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# Application of Lectins for Detection of Glycomic Differences in the Epididymal Duct in Water Buffalo (*Bubalus bubalis*) and Dromedary Camel (*Camelus dromedarius*)

## Aplicación de Lectinas para la Detección de Diferencias Glucómicas en el Conducto Epididimario en Búfalos de Agua (*Bubalus bubalis*) y Dromedarios (*Camelus dromedarius*)

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**SUMMARY:** The present study was conducted to detect the differences in glycohistochemical features in the epididymal duct of the dromedary camel and the water buffalo. Epididymal sections (caput, corpus and cauda) from both species were stained with fluorescein isothiocyanate (FITC) conjugated lectins. Binding sites for five lectins (DBA, GSA-1, HPA, PNA and WGA) have been found in both species. The binding sites of different lectins showed significant variations in the pattern of distribution in both a species. This included both species-specific and region-specific order. Additionally, only three (GSA-1, PNA and WGA) out the five lectins studied exhibited binding sites in all epididymal regions in both species. The other two lectins (DBA and HPA) followed the same order recorded for the other three (GSA-1, PNA and WGA) in buffalo, but failed to show any binding sites in cauda epididymis in camel. In conclusion, the variable regional and species-specific distribution features of lectins revealed that both species have diverse glycomic characteristics that may be related to their different reproductive patterns. However, the glycome-associated functional capacities remain obscured and need further profound investigations.

**KEY WORDS: Buffalo; Camel; Epididymis; Lectins.**

## INTRODUCTION

Despite the economic value of dromedary camels and buffaloes, relatively little is known about their reproductive biology. Data available about the buffalo (Abdou *et al*., 1985; Cruzana *et al*., 2003; Alkafafy *et al*., 2011a) and the dromedary camel (Tingari & Moniem, 1979; Singh & Bharadwaj, 1980; Abd El-maksoud, 2010; Alkafafy *et al*., 2011b) are still relatively scarce.

Lectins are specific types of proteins, which have the capacity to specifically bind carbohydrates. Though this binding displays similarity to antigen-antibody reaction specificity, it rather has been built on non-immunologic basis. They have a wide range of distribution in nature and possess the capacity to specifically bind certain sugar residues via carbohydrate-binding sites (Goldstein & Poretz, 1986). Lectins are valuable means for recognition of sugar residues in different histological sections (Lohr *et al*., 2010).

Lectin histochemical studies on the epididymis have been carried out in different species including human (Arenas *et al*., 1998), rat (Arya & Vanha-Perttula, 1984), mouse (Lohr *et al*., 2010), hamster (Calvo *et al*., 1995), dog (Schick *et al*., 2009), bull (Arya & Vanha-Perttula, 1985; Alkafafy, 2005), boar (Calvo *et al*., 2000), horse (Ha *et al*., 2003) and alpaca (Parillo *et al*., 2009). However, the data about the patterns of lectin binding to their specific glycoconjugates in the epididymis of the camel and the buffalo are little or even unavailable.

The present study aimed to shed light on the regional differences of the epididymal duct in both the buffalo and the dromedary camel. As far as we are aware, the present study is the first to use lectins in a comparative study to describe the glycoconjugates distribution in the different epididymal segments in two economically valuable species: dromedary

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camel and water buffalo, in order to expose species-specific regional differences of their epididymal glycome.

## MATERIAL AND METHOD

**Animals and tissues.** The epididymal tissues were collected from seven adult male buffalos (*Bubalus bubalis*) (5 years old) and from seven adult camels (*Camelus dromedarius*) (6 years old) after slaughter (Cairo abattoir, Egypt). The camel's samples were collected during the breeding season (January-March) in Egypt. Specimens from different regions of the epididymal duct (caput, corpus and cauda) were immediately immersed in the fixative.

**Histology and lectin histochemistry.** Specimens were immersed overnight in Bouin's fluid, dehydrated in a graded series of ethanol, cleared in xylene, embedded in Paraplast wax (Sigma-Aldrich, St. Louis, MO, USA) and sectioned at 5mm thickness. Tissue sections were mounted on positively charged, coated slides (Thermo Scientific). Distribution of glycoconjugates in the epididymal tissues was investigated using five different fluorescein isothiocyanate (FITC) conjugated lectins (Sigma-Aldrich, Munich, Germany) (Table III). Dewaxed and rehydrated sections were subjected to lectin histochemical procedures as described by Alkafafy (2005).

**Scoring and photomicrography.** Lectin-stained epididymal tissues and their controls were evaluated (a semi-quantitative subjective scoring performed by three independent observers) by using a Dialux 20 fluorescent microscope (Leitz GmbH, Wetzlar, Germany). The photos were taken using Kodak Elite 400 film.

