Lectin histochemistry of the boar bulbourethral glands

E. Badia,[1] E. Pinart,[1] M. Briz,[1] L.M. Pastor,[2] S. Sancho,[1] N. Garcia-Gil,[1] J. Bassols,[1] E. Kádár,[1] A. Pruneda,[1] E. Bussalleu,[1] M. Yeste,[1] S. Bonet[1]

[1]Biotechnology of Porcine Reproduction, Department of Biology, Faculty of Sciences, University of Girona, Campus de Montilivi, Girona; [2]Department of Cell Biology, School of Medicine, University of Murcia, Campus de Espinardo, Murcia, Spain

The present study describes, for the first time, the glycosidic content of boar bulbourethral glands using lectin histochemistry. Fourteen horseradish peroxidase- or digoxigenin-labelled lectins with different carbohydrate specificities were used in samples obtained from 3 healthy Landrace boars. The results obtained indicate that endpiece and duct cells synthesize and secrete mainly *O*-glycoproteins with α- and β-D-*N*-acetylgalactosamine, β-D-galactose-β(1→3)-D-N-acetylgalactosamine, D-N-acetylglucosamine and neuraminic acid residues. Glycoproteins secreted by bulbourethral glands have a role in the protection and lubrication of the urethra. In addition, they may be also involved in the regulation of the sperm metabolic activity and in the maintenance of the structural integrity of acrosomal and plasma membranes.

Key words: Lectin histochemistry, bulbourethral glands, boar.

Correspondence: Dr. Elena Badia
Biotechnology of Porcine Reproduction,
Department of Biology, Faculty of Sciences, University of Girona, Campus de Montilivi 17071, Girona, Spain.
Tel: +34 972 41 83 66.
Fax: +34 972 41 81 50.
E-mail: elena.badia@udg.es

Paper accepted on December 23, 2004

European Journal of Histochemistry
2005; vol. 49 issue 2 (Apr-Jun): 25-32

The bulbourethral (Cowper's) glands are male accessory sex glands present in most mammals but absent in aquatic mammals and a few carnivores (Price and Williams-Ashman, 1961; Dyce *et al.*, 1999). In general, these glands are formed of several lobules divided by fibromuscular septa; each lobule consists of endpieces and tubular ducts separated by interstitial tissue, both lined by a single epithelium of mucous cells (Barone, 1983; Badia *et al.*, 2003). However, there are considerable variations in the development, contribution to the ejaculate, and composition of the secretion of the bulbourethral glands among the different mammal species (Nielsen *et al.*, 1977; Setchell *et al.*, 1994; Dyce *et al.*, 1999). In boars, bulbourethral glands are very developed and their secretion, mainly released during the postspermatic phase, contributes with 10-25% to the total ejaculatory volume (Dyce *et al.*, 1999). In contrast, human bulbourethral glands are poorly developed and their secretion, emitted during the prespermatic phase as a result of sexual stimulation, only forms 2-5% of the total ejaculate (Jequier, 1995).

In the boar (Boursnell *et al.*, 1970), goat (Tsukise and Yamada, 1987), man (Riva *et al.*, 1981), guinea pig (Nittinger, 1973), hamster (Feagans and Robertson, 1964), and rat (Tsukise *et al.*, 1979), bulbourethral glands have been reported to secrete a great amount of glycoproteins (Moré, 1991). In general, these glycoproteins serve to lubricate and clean the urethra (Mann and Lutwak Mann, 1981; Krstic, 1984; Cossu *et al.*, 1988), even though in pigs they also form a gelatinous plug in the uterine cervix of the sow to prevent black flow of semen (Dyce *et al.*, 1999). Besides these functions, in boars (Larsson *et al.*, 1976), as well as in goats (Yamada, 1985), it has been suggested that bulbourethral glycoproteins could also play an important role for the activity and metabolism of spermatozoa. Nevertheless, little attention has been paid to boar bulbourethral glycoproteins.

Table 1. Concentration, specificity and inhibitor sugars for the lectins used.

