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Lectin histochemistry of the olfactory mucosa of Korean native cattle, *Bos taurus coreanae*

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ABSTRACT

Background: The olfactory mucosa (OM) is crucial for odorant perception in the main olfactory system. The terminal carbohydrates of glycoconjugates influence chemoreception in the olfactory epithelium (OE).
Objectives: The histological characteristics and glycoconjugate composition of the OM of Korean native cattle (Hanwoo, *Bos taurus coreae*) were examined to characterize their morphology and possible functions during postnatal development.
Methods: The OM of neonate and adult Korean native cattle was evaluated using histological, immunohistochemical, and lectin histochemical methods.
Results: Histologically, the OM in both neonates and adults consists of the olfactory epithelium and the lamina propria. Additionally, using periodic acid Schiff and Alcian blue (pH 2.5), the mucus specificity of the Bowman's gland duct and acini in the lamina propria was determined. Immunohistochemistry demonstrated that mature and immature olfactory sensory neurons of OEs express the olfactory marker protein and growth associated protein-43, respectively. Lectin histochemistry indicated that numerous glycoconjugates, including as N-acetylglucosamine, mannose, galactose, N-acetylgalactosamine, complex type N-glycan, and fucose groups, were expressed at varied levels in the different cell types in the OMs of neonates and adults at varying levels. According to our observations, the cattle possessed a well-developed olfactory system, and the expression patterns of glycoconjugates in neonatal and adult OMs varied considerably.
Conclusions: This is the first study to describe the morphological assessment of the OM of Korean native cattle with a focus on lectin histochemistry. The findings suggest that glycoconjugates may play a role in olfactory chemoreception, and that their labeling properties may be closely related to OM development and maturity.

Keywords: Glycoconjugate; immunohistochemistry; Korean native cattle (Hanwoo); lectin histochemistry; olfactory mucosa

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Conflict of Interest

The authors declare no conflicts of interest.

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INTRODUCTION

Olfaction is important for animals because it allows them to explore their surroundings for odorous signals from food sources and environments, as well as detect chemical compounds that influence social interaction and reproductive behavior [1]. This perception is mediated by two chemosensory systems: the main olfactory system and the vomeronasal system. The olfactory mucosa (OM), positioned at the caudal/posterior roof of the nasal cavity, and the vomeronasal organ (VNO), located at the base of the nasal septum or on the roof of the mouth, are the organs comprising these two systems [2,3]. Traditionally, the OM and VNO have been considered functionally and anatomically distinct, with the OM detecting conventional volatile odorants and the VNO receiving pheromones [4,5]. These two organs share some common features but differ in neuron types, primary structures of receptor proteins, physiological pathways, and central neuroanatomical projections into the brain [6]. However, despite the anatomical and functional differences, previous studies indicate that the OM and VNO play a synergistic role in the regulation of various olfactory-induced behaviors and reproductive and social interactions in mammals [1,5,7-9].

The OM is critical for chemical signal acquisition in the main olfactory system, conveying signals to the main olfactory bulb [4]. In mammals, the OM is composed of the olfactory epithelium (OE) and the lamina propria. The OE is predominantly composed of chemosensory neurons, supporting cells, and basal cells [10]. The lamina propria is constituted by loose connective tissue in which olfactory nerve axon bundles, Bowman's glands, and blood and lymph vessels [10,11]. In mammals, odorant reception occurs via the chemosensory neurons of the OE, which contain dendrites that extend beyond the apical surface, where the cilia protrude into the mucus layer, and a basally projecting axon process [12]. Glycoconjugates (terminal carbohydrates) have been shown to play a crucial role in the chemoreception of the OE [13].

Cell surfaces are densely packed with a diverse array of glycoconjugates, each of which provides considerable biological information [14]. Lectins are naturally occurring glycoconjugate-binding molecules, and a large number of purified lectins are regarded as the primary analytical tool for studying glycoconjugates in the olfactory system [13,15,16]. Specific lectin binding is closely related to the olfactory neuron function, as evidenced by the abundance of glycoconjugates in the mucosensory compartment of the chemosensory epithelia, where receptor-specific events associated with transduction occur [13]. Numerous animals, including rats [17,18], mice [19,20], marmosets [21], sheep [15,22], camels [22], horses [23], and humans [24], express the carbohydrate (lectin-binding) moiety in their OM.

Despite the fact that cattle are one of the best species for biochemical investigations on olfaction [25], there is little published information on the immunohistochemical and lectin histochemical properties of the OM in bovines. We first evaluated the histological characteristics and lectin histochemistry of the OM in Korean native cattle, and then we compared neonate to adult OMs to determine the features associated with maturity. To our knowledge, this is the first study that provides a comprehensive outlook on the expression levels of lectin-binding glycoconjugates in bovine OM.

MATERIALS AND METHODS

Tissue preparation

Three neonatal OM samples (1 to 3 day old) and three adult OM samples (2.5-year-old) of Korean native cattle (Hanwoo, *Bos taurus coreanae*) were obtained from local farms and a local slaughterhouse in Chungcheongbuk-do, South Korea, respectively. Both sexes of all animals were utilized in this study (male: four neonates and five adults; female: three neonates and three adults). The OM used in this study belongs to a small area on the caudal roof of the nasal cavity, where it completely covered the ethmoturbinates and caudal portions of the dorsal and middle nasal conchae (**Fig. 1A**). Morphological exams of the nasal cavity indicated that none of them had underlying respiratory diseases. All experimental and animal handling procedures were conducted in accordance with the guidelines of Institutional Animal Care and Use Committee of Chonnam National University (26 October 2021; CNU IACUC-YB-2021-131).