## RESULTS

1. Lectin-binding sites in the caput region (Fig. 1, Table I).

**Basal cells.** In sections from buffalo epididymal head, the basal cells displayed binding sites for WGA, PNA and HPA. This binding was weak (WGA and PNA) or negative to weak

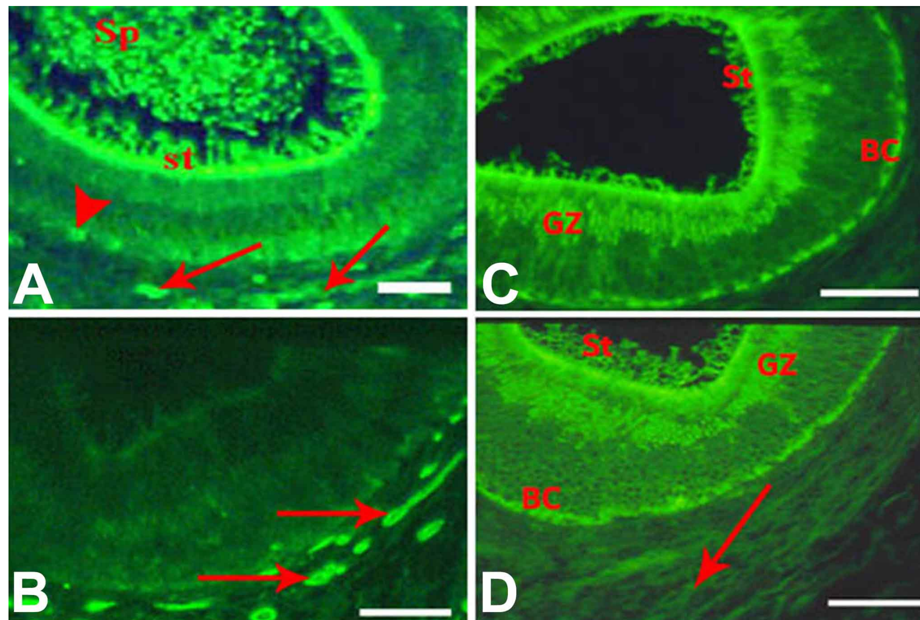


Fig. 1. Distribution of binding sites of FITC-lectins in the epididymal caput: (a) PNA-binding sites in buffalo showing strong [apical cytoplasm and stereocilia (St), and in sperm cell mass (Sp)], moderate in IELs (arrowhead) and vascular endothelium (arrows) and weak labeling in Golgi zone (GZ); (b) GSA-1-binding sites in buffalo displaying distinct staining in the interstitial blood vessels (arrows); (c) WGA-binding sites in camel showing distinct staining in stereocilia (st), apical cytoplasm, Golgi zone (GZ) and basal cells (BC); (d) GSA-1-binding sites in camel displaying distinct labeling in Golgi zone (GZ), apical cytoplasm, stereocilia (st) and basal cells (BC) but not in vascular endothelium (arrow); Scale bars: 50 (a and b) and 100 µm (c and d).

Table I. Distribution of lectin-binding sites in the caput epididymis in the buffalo and the camel.

| Lectins | Buffalo | | | | | | | Camel | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BC | PC | | | IEL | Sp | VE | BC | PC | | | IEL | Sp | VE |
| | | St | A Cyt | GZ | | | | | St | A Cyt | GZ | | | |
| DBA | - | ± | ± | ± | +/++ | + | ± | - | +/++ | ++ | ++ | + | + | - |
| HPA | ± | ++ | ++ | + | - | + | - | + | + | + | ++ | - | - | - |
| WGA | + | + | ++ | +++ | +/++ | +++ | + | +++ | ++ | ++ | ++ | +/++ | ++ | - |
| PNA | + | +++ | +++ | ± | - | +++ | ++ | - | - | - | - | - | ± | - |
| GSA-1 | - | - | - | - | ++ | - | +++ | ±/+ | +/++ | ++ | ++ | ±/+ | +/++ | - |

Negative (-), negative to weak (±), weak (+), moderate (++), strong (+++) labeling.

(HPA and GSA-1). No binding sites to either DBA or GSA-1, could be found in the basal cells at the region of buffalo epididymal head. On the other hand, sections from camel epididymal head, manifested basal cells with strong (WGA), weak (HPA), negative to weak (GSA-1 reaction) or negative reaction (DBA and PNA).

**Principal cells**

**Stereocilia.** The stereocilia of the principal cells in sections from buffalo epididymal head showed binding sites for each of WGA, HPA, DBA and PNA. The lectin distribution pattern in the buffalo epididymal head displayed strong (PNA), moderate (HPA), weak (WGA), negative to weak (DBA) and negative reaction (GSA-1). Stereocilia of the principal cells in sections from camel epididymal head showed binding sites for each of WGA, DBA, HPA and GSA-1. The intensity of lectins binding exhibited moderate (WGA), weak to moderate (DBA and GSA-1), weak (HPA), and negative reactivity (PNA).

**Apical Cytoplasm.** The apical cytoplasm of the principal cells in sections from buffalo epididymal head showed binding sites for each of WGA, DBA, HPA and PNA. The lectin distribution pattern in the buffalo epididymal head displayed strong (PNA), moderate (WGA and HPA), negative to weak (DBA) and negative reaction (GSA-1). Stereocilia of the principal cells in sections from camel epididymal head showed moderate binding for each of WGA, DBA, HPA and GSA-1.

**Golgi zone.** The Golgi zone of the principal cells in sections from buffalo epididymal head showed binding sites for each of WGA, DBA, HPA and PNA. The lectin distribution pattern in the buffalo epididymal head displayed strong (WGA), weak (HPA), negative to weak (DBA and PNA) and negative reaction (GSA-1). Golgi zone of the principal cells in sections from camel exhibited binding sites for each of WGA, DBA, HPA and GSA-1. The intensity of lectins binding exhibited moderate (DBA, HPA, WGA and GSA-1) and negative reactivity (PNA).