Lectin	Concentration (μg/mL)	Specificity*	Inhibitor sugar and dilution used
Lotus tetragonolobus (LTA)	25	α-L-Fuc	L-Fuc (0,2 M)
Ulex europaeus (UEA-I)	25	α-L-Fuc	L-Fuc (0,2 M)
Aleuria aurantia (AAA)	10	α(1#→6)Fuc	L-Fuc (0,2 M)
Galanthus nivalis (GNA)	10	α-D-Man	Methyl α-Man (0,2 M)
Canavalia ensiformis (Con A)	20	α-D-Man > α-D-Glc	Methyl α-Man (0,2 M)
Helix pomatia (HPA)	15	α-D-GalNac	D-GalNac (0,2 M)
Dolichos biflorus (DBA)	20	α-D-GalNac	D-GalNac (0,2 M)
Glycine max (SBA)	15	α- / β-D-GalNac	D-GalNac (0,4 M)
Arachis hypogea (PNA)	15	β-D-Galβ(1→3)-D-GalNAc > α-D-Gal	β-D-Gal (0,2 M)
Ricinus communis (RCA-I)	10	Galβ(1?4)-GlcNAc	Gal (0,4 M)
Triticum vulgaris (WGA)	10	Galβ(1?4)-GlcNAc > D-GlcNAc > NeuAc NeuAc	D-GlcNAc (0,2 M)
Limax flavus (LFA)	125	NeuAc	NeuAc (0,2 M)
Sambucus nigra (SNA)	10	NeuAcα2,6-Galβ(1→4)GlcNAc > NeuAcα2,6-Galβ(1?4)GalNAc	NeuAc (0,2 M)
Maackia amurensis (MAA)	10	NeuAcα2,3-Galβ(1?4)GlcNAc	NeuAcα2,3Lac (0,2 M)

** Fuc, fucose; Gal, galactose; GalNAc, N-acetylgalactosamine; Glc, glucose; GlcNAc, N-acetylglucosamine; Lac, lactose; Man, mannose; NeuAc, neuraminic acid.*

Lectin histochemistry is used as a histochemical probe to identify and localise specific carbohydrate residues of glycoconjugates in order to characterize them (Roth, 1978; Chan and Wong, 1992). To our knowledge, there are only a few reports on the carbohydrate histochemistry of boar bulbourethral glands using less specific conventional histochemical methods (Aitken, 1960; Nielsen *et al*., 1977), while no lectin histochemistry studies are available. Therefore, the present work is a first attempt to elucidate the glycan composition of boar bulbourethral glands secretion by means of lectin histochemistry. This work also aims to get some insight into the possible physiological functions of the glycoprotein secreted by boar bulbourethral glands.

Material and methods

Tissue preparation

The study was performed using three adult Landrace boars. The animals were maintained in a controlled environment with and average temperature of 18°C and fed with a nutritious diet. The males were slaughtered at 9.5 month of age, and their bulbourethral glands were immediately removed and processed for light microscopy.

Bulbourethral glands samples were fixed in Bouin's fluid, dehydrated through increasing ethanol series and embedded in paraffin wax (Kiernan, 1990). Eventually, 5 μm-thick sections were cut.

Lectin histochemistry

Lectin binding pattern was established using horseradish peroxidase (HRP)- and digoxigenin (DIG)-conjugated lectins. The carbohydrate binding specificity and the concentration used of each lectin are summarized in Table 1.

Histochemical staining using HRP-conjugated lectins was performed according to Calvo *et al*. (2000). In hydrated cross sections, endogenous peroxidase was blocked with 0.3% (v/v) hydrogen peroxide (H2O2) in Tris-buffered saline (TBS) during 30 minutes. After washing in TBS, the sections were incubated for 90 minutes in a moist chamber at room temperature with the following HRP-conjugated lectins: LTA, UEA-I, Con A, HPA, DBA, SBA, PNA, RCA-I, WGA (Sigma Chemical Co., St. Louis, MO) and LFA (ICM, Biomedicals Ltd, UK).

Peroxidase was developed with 0.05% (w/v) 3,3′-diaminobenzidine and 0.015% (v/v) H_2O_2 in TBS. Finally, sections were counterstained with haematoxylin for 3 minutes.

Histochemical staining using DIG-labeled lectins was performed applying a two-step technique previously reported by Calvo *et al*. (2000). Briefly, endogenous peroxidase was inhibited by a 30 minutes treatment with 0.3% (v/v) H2O2 in TBS. Then, sections were rinsed in TBS and covered with 1% bovine serum albumin (BSA) in TBS for 10 minutes, and incubated with the following DIG-labelled lectins for 90 minutes at room temperature in a moist chamber: AAA, GNA, SNA and MAA

Table 2. Lectin binding pattern in boar bulbourethral glands*.

Lectins	Epithelium		Luminal secretion	Stroma
	Cytoplasm of endpiece cells	Cytoplasm of tubular duct cells		
LTA	-	-	-	-
UEA-I	-	-	-	-
AAA	-	-	-	-
GNA	-	-	-	-
Con A	-	-	-	-
HPA	++	++	++	-
HPA + ,-elimination	-	-	-	-
DBA	-/+[1]	-	-	-
SBA	+/++/+++[1]	++	+/++	-
PNA	+/++	+	+/++	-
PNA + Endo F digestion	+/++	+	+/++	-
RCA-I	-	-	-	-
WGA	++	++	++	-
WGA + ,-elimination	+	+	+	-
WGA + Endo-F digestion	+	+	+	-
LFA	+/++	-/+	++	-
SNA	-	-	-	-
MAA	-	-	-	-

**Staining intensity: - negative, + weak, ++ moderate, +++ strong 1Reactivity present in a few cells*

(Boehringer Mannheim, Spain). After two rinses for 5 minutes in TBS, slides were incubated with HRP-conjugated anti-DIG-antibody (Roche, Switzerland) at 0.6 U/mL in TBS supplemented with 1% (w/v) BSA for 90 minutes. Peroxidase was developed as described above. The sections were counterstained with haematoxylin for 3 minutes.

