig. 3C) and WGA (Fig. 3E) were also detected in the spermatocytes. WGA was strongly detected in the connective tissue (Fig. 3E), and UEA-I was weakly detected in the sterocilia (Fig. 2F).

In the corpus epididymis, the stereocilia in particular was positive for DBA (Fig. 4A), SBA (Fig. 4B), PNA (Fig. 4D), WGA (Fig. 4E) and UEA-I (Fig. 4F), but no isolectin B4 (Fig. 4C) was detected. Isolectin B4 (Fig. 4C) and WGA (Fig.

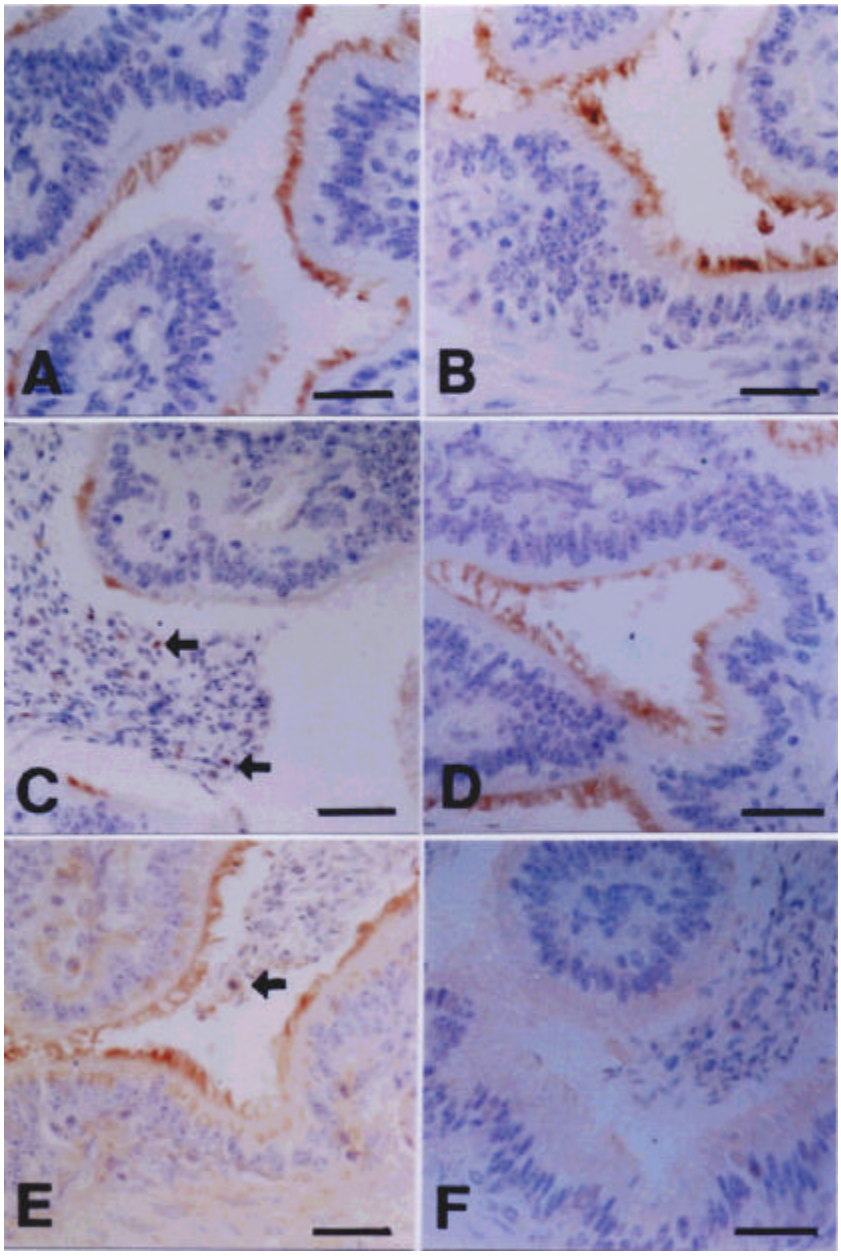

Fig. 3. Immunohistochemical detection of DBA (A), SBA (B), isolectin B4 (C), PNA (D), WGA (E), and UEA-I (F) in the caput epididymis of thoroughbred horses. DBA (A), SBA (B), PNA (D), and WGA (E) were strongly detected in the stereocilia. Isolectin B4 (C) and WGA (E) were also detected in the spermatocytes (arrow), and WGA was strongly detected in the connective tissue (E). UEA-I was detected in a few sterocilia (F). Counterstaining was with hematoxylin. Scale bar = 30 μm.

4E) were detected in the connective tissue, and UEA-I was detected in the spermatocytes (Fig. 4F). Some basal cells were weakly positive for WGA (Fig. 4E).

In the cauda epididymis, some stereocilia were intensely positive for SBA (Fig. 5B) and UEA-I (Fig. 5F). In the epithelial cells, only UEA-I was detected (Fig. 5F). DBA (Fig. 5A) and PNA (Fig. 5D) were not detected in any cauda epididymis cells. Isolectin B4 (Fig. 5C) and WGA (Fig. 5E) were detected in the connective tissue.

In the ductus deferens, all lectins (Fig. 6) except isolectin B4 (Fig. 6C) and UEA-I (Fig. 6F) were detected in the stereocilia. No lectins were detected in the epithelial cells. The histochemical reactions of the lectins in the testis, epididymis, and ductus deferens are summarized in Table 1.