Histological examination

For light microscopic examination, the ethmoturbinates were removed immediately after death, cut into 5-cm-thick cross sections, and fixed for 3–5 days in 10% buffered formalin. Following fixation, the ethmoturbinates were trimmed and decalcified through multiple solution changes of sodium citrate-formic acid solution. When a needle could readily penetrate the bone with very little force, the decalcification process was stopped. The samples were then washed for 24 h with running tap water, dehydrated in a succession of ethanol concentrations (70%, 80%, 90%, 95%, and 100%), cleaned in xylene, embedded in paraffin, and sectioned into 4 μm slices. Following deparaffinization, the sections were utilized for hematoxylin and eosin staining, mucus and lectin histochemistry, and immunohistochemistry.

Mucus histochemistry

The acidic mucin was distinguished from the neutral epithelial mucin using periodic acid Schiff (PAS) and Alcian blue (pH 2.5) staining. For PAS staining, the sections were subjected to 0.5% periodic acid for 15 min, then washed and incubated in Schiff reagent for 30 min. After 10 min of washing with running tap water, the sections were counterstained with hematoxylin. For Alcian blue staining, the sections were first incubated in 3% acetic acid for 3 min, followed by 30 min in 1% Alcian blue solution in 3% acetic acid (pH 2.5). After 1 min of washing with running tap water, the sections were counterstained with neutral red.

Immunohistochemistry

To retrieve the antigens, the sections were heated for 1 h at 90°C in citrate buffer (0.01 M, pH 6.0). After cooling, the sections were treated for 20 min with 0.3% hydrogen peroxide in distilled water to suppress the endogenous peroxidase activity. To avoid non-specific binding, the sections were treated with normal goat serum (Vectastain Elite ABC kit; Vector Laboratories, USA) for 1 h before being incubated overnight at 4°C with rabbit monoclonal anti-olfactory marker protein (OMP) (1:1,000 dilution, Cat. No. ab183947; Abcam, UK) and rabbit polyclonal anti-growth associated protein-43 (GAP-43) (1:1,000 dilution, Cat. No. PA5-34943; ThermoFisher Scientific, USA) antibodies. The primary antibodies were excluded from the procedure as negative controls. The sections were rinsed with phosphate-buffered saline (PBS), treated with biotinylated goat anti-rabbit IgG (Vectastain Elite ABC kit) for 1 h, and then reacted with the avidin-biotin-peroxidase complex (Vectastain Elite ABC kit) for 1 h at room temperature (RT). Immunoreactivity was detected using a diaminobenzidine