**Intraepithelial leukocytes.** The intraepithelial leukocytes (IELs) in sections from both buffalo and camel epididymal head regions, displayed binding sites for WGA, DBA and GSA-1. However, the intensity of binding was different in both species. The intensity in sections from buffalo was either moderate (GSA-1) or weak to moderate (WGA and DBA). On the other hand, the reaction in sections from camel was either weak to moderate (WGA), weak (DBA) or negative to weak (GSA-1). No binding sites to either HPA or PNA, could be found in the IELs at the head region in both species.

**Sperm cell mass.** The sperm cell mass (SCM) in sections from both buffalo and camel epididymal head regions, displayed binding sites for WGA, DBA and PNA. Whereas, HPA-binding sites could be found only in sections from buffalo, binding sites for GSA-1 have been found only in sections from camel. The intensity in sections from buffalo was either strong (WGA and PNA) or weak (HPA and DBA). On the other hand, the reaction in sections from camel was either moderate (WGA), weak to moderate (GSA-1), weak (DBA) or negative to weak (PNA).

**Blood vessels endothelium.** The endothelium of the interstitial blood vessels in sections from buffalo epididymal head, showed binding sites for WGA, DBA, GSA-1 and PNA. Whereas, in sections from camel no bindings sites could be found. The binding intensity in sections from buffalo was either strong (GSA-1), moderate (PNA) or weak (WGA and DBA). On the other hand, the reaction in sections from camel was almost negative.

Lectin-binding sites in the corpus region (Fig. 2, Table II).

**Basal cells.** In sections from buffalo epididymal body, the basal cells displayed binding sites for WGA, PNA and HPA. This binding was weak (WGA and PNA) or negative to weak (HPA). No binding sites to either DBA or GSA-1, could be found in the basal cells at the region of buffalo epididymal body. On the other hand, sections from camel epididymal body, manifested basal cells with weak (GSA-1), negative to weak (WGA and HPA) or negative reaction (DBA and PNA).

**Principal cells**

**Stereocilia.** The stereocilia of the principal cells in sections from buffalo epididymal body showed binding sites for each of WGA, HPA, DBA and PNA. The lectin distribution pattern in the buffalo epididymal body displayed strong (HPA), moderate (WGA), negative to weak (DBA and PNA) and negative reaction (GSA-1). Stereocilia of the principal cells in sections from camel epididymal body showed binding sites for each of DBA, WGA, HPA and GSA-1. The intensity of lectin binding exhibited negative (PNA) to weak (WGA), weak (HPA and GSA-1), and moderate reactivity (DBA).

**Apical Cytoplasm.** The apical cytoplasm of the principal cells in sections from buffalo epididymal body showed binding sites for each of WGA, HPA and PNA. The lectin distribution pattern in the buffalo epididymal body displayed strong (HPA), weak (PNA), negative to weak (WGA and DBA) and negative reaction (GSA-1). The