Discussion

Lectins are useful histochemical markers for examining

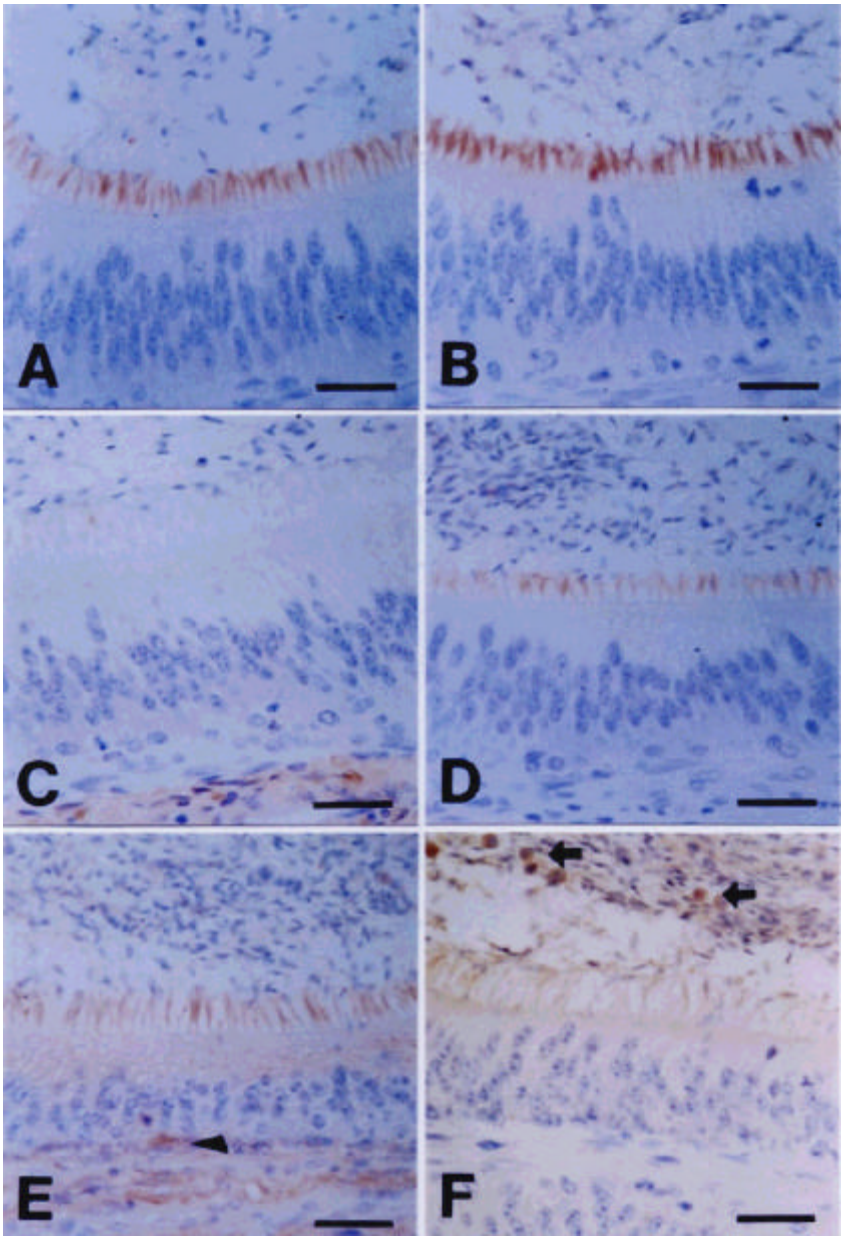

Fig. 4. Immunohistochemical detection of DBA (A), SBA (B), isolectin B4 (C), PNA (D), WGA (E), and UEA-I (F) in the corpus epididymis of the thoroughbred horses. Stereocilia were positive for DBA (A), SBA (B), PNA (D), WGA (E), and UEA-I (F), but no isolectin B4 (C) was detected. Isolectin B4 (C) and WGA (E) were detected in the connective tissue. UEA-I (F, arrow) was detected in the spermatocytes. Some basal cells were weakly positive for WGA (E, arrowhead). Counterstaining was with hematoxylin. Scale bar = 30 μm.

the emergence and distribution of glycoconjugates during cellular differentiation [1] and they can be used to detect differences between morphologically different, or even similar, cell types in numerous tissues [11].

This is the first study to examine the binding of six different lectins, including DBA, SBA, isolectin B4, WGA, PNA and UEA-I, in thoroughbred horses. The lectins showed consistent binding in the sterocilia of seminiferous tubules, while only limited binding to spermatocytes was seen. WGA lectin was strongly detected in the reproductive ducts. These findings suggest that each glycoprotein has a specific pattern depending on its location in the male reproductive tubules.

Several studies have shown that lectins have binding sites in the covering epithelia found in the male reproductive systems of horses [9], bulls [1], and rats [2]. The present study is largely consistent with these studies.

There is a general agreement that in the thoroughbred horse, the detection of particular glycoproteins by lectins