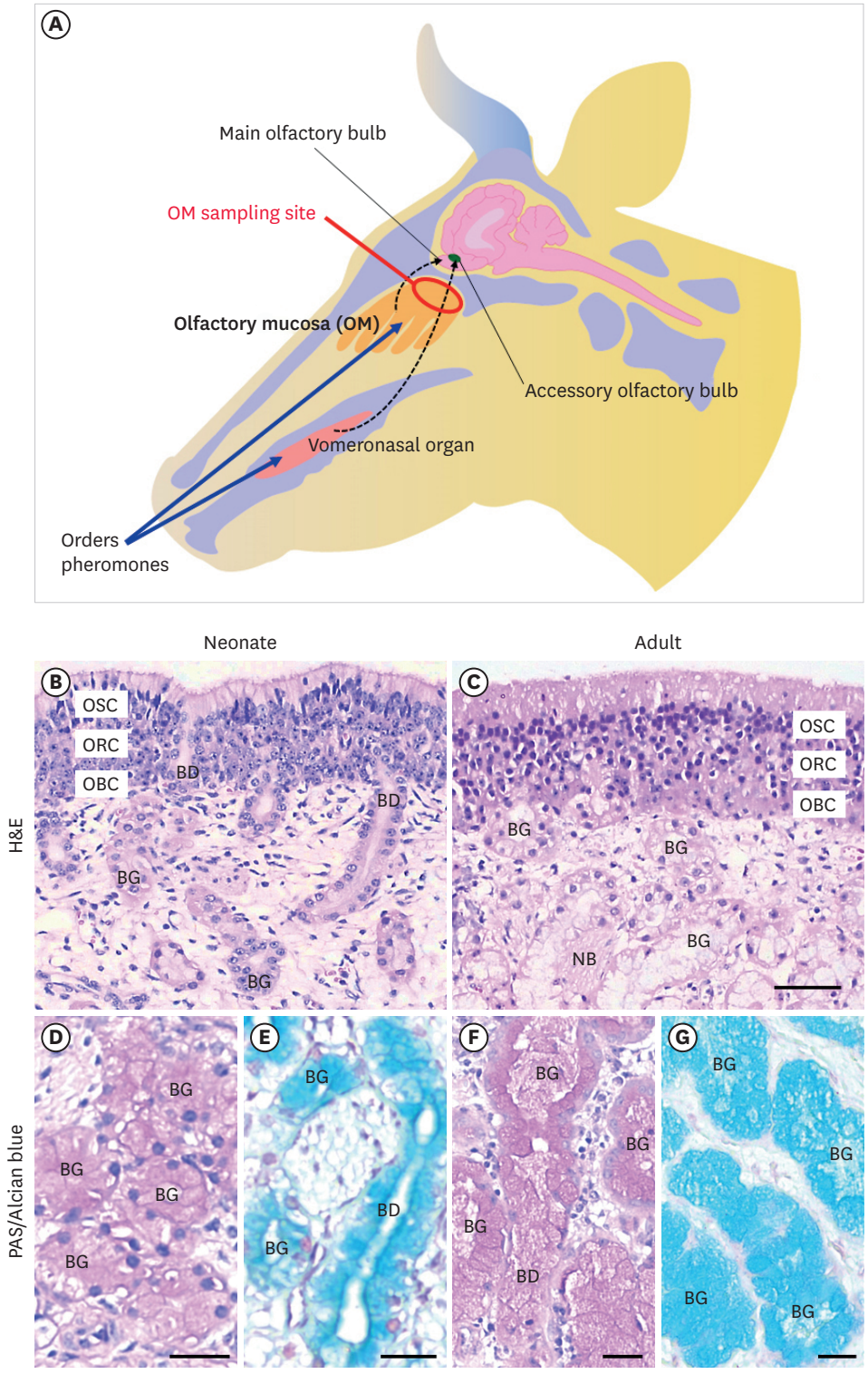

Fig. 1. Schematic of the OM employed in this study and histological characteristics of the cattle OM. Schematic shows the location of the OM sampling in the nasal cavity of Korean native cattle (A). Transverse sections of the OM of neonates (B) and adults (C) stained with hematoxylin and eosin. High-magnification views of the Bowman's glandular acini of neonates (D and E) and adults (F and G), stained with PAS (D and F) and Alcian blue (pH 2.5) (E and G), respectively. Scale bars in A and B = 50 μm. Scale bars in C–F = 25 μm.
OM, olfactory mucosa; BD, Bowman's glandular duct; BG, Bowman's gland; NB, nerve bundle; OBC, olfactory basal cells; OSC, olfactory supporting cells; ORC, olfactory receptor cells.

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substrate kit (DAB Substrate Kit SK-4100; Vector Laboratories), followed by counterstaining with hematoxylin.

Lectin histochemistry

Biotinylated lectin kits I, II, and III (Cat. No. BK-1000, BK-2000, and BK-3000) were acquired from Vector Laboratories. The acronyms, sources, and specificities of the used lectins are shown in **Table 1**. On the basis of their binding specificity and inhibitory sugars, lectins were categorized as N-acetylglucosamine, mannose, galactose, N-acetylgalactosamine, complex type N-glycan, and fucose-binding lectins. For competitive inhibition, the following sugars were purchased from Sigma-Aldrich (USA) and Vector Laboratories (**Table 1**): N-acetyl-D-glucosamine (β-D–GlcNAc; Sigma-Aldrich), Chitin Hydrolysate (Vector Laboratories), α-methyl mannoside/α-methyl glucoside (Sigma-Aldrich), lactose (Galβ1, 4Glc; Sigma-Aldrich), N-acetyl-D-galactosamine (α-D-GalNAc; Sigma-Aldrich), melibiose (Galα1, 6Glc; Sigma-Aldrich), and β-D-galactose (Sigma-Aldrich).

To eliminate the endogenous peroxidase activity, the sections were treated with 0.3% hydrogen peroxide in methanol. To prevent non-specific reactions, the sections were rinsed with PBS and then incubated with 1% bovine serum albumin in PBS. The sections were then treated overnight at 4°C with biotinylated lectins and reacted for 45 min at RT with an avidin-biotin-peroxidase complex (Vectastain Elite ABC kit). The sections were rinsed with PBS, treated with a diaminobenzidine substrate kit (DAB Substrate Kit SK-4100;

Table 1. Binding specificities of the lectins used in the present study[a]

Lectin	Source	Sugar specificity	Concentration (μg/mL)	Inhibitor or eluting sugar
N-acetylglucosamine-binding lectins				
s-WGA	Succinylated-Wheat germ agglutinin	GlcNAc	1.0×10^{-2}	0.2 M GlcNAc
WGA	Wheat germ agglutinin	GlcNAc NeuAc, SA	1.0×10^{-2}	0.2 M GlcNAc
BSL-II	*Bandeiraea simplicifolia* lectin II	α or β GlcNAc	4.0×10^{-3}	0.2 M GlcNAc
DSL	*Datura stramonium* lectin	$(GlcNAc)_{2\text{-}4}$	2.0×10^{-2}	0.5 M chitin hydrolysate
LEL	*Lycopersicon esculentum* lectin	$(GlcNAc)_{2\text{-}4}$	1.0×10^{-2}	0.5 M chitin hydrolysate
STL	*Solanum tuberosum* lectin	$(GlcNAc)_{2\text{-}4}$	1.0×10^{-2}	0.5 M chitin hydrolysate
Mannose-binding lectins				
ConA	*Canavalia ensiformis* (concanavalin A)	αMan, αGlc, 4GlcNAc	3.3×10^{-3}	0.2 M MeαMan/0.2 M MeαGlc
LCA	*Lens culinaris* agglutinin	αMan, αGlc, 4(Fucα1,6)GlcNAc	4.0×10^{-3}	0.2 M MeαMan/0.2 M MeαGlc
PSA	*Pisum sativum* agglutinin	αMan, αGlc, 4(Fucα1,6)GlcNAc	4.0×10^{-3}	0.2 M MeαMan/0.2 M MeαGlc
Galactose-binding lectins				
RCA_{120}	*Ricinus communis* agglutinin I	Gal	2.0×10^{-3}	0.2 M lactose
BSL-I	*Bandeiraea simplicifolia* lectin I	αGal, αGalNAc	4.0×10^{-3}	0.2 M GalNAc
Jacalin	*Artocarpus integrifolia* (Jacalin)	Galβ3GalNAc	5.0×10^{-4}	0.2 M melibiose
PNA	*Arachis hypogaea* (peanut) agglutinin	Galβ3GalNAc	4.0×10^{-3}	0.2 M βGal
ECL	*Erythrina cristagalli* lectin	Galβ3GalNAc	2.0×10^{-2}	0.2 M lactose
N-acetylgalactosamine-binding lectins				
VVA	*Vicia villosa* agglutinin	GalNAc	4.0×10^{-3}	0.2 M GalNAc
DBA	*Dolichos biflorus* agglutinin	αGalNAc	1.0×10^{-2}	0.2 M GalNAc
SBA	*Glycine maxi* (soybean agglutinin)	α > βGalNAc	1.0×10^{-2}	0.2 M GalNAc
SJA	*Sophora japonica* agglutinin	βGalNAc	2.0×10^{-2}	0.2 M GalNAc
Complex type N-glycans (complex oligosaccharides)-binding lectins				
PHA-E	*Phaseolus vulgaris* E	Galβ3GalNAcβ 2 Man α6 (GlcNAcβ4) (GlcNAcβ4Manα 3) Manβ4	5.0×10^{-3}	0.1 M acetic acid
PHA-L	*Phaseolus vulgaris* L	Galβ4GlcNAcβ6 (GluNAcβ2Man α3) Man α3	2.5×10^{-3}	0.1 M acetic acid
Fucose-binding lectin				
UEA-I	*Ulex europaeus* I	αFuc	2.0×10^{-2}	0.1 M L-fucose

