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Glycohistochemical Study on the Denizli Cock Testis

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Abstract: Denizli cock is a special race which has been breeding in Denizli province of Turkey for a long time. It is famous with its long, beautiful and harmanious crowing and beautiful appearance. In this study, the testis of the Denizli cock was investigated using histological and lectin histochemical methods by light microscopy. The distribution of lectin bindings in the seminiferous epithelium of testis was studied using five digoxigenin labelled lectins including Peanut Agglutinin (PNA), *Datura stramonium* Agglutinin (DSA), *Galanthus nivalis* Agglutinin (GNA), *Maackia amurensis* Leucoagglutinin (MAL) and *Sambucus nigra* Agglutinin (SNA). Some differences in lectin staining density in interstitial space, basement membrane, spermatogenic cells and sertoli cells have been detected. Interstitial space, basement membrane, spermatogenic cells with DSA and sertoli cells with GNA showed the most intense stainings. In spermatogenic cells, GNA, MAL and SNA displayed moderately stainings. However, they were weakly stained with PNA. In general, PNA, GNA, MAL and SNA stainings were similar moderate stainings in interstitial space and basement membrane. In sertoli cells, weak to moderate staining density of PNA, MAL and SNA but strong staining density of DSA according to stainings of PNA, MAL and SNA were observed. The results demonstrated the distribution of some glycoconjugates in the testis of Denizli cock. These lectin-binding properties in Denizli cock testis were provided in reference to glycohistochemical investigations in birds.

Key words: Denizli cock, seminiferous tubules, glycoconjugates, lectin histochemistry, Turkey

INTRODUCTION

The avian reproductive tract consists of the paired testes, epididymis and ductus deferens. The testes resemble a bean shape and light yellow in color and are located in the peritoneal cavity, adjacent to the adrenal glands and ventral to the kidneys.

There are a bundle of tubules called seminiferous tubules that are separated by connective tissue in testes. They are very active parts and mainly the site for sperm production. Seminiferous epithelium consists of the multiple cells layer that contains differenting germ cells and sertoli cells. Sertoli cells are large cells extended from the base of the seminiferous epithelium to the interior of the tubules between spermatogonia. The specific relationship between sertoli cells and spermatids cause the spermatids to develop into active sperm. Interstitial spaces between seminiferous tubules contain capillaries, lymphatics, macrophage and Leydig cells which produce testosterone in response to the maturation of germ cells.

Glycoconjugates have an important role for cell differentiation (Roth, 1996) and maturation, cell to cell interaction (Varki *et al*., 1999). As it has been known that lectin histochemistry is a useful tool to investigate of the sugar residues of glycoconjugates in cells and tissues. Lectin binding patterns in testes and functions of glycoconjugates in seminiferous epithelium of mammals (Kurohmaru *et al*., 2000; Verini-Supplizi *et al*., 2000; Calvo *et al*., 2000; Gheri *et al*., 2004; Agungpriyono *et al*., 2007, 2009; Parillo *et al*., 2009) have been studied extensively. In the seminiferous epithelium, they play a pivotal role in germ cell differentiation related with the sexual hormon concentrations (Malmi *et al*., 1990; Liguoro *et al*., 2004). In human germ cells, specific alterations occur in cellular glycoconjugates during germ cell differentiation (Malmi *et al*., 1987). In hamster testis, histological, morphological and hormonal alterations in gonadally active and inactive states are reflected in altered patterns of expression and distribution of N and O-linked glycans (Pastor *et al*., 2003). In the camel testis, the topographical distribution of the sugar moieties may indicate that the necessity of specific carbohydrate structures for spermatogenesis during periods of sexual activity (Abd-Elmaksoud *et al*., 2008). Unlike mammals,

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there are a few studies about on the histochemical evaluation of glycoconjugates in testes of birds. Several studies have been carried out on histochemical, immunohistochemical and lectin histochemical examinations in avian male reproductive tract (Aire and Ozegbe, 2007; Bakst *et al*., 2007; Tingari and Lake, 1972; Abd-Elmaksoud, 2009).

Denizli cock is a special race which has been breeding in Denizli province of Turkey since the long times before. It is famous with its long, beautiful and harmanious crowing (15-35 sec) and beautiful appearance. The histological features of Denizli cock testes have not yet been investigated in details. Therefore, in order to provide additional data on the histomorphology and the localization and characterization of oligosaccharide moieties in the testes of birds, the present study reports the demonstrations of the histomorphologic structures and the glycoconjugates in the testes of Denizli cock.