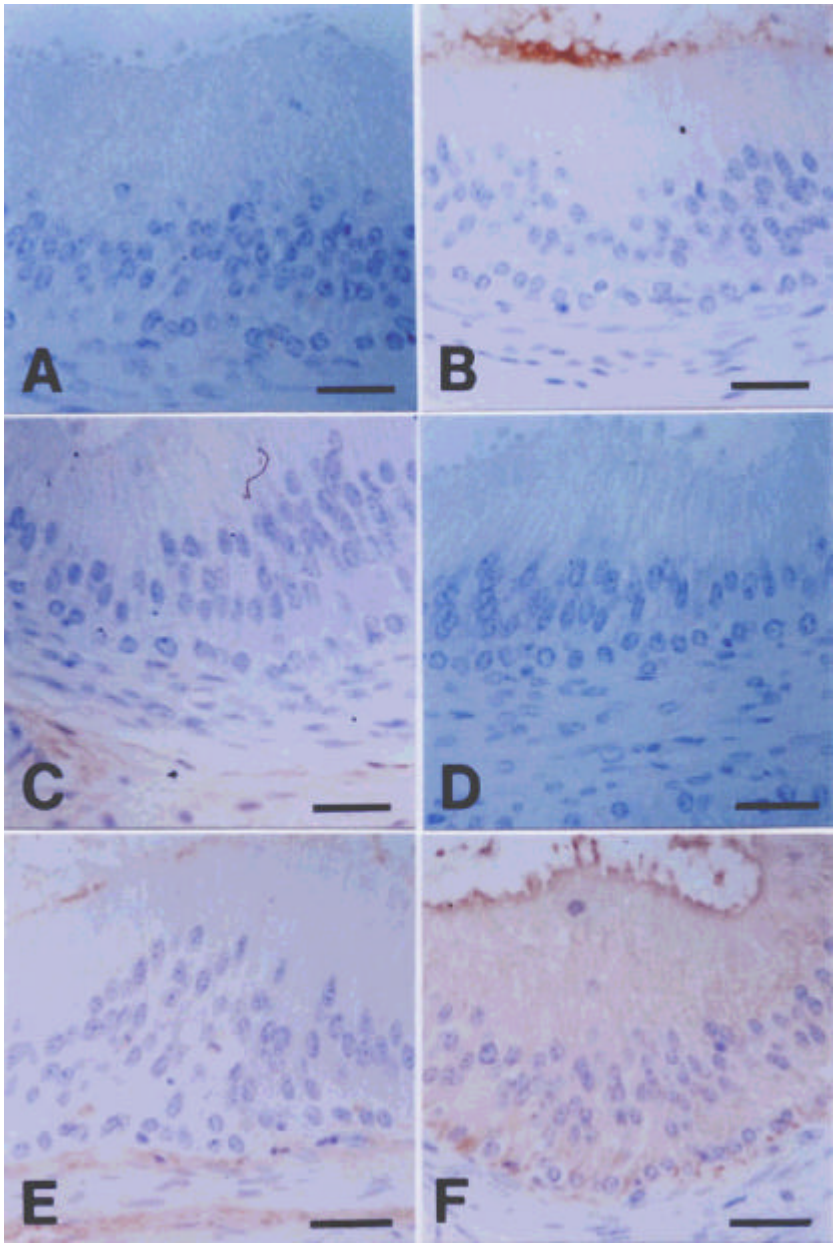

Fig. 5. Immunohistochemical detection of DBA (A), SBA (B), isolectin B4 (C), PNA (D), WGA (E), and UEA-I (F) in the cauda epididymis of thoroughbred horses. Some stereocilia were intensely positive for SBA (B) and UEA-I (F). In the epithelial cells, only UEA-I was detected (F). DBA (A) and PNA (D) were not detected in any cauda epididymis cells. Isolectin B4 (C) and WGA (E) were detected in the connective tissue. Counterstaining was with hematoxylin. Scale bar = 30 μm.

reflects the presence of terminal sugar residues. The sequential biosynthetic steps might depend on glycosyltransferase activity, which changes during cell maturation [11].

The six lectins examined in the present study were also expressed in cell groups in the tissues of the reproductive tubules. Based on the distribution of the lectins, DBA, SBA, isolectin B4, WGA, PNA and UEA-I, these cells may be active in the digestion of absorbed material and are probably derived from the principal cells, which may be active in transporting absorbed material in the testis, epididymis and ductus deferens in the horse.

In the present study, it was confirmed that degenerating cells in the testis, epididymal tubules, and ductus deferens showed a differential affinity for lectins. This suggests that in the horse, some lectins are involved in cell degeneration, either as a cause or a consequence. Additionally, the heterogeneity of the lectin staining patterns in the reproductive tubules of the adult thoroughbred horse suggests that the

