Histol Histopathol (2003) 18: 787-795

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Lectin binding patterns in normal canine endometrium and in bitches with pyometra and cystic endometrial hyperplasia

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Summary. Cystic endometrial hyperplasia (CEH) and pyometra in the bitch are dioestral syndromes, supposed to be caused by hormonal disturbances and changes in endometrial steroid hormone receptor levels. Histologically, the endometria show cystic dilated glands and, if bacteria succeed in invading the uterus, pyometra may develop in the following metoestrus. In this study, lectin histochemistry was performed on paraffin sections to compare carbohydrate expression of uterine glands and surface epithelium in healthy dogs and in dogs with CEH and pyometra. Lectin binding is a useful tool to identify glycoconjugates, especially of the glycocalyx, which has essential functions in the endometrium during reproduction. Uterine tissue was obtained from 18 healthy bitches in metoestrus or anoestrus and 18 bitches with a clinical diagnosis of CEH or pyometra. Normal endometria showed cycle-dependent changes in SBA, PNA, HPA and UEA binding during metoestrus and anoestrus. LCA did not show cycle-dependent changes and WGA bound to Golgi regions in the apical parts of surface epithelial cells only in metoestrous. Endometria with inflammatory alterations lost cycle-specific lectin binding patterns and, with increasing severity of pathological changes, showed a marked decrease in binding intensity to the glandular and surface epithelial glycocalyx and secretions. In dogs with CEH, unaltered glands with generally strong lectin binding to the glycocoalyx and Golgi regions were found adjacent to altered glands. The decrease of lectin binding in pyometra cases is supposed to be a result of glandular exhaustion after cystic hyperplasia. In addition, bacterial adhesion to sugar residues on the uterine surface epithelium might impede lectin binding.

Key words: Pyometra, Dog, Endometrium, Lectins, Glycocalyx

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Introduction

Pyometra and cystic endometrial hyperplasia (CEH) are hormonally-mediated uterine disorders of middle-aged and aged bitches (Dow, 1957, 1958). Pyometra results from bacterial interaction with an endometrium that has undergone pathological changes caused by prolonged and/or repeated progesterone influence (Feldman and Nelson, 1987; De Bosschere et al., 2001). It has been generally accepted that CEH precedes pyometra. CEH is supposed to be an abnormal uterine response to progesterone with excessive proliferation of mucus-producing glands (Dow, 1958; Sevelius et al., 1990; De Bosschere et al., 2001). The endometrial response to progesterone depends on a preceding oestrogen priming, i.e. oestrogens induce the expression of progesterone receptors (Kennedy and Miller, 1993). The endometrium then converts from proliferatory to secretory mode. Disturbances in the timing and/or duration of progesterone or oestrogen receptor expression may affect endometrial growth and give rise to endometrial hyperplasia (De Cock et al., 1997). At the end of metoestrus, CEH can either resolve or worsen with subsequent dioestrual periods (Maretta et al., 1989). Inflammatory complications may lead to cystic endometritis (Dow, 1957, 1958; Kennedy and Miller, 1993). With invading bacteria, pus may accumulate in the uterus and result in pyometra (Sevelius et al., 1990). Although pyometra and CEH bear similarities such as glandular cyst formation, recent studies assume that pyometra does not always develop from CEH but can also arise de novo (De Bosschere et al., 2001).

Only limited information is available on uterine gland secretions in healthy dogs and dogs with CEH and pyometra. Therefore, in this study glycoconjugate patterns of the endometrium in healthy bitches and dogs with CEH or pyometra were analysed. Lectin histochemistry was used to demonstrate carbohydrate residues on the glycocalyx of endometrial epithelia. The glycocalyx as part of the apical plasma membrane plays an important role in cell to cell recognition processes

which are important during early embryonic development. Altered uterine secretions due to inflammation or hormonal abnormalities lead to changes in the uterine milieu and subsequent reproductive problems. Furthermore, sugar residues on apical cell membranes are binding targets for bacteria and therefore play a role in infections (King et al., 2000). Steroid hormones may support adherence of bacteria and thus may participate in the pathogenesis of pyometra (Beachey, 1981; Nishikawa et al., 1984). Therefore, we have studied sugar residues known to bind E. coli on epithelial surfaces such as mannose and N-acetyl-galactosamine. HPA, SBA and PNA are lectins that bind to N-acetyl-galactosamine and LCA was used to detect mannose moieties. Due to the clinical occurrence of CEH and pyometra during metoestrus and anoestrus, investigations of healthy bitches were also restricted to these two stages of the oestrous cycle.