The staining intensity of the different lectins in cell cytoplasm, luminal secretion and stroma was subjectively evaluated and classified into four categories: no labelling (-), weak (+), moderate (++) and strong (+++) labelling.

Chemical and Enzymatic treatments

Chemical and enzymatic treatments of the tissue preparations were carry out with some lectins in order to obtain more information about the type of the glycans present in boar bulbourethral glands. The paraffin sections were incubated with 0.5 N NaOH in 70% (v/v) ethanol, at 4°C, for 5 days (Arenas *et al*., 2001) to remove *O*-linked oligosaccharides (β-elimination reaction). After this treatment, the incubated sections were stained with HPA, a specific marker for *O*-linked oligosaccharides (Arenas *et al*, 1998) and WGA, which detects both *N*- and *O*-linked oligosaccharides (Saez *et al*., 2001).

Hydrolysis of *N*-linked oligosaccharides was carried out by enzymatic treatment with Endo-β-acetylglucosaminidase F/peptide *N*-glycosidase F (Roche, Switzerland) as previously reported (Arenas *et al*., 2001). After incubation in 0.1M Tris, 150 mM NaCl, 2.5 mM EDTA (pH 9) buffer containing 1% (w/v) BSA for 10 minutes, and a brief washing in the buffer without BSA, the sections were incubated with the enzyme at 6U/mL for 3 days. Eventually, the sections treated were stained with PNA and WGA, both markers for *N*- and *O*-linked oligosaccharides (Saez *et al*., 2001).

Histochemical controls

Two types of controls were used: (1) Substitution of the conjugated lectins by the buffer alone; (2) preincubation of the lectins with the corresponding hapten-sugar inhibitor (Sigma Química, Spain) used at appropriate concentration (Table 1).

Results

The results concerning the lectin binding pattern in the bulbourethral glands are summarized in Table 2. No labelling was found in control sections for all lectins used. The cytoplasm of endpiece and tubular duct cells, as well as the luminal secretion and the stroma were stained negatively with all three fucose binding lectins, LTA, UEA-I, and AAA (Figure 1A).

Con A and GNA, both specific for mannose, were unreactive in the bulbourethral glands (Figure 1B).

The three *N*-acetylgalactosamine binding lectins, HPA, DBA, and SBA, showed different patterns of staining. HPA presented a moderate reactivity in the