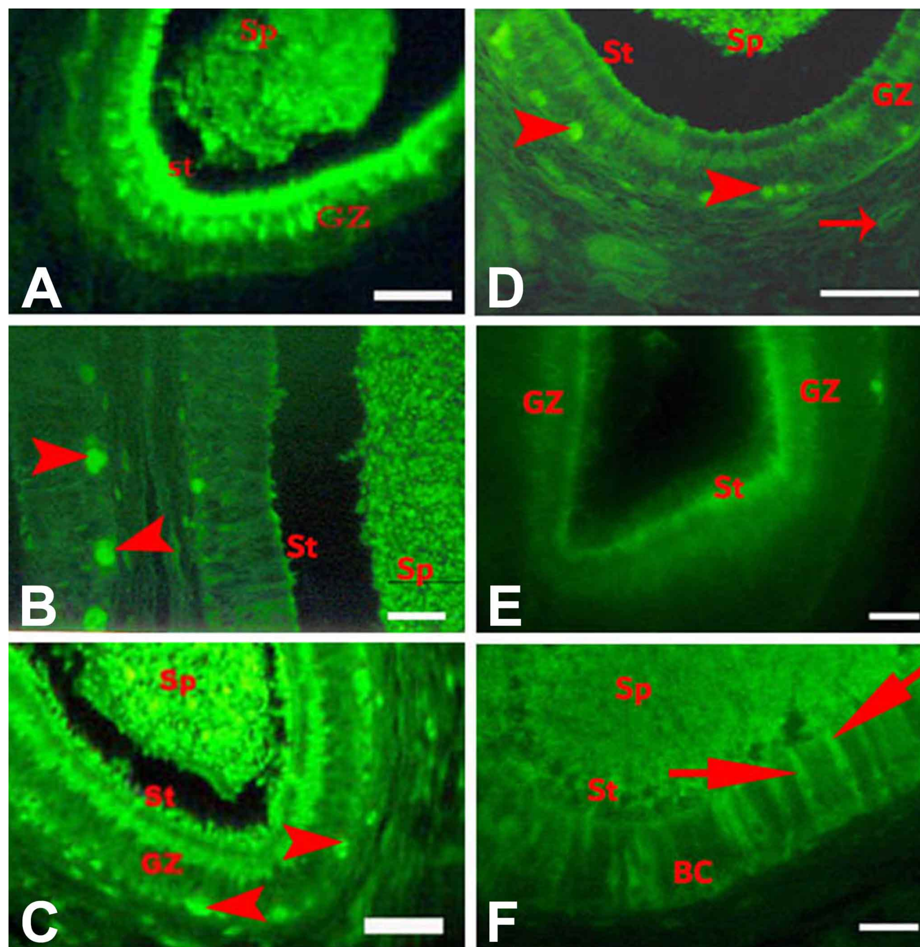


Fig. 2. Distribution of binding sites of FITC-lectins in the epididymal corpus: (a) HPA-binding sites in buffalo displaying distinct staining in apical cytoplasm, stereocilia (st) and in Golgi zone (GZ), and a weak to moderate reaction in sperm cell mass (Sp); (b) DBA-binding sites in buffalo showing negative to weak staining in apical cytoplasm and stereocilia (st), and moderate reaction in sperm cell mass (Sp) and IELs (arrowhead); .(c) WGA-binding sites in buffalo displaying distinct labeling in Golgi zone (GZ), stereocilia (st), in sperm cell mass (Sp) and IELs (arrowhead); (d) GSA-1-binding sites in camel exhibiting negative to weak reactivity in vascular endothelium (arrows), weak labeling in stereocilia (st) and in Golgi zone (GZ), moderate staining in sperm cell mass (Sp) and IELs (arrowhead); (e) DBA-binding sites in camel displaying weak staining in Golgi zone (GZ) and moderate reaction in stereocilia (st); (f) WGA-binding sites in camel showing weak labeling in narrow cells (longhead arrow), sperm cell mass (Sp) and negative to weak staining in stereocilia (st) and basal cells (BC). Scale bars: 25 (e), 50 (a, b, c and f) and 100 μm (d).

Table II. Distribution of lectin-binding sites in the corpus epididymis in the buffalo and the camel.

| Lectins | Buffalo | | | | | | | Camel | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BC | PC | | | IEL | Sp | VE | BC | PC | | | IEL | Sp | VE |
| | | St | A Cyt | GZ | | | | | St | A Cyt | GZ | | | |
| DBA | - | ± | ± | - | +/++ | +/++ | ± | - | ++ | - | ± | +/++ | ± | - |
| HPA | ± | +++ | +++ | ++ | - | +/++ | - | ± | + | + | + | - | - | - |
| WGA | + | ++ | ± | ++ | ++ | ++ | + | ± | ± | ± | - | ± | + | - |
| PNA | + | ± | + | +++ | ++ | +++ | ++ | - | - | - | - | ± | + | ± |
| GSA-1 | - | - | - | - | ++ | - | +++ | + | + | + | ± | +/++ | ++ | ± |

Negative (-), negative to weak (±), weak (+), moderate (++), strong (+++) labeling.

apical cytoplasm of the principal cells in sections from camel epididymal body showed binding sites for each of WGA, HPA, and GSA-1. The intensity of lectins binding exhibited weak (HPA and GSA-1), negative to weak (WGA and DBA) and negative reactivity (WGA and PNA).

**Golgi zone.** The Golgi zone of the principal cells in sections from buffalo epididymal body showed binding sites for each of WGA, HPA and PNA. The lectin distribution pattern in the buffalo epididymal body displayed strong (PNA), moderate (HPA and WGA), and negative reaction (DBA and GSA-1). Golgi zone of the principal cells in sections from camel exhibited binding sites for each of HPA and GSA-1. The intensity of lectins binding exhibited weak (HPA), negative to weak (GSA-1 and DBA) and negative reactivity (WGA and PNA).

**Intraepithelial leukocytes.** The intraepithelial leukocytes (IELs) in sections from both buffalo and camel epididymal body regions, displayed binding sites for WGA, DBA, PNA

and GSA-1. However, the intensity of binding was different in both species. The intensity in sections from buffalo was either moderate (GSA-1, WGA and PNA) or weak to moderate (DBA). On the other hand, the reaction in sections from camel was either weak to moderate (DBA and GSA-1) or negative to weak (WGA, PNA). No binding sites to HPA, could be found in the IELs at the body region in either species.

**Sperm cell mass.** The sperm cell mass (SCM) in sections from both buffalo and camel epididymal body regions, displayed binding sites for WGA, DBA and PNA. Whereas, HPA-binding sites could be found only in sections from buffalo, and the binding sites for GSA-1 have been found only in sections from camel. The intensity in sections from buffalo was either strong (PNA), moderate (WGA) or weak to moderate (HPA and DBA). On the other hand, the reaction in sections from camel was either moderate (GSA-1), weak (PNA and WGA) or negative to weak (DBA).

**Blood vessels endothelium.** The endothelium of the interstitial blood vessels in sections from buffalo epididymal body, showed binding sites for WGA, DBA, GSA-1 and PNA. Whereas, in sections from camel bindings sites could be found only for GSA-1 and PNA (negative to weak reactivity). The binding intensity in sections from buffalo was either strong (GSA-1), moderate (PNA) or weak (WGA) and negative to weak (DBA).

Lectin-binding sites in the cauda region (Fig. 3, Table III).

**Basal cells.** In sections from buffalo epididymal tail, the basal cells displayed a weak binding with both of WGA, PNA and HPA. No binding sites to either DBA or GSA-1, could be found in the basal cells at the region of buffalo epididymal tail. On the other hand, sections from camel epididymal tail, manifested basal cells with a moderate reaction only with WGA.

