Lectin Histochemical Study on the Reproductive Tract in Normal and *Brucella ovis*-infected Rams

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(Received 13 February 1995/Accepted 9 May 1995)

ABSTRACT. Histochemical studies on tissue sections showed alterations of lectin-binding reactivities in the epididymis, seminal vesicle and ampulla of *Brucella ovis*-infected rams. These modifications in the carbohydrate composition of organs participating in maturation, transport, and storage of spermatozoa, could be involved in the impaired fertility observed in this disease.—**KEY WORDS:** *Brucella ovis*, lectin histochemistry, ram.

J. Vet. Med Sci. 57 (5): 935–938, 1995

Synthesis, secretion and absorption of glycoproteins have a paramount functional importance in the male reproductive tract [6] like in the respiratory and alimentary tracts [9, 26]. Lectin histochemistry has been employed in the evaluation of glycoprotein expression in the male reproductive tract in several animal species [14, 15, 17, 24] and changes of glycoproteins have been suggested in some pathological conditions (reproductive disorders) [27, 28]. However, very few have been conducted in rams [18]. *Brucella (B.) ovis* infection is a frequent and serious cause of reduced fertility in sheep [7]. It brings about chronic interstitial inflammation with formation of spermatoc granuloma in the epididymis, seminal vesicle, and ampulla, in which *B. ovis* can persist for a long period without apparent clinical signs [15, 23]. The aim of this study is to clarify the lectin binding pattern in the epididymis, seminal vesicle and ampulla of *B. ovis*-infected rams.

The epididymis, seminal vesicle and ampulla were collected from 2 Corriedale rams, aged 3 years, which have been diagnosed as *B. ovis* infection by bacteriological, serological and pathological examinations, and from 2 age-matched healthy rams of the same breed. By semen examination, both infected rams exhibited slight oligospermia and severe necroteratospermia (viability: 50–60%; motility: 10–15%; high number of immature spermatozoa), suggesting severely impaired fertility. Also, higher than 1:8 anti-spermatozoa immobilizing antibody titer was detected in serum of one infected ram. Macroscopic enlargement was observed in the epididymides of both infected rams. The epididymal tail, and the middle part of the seminal vesicles and ampulla were fixed in 10% neutral buffered formalin, and serial 5 μ m-paraffin sections were cut, and stained with haematoxylin and eosin. Fibrosis and granuloma formation were observed in all the organs of the infected rams. Epididymal sections free of granulomas were selected for lectin binding analysis. Following 7 lectins were used in this study: *Arachis hypogaea* (PNA), *Ricinus*

communis (RCA)-I, *Dolichos biflorus* (DBA), *Concanavalina ensiformis* (Con A), *Triticum vulgaris* (WGA), *Ulex europaeus* (UEA)-I and *Glycin max* (SBA). Six lectins except Con A were used in their horseradish-peroxidase (HRP)-conjugated form (E-Y Laboratories, San Mateo, CA) [12]. Con A (Sigma) affinity was investigated according to the method of Katsuyama and Spicer [16]. Histochemical control sections for all lectins were also processed according to Itagaki *et al.* [13]. Following lectin staining pattern observed in this study was essentially same in each animal of normal or infected group, although there were subtle differences in the binding intensities of some lectins.

Mammalian epididymis has secretory and absorptive functions, both of which play an important role in the final maturation of spermatozoa, and principal cells are responsible for the biosynthesis and secretory pathway for glycoproteins in the epididymal epithelium [1]. As shown in Table 1, the apical surface of the principal cell in *B. ovis*-infected animals lost their binding sites for PNA and DBA while it developed binding sites for UEA-I (Figs. 1 and 2). Such increase of α -L-fucose terminals in the apical surface of principal cells which was indicated by UEA-I stainability has also been observed in castrated rats [2]. Besides, the congenital lack of α -L-fucosidase in male dogs modify the carbohydrate moieties with which spermatozoa are coated during transit and these animals become infertile [27]. Glycoproteins with fucose residues play a crucial role in spermatozoa maturation and fertilizing capacity [12, 27]. Thus, the modifications in UEA-I receptor sites demonstrated in the epididymis of *B. ovis*-infected rams, should be considered in relation with their failures in fertility.

It is said that light and narrow cells, the other major cell type of the epididymal epithelium, appear to be active in the absorption of glycoproteins from the tubular lumen [21, 28]. In control rams, these cells were not clearly identified even by lectin histochemistry (Fig. 3) in coincidence with Nicander's report that they are not present in rams [22]. However, they could be detected as SBA- and DBA-positive cells in *B. ovis*-infected rams (Table 1 and Fig. 4). This

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Fig. 1. UEA-I staining of a normal ram epididymis. Epithelial cells are negative to UEA-I. $\times 400$.

Fig. 2. UEA-I staining of a *B. ovis*-infected ram epididymis. Apical surface of epithelial cells is strongly positive to UEA-I. $\times 400$.

Fig. 3. SBA staining of a normal ram epididymis. Narrow cells can not be identified, compared with Fig. 4. $\times 400$.