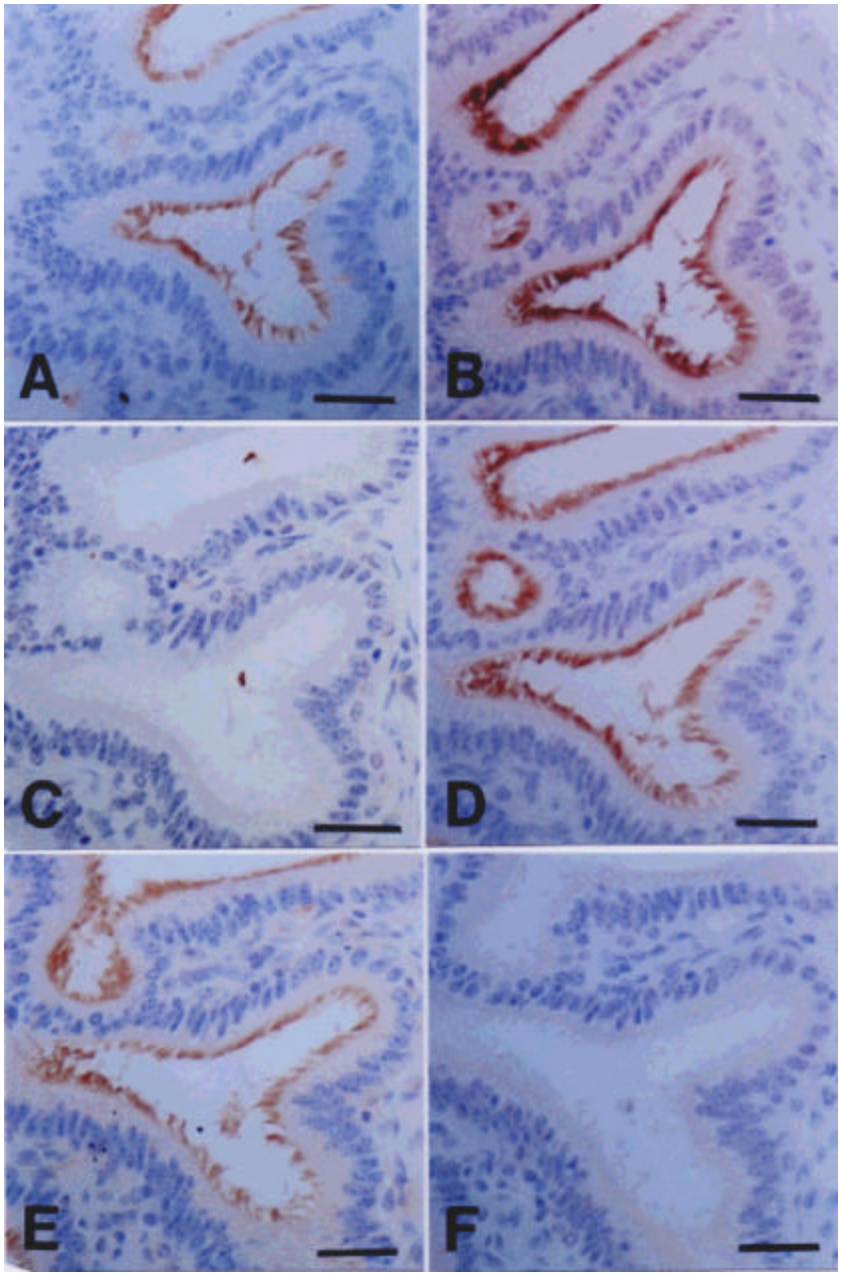

Fig. 6. Immunohistochemical detection of DBA (A), SBA (B), isolectin B4 (C), PNA (D), WGA (E), and UEA-I (F) in the ductus deferens of the thoroughbred horses. All lectins were detected in the stereocilia (A-B and D-E), except isolectin B4 (C) and UEA-I (F). No lectins were detected in the epithelial cells. Counterstaining was with hematoxylin. Scale bar = 30 μm.

carbohydrate composition of each cell type is different. The functional role of each lectin needs further study.

Taken together, these results suggest that covering epithelia have a specific glycoprotein in the reproductive system of male horses, which may function to control germ cell development and maturation along the ductal system.