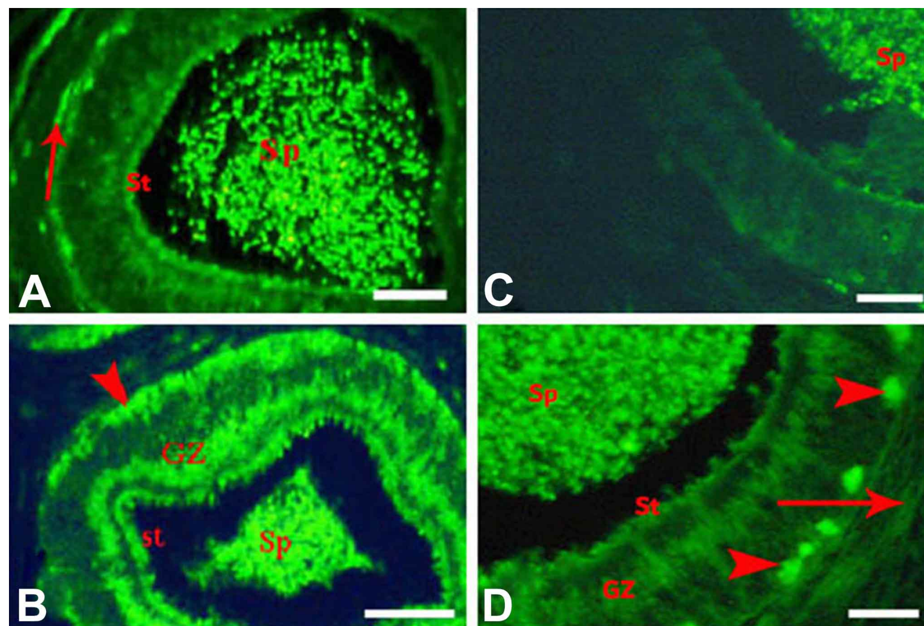


Fig. 3. Distribution of binding sites of FITC-lectins in the epididymal cauda: (a) PNA-binding sites in buffalo displaying distinct staining in sperm cell mass (Sp), weak staining in apical cytoplasm and stereocilia (st), and weak to moderate reaction in the interstitial blood vessels (arrows); (b) HPA-binding sites in buffalo showing distinct labeling Golgi zone (GZ) and in apical cytoplasm and stereocilia (st) and in sperm cell mass (Sp) and IELs (arrowhead) (c) PNA-binding sites in camel displaying a moderate staining only in sperm cell mass (Sp); (d) WGA-binding sites in camel showing strong labeling in IELs (arrowhead) and sperm cell mass (Sp), and weak staining in stereocilia (st) and in Golgi zone (GZ). Scale bars: 25 (d) and 50 μm (a, b and c).

Table III. Distribution of lectin-binding sites in the cauda epididymis in the buffalo and the camel.

| Lectins | Buffalo | | | | | | | Camel | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BC | PC | | | IEL | Sp | VE | BC | PC | | | IEL | Sp | VE |
| | | St | A Cyt | GZ | | | | | St | A Cyt | GZ | | | |
| DBA | - | - | - | - | ++ | ++ | ± | - | - | - | - | - | - | - |
| HPA | + | + | - | ++ | - | ++ | - | - | - | - | - | - | - | - |
| WGA | + | + | ± | ± | ++ | ++ | + | ++ | + | ± | ± | + | ++ | ± |
| PNA | + | ± | ± | ± | ++ | +++ | ++ | - | - | - | - | - | ++ | - |
| GSA-1 | - | - | - | - | +/++ | - | +++ | - | - | - | - | ± | +/++ | ± |

Negative (-), negative to weak (±), weak (+), moderate (++), strong (+++) labeling. Apical cytoplasm (A Cyt), basal cell (BC), Golgi zone (GZ), intraepithelial leukocytes (IEL), principal cell (PC), sperm cell mass (Sp), stereocilia (st) and vascular endothelium (VE).

### Principal cells

**Stereocilia.** The stereocilia of the principal cells in sections from buffalo epididymal tail showed binding sites for each of WGA, HPA and PNA. The lectin distribution pattern in the buffalo epididymal tail displayed weak (HPA and WGA) or negative to weak (PNA) and negative reaction (DBA and GSA-1). Stereocilia of the principal cells in sections from camel epididymal tail showed binding sites only for WGA (weak reactivity).

**Apical Cytoplasm.** The apical cytoplasm of the principal cells in sections from buffalo epididymal tail showed negative to weak binding only with WGA and PNA. The apical cytoplasm of the principal cells in sections from camel epididymal tail showed binding sites only with WGA (negative to weak reactivity).

**Golgi zone.** The Golgi zone of the principal cells in sections from buffalo epididymal tail showed binding sites for each of WGA, HPA and PNA. The lectin distribution pattern in the buffalo epididymal tail displayed moderate (HPA), negative to weak (WGA and PNA), and negative reaction (DBA and GSA-1). Golgi zone of the principal cells in sections from camel exhibited binding sites only for WGA (negative to weak reactivity).

**Intraepithelial leukocytes.** The intraepithelial leukocytes (IELs) in sections from buffalo epididymal tail, displayed moderate (WGA, DBA and PNA), weak to moderate (GSA-1) or negative reactivity (HPA). On the other hand, the reaction in sections from camel was either weak (WGA) or negative to weak (GSA-1). No binding sites for DBA, PNA or HPA could be found in the IELs at the tail region in camel.

**Sperm cell mass.** The sperm cell mass (SCM) in sections from buffalo epididymal tail displayed binding sites for WGA, DBA, HPA and PNA. Binding sites for GSA-1 could not be found in SCM within the buffalo epididymal tail. The intensity in sections from buffalo was either strong (PNA) or moderate (WGA, HPA and DBA). On the other hand, the SCM in sections from camel epididymal tail displayed moderate reactivity with both of WGA, GSA-1 and PNA. No binding sites could be found for either DBA or HPA in camel epididymal cauda.