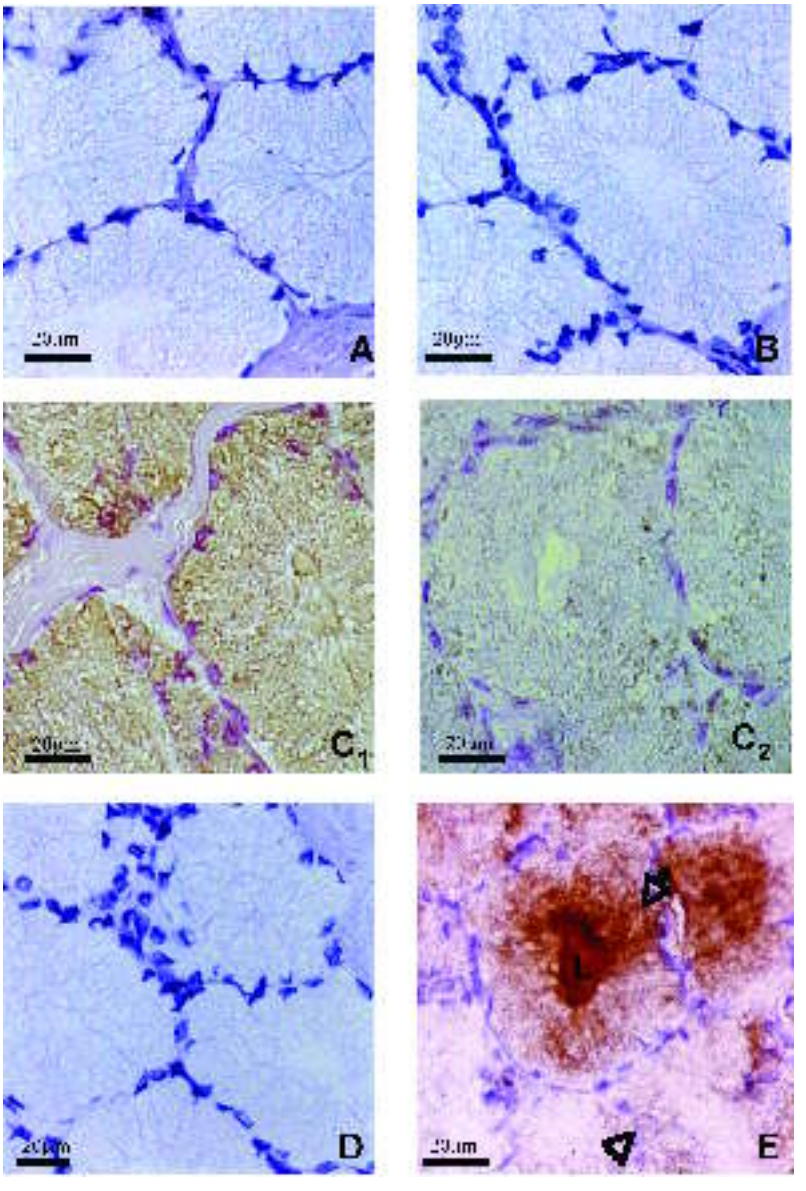
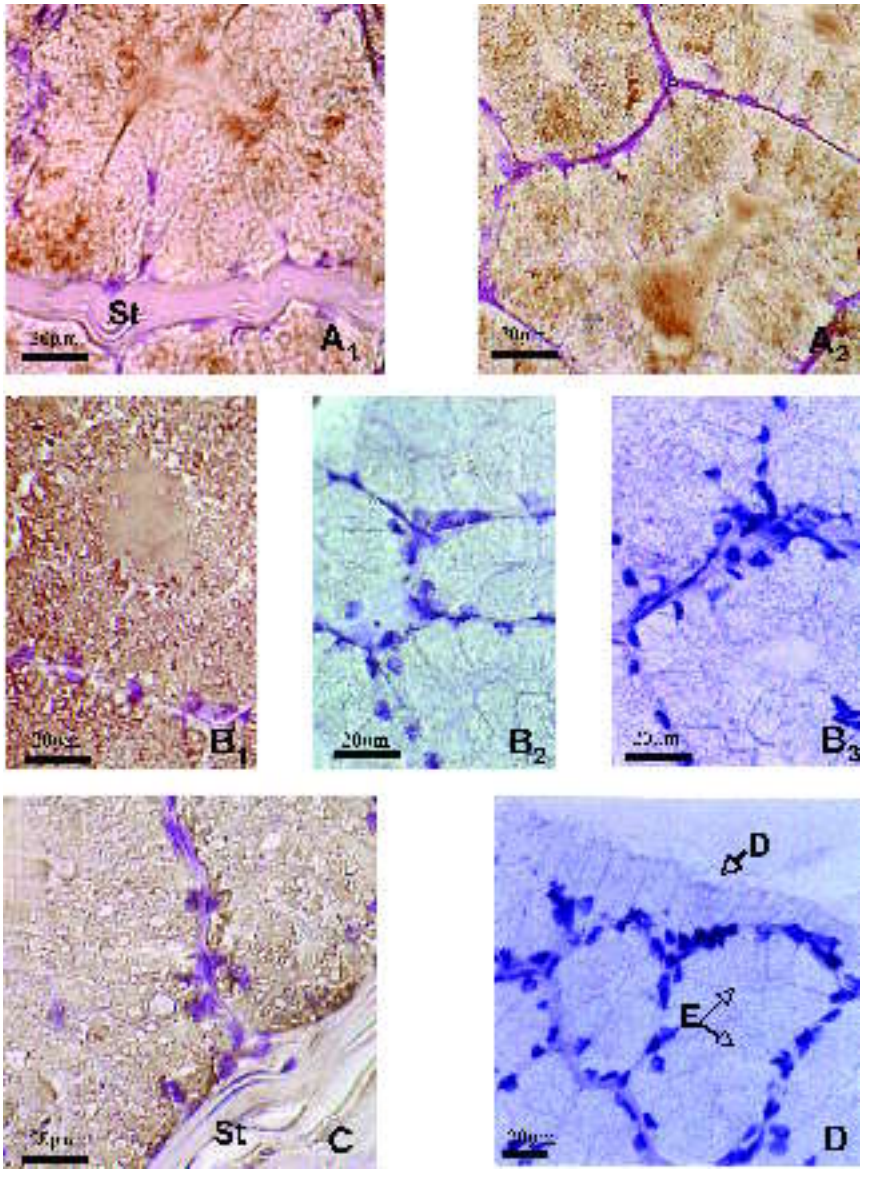


Figure 1. Lectin labelling of boar bulbourethral glands. A) LTA labelling. No reaction is observed in the cytoplasm of epithelial cells. B) GNA labelling. Epithelial cells are negatively stained. C) HPA labelling. C1: Epithelial cells are moderately stained. C2: HPA labelling decreases after ,-elimination treatment. D) DBA labelling. No staining is observed in epithelial cells. E) SBA labelling. The cytoplasm of epithelial cells is weakly (arrowhead) or moderately (arrow) stained. The luminal secretion (L) shows a moderate reaction.