MATERIALS AND METHODS

Sexually mature Denizli cocks (n =15) were obtained from the Agricultural Directory of Denizli province. In order to demonstrate the general histology of the testes, tissues taken from anesthetized cocks were fixed in 10% formalin, dehydrated through increasing concentrations of ethyl alcohol and embedded in paraffin. Sections (5 μm) mounted on glass slides were deparaffinized and rehydrated in a series of decreasing concentrations of ethanol solutions and distilled water, respectively. Slides were then stained with Hematoxylin and Eosin (H and E) stain. In order to demonstrate glycoconjugates of the testes, tissue pieces were rapidly frozen in liquid nitrogen for lectin histochemistry. Sections (6 μm) were cut in a cryostat and maintained at -25°C. Lectins used were Digoxigenin (DIG) labelled plant lectins, DSA, SNA, GNA, PNA, MAL (DIG glycan differentiation kit, Roche Diagnostics, Germany). The common names, sources and sugar specificity for each lectin are shown in Table 1. For the negative controls in which they were run by omitting the lectins were included in the analysis. All sections were photographed with a photomicroscope.

Table 1: Lectin characteristics

Abbreviation	Lectin sources	Carbohydrate binding specificity
GNA	*Galanthus nivalis*	α 1-3 and α 1-6 linked high mannose structures
MAL	*Maackia amurensis*	NeuAc α 2-3Gal
PNA	*Arachis hypogaea*	Gal β1-3GalNAc
DSA	*Datura stramonium*	Gal β1, 4 GlcNAc
SNA	*Sambucus nigra*	NeuAc α 2-6Gal

RESULTS AND DISCUSSION

The testis is formed of oval shaped seminiferous tubules (Fig. 1a) which are surrounded by tunica albuginea layer (Fig. 1b). The seminiferous tubules are covered by a basement membrane and connective tissue. In interstitial spaces between seminiferous tubules, leydig cells are distinguished with their round nuclei (Fig. 1c). Various spermatogenic cells and sertoli cells are located in seminiferous tubules (Fig. 1c and d) and dead cells are found at the center of tubule (Fig. 1d).

About 5 lectins applied, PNA, DSA, MAL, GNA and SNA showed a variety of staining patterns in the seminiferous epithelium (Table 2). In general, interstitial space, basement membran and round shaped spermatogenic cells rested upon the basement membrane were positive for all lectins but different in their staining intensities.

With PNA, spermatogenic cells showed weakly stainings. Regular lined dot-like granules were visualized around spermatogenic cells (Fig. 2a). Strong reactions to

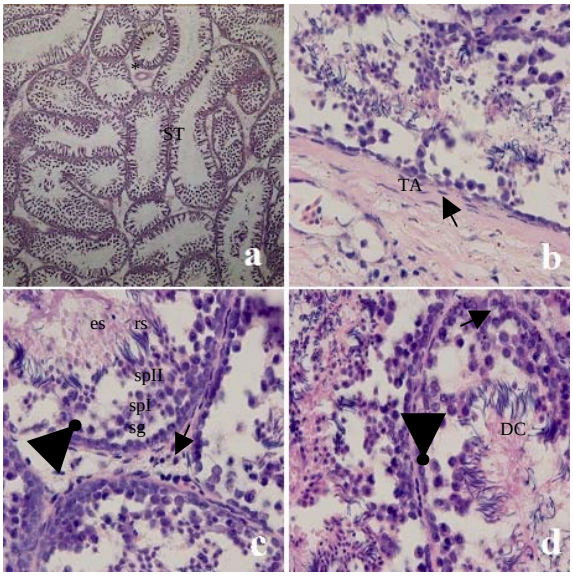

Fig. 1: Light micrograph sections stained hematoxylin and eosin; a) Oval shaped Seminiferous Tubules (ST), interstitial region (*) including capillary, x200; b) Tunica Albuginea layer (TA) containing myoid cells (arrow), x1000; c) Spermatogenic cells; spermatogonia (sg), primary spermatocyte (spI), secondary spermatocyte (spII), round spermatids (rs), elongated spermatids (es) and basement membrane (arrowhead), leydig cell with round nucleus (arrow), x1000; d) Pre-Leptotene spermatocyte (arrowhead) in meiosis with a pair of nuclei, sertoli cell with clear cytoplasm (arrow), Dead Cells (DC), x1000