Fuc, fucose; Gal, galactose; GalNAc, N-acetylgalactosamine; Glc, glucose; GlcNAc, N-acetylglucosamine; Man, mannose.
[a]The acronyms and specificities of the 21 lectins were obtained from the data sheep (Vector laboratory) and grouped, as shown in a previous paper [23].

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Vector Laboratories), and counterstained with hematoxylin. Negative controls for lectin histochemistry were generated by removing the biotinylated lectins and preincubating the lectins with appropriate inhibitors for 1 h at RT.

RESULTS

Histological findings of the OM

In neonatal (**Fig. 1A**) and adult cattle (**Fig. 1B**), the OM was composed of the OE and the lamina propria. The OE is predominantly constituted of basal cells, receptor cells, and supporting cells. The nuclei of the basal, receptor, and supporting cells were located in the basal, middle, and apical regions, respectively, of the OE (**Fig. 1A and B**). The basal cells were round, pallid, and had little nuclei. The nuclei of the supporting cells were elongated and spindle-shaped, but the nuclei of receptor cells were more rounded. While neonatal and adult OMs displayed comparable histological features, adult OMs appeared thicker than neonatal OMs.

The lamina propria included Bowman's glands and olfactory axon bundles. Bowman's glands were composed of clustered acinar cells that were connected through ducts to the apical surface (**Fig. 1A and B**). The Bowman's glandular ducts and/or acini stained positively for PAS (**Fig. 1C and E**) and Alcian blue stains (pH 2.5; **Fig. 1D and F**) in neonates (**Fig. 1C and D**) and adults (**Fig. 1E and F**); however, the reactivity of duct cells was relatively lower than that of gland cells. Additionally, the histological features of the OM did not differ between males and females at both ages.

Immunohistochemical analysis of OMP in the OM

In neonatal (**Fig. 2A**) and adult (**Fig. 2B**) cattle, OMP, a marker for mature olfactory sensory neurons, immunoreactivity was identified in the majority of the mature receptor cells in the middle region of the OE, with dendrites reaching to the OE free border, but not in basal and supporting cells. Their reactivity was more prominent in adults. GAP-43, a marker of immature olfactory sensory neurons, was found in certain receptor cells in the basal region of the OE, and some dendrites were immunopositive for GAP-43 (**Fig. 2C and D**). In addition, OMP and GAP-43 immunoreactivities were observed in the nerve bundles of the lamina propria (**Fig. 2A and C**).

Lectin histochemistry in the OM

The intensity values of 21 lectin-binding sites in the OE and lamina propria of the OM of Korean native cattle are summarized in **Tables 2** and **3**, respectively.

N-acetylglucosamine-binding lectins

All lectins except BSL-II were highly present in the free border of neonatal and adult OEs (**Fig. 3A-F**, **Table 2**). In neonates and adults, most OE cells lacked BSL-II reactivity (**Table 2**). Neonatal OE cells (especially receptor cells) responded more strongly to DSL (**Fig. 3C and D**), LEL, and STL (**Fig. 3E and F**) than adult ones, but they had no remarkable differences in s-WGA (**Fig. 3A and B**) and WGA labeling (**Table 2**). Except for BSL-II, all these lectins were present in different amounts in the nerve bundles, but they did not vary with age (**Fig. 3A-F**, **Table 3**). Neonatal Bowman's gland ducts displayed various reactivities to these lectins although reactivity in adults was faint (**Fig. 3A-F**, **Table 3**). The intensities of s-WGA (**Fig. 3A and B**), BSL-II, DSL (**Fig. 3C and D**), LEL, and STL (**Fig. 3E and F**) labeling were greater in neonates than in adults in Bowman's gland acini (**Table 3**).