cytoplasm of endpiece and tubular duct cells and in the luminal secretion (Figure 1C$_1$); HPA labelling decreased after β-elimination reaction (Figure 1C$_2$). DBA was unreactive in endpiece cells although some of them were weakly stained. In contrast, tubular duct cells and luminal secretion were always unreactive (Figure 1D). SBA showed a variable reactivity in the cytoplasm of endpiece cells. Most cells were weakly or moderately stained and only few cells showed a strong reactivity. SBA reaction was moderate in the cytoplasm of tubular duct cells and weak or moderate in the luminal secretion (Figure 1E). Labelling with HPA, DBA, and SBA was negative in the stroma.

PNA and RCA-I, both galactose-binding lectins, showed different staining patterns in the bulbourethral glands. Reaction with PNA was weak or moderate in the cytoplasm of endpiece cells and in the luminal secretion, weak in the cytoplasm of tubular duct cells and, negative in the stroma (Figure 2A$_1$). The labelling with PNA was not modified after Endo-F-digestion (Figure 2A$_2$). RCA-I was unreactive in the bulbourethral glands.

Reaction with WGA, specific for galactosyl, *N*-acetylglucosamine and neuraminic acid residues, was moderate in all epithelial cells and in luminal secretion, whereas no staining was observed in the stroma (Figure 2B$_1$). Labelling with WGA



decreased after β-elimination reaction (Figure 2B2) and Endo-F-digestion (Figure 2B3).

Concerning the lectins that bind neuraminic acid, LFA reaction was weak or moderate in the cytoplasm of endpiece cells, weak or negative in the cytoplasm of tubular duct cells and moderate in the luminal secretion. No labelling was observed in the stroma (Figure 2C). SNA and MAA were unreactive in the bulbourethral glands (Figure 2D).

Discussion

Lectins are an excellent tool for the characterization of the sugar residues of glycoconjugates, which include glycoproteins as well as glycolipids and glycosaminoglycans (Spicer and Schulte, 1992). However, the majority of the sugar residues detected by lectins in the present study correspond to glycoproteins, as glycolipids are extracted during the dehydration process and glycosaminoglycans are known to have a low affinity for common lectins (Zanetta *et al.*, 1994). The results of this study show there are no differences in the glycosidic composition between endpiece and tubular duct cells and the luminal secretion, thus, luminal glycoproteins are synthesised and secreted by both types of epithelial cells. Therefore, endpieces and ducts present both the same secretory function showing only diameter

differences between them. The lectin binding pattern obtained suggests that luminal secretion of boar bulbourethral glands contains α- and β-D-*N*-acetylgalactosamine, β-D-galactose-β(1→3)-D-*N*-acetylgalactosamine, D-*N*-acetylglucosamine and neuraminic acid residues; whereas α-L-fucose, α(1→6)fucose, α-D-mannose, α-D-glucose, galactose-β(1→4)-*N*-acetylglucosamine, neuraminic acid-α-2,6-galactose-β(1→4)-*N*-acetylglucosamine, neuraminic acid-α-2,6-galactose-β(1→4)-*N*-acetylgalactosamine and neuraminic acid-α-2,3-galactose-β(1→4)-*N*-acetylglucosamine residues are absent. These results are different from those reported in goat (Tsukise and Yamada, 1987), water buffalo (Abou-Elmagd, 1989), and calf (Moré, 1991) in which fucose and mannose residues are present in the bulbourethral secretion. Oligosaccharide chains of glycoproteins have been classified into two families (Kornfeld and Kornfeld, 1985). The so-called *N*-linked oligosaccharides are those in which an *N*-glycosidic bond is established between *N*-acetylglucosamine and the amine nitrogen of asparagine (Kornfeld and Kornfeld, 1985); the *N*-linked oligosaccharides exhibit mannosyl, galactosyl, fucosyl, *N*-acetylglucosamine and neuraminic acid residues (Spicer and Schulte, 1992; Wheatley and Hawtin, 1999). The second group, the named *O*-linked oligosaccharides, comprises the sugar chains linked to the hydroxyl group of serine or threonine via an *N*-acetylgalactosamine (Strous and Dekker, 1992); these *O*-linked glycans contain galactosyl, fucosyl, *N*-acetylgalactosamine, *N*-acetylglucosamine, and neuraminic acid residues (Wheatley and Hawtin, 1999). The presence of *N*-acetylgalactosamine residues, detected with HPA, DBA, and SBA, and the absence of mannosyl residues in the luminal secretion, as shows the negative reaction obtained with Con A and GNA, indicate that epithelial cells of the bulbourethral glands synthesise and secrete *O*-glycoproteins. The negative reactivity to HPA after the ,-elimination pre-treatment corroborates the occurrence of *O*-glycosilated glycoproteins. On the other hand, the presence of *N*-glycosilated proteins is not able to be rejected despite the unreactivity of Con A and GNA. Con A binds tightly to high-mannose-type N-glycans, and with weaker affinity to hybrid-type and biantennary complex-type *N*-glycans. However, Con A does not bind detectably to more highly branched complex-type *N*-glycans (Varki *et al.*, 1999). Moreover, the staining with WGA, which detect many *N*- and *O*-glycans (Varki *et al.*, 1999), decrease not only with β-elimination but also with Endo-F digestion confirming the presence of *N*-glycoproteins. Therefore, the epithelial cells of the bulbourethral glands synthesise and secrete mainly *O*-glycoproteins even thought some *N*-glycosilated proteins are also secreted.