Table 1. Histochemical lectin staining pattern in various cell types in the reproductive system of two-year-old thoroughbred horses (+, weak; ++, moderate; +++, intense)

		DBA	SBA	Isolectin B4	PNA	WGA	UEA-I
Testis	Primary Spermatid	−	−	−	−	+	−
	Secondary Spermatid	−	+++	+	−	+++	+
	Spermatocyte	−	+	−	−	+	−
	Sertoli cell	−	−	+	−	−	−
	Interstitial cell	−	−	+++	−	++	−
Caput epididymis	Stereocilia	++	++	+	+++	+++	+
	Epithelium	−	−	−	−	−	−
	Basal cell	−	−	−	−	−	−
	Spermatocyte	−	−	+	−	+	−
	Connective tissue	+	−	+++	−	++	+
Corpus epididymis	Stereocilia	++	+++	−	++	+++	+
	Epithelium	−	−	−	−	−	−
	Basal cell	−	−	−	−	+	−
	Spermatocyte	−	−	−	−	−	++
	Connective tissue	−	−	++	−	++	+
Cauda epididymis	Stereocilia	−	++	−	−	+	++
	Epithelium	−	−	−	−	−	++
	Basal cell	−	−	−	−	+	−
	Connective tissue	−	−	+	−	+	−
Ductus deferens	Stereocilia	+	+++	−	+++	++	−
	Epithelium	−	−	−	−	−	−
	Serosa	−	−	++	−	+	+

References

1. **Arya, M. and Vanha-Perttulla, T.** Lectin binding pattern of bull testis and epididymis. J. Androl. 1985, **6**, 230-242.
2. **Arya, M. and Vanha-Perttulla, T.** Distribution of lectin binding in rat testis and epididymis. J. Androl. 1984, **16**, 495-508.
3. **Breer, H.** Molecular reaction cascades in olfactory signal transduction. J. Steroid Biochem. Mol. Biol. 1991, **39**, 621-625.
4. **Franceschini, V., Lazzari, M., Revoltella, R. P. and Ciani, F.** Histochemical study by lectin binding of surface glycoconjugates in the developing olfactory system of rat. Int. J. Dev. Neurosci. 1994, **12**, 197-206.
5. **Kawakami, E., Morita, Y., Hori, T. and Tsutsui, T.** Lectin-binding characteristics and capacitation of canine epididymal spermatozoa. J. Vet. Med. Sci. 2002, **64(7)**, 543-549.
6. **Malika, B. and Aifa, A.-H.** Characterization of glyco-conjugates in the epididymal epithelium and luminal fluid during postnatal development of the mouse. Cell Tissue Res. 1997, **287**, 611-619.
7. **Nakajima, T., Shiratori, K., Ogawa, K., Tanioka, Y. and Tahiguchi, K.** Lectin-binding patterns in the olfactory epithelium and vomeronasal organ of the common marmoset. J. Vet. Med. Sci. 1998, **60(9)**, 1005-1011.
8. **Ong, C. N., Shen, H. M. and Chia, S. E**. Biomarkers of male reproductive health hazards: Are they available? Toxicol. Lett. 2002, **134**, 17-30.
9. **Parillo, F., Stradaioli, G., Supplizi, A. V. and Monaci, M.** Detection of glycoconjugates in the ductus epididymis of the prepubertal and adult horse by lectin histochemistry. Histol. Histopathol. 1997, **12(3)**, 691-700.
10. **Pinart, E., Bonet, S., Briz, M., Pastor, L. M., Sancho, S., Garcia, N., Badia, E. and Bassols, J.** Histochemical study of the interstitial tissue in scrotal and abdominal boar testes. Vet. J. 2002, **163**, 68-76.
11. **Spicer, S. S. and Schulte, D. A.** Diversity of cell glycoconjugates shown histochemically; a perspective. J. Histochem. Cytochem. 1992, **40**, 1-38.
12. **Wrobel, K. H.** Morphogenesis of the bovine rete testis: the intratesticular rete and its connection to the seminiferous tubules. Anat. Embryol. 2000, **202**, 475-490.