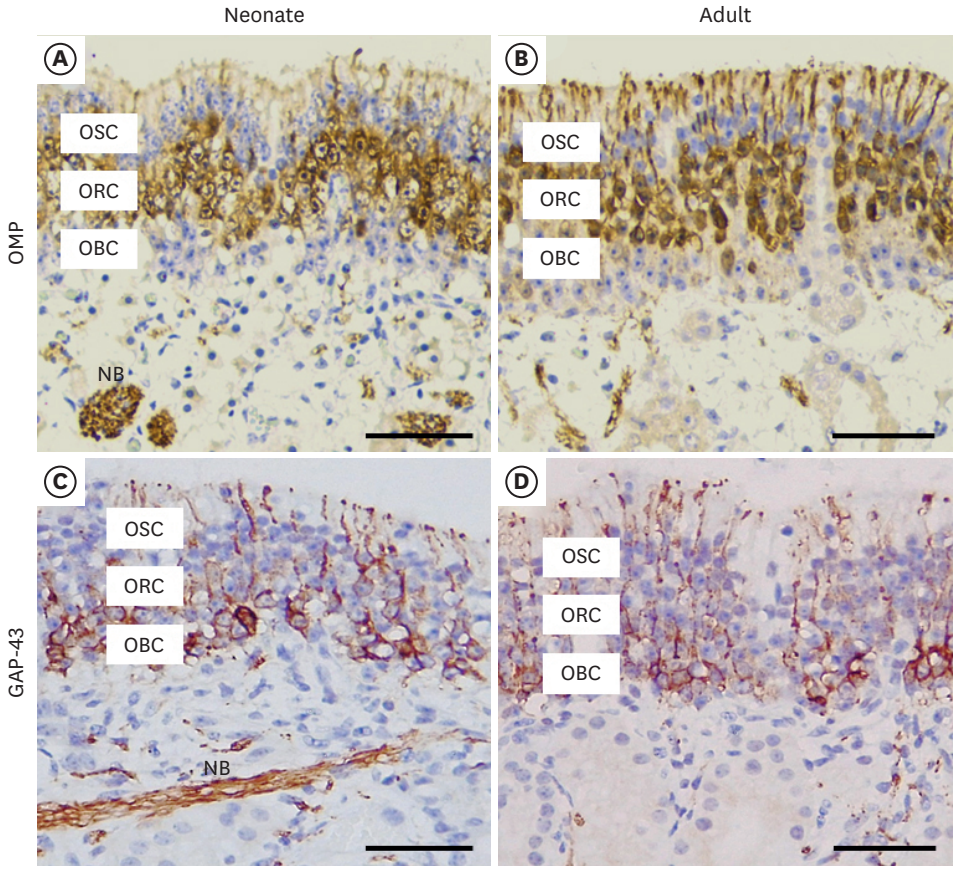

Fig. 2. Immunohistochemical localization of OMP and GAP-43 in the cattle OM. The adult receptor cells in the middle region of the OE are positive for OMP in neonates (A) and adults (B), and the dendritic processes of OMP-positive cells reach the free mucosal border at both ages. The immature receptor cells in the basal region of OE are positive for GAP-43 in neonates (C) and adults (D), and some dendritic processes are positive for GAP-43 at both ages. OMP and GAP-43 are also expressed in the nerve bundle. Counterstained with hematoxylin. Scale bars = 50 μm.
OMP, olfactory marker protein; GAP-43, growth associated protein-43; OM, olfactory mucosa; OE, olfactory epithelium; NB, nerve bundle; OBC, olfactory basal cells; OSC, olfactory supporting cells; ORC, olfactory receptor cells.

Mannose-binding lectins

ConA, LCA (**Fig. 3G and H**), and PSA were labeled with varying intensities across the OE layers, except the LCA in the supporting cells of neonates; their intensities were greater in adults than in neonates (**Table 2**). ConA labeling was more intense in nerve bundles and Bowman's gland duct/acini of the lamina propria in neonates than in adults (**Table 3**). LCA (**Fig. 3G and H**) labeling was more intense in Bowman's gland duct/acini of the lamina propria in neonates than in adults, but PSA labeling was more intense in the nerve bundles of the lamina propria in adults than in neonates (**Table 3**).

Galactose-binding lectins

In the epithelial free border, most lectins were tagged similarly in neonates and adults (**Table 2**), but the intensity of PNA labeling was greater in neonates than in adults (**Fig. 3I and J**). RCA_{120}, PNA (**Fig. 3I and J**), and ECL reactivities were observed in all OE layers except for supporting cells in neonates and/or adults (**Table 2**). The results of BSL-I labeling of the components of the lamina propria did not differ between neonatal and adult OM (**Table 3**). Jacalin had no reactivity in any of the OE layers in neonates and adults (**Table 2**) although it was tagged in Bowman's gland duct and acini in the lamina propria (**Table 3**).

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Table 2. Lectin-binding patterns in the OE of the OM in Korean native cattle

Lectin	Neonatal structures				Adult structures			
	Free border	Receptor cell	Supporting cell	Basal cell	Free border	Receptor cell	Supporting cell	Basal cell
N-acetylglucosamine-binding lectins								
s-WGA	+++	++[a]	–	+	+++	++[a]	–	+
WGA	+++	++	++	+	+++	++	–	+
BSL-II	–	–	–	–	–	–	–	–
DSL	+++	++	+	++	+++	+	++	+
LEL	+++	++	+	+	++	+	+	+
STL	+++	++[a]	+	++	+++	+	+	+
Mannose-binding lectins								
ConA	+++	+	+	+	+++	+	+	+
LCA	+++	+[a]	–	+	++	+++[a]	+	++
PSA	++	++[a]	+	+	+++	++[a]	+	++
Galactose-binding lectins								
RCA_{120}	+++	+	–	+	++	++	+	+
BSL-I	++	–	–	–	++	++[a]	–	+++
Jacalin	–	–	–	–	–	–	–	–
PNA	+++	++[a]	+	++	+	+[a]	–	–
ECL	+++	++	–	+	++	+	–	+
N-acetylgalactosamine-binding lectins								
VVA	+++	+[a]	–	–	+	+++[a]	–	+
DBA	++	+	+	+	++	+	+	+
SBA	+++	++[a]	–	–	++	+++[a]	–	+
SJA	+	+	–	–	+	+	–	+
Complex type N-glycans (complex oligosaccharides)-binding lectins								
PHA-E	+++	++	++	++	++	+	–	+
PHA-L	+	+	++	+++	+	+	++	++
Fucose-binding lectin								
UEA-I	+++	+	–	–	++	+	–	–

–, negative staining; +, faint staining; ++, moderate staining; +++, intense staining.
[a]Apical perinuclear labeling.