Mucins are heavily *O*-glycosilated glycoproteins (Van Klinken *et al.*, 1995) that form extremely large and viscous gels when they are secreted (Forestmer, 1995). The dense packing of the *O*-linked glycans protects a large part of mucin polypeptide from proteolytic attack (Jentoff, 1990). In general, mucins provide lubrication and prevent dehydration of luminally disposed cell surfaces (Jentoff, 1990; Devine and McKenzie, 1992) but, in the male reproductive tract they also serve as a protective barrier against bacterial colonization and/or infection (Cohen *et al.* 1984; Lamblin and Roussel, 1993). Therefore, bulbourethral secretion likely takes part in the protection and lubrication of the urethral epithelium.

The secretion of boar bulbourethral glands contains great amounts of neutral and carboxylated acid mucosubstances, whereas sulfated acid mucosubstances are scarce (Badia *et al.*, 2003). These results coincide with those obtained in goats (Wrobel, 1970; Tsukise and Yamada, 1987) but differ from those of cats (Wrobel, 1969) and rats (Nielsen, 1976), which have a bulbourethral secretion rich in sulfated acid mucosubstances. Bulbourethral mucosubstances secreted in boars (Mann, 1974), goats (Wrobel, 1970), rats (Nielsen, 1976; Tsukise *et al.*, 1979) and humans (Riva *et al.*, 1981; Sirigu *et al.*, 1993) have been classified as sialoproteins. According to Hartree (1962) and Boursnell *et al.* (1970), the secretion of boar bulbourethral glands contains on a dry weight basis 27% of sialic acid, 13% of *N*-acetylgalactosamine and 4% of neutral sugars. Because of the high amount of sialic acid in the secretion, it is used as a marker of the boar bulbourethral glands activity (Hartree, 1962). Lack of neuraminic acid α-2,6 and α-2,3 galactose complexes as shows the unreactivity of SNA and MAA, suggests that LFA detects neuraminic acid residues with other type of linkages to the underlying sugar chain or with a different structure of the sugar chain. In this way, Rana *et al.* (1984) demonstrated by protein purification techniques that neuraminic acid residues present in the boar bulbourethral gland secretion are neuraminic acid-α-2,6-*N*-acetylgalactosamine and neuraminic acid-α-2,3 galactose-,(1→3)-*N*-acetylgalac-

tosamine. Nevertheless, the negativity of these lectins could be also attributed to the binding of SNA and MAA lectins being hampered by the water strongly bound to the high amount of secretory sialoglycoderivatives (Accili *et al.*, 2001). Moreover, the high molecular weigh of these lectins (Mandal and Mandal, 1990), as well as the masking effect of other glucidic residues present in the same or neighbouring oligosaccharide chains (Menghi *et al.*, 1985), may hamper lectin internalization (Accili *et al.*, 2001). Neuraminic acid residues have been localised in ejaculated glycoproteins which attach to the sperm plasma membrane, where they play an important role in the regulation of sperm metabolic activity and the maintenance of the structural integrity of the acrosomal and plasma membranes (Yamada, 1985; Du Toit *et al.*, 1992). Galactose, *N*-acetylgalactosamine and D-*N*-acetylglucosamine residues are present in glycoproteins that participate in sperm-egg interactions (Nimtz *et al.*, 1999; Töpfer-Petersen, 1999). Besides, galactose residues are involved in sperm-oviduct attachment which increases sperm viability, suppresses sperm motility and modulates the process of capacitation until the egg is ovulated into the ampulla (Smith, 1998; Suarez, 1998; Töpfer-Petersen, 1999). The bulbourethral gland stroma in boars, as well as in goats (Tsukise and Yamada, 1987), is rich in fucose residues whereas no other glycans have been detected. The low lectin affinity of the stroma could be attributed to the abundance of proteoglycans in the extracellular matrix (Ruoslahti, 1988) which have a low affinity by common lectins (Zanetta *et al.*, 1994). Fucose residues are known to participate in structural functions, cell-cell interaction and regulation of substrate diffusion across the extracellular matrix (Parillo *et al.*, 1998).