Materials and methods

Animals and clinical examination

A total of 36 bitches from various breeds were included in the study. Animals were presented either for routine ovariohysterectomy (18 dogs, average age 3.05±3.2 years) or because of clinical symptoms of pyometra (18 dogs, average age 9.03±4.02 years). In all animals a general physical (nutritional status, hair and skin, inner body temperature, lymph nodes, mucuous membranes, pulse, respiratory rate, auscultation of heart and lungs) and gynaecological examination (adspection of external genitalia, palpation of abdomen and mammary glands, vaginal inspection, vaginal smear for exfoliative cytology) were performed. In case of pathological findings, transabdominal ultrasonography of the uterus was added. Blood samples were taken from all animals for haematology and blood chemistry (urea, creatinine, alkaline phosphatase, alanine amino transferase) and determination of plasma progesterone and oestradiol-17ß concentrations by commercial Elisa kit (SR 1 Analyzer, Serono, Vienna, Austria) were performed. The detection limits were 0.64 nmol/l for progesterone and 5 pg/ml for oestrogen. For the progesterone assay the intra-assay coefficient of variation was 6.8% and the inter-assay coefficient of variation was 9.0%. All dogs underwent ovariohysterectomy under general anesthesia using routine surgical techniques.

Tissue collection

Immediately after ovariohysterectomy, uterine tissue samples of 1 cm^3 were taken from the middle region of each uterine horn. Samples were immersed into 4% buffered formaldehyde for at least 24 hours and then embedded in Histo-Comp® (Vogel, Giessen, Germany) with an automated embedding equipment (Tissue Tek V.I.P. 2000; Miles Scientific, Mishikawa, IN, USA). Serial sections of 5 μm thickness were cut and stained with haematoxylin and eosin (H&E) for general evaluation according to Romeis (1989).

Lectin histochemistry

All lectins used were biotinylated and purchased from Sigma Chemicals (Vienna, Austria). Paraplast® sections of 5 μm thickness were mounted on poly-L-lysine-coated slides. After rehydration, endogenous peroxidase activity was blocked by incubation in 0.6% H_2O_2 in methanol for 10 min at room temperature (RT). Sections were then rinsed in tap water and incubated in 1% BSA (Sigma) in 0.1 M phosphate-buffered saline (PBS), pH 7.4, for 30 min at RT to inhibit unspecific staining. Afterwards, slides were incubated with the respective biotinylated lectin (diluted in 0.1% BSA in 0.1 M PBS) for one hour at RT. For LCA, instead of PBS, 10 mM Hepes buffer (Sigma) was used. Optimal concentration for each lectin was as follows: HPA 25 μg/ml; and WGA, UEA, SBA, PNA and LCA 20 μg/ml. Sugar-specificity of lectins is summarized in Table 1. To intensify the signal, sections were incubated with avidin-biotin-peroxidase-complex (ABC, Vector Laboratories, Burlingame, CA, USA) in PBS, pH 7.4, for 30 min at RT. After washing in PBS, slides were developed for 10 min at RT with DAB (0.2 mg 3,3´-Diaminobenzidine/ml 0.03 % H_2O_2 in 0,1 M Tris-HCl buffer, pH 7.4). Then slides were washed in distilled water, counterstained with Mayer´s haemalumn for 2 min, dehydrated, and mounted with DPX (Fluka Chemicals, Buchs, Switzerland). Neuraminidase (1U, Calbiochem®, Merck, Darmstadt, Germany) digestion for 24h at 37 °C was performed on selected slides and followed by WGA or PNA binding. Specificity of lectin binding was tested by preincubation of the lectin solution with 0.3 M of the corresponding sugar before incubation of control

Table 1. Full names of lectins, their source, and carbohydrate binding specificities.

LECTIN (ABBREVIATION)	SOURCE	SUGAR SPECIFICITY
Helix pomatia (HPA)	Roman or edible snail	D-N-acteyl-galactosamine
Triticum vulgaris (WGA)	Wheatgerm	ß-D-N-acetyl-glucosamine
Ulex europaeus I (UEA I)	Gorse or Furze	α-L-fucose
Glycine max (SBA)	Soybean	D-N- acetyl-galactosamine, galactosamine
Arachis hypogaea (PNA)	Peanut	ß-D-galactose, (1-3)-D-N-acetyl-galactosamine
Lens culinaris (LCA)	Lentil	α-D-mannose, D-glucose

sections. Lectin binding was completely abolished after the control procedure except for WGA, where light apical staining of epithelial cells remained. Negative controls were performed by replacing the lectin solution with PBS. No background staining was observed.