**Blood vessels endothelium.** The endothelium in sections from buffalo epididymal cauda, showed binding sites for WGA, DBA, GSA-1 and PNA. Whereas, in sections from camel bindings sites could be found only for GSA-1 and WGA (weak reactivity). The binding intensity in sections from buffalo was either strong (GSA-1), moderate (PNA), weak (WGA and DBA). Moreover, HPA-binding sites could not be found in the vascular endothelium throughout the whole length of the epididymis in either species.

**Controls.** None of the negative control sections that were carried out by omission of the lectin presented any positive labeling. Likewise, all of the sections that were treated by pre-incubating lectins with the corresponding hapten sugar inhibitor displayed no labeling.

## DISCUSSION

Because of their specific binding affinity for sugar residues in glycoconjugates, lectins are widely used as markers for detection of reactive saccharide groups in histological sections (Lohr *et al*., 2010).

The main glycohistochemical findings are shown in Tables I, II and III and representative photomicrographs are displayed in Figures 1, 2 and 3. The current results displayed that all the lectins used (GSA-I, PNA, WGA, HPA, DBA) exhibited an inconstant pattern of distribution in the epididymis of buffalo and camel, exposing resemblances and discrepancies to those observed in other mammals (Arya & Vanha-Perttula, 1984; Calvo *et al*., 1995; Arenas *et al*., 1998; Calvo *et al*., 2000; Ha *et al*., 2003; Alkafafy, 2005; Parillo *et al*., 2009; Schick *et al*., 2009; Lohr *et al*., 2010).

Though some lectins have the specificity to the same monosaccharide, they show divergent binding features. For instance, both PNA and GSA-1 are N-acetyl-galactosamine-binding lectins. They however, displayed evident variability in their distribution pattern throughout the epididymal duct. This goes in line with earlier studies on the bovine epididymis (Alkafafy, 2005). Therefore, lectins with analogous specificity to a certain monosaccharide can detect diversities in the structural complexity of receptors (Kunz *et al*., 1984). Consequently, lectins with identical specificity to a sugar may not link to the same glycoconjugates (Malmi & Söderström, 1988). This may be attributed to the notion that the binding of a monosaccharide with its specific lectin may be affected by numerous actors, for example the impact of the adjacent sugars.

### Lectin-binding sites in principal cells

**Stereocilia and apical cytoplasm.** The SC and the apical cytoplasm of the principal cells in sections from buffalo epididymis showed a strong PNA-labelling (caput), which has been reduced but still expressed in the distal epididymal segments. On the other hand, no PNA-labelling could be found either in SC or in the apical cytoplasm throughout the

whole length of the camel epididymis. A similar pattern was reported for HPA-labelling in SC of PCs, which was moderate (caput), strong (corpus) and weak (cauda) in the buffalo, but the camel's epididymis revealed a weak (caput and corpus) and negative (cauda) HPA-labelling. On the other hand, the apical cytoplasm of PCs in the caput presented either moderate (in buffalo) or weak (in camel) HPA-labelling. While, those in the corpus displayed either strong (in buffalo) or weak (in camel) HPA-labelling. Additionally, HPA-reactivity was totally absent in the apical cytoplasm of PCs in cauda. On the other hand, DBA-labelling in SC and the apical cytoplasm from buffalo epididymis reduced from weak in caput and corpus to negative in cauda, while those from camel showed moderate (in caput) reduced to negative DBA-labelling in the distal segments. Regarding the WGA-binding, SC showed weak (caput and cauda) to moderate labelling (corpus) in buffalo, whereas in camel they displayed moderate (caput) reduced to weak labeling (corpus and cauda). Additionally, WGA-labelling in the apical cytoplasm of PCs were consistent in both species, where a moderate labelling was reported in the caput region with gradual reduction (weak to negative) in reactivity in corpus and cauda in both species. Though both DBA and HPA are N-acetyl-D-galactosamine-binding lectins, they exhibited a variable pattern of labelling in the SC and apical cytoplasm, especially in the caput from both species with remarkable reactivity of HPA in buffalos and DBA in camels. Additionally, both N-acetylglucosamine-binding lectin WGA and the N-acetyl-galactosamine-binding lectin PNA exhibited a similar pattern where PNA was highly reactive to SC and apical cytoplasm in caput from buffalo, while WGA was more reactive to SC and apical cytoplasm in caput from camel. Though GAS-1 and PNA are N-acetyl-galactosamine-binding lectins, they behaved differently. In contrast to PNA, GSA-1-labelling in the SC and apical cytoplasm of the PCs was totally absent from the epididymis of buffalo, but in the camel epididymis it revealed moderate (caput), weak (corpus) and negative (cauda) GSA-1-labelling. The current results presented resemblances and discrepancy with those stated in other mammals (Calvo *et al*., 2000; Ha *et al*., 2003; Alkafafy, 2005; Parillo *et al*., 2009; Schick *et al*., 2009).

It is well noted that the lectin biding sites in the SC of PCs showed either a comparable (WGA) or a different (DBA, GSA-1, HPA and PNA) pattern of distribution and intensity. Thus, both GSA-1 and DBA were more reactive to SC from camel, whereas both HPA and PNA were more reactive to SC from buffalo. This discrepancy indicates that the SC of the buffalo have a glycomic pattern diverse from that of the camel reflecting divergent species-specific absorptive capacities. In general, the labeling of SC with different lectins was significantly decreased and became limited to certain lectins in the distal epidydimal segments (corpus and cauda) in both species. So, the regional variances in the pattern of lectin binding sites on the SC of the epididymal epithelium may lend support to the results stated by Sinowatz (1981), who mentioned that the SC in the caput region express marked activity of alkaline phosphatase (ALPase); while those of the distal regions were ALPase-negative. Moreover, the marked ALPase-activity may be related to absorptive role of PCs (Goyal *et al*., 1980). Additionally, most of the rete testis fluid is reabsorbed in the region of the caput epididymis (Dacheux *et al*., 1989).