In summary, this work provides the first detailed characterization of the glycosidic content of the boar bulbourethral glands. Knowing the glycan composition of the bulbourethral gland secretion will allow a much better understanding of its role in the ejaculate.

Acknowledgements

This work was supported in part by grant FPU-AP99 40325284 awarded to E. Badia. This research was further supported by projects AGL2002-01924, RZ02-013 and RZ03-29 with funds provided by the "Ministerio de Ciencia y Tecnología"-MCYT (Spanish Government), and by project 2001SGR-00294 from the "Direcció General de Recerca" (Autonomous Government of Catalonia).

References

Abou-Elmagd A, Wrobel KH. The periurethral glandular complex in the water buffalo: an ultrastructural, histological and lectin-histochemical study. Arch Histol Cytol 1989; 52(5):501-12.

Accili D, Menghi M, Materazzi G, Menghi G. Sialic acid derivatives and their distribution in rat sublingual gland acini during pre- and postnatal development. Histochem J 2001; 33:363-71.

Arenas MI, Madrid JF, Bethencourt FR, Fraile B, Paniagua R. Lectin histochemistry study in the human vas deferens. Glycoconjugate J 1998; 15:1085-91.

Arenas MI, Royuela M, Fraile B, Paniagua R, Wilhelm B, Aumuller G. Identification of N- and O-linked oligosaccharides in human seminal vesicles. J Androl 2001; 22(1):79-87.

Badia E, Pinart E, Briz M, Sancho S, Garcia N, Kádár E et al. Les glàndules bulbouretrals de porcí: estudi estructural i histoquímic de lectines. Biologia de la Reproducció 2003, 8:23-6.

Barone R. Anatomia comparada dei mammiferi domestici. In: Bartolami R, Callegari E, eds. Splancnologia: Apparecchio Uro-Genitale, Feto e Suoi Annessi, Peritoneo e Topografia Abdominale, vol. 4. Edagricole, Bologna, 1983. pp.156-65.

Boursnell JC, Hartree EF, Briggs PA. Studies of the bulbo-urethral (Cowper's)-gland mucin and seminal gel of the boar. Biochem J 1970;117(5):981-8.

Calvo A, Pastor LM, Bonet S, Pinart E, Castells MJ. Lectin affinity of the boar testis and epididymis. J Reprod Fertil 2000; 120:325-35.

Chan L, Wong, YC. Localization of prostatic glycoconjugates by the lectin-gold method. Acta Anat (Basel) 1992; 143(1):27-40.

Cohen MS, Black JR, Proctor RA, Sparling PF. Host defenses and the vaginal mucosa. Scand J Urol Nephrol 1984; 86:13-22.

Devine PL, Mckenzie IFC. Mucins: structure, function and association with malignancy. BioEssays 1992;14:619-25.

Du Toit D, Bornman MS, Van Der Merwe MP, Du Plessis DJ. High seminal sialic acid concentrations in patients with severe teratozoospermia. Arch Androl 1992; 28:177-9.

Dyce KM, Sack WO, Wensing CJG. Anatomía Veterinaria. McGraw-Hill Interamericana, México, 1999.

Feagans WM, Robertson ZK. Histochemical and electron microscopical observations of the hamster bulbourethral duct and associated glands. Anat Rec 1964;148:280-1.

Forestmer G. Signal transduction, packaging and secretion of mucins. Annu Rev Physiol 1995; 57:585-605.

Hartree EF. Sialic acid in the bulbo-urethral glands of the boar. Nature (Lond) 1962; 196:483-4.

Jentoff N. Why are proteins O-glycosylated? Trends Biochem Sci 1990;15:291-4.

Jequier AM. Clinical disorders affecting semen quality. In: Grudzinskas JG, Yovich JL, eds. Gametes-The Spermatozoon. University Press, Cambridge, 1995, pp. 175-91.

Kiernan JA. Histological and Histochemical Methods. Theory and Practice. Pergamon Press, London, 1990.

Kornfeld R, Kornfeld S. Assembly of asparagine-linked oligosaccharides. Annu Rev Biochem 1985; 54:631-64.

Lamblin G, Roussel P. Airway mucins and their role in defense against micro-organism. Respir Med 1993; 87:421-6.

Larsson K, Eirnarsson S, Nicander L. Influence of thawing diluents on vitality acrosome morphology, ultrastructure and enzyme release of deep frozen boar spermatozoa. Acta Vet Scand 1976; 17:83-100.

Mandal C, Mandal C. Sialic acid binding lectins. Experientia 1990; 46:433-41.

Mann T. Secretory function of the prostate, seminal vesicle and other male accessory organs of reproduction. J Reprod Fertil 1974; 37(1):179-88.

Menghi G, Bondi AM, Accili D, Fumagalli L, Materazzi G.

Characterization in situ of the complex carbohydrates in rabbit oviduct using digestion with glycosidases followed by lectin binding. J Anat 1985; 140:613-25.