N-acetylgalactosamine-binding lectins

All the lectins produced a reaction of different intensities in neonates and adults at the epithelial free border (**Fig. 4A-F**, **Table 2**). In receptor cells, although all these lectins were detected faintly in neonates, the intensities of VVA (**Fig. 4A and B**) and SBA (**Fig. 4E and F**) were enhanced in adults (**Table 2**). Additionally, except for DBA, none of these lectins reacted with neonatal basal cells, whereas all lectins were faintly labeled in adults (**Table 2**). In nerve bundles, DBA (**Fig. 4C and D**) staining was more intense in neonates than in adults, whereas other lectins were absent (**Table 3**). In adults, the reactivities for VVA (**Fig. 4A and B**), SBA (**Fig. 4E and F**), and SJA were moderate to intense in Bowman's gland duct and acini but were relatively lower in adults (**Table 3**).

Complex type N-glycan-binding lectins

In the OE (**Table 2**) and lamina propria (**Table 3**), the reactivities of PHA-E and PHA-L (**Fig. 4G and H**) were extensively higher in neonates than in adults. Bowman's gland duct and acini were positive for both lectins (**Fig. 4G and H**).

Fucose-binding lectin

In the OE of neonates (**Fig. 4I**) and adults (**Fig. 4J**), UEA-I was identified in the free border and receptor cells but not in the supporting cells (**Table 2**). UEA-I was labeled extensively in Bowman's gland duct/acini in the lamina propria but was relatively lower in adults than in neonates (**Table 3**).

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Table 3. Lectin-binding patterns in the lamina propria of the OM in the Korean native cattle

Lectin	Neonatal structures			Adult structures		
	Nerve bundle	Bowman's gland duct	Bowman's gland acini	Nerve bundle	Bowman's gland duct	Bowman's gland acini
N-acetylglucosamine-binding lectins						
s-WGA	+++	–	++	+++	+	+
WGA	+++	+	++	++	+	++
BSL-II	–	+	++	–	+	+
DSL	++	+++	+++	+	+	+
LEL	++	++	++	++	+	+
STL	+++	+++	+++	++	+	+
Mannose-binding lectins						
ConA	++	++	++	+	+	+
LCA	++	+++	+++	++	++	+++
PSA	++	++	++	+++	++	++
Galactose-binding lectins						
RCA_{120}	++	+++	+++	++	+	+++
BSL-I	–	+++	+++	–	+++	+++
Jacalin	–	+	++	–	++	++
PNA	++	++	+++	+	+++	+++
ECL	–	+++	+++	–	++	+++
N-acetylgalactosamine-binding lectins						
VVA	–	++	++	–	++	++
DBA	+	+	+	+	+	+
SBA	+	++	+++	–	++	++
SJA	–	++	+++	–	+	+
Complex type N-glycans (complex oligosaccharides)-binding lectins						
PHA-E	++	++	++	++	+	+
PHA-L	–	+++	+++	–	++	++
Fucose-binding lectin						
UEA-I	–	+++	+++	+	+	+

–, negative staining; +, faint staining; ++, moderate staining; +++, intense staining.

DISCUSSION

This is the first study that visualized individual glycoconjugate (carbohydrate residues) during the postnatal development of bovine OM. We employed specific antibodies for olfactory sensory neurons against OMP and 21 lectins specific for the glycoconjugate sugar residues. Finally, we found that the distribution and expression levels of glycoconjugates varied remarkably between the OMs of neonatal and adult cattle.

In the present study, histological examination revealed that the cattle OM is composed of the OE and the underlying lamina propria, which contains Bowman's glands, bundles of olfactory axons, and ensheathing glia, as observed in other mammals, such as rats [26], sheep [15], and dogs [22]. A previous study established that age-related thickening results from a rapid increase in olfactory density and contributes to an improved odor sensitivity in domestic dogs and sheep [27]. This study evaluated differences in OM morphology between neonate and adult cattle to test the notion that mucosal refinement changes with age. Our findings indicated that adult cattle OM had a more complete structural shape than that of the neonate. Additionally, PAS and Alcian blue (pH 2.5) staining were utilized to confirm the mucus specificity of the Bowman's gland duct/acini in the lamina propria. In neonates and adults, Bowman's gland ducts/acini were stained positively with PAS and Alcian blue to varied degrees of intensity. These findings imply that the Bowman's glands release neutral and acidic components in the lamina propria in both the neonatal and adult OM, which may be implicated in the perception of scents or in protection against infectious agents and particles [28].