Fig. 4. SBA staining of a *B. ovis*-infected ram epididymis. Cytoplasm of narrow cells (arrows) are strongly positive to SBA. $\times 400$.

may be dependent on partial or total blockage of the sperm transit and an uptake of glycoproteins from disintegrating spermatozoa. The increased lectin stainability of light and narrow cells caused in rats by the ligation of vas deferens supports this hypothesis [1].

The lectin binding activity of spermatozoa in control rams was similar to the pattern reported in 6 animal species by Arya and Vanha-Pertula [3, 4, 28]. However, in *B. ovis*-infected rams, spermatozoa were stained strongly with UEA-I, which indicates a high expression of α -L-fucose residues. This change is coincident with the heavy expression of UEA-I receptors observed in principal cells of the epithelium in infected rams (Table 1).

In the seminal vesicle, as shown in Table 2, a clear difference was seen in the binding activities of the cytoplasm of epithelial cells for SBA and DBA; they were negative in controls and became strong positive in affected animals.

In the ampulla, a distinct difference in lectin binding activity between control and *B. ovis*-infected rams was observed in the apical surface of epithelial cells and in the luminal secretion (Table 3). Namely, control animals were negative for PNA, while *B. ovis*-infected ones were positive. It has been reported that terminal sialidation does not take place in the cell membrane, in inflammatory and neoplastic

lesions, leaving PNA receptors available for binding [8, 14, 20]. There are possibilities that the above-mentioned changes of PNA-stainability might be explained by the same reason. Luminal secretion in the ampulla showed clear variations in the lectin pattern of PNA, SBA and Con A (Table 3).

Macrophages of spermatic granuloma in all the seminal vesicle and ampullary gland sections of infected animals, were positive for almost all lectins examined (Tables 2 and 3). This may suggest that they contained a complex mixture of carbohydrates, probably derived from phagocytized and disintegrated spermatozoa.

In conclusion, a marked modification of lectin binding pattern was observed in the reproductive tract of the affected animals. These alterations in the carbohydrate composition of organs participating in maturation, transport and storage of spermatozoa, could be involved in the impaired fertility observed in *B. ovis*-infected rams.

ACKNOWLEDGEMENTS. The present study was carried out as part of research cooperation between The University of Tokyo, Japan, and The National University of La Plata, Argentina, and partially financed by JICA (Japan International Cooperation Agency).

Table 1. Lectin staining pattern in epididymis of normal and *B. ovis*-infected rams

Part of tissue	Lectins									
	UEA-I		PNA		RCA-I		SBA		DBA	
	N	I	N	I	N	I	N	I	N	I
Epithelium										
Principal -cytoplasm	0	2	1	0	0	0	1	2	1	1
Cells -apical surface	0	3	3	0	3	3	3	3	2	0
Light/narrow cells	0	0	0	0	0	0	0	3	0	2
Connective tissue	0	0	0	0	1	2	0	0	0	0
Endothelium	0	0	0	0	2	2	0	0	0	0
Spermatozoa	0	3	1	3	2	1	2	2	0	0

Numbers indicate staining intensity on a subjectively estimated scale from 0, unreactive to 3, most reactive. N=normal rams, I=infected rams.

Table 2. Lectin staining pattern in seminal vesicles of normal and *B. ovis*-infected rams

Part of tissue	Lectins									
	UEA-I		PNA		RCA-I		SBA		DBA	
	N	I	N	I	N	I	N	I	N	I
Epithelium										
-cytoplasm	0-1	0	0	0	0	1	0	3	0	3
-apical surface	0	0	2-3	0-1	3	3	3	3	3	2
Connective tissue	0	0	0	0	1	1	0	0	1	0
Endothelium	0	0	0	0	0	0	0	0	0	0
Luminal secretion	0	0	0	1	3	3	2	2	3	3
Mononuclear cells	0	1-2	0	0	0	2	0	1	0	1
-macrophages	0	3	0	0	0	2	0	1	0	2

Numbers indicate staining intensity on a subjectively estimated scale from 0, unreactive to 3, most reactive. N=normal rams, I=infected rams.

Table 3. Lectin staining pattern in ampullae of normal and *B. ovis*-infected rams

Part of tissue	Lectins									
	UEA-I		PNA		RCA-I		SBA		DBA	
	N	I	N	I	N	I	N	I	N	I
Epithelium										
-cytoplasm	0	1	0	0-1	0	0	3	1	0-1	3
-apical surface	2	0	0	3	2	3	3	3	3	2
Connective tissue	0	0	0	0	0	2	0	0	0	1
Endothelium	0	0	0	0	0	1	0	0	0	0
Luminal secretion	0	0	0	3	1	3	2	0	3	3
Mononuclear cells	0	2	0	0	0	2	0	0	0	1
-macrophages	0	3	0	1	0	2	0	0	0	2

Numbers indicate staining intensity on a subjectively estimated scale from 0, unreactive to 3, most reactive. N=normal rams, I=infected rams.

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