Moré J. Lectin histochemistry of mucus-secreting cells in the calf bulbourethral gland. Acta Anat (Basel) 1991; 142(2):147-51.

Nielsen EH. The bulbourethral gland of the rat. Fine structure and histochemistry. Anat Anz 1976; 139(Suppl):254-63.

Nimtz M, Grabenhorst E, Conradt HS, Sanz L, Calvete JJ. 1999. Structural characterization of the oligosaccharide chains of native and crystallized boar seminal plasma spermadhesin PSP-I and PSP-II glycoforms. Eur J Biochem 1999;265:703-18.

Nittinger H. Untersuchungen zur histologie, histochemie and ultrastruktur der cowperschen drüse und der prostata normaler und stilboestrolbehandelter meerschweinchen. Cavia porcellus. Zool Jb Anat 1973; 90:255-310.

Parillo F, Stradaioli G, Verini-Supplizi A, Monaci M. Lectin staining pattern in extratesticular rete testi and ductuli efferentes of prepuberal and adult horses. Histol Histopathol 1998; 13:307-14.

Price D, Williams-Ashman HG. The accessory reproductive glands of mammals. In: Young WC, ed. Sex and internal secretions, vol. 1. Williams and Wilkins, Baltimore, 1961 pp. 366-448.

Rana SS, Chandrasekaran EV, Kennedy J, Mendicino J. Purification and structure of oligosaccharide chains in swine trachea and Cowper's gland mucin glycoproteins. J Biol Chem 1984; 259(20):12899-907.

Riva A, Sirigu P, Testa-Riva F, Usai E. Fine structure and histochemistry of epithelial cells of the human bulbourethral glands. Acta Anat 1981;11:125-6.

Roth J. The lectins: Molecular probes in cell biology and membrane research. Exp Path Suppl 1978;3:186.

Sáez FJ, Madrid JF, Aparicio R, Hernández F, Alonso E. Carbohydrate moieties of the interstitial and glandular tissues of the amphibian Pleurodeles waltl testis shown by lectin histochemistry. J. Anat 2001;198:47-56.

Setchell BP, Maddocks S, Brooks DE. Anatomy, vasculature, innervation, and fluids of the male reproductive tract. In: Knobil E, Neill JD, eds. The Physiology of Reproduction, vol. 1. Raven Press, New York, 1994, pp. 1063-75.

Sirigu P, Turno F, Usai E, Perra MT. Histochemical study of the human bulbourethral (Cowper's) glands. Andrologia 1993;25(5):293-99.

Smith TT. The modulation of sperm function by the oviductal epithelium. Biol Reprod 1998; 58:1102-4.

Spicer SS, Schulte BA. Diversity of Cell Glycoconjugates shown histochemically: A perspective. J Histochem Cytochem 1992; 40(1):1-38.

Strous GJ, Dekker J. Mucin-type glycoproteins. CRC Crit Rev Biochem Mol Biol 1992; 27:57-92.

Suarez SS. The oviductal sperm reservoir in mammals: mechanism of formation: Biol Reprod 1998; 58:1105-7.

Tsukise A, Sugawa Y, Yamada K. Histochemistry of carbohydrates in the epithelium lining the bulbourethral gland of the rat. Acta Anat 1979; 105:529-38.

Tsukise A, Yamada K. Histochemistry of glycoconjugates in the secretory epithelium of the goat bulbourethral gland. Acta Anat (Basel) 1987; 129(4):344-52.

Van Klinken BJ, Dekker J, Buller HA, Einerhand AW. Mucin gene structure and expression: protection vs. adhesion. Am J Physiol 1995; 269:613-27.

Varki A, Cummings R, Esko J, Freeze H, Hart G, Marth J. Essentials of Glycobiology. Cold Spring Harbor Laboratory Press, New York, 1999.

Wheatley M, Hawtin S. Glycosilation G-protein coupled receptors for hormones central to normal reproductive functioning: its occurrence and role. Hum Reprod Update 1999; 5: 356-64.

Wrobel KH. Morphological study of the bulbourethral gland in the cat. Z Zellforsch Mikrosk Anat 1969; 101: 607-20.

Wrobel KH. Studies on the ultrastructure and histochemistry of the bulbourethral gland in the goat. Z Zellforsch Mikrosk Anat, 1970; 108(4): 582-96.

Töpfer-Petersen E. Carbohydrate-based interactions on route of a spermatozoon to fertilization. Hum Reprod Update 1999; 5:314-29.

Yamada K. Bulbourethral gland. In: Ogawa et al., ed. Human Histology. Endocrine and Reproductive Organs, vol. 6.Asakura Shoten, Tokio, 1985, pp. 325-30.

Zanetta JP, Badache A, Maschke S, Marschal P, Kuchler S. Carbohydrates and soluble lectins in the regulation of cell adhesion and proliferation. Histol Histopathol 1994; 9:385-412.