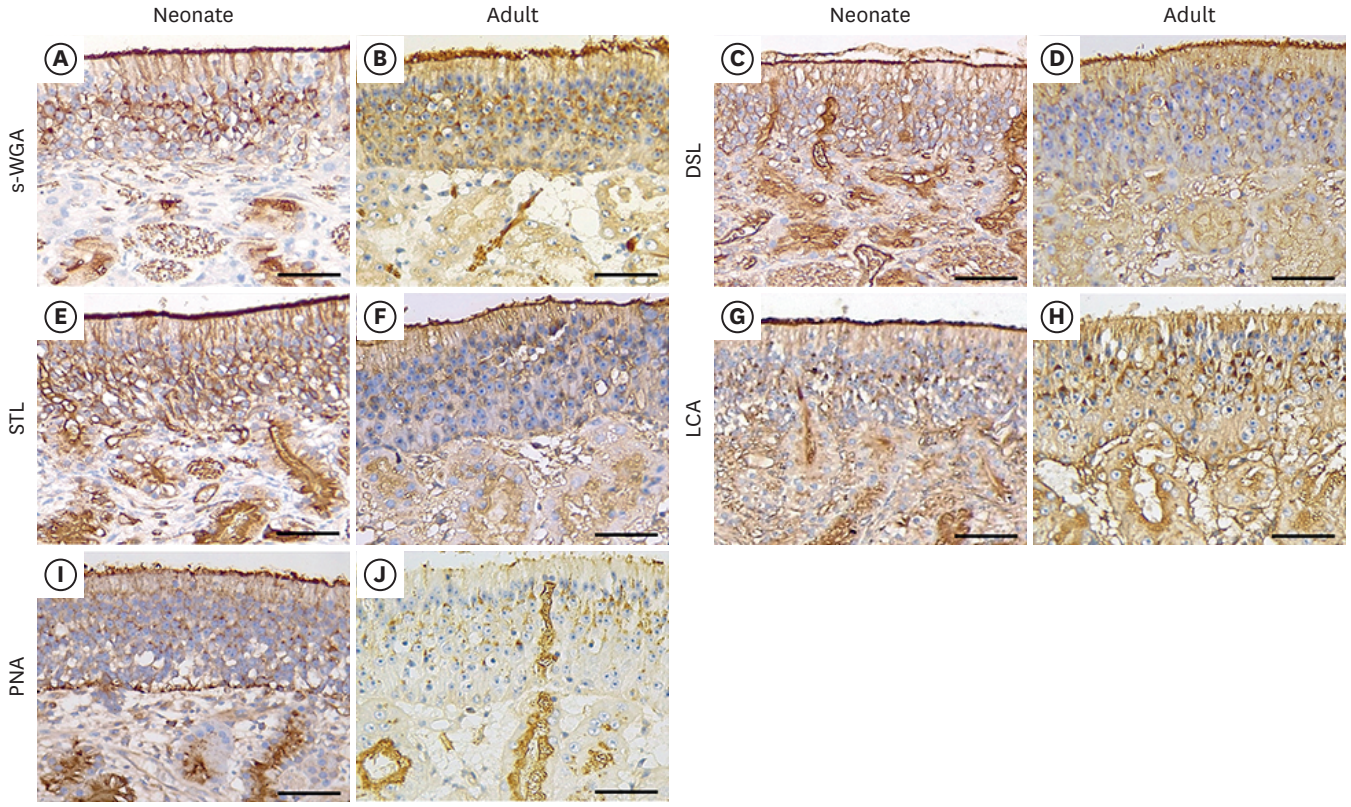

Fig. 3. Histochemistry of lectins binding to N-acetylglucosamine, mannose, and galactose in the OM of Korean native cattle. (A-F) The reactivities of N-acetylglucosamine-binding lectins, such as s-WGA (A and B), DSL (C and D), and STL (E and F), in neonatal (A, C and E) and adult OMs (B, D, and F). (G-J) The reactivities of mannose-binding lectin for LCA (G and H) and of galactose-binding lectin for PNA (I and J) in neonatal (G and I) and adult OMs (H and J). Counterstained with hematoxylin. Scale bars = 50 μm.
OM, olfactory mucosa.

OMP has been implicated in olfactory transduction [29], and its expression is restricted to mature chemosensory neurons in the OE [30]. We assessed the OMP expression in the OM to verify whether it is associated with mature sensory neurons that extend their dendritic knobs to the OE mucociliary layer or free border [31]. In contrast to OMP, GAP-43 expression is detected in the basal region of the OE, indicating that GAP-43 is expressed in immature neurons [32]. In neonatal and adult cattle, mature receptor cells in the middle region and immature receptor cells in the basal region of the OE were markedly positive for OMP and GAP-43, respectively. Therefore, OMP-positive mature and GAP-43-positive immature sensory neurons may be essential for the development of the main olfactory system [32,33]. However, the precise roles of OMP and GAP-43 in cattle OM should be further verified.

Tables 2 and **3** demonstrate the results of lectin-binding patterns in the OM of neonatal and adult cattle. In all regions of the OM, including the OE free border, receptor cells, supporting cells, basal cells, nerve bundles, and Bowman's glands, lectin-reactive glycoconjugates were found at varying intensities. Except for BSL-II and jacalin, all lectins utilized in this study were expressed in the free border of both neonatal and adult cattle, implying that the majority of the carbohydrates detected by these lectins are present in the mucociliary layer of the OE. This finding somewhat contrasts with previously published research on sheep [15] and horses [23], wherein the free borders were labeled with 13 and 19 of the 21 lectins, respectively. Except for BSL-II and jacalin, 19 of the 21 lectins were labeled in receptor cells with varying intensities in the cattle OE, indicating that sugar residues play a role in the cell biology of