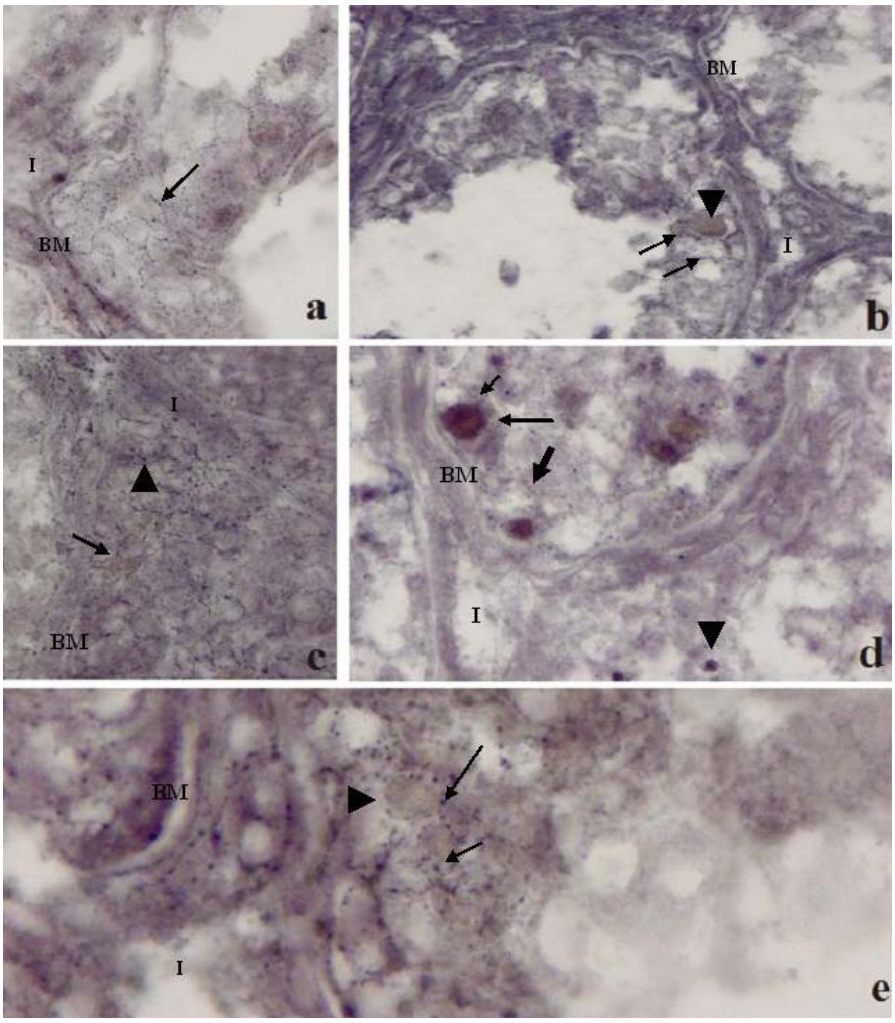

Fig. 2: Micrographs showing the PNA, DSA, GNA, MAL and SNA stainings in seminiferous tubules; a) Reaction with PNA. Spermatogenic cells present a weak reactivity. PNA positive dot-like granules (arrow) are visualized around cell membranes of spermatogenic cells; b) DSA stained strongly the Basement Membrane (BM), Interstitial space (I) and spermatogenic cells. Intense stained dot-like granules (arrow) are observed in the sertoli cell (arrowhead) cytoplasm and around cell membranes of spermatogenic cells; c) With MAL, basement membrane and spermatogenic cells were moderately stained and dot-like granules (arrowhead) surrounding spermatogenic cells gave intense positive reactions. The weakly stained sertoli cell is visualized on basement membrane (arrow); d) Basement membrane, interstitial space and spermatogenic cells were moderately stained with GNA. The intense stained large cell containing dot-like granules (short arrow) in cytoplasm indicates the sertoli cell (arrow) and the smaller ones the types of spermatogenic cells (arrowhead). Several GNA positive granules in cytoplasm of some spermatogenic cells are seen (thick arrow); e) SNA moderately stained basement membrane and spermatogenic cells. SNA positive granules are found in cell membrane and cytoplasms of spermatogenic cells. Sertoli cell with clear cytoplasm is observed (arrowhead), x1000