**Golgi Bodies.** Golgi bodies are membranous cytoplasmic organelles which are concerned by the modifications of proteins by glycosylation (Ramakrishnan *et al*., 2001). In the present work, there was a conspicuous segment-specific variance regarding the binding sites of different lectins as exhibited by the GZ of the PCs involving the whole epididymal duct in buffalo and camel. The GZ of the PCs in sections from buffalo epididymis showed a strong WGA-labelling (caput), which gradually reduced in the distal epididymal segments. However, the GZ in the camel epididymis behaves somewhat differently. It revealed a moderate (in caput), negative (in corpus) and weak WGA-labelling (in cauda). Though, the GZ of PCs in buffalo epididymis displayed weak PNA-labelling in the proximal (caput) and distal (cauda) segments, those in the middle segment (corpus) showed a strong reactivity. On the other hand, the GZ of PCs in camel epididymis didn't show any PNA-labelling. The pattern of HPA-labelling in GZ of PCs from buffalo epididymis was weak (in caput) and increased to moderate labelling at the more distal segments. Contradicting to the case in buffalo, GZ of PCs from camel epididymis showed moderate (in caput), weak (in corpus) and negative (in cauda) HPA-labelling. DBA-labelling in GZ of PCs from both species was only found in the caput region, however the intensity was more evident in camel epididymis (moderate reactivity) than in the buffalo epididymis (weak reactivity). The GZ of PCs from buffalo epididymis didn't show any binding sites for GSA-1, however those from camel epididymis displayed moderate GSA-1-labelling (in caput), which gradually vanished in the distal segments.

These results displayed that the labeling of the GZ was evident in the caput epididymis in contrast with the corpus and cauda epididymis in both species. Yet, it demonstrated a species-specific pattern as denoted by unbinding GZ of PCs to certain lectins (PNA in camel) and (GSA-1in buffalo). However, the other lectins (WGA, HPA and DBA) used in the study displayed a pattern of labelling characteristic for each species. Furthermore, the labeling of GZ in the corpus epididymis was more intense in the buffalo

than in the camel. These findings go in line, with previous studies in large ruminants (Alkafafy, 2005), llama (Parillo *et al*., 2009), boar (Calvo *et al*., 2000), and dog (Schick *et al*., 2009). Yet, the present results contradict those reported in humans, where segmental variances were indistinct (Arenas *et al*., 1996). Although the labeling nearly faded out in the cauda from both species, the GZ in buffalo cauda still exhibit a distinct HPA-labelling. Comparable results were found in the cauda epididymis from boar (Calvo *et al*., 2000), in which the GZ showed noticeable HPA-binding. These findings propose that, in addition to the marked secretory potentials of PCs in the caput epididymis, the distal segments of buffalo epididymis may hold some secretory capacity. It is worth noting that PCs may possess either secretory or absorptive activities or both (Moore & Bedford, 1979).

**Lectin-binding sites in basal cells.** In parallel to the findings reported in previous studies on the epididymis in different species (Calvo *et al*., 2000; Ha *et al*., 2003; Alkafafy, 2005; Parillo *et al*., 2009; Schick *et al*., 2009), the BCs displayed variable reactivity with most of lectins used in the present study. This inconsistency included both the intensity and the distribution pattern of binding. For example, the BCs of the camel epididymis revealed a strong WGA binding in the caput, weak in the corpus and moderate in the cauda. On the contrary, BCs of the buffalo epididymis showed a weak WGA-labeling throughout the entire length of the epididymis. Additionally, HPA-labeling was generally weak and rather contradictory in BCs from both species. BCs from camel's epididymal caput displayed a weak HPA-labeling, which diminished distally until disappeared in cauda. On the other hand, BCs from buffalo's epididymis exhibited more or less negative HPA-labeling, which increased distally to be weak labelling at the cauda. However, the BCs from both species showed negative DBA-labelling throughout the entire length of the epididymal duct. The BCs showed either a weak PNA-labelling in buffalo or totally negative PNA-labelling in camel throughout the whole length of the epididymis. The BCs from camels showed more or less weak (caput and corpus) or negative (cauda) GSA-1-labelling, however those from buffalo were negative throughout the duct. It is worth noting that the BCs embody the second constant cell population of the epithelium lining the epididymal duct. Though their role is to a great extent unidentified (Amann, 1989), BCs are supposed to be reserve cells (Bidwai & Bawa, 1981) for the renewal of the epididymal epithelium, yet, this hypothesis may be inacceptable (Arrighi *et al*., 1993).