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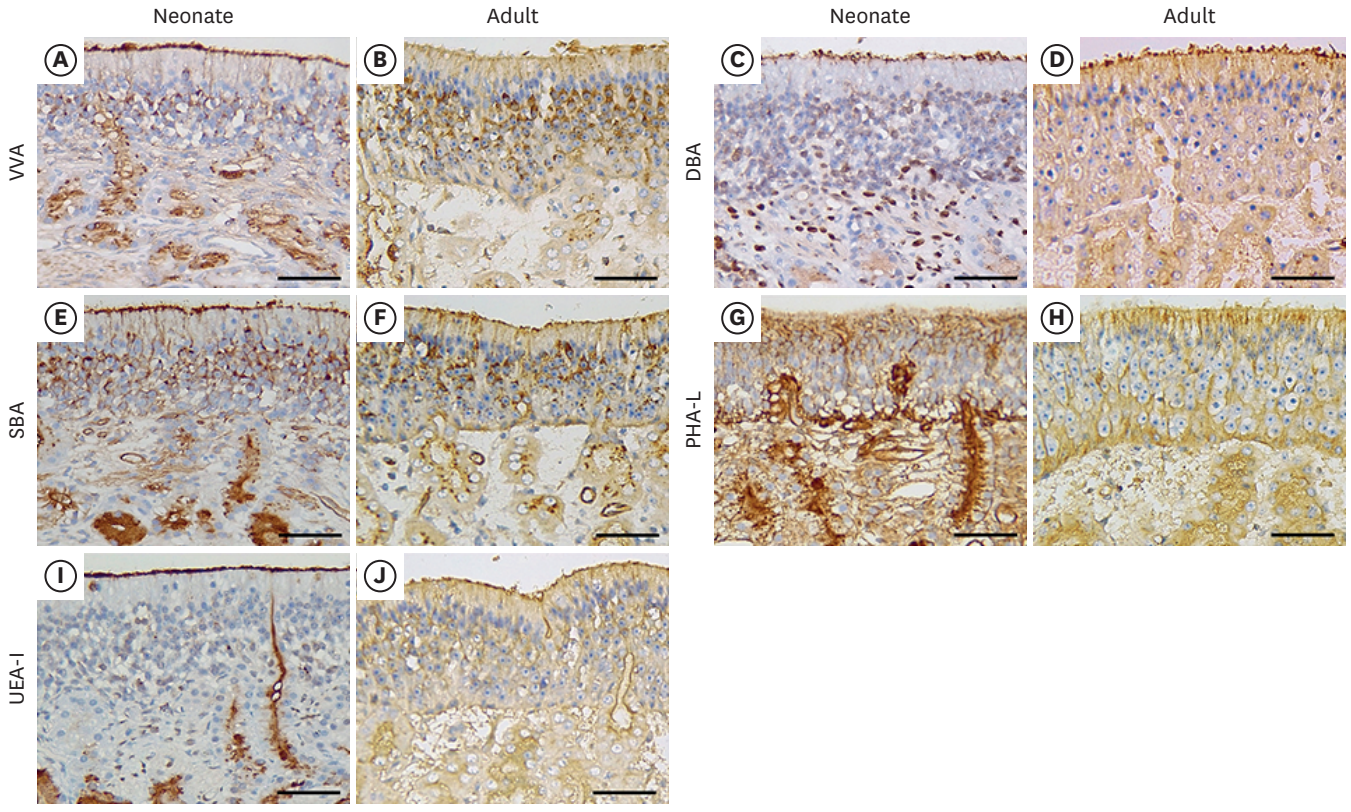

Fig. 4. Histochemistry of lectins binding to N-acetylgalactosamine, complex type N-glycans (complex oligosaccharides), and fucose in the OM of Korean native cattle. (A-F) The reactivities of N-acetylgalactosamine-binding lectins, such as VVA (A and B), DBA (C and D), and SBA (E and F), in neonatal (A, C and E) and adult OMs (B, D, and F). (G-J) The reactivities of complex type N-glycans-binding lectin for PHA-L (G and H) and of fucose-binding lectin for UEA-I (I and J) in neonatal (G and I) and adult OMs (H and J). Counterstained with hematoxylin. Scale bars = 50 μm.
OM, olfactory mucosa.

receptor cells. The results of this study were consistent with those of a previous study in horses [23]. However, DBA, SJA, PHA-L, and UEA-I were found in cattle but not in horses [23]. Furthermore, similar lectins have been identified in the olfactory receptor neurons and axons of other animals [34-37], which is consistent with our findings. Among the 21 lectins, 10 and 9 lectins were labeled as supporting cells in neonates and adults, respectively, and the labeling patterns were relatively comparable to those reported previously [22,38], but differed from the results obtained by a previous study in the horse [23]. In the lamina propria, 14 of the 21 lectins were found in the nerve bundles with varying intensities, whereas 3 lectins that were not found in the nerve bundles were found in the OE receptor cells. We hypothesize that sugar residue modifications cause this disparity as a result of positional changes. This event also occurred in the Bowman's gland in the lamina propria, where the expression patterns of lectin were altered as the Bowman's gland ducts exited through the OE. In the Bowman's glands, the majority of lectins were expressed more strongly in acinar cells than in ductal cells, implying that the acini contain a greater variety of sugar residues than the secretory ductal epithelium.

A previous study suggested a link between the expression pattern of DBA-binding glycoconjugates and the development of the olfactory system [36]. Similarly, previous studies hypothesized that the expression patterns of lectins, such as VVA and PNA, may be associated with the development of the OM [39,40]. The majority of the lectin-binding sites in the cattle OM were identical or stronger in neonates than in adults, implying that the labeling of lectins

may be closely related to the development and maturation of the OM. Thus, developmental changes in the expression patterns of lectin-binding glycoconjugates were evident in the OM of Korean native cattle. However, further functional investigations are required to determine how each glycoconjugate contributes to olfactory sensory processing.

Globally, cattle is important for animal protein production and may be a desirable animal model for studying olfaction and its effect on animal behavior. The Korean native cattle used in this study is part of a national breeding and selection program for beef production and thus subjected to artificial selection for intensive production in South Korea [41]. For example, in the United States, artificial insemination (AI) accounts for 50% of beef cattle breeding; in South Korea, it accounts for more than 90% [42]. AI timing is an essential component of conception, and the role of pheromones and olfaction in this process is being investigated [43]. Additional factors, such as irregular or prolonged estrous cycles and anestrus, which contribute to lower conception rates during AI, have been linked to pheromones and their receptors [44]. Consequently, olfaction may differ between Korean native cattle and other cattle according to these various factors [45]; however, information about the association between fertility-related behaviors and olfaction in Korean native cattle is limited. Still, we suggest that our data on the histology and lectin histochemistry in the OM of Korean native cattle may serve as a basis for further investigating their olfactory behavior and physiology.

In conclusion, we have performed a comprehensive characterization of the Korean native cattle OM at the histological, immunohistochemical, and lectin histochemical levels, thereby revealing new information about the main olfactory system. Additionally, the expression levels of glycoconjugates were considerably different between the OMs of neonatal and adult cattle, with adult OMs exhibiting higher intensities than neonates. Thus, this information can help clarify the role of the OM and its chemo-communication pathways in cattle. Further functional studies, however, are required to demonstrate the role of lectin-binding glycoconjugates in olfactory sensory processing and its associated behaviors.

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