Table 2: Intensity of lectin binding in seminiferous epithelium of testis

Lectins	Interstitial regions	Basement membrane	Spermatogenic cells	Sertoli cells
PNA	+++	+++	+	++
DSA	++++	++++	++++	+++
GNA	+++	+++	+++	++++
MAL	+++	+++	+++	++
SNA	+++	+++	+++	++

Intensity of reaction: + (pale reaction) to ++++ (strongest reaction)

DSA were appeared in the basement membrane, interstitial space and spermatogenic cells. Sertoli cell with clear cytoplasm and intense stained granular structures surrounding cell was distinguished. Its staining pattern was stronger than the staining patterns of PNA, MAL and SNA (Fig. 2b). In MAL staining pattern, intensely stained granules surrounding spermatogenic cells were visualized.

Interstitial space, basement membrane and spermatogenic cells were moderately stained. Weakly stained sertoli cell was also observed (Fig. 2c). With GNA, basement membrane, interstitial space and spermatogenic cells were moderately stained. Various sized of intensily stained cells were visualiazed upon the basement membrane and in the lumen of tubules. The large ones may indicate the sertoli cells having dot-like granules within the cytoplasm, the smaller ones may indicate different types of spermatogonia and spermatids. Several dot-like granules in cytoplasm of some spermatogenic cells were also observed (Fig. 2d). SNA positive reaction was observed in granular structures around cell membranes of spermatogenic cells. Besides, SNA moderately stained interstitial space, basement membrane and spermatogenic cells but weakly stained sertoli cell (Fig. 2e).

According to the histochemical analyses, Denizli cock testis were resemble the Jungle Crow (*Corvus macrorhynchos*) testis whose seasonal testicular variations were studied. Similarly, the cells demonstrated as dead cells at the center of the seminiferous tubule in the Jungle Crow (Islam *et al*., 2010) were resemble the cells found at the center of the seminiferous tubule in Denizli cock. Also, the histological feature of tunica albuginea layer of testicular capsule was similar as described in the domestic fowl by Aire and Ozegbe (2007).

The glycoconjugate characteristics in testis have been demonstrated using lectins as histochemical markers not only in mammals but also in amphibians (Saez *et al*., 2000, 2001, 2004; Valbuena *et al*., 2010), shark (Kassab *et al*., 2009) and spotted ray (Liguoro *et al*., 2004). However, several studies have been conducted for birds on this topic as mentioned above.

The present research was carried out in order to demonstrate the glycoconjugates in Denizli cock testis using digoxigenin labelled PNA, DSA, GNA, MAL and SNA lectins. In general, intertitial space, tubular wall and spermatogenic cell lines in seminiferous tubules were reactive to all lectins. The differences were in their staining patterns.

Researchers observed some granular-like structures surrounding the cells and also in their cytoplasms reacted with lectins. For example, PNA with affinity for Gal β1-3GalNAc residues showed regular lined granules around the spermatogenic cells. Similar carbohydrate moieties were reported in the seminiferous epithelium of the common tree shrew (*Tupaia glis*) as a granular reaction in spermatocyte cytoplasm with *Griffonia simplicifolia* agglutinin II (Kurohmaru *et al*., 1996) and in primary spermatocytes of the bovine testis during postnatal ontogenesis as faintly stained small granules with horseradish peroxidase labelled-PNA (Ertl and Wrobel, 1992).

Sertoli cell and spermatogenic cells reacted positively with DSA that specifically binds to Gal β1,4 GlcNAc. It also displayed strong staining in the tubular wall. Likewise, more intense reaction with DSA was reported in spermatogenetic cells of the urodele amphibian *pleurodeles waltl* (Saez *et al*., 2000). In GNA staining pattern, it can be suggested that the cells appeared to be densely stained with different sizes in seminiferous tubules contain more mannose residues. The positive reactions of MAL and SNA observed in basement membrane, sertoli cells, cell membranes of spermatogenic cells and intertitial space indicated the presence of N-acetylneuraminic acid-binding galactose. Similarly, using MAA (MAL) and SNA lectins (as well as PNA, GNA, DSA lectins), some differences in glycan composition between the interstitial and the glandular tissue were shown in the amphibian *Pleurodeles waltl* testis (Saez *et al*., 2001).

CONCLUSION

The present study is an original one to describe the histology and some glycoconjugate characteristics of the seminiferous epithelium in Denizli cock testis. Since, there is little fundamental information about glycohistochemical aspects of testis in birds unlike mammals, this study may provide the glycoconjugate research for testicular tissue in birds.

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