**Lectin binding sites in intraepithelial leukocytes.** The IELs in sections from both buffalo and camel epididymal head regions, displayed binding sites for WGA, DBA and GSA-1. However, the intensity of binding was different in both species. The IELs from buffalo epididymis displayed moderate GSA-1-labelling in caput and corpus, which was reduced to weak in cauda, whereas in camel they showed negative to weak in caput and cauda and weak to moderate reactivity in corpus. Regarding the WGA-labelling, IELs from buffalo epididymis showed weak to moderate in caput or moderate in distal regions, while in camel epididymis the WGA-labelling was comparable to that in buffalo in caput but was much weaker in distal segments. The IELs from buffalo epididymis exhibited weak to moderate (in caput and corpus) and moderate (in cauda) DBA-labelling, whereas IELs from camel epididymis revealed weak (in caput), weak to moderate (in corpus) and negative DBA-labelling (in cauda). Moreover, no binding sites to HPA had been found in the IELs in all epididymal segments from both species. Also, no PNA-binding sites could be found in the IELs at the caput region in both species, however the situation showed more discrepancy in other two segments (corpus and cauda) in both species. So, the IELs in corpus and cauda epididymis from buffalo displayed a moderate PNA-labelling, while in camel they showed negative to weak (in corpus) and negative PNA-labelling in cauda.

Comparable to the instance in other mammals (Alkafafy, 2005; Parillo *et al*., 2009), the IELs showed inconstant binding with the diverse lectins. Furthermore, this binding displayed a distribution pattern characteristic for both region and species. This specific pattern of the lectin binding sites distribution in the diverse populations of epididymal cells may indicate their harmonized interactivity in absorption and destruction of certain ingredients from the epididymal lumen (Sinowatz, 1981). Also, BCs in the human epididymis reacted positively with antibodies specific to macrophages and hypothesized that they may convert into intraepithelial macrophages, contributing in the absorptive potentials in the epididymis (Yeung *et al*., 1994). On the other hand, IELs destroy the resorbed ingredients by their hydrolytic enzymes and then migrate back into the interstitium (Sinowatz, 1981).

**Lectin binding sites in sperm cell mass.** The sperm cell mass (SCM) in sections from both buffalo caput and corpus epididymis, displayed binding sites for WGA, DBA, HPA and PNA, but not for GSA-1. The intensity of reaction was either strong in cases of PNA or weak to moderate in cases of HPA, DBA and WGA. On the other hand, the SCM in sections from camel caput and corpus showed binding sites for WGA, DBA, PNA and GSA-1, but not for HPA. Additionally, the SCM in camel cauda failed to display binding sites for both DBA and HPA. The intensity of reaction in camel epididymis was mostly weak; however, it was moderate in caput (WGA and GSA-1), corpus (GSA-1) and in cauda (WGA, GSA-1 and PNA). The discrepancy in

the intensity and pattern of distribution of bindings of different lectins exhibited by SCM in buffalo and camel potentiate previous studies in bovines (Arya & Vanha-Perttula, 1985; Alkafafy, 2005). The modifications of the sperm membranes affinity to lectins may be due to the integration of species-specific glycoproteins produced by the epididymal epithelium (Kawakami *et al*., 2002; Srivastav *et al*., 2004).

**Lectin binding sites in vascular endothelium.** The endothelium of the interstitial blood vessels in sections from buffalo epididymis (caput, corpus and cauda) showed binding sites for WGA, DBA, GSA-1 and PNA. The binding intensity was either strong (GSA-1), moderate (PNA) or weak (WGA and DBA). Whereas, sections from camel caput exhibited no bindings sites for any lectin; however, the sections from other distal segments displayed a limited reactivity only for PNA and GSA-1 in corpus and only for GSA-1 and WGA in cauda. Our findings of the lectins bindings sites in the epididymal vascular endothelium are consistent with previous reports on bovine (Alkafafy, 2005) and equine (Ha *et al*., 2003) epididymis.

In conclusion, the binding sites for the most studied lectins exhibited a variable regional as well as species-specific distribution patterns. So, the glycohistochemical findings revealed that both species have different glycomic characteristics that may be related to their different patterns of reproductive activities. However, the glycome-associated functional capacities remain obscured and need more profound investigations.

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**ALKAFAFY, M. E.** Aplicación de lectinas para la detección de diferencias glucómicas en el conducto epididimario en búfalos de agua (*Bubalus bubalis*) y dromedarios (*Camelus dromedaries*). *Int J. Morphol, 40(3)*:662-671, 2022.

**RESUMEN:** El presente estudio se realizó para detectar las diferencias en las características glicohistoquímicas del conducto epididimal del dromedario y el búfalo de agua. Las secciones del epidídimo (cabeza, cuerpo y cola) de ambas especies se tiñeron con lectinas conjugadas con isotiocianato de fluoresceína (FITC). Se encontraron sitios de unión para cinco lectinas (DBA, GSA-1, HPA, PNA y WGA) en ambas especies. Los sitios de unión de diferentes lectinas mostraron variaciones significativas en el patrón de distribución en ambas especies. Esto incluía tanto el orden específico de la especie como el específico de la región. Además, solo tres (GSA-1, PNA y WGA) de las cinco lectinas estudiadas exhibieron sitios de unión en todas las regiones del epidídimo en ambas especies. Las otras dos lectinas (DBA y HPA) siguieron el mismo orden registrado para las tres restantes (GSA-1, PNA y WGA) en búfalos, pero no mostraron ningún sitio de union en la cola del epidídimo en camellos. En conclusión, las características de distribución regionales y específicas de especies variables de las lectinas revelaron que ambas especies tienen características glucómicas diversas que pueden estar relacionadas con sus diferentes patrones reproductivos. Sin embargo, las capacidades funcionales asociadas con el glicoma permanecen desconocidas y requieren mayor investigación.

**PALABRAS CLAVE: Búfalo; Camello; Epidídimo; Lectinas.**

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