Results

Groups of animals were classified according to their gynaecological clinical findings, and their hormonal status. The healthy dogs were classified as being in middle metoestrus = progesterone (P) >10 nmol/l (n=4), late metoestrus = P 1 to 10 nmol/l (n=6) and anoestrus = P <1 nmol/l (n=8). Oestrogen levels were in a range between 5 and 15pg/ml during luteal phase. In all dogs with clinical disorders, pyometra (n=13) or CEH (n=5) were confirmed after surgery.

Table 2. Lectin binding results of healthy dogs.

	SURFACE EPITHELIUM		GLANDULAR EPITHELIUM						SECRETIONS	STROMA
			Basal zone		Intermediate zone		Crypt zone			
	Glycocalyx	Golgi region	Glycocalyx	Golgi region	Glycocalyx	Golgi region	Glycocalyx	Golgi region		
Middle Metoestrous										
HPA	-	-	-	-	-	-	+++	-	-	-
PNA	-	-	-	-	-/+	-	+++	-	-	-
SBA	-	-	-	-	-	-	+++	-	-	-
WGA	+++	+++	+++	-	++	-	++	-	+++	+
UEA I	-	-	-	-	+	-	+	-	-	-
LCA	-	-	-	-	-	-	-	-	-/+	+
Late Metoestrous										
HPA	-/+	-	+++	-	+++	-	+++	-	+	-
PNA	-/+++	-	+++	-	+++	-	+++	-	+	-
SBA	-/+	-	+++	-	+++	-	+++	-	+	-
WGA	+++	-	+++	-	+	-	+	-	+	+
UEA I	-/+++	-	++	-	++	-	++	-	+++	-
LCA	-/+	-	+/++	-	-	-	-	-	+/++	+
Anoestrous										
HPA	+++	-	+++	-	+++	-	+++	-	++	-
PNA	+++	-	+++	-	+/++	-	+++	-	++	-
SBA	+++	-	+++	-	+/++	-	+++	-	++	-
WGA	+++	-	+++	-	+	-	+	-	+++	+
UEA I	-	-	-	-	-	-	-	-	-	-
LCA	-	-	+/++	-	-	-	-	-	+/++	+

Staining intensity score: +++, strong lectin binding; ++ moderate lectin binding; + weak lectin binding; - negative.

Table 3. Lectin binding results of diseased dogs.

	SURFACE EPITHELIUM		GLANDULAR EPITHELIUM				SECRETIONS	STROMA
			Normal glands		Dilated glands			
	Glycocalyx	Golgi regions	Glycocalyx	Golgi regions	Glycocalyx	Golgi regions		
CEH								
HPA	+++	-	+++	+++	+/++	-	+/++	-
PNA	++	-	+++	+++	+/++	-	+	-
SBA	++	-	+++	+++	+/++	-	+	-
WGA	++	-	++	++	++	++	-	-
UEA I	+/+++	+/+++	++/+++	+/+++	++/+++	++/+++	++/+++	-
LCA	-/+	-	+++	+++	-/++	-	-/++	-/++
Pyometra								
HPA	-	-/+	-/+++	-	+	-	+/+++	-
PNA	-/++	-/+	-/+++	-	-/+	-	-	-
SBA	-/++	-/+	-/+++	-	-/+	-	-	-
WGA	+	-	++		-/++	-	-/++	-
UEA I	-/++	-/+	++	-	+++	-	-/++	-
LCA	-/+	-/+	-/+	-	+/-	-	-/+	+/++

Staining intensity score: +++, strong lectin binding; ++ moderate lectin binding; +, weak lectin binding; -, negative.

Haematoxylin and eosin staining

Histologically, the endometrium in healthy bitches during middle metoestrus was thickened and the stroma oedematised. The uterine mucosa had a columnar surface epithelium which also covered the deep crypts. Uterine gland lumina were extended and the glandular epithelium was flat to cuboidal. Secretions were frequently present in the glands. In late metoestrus, surface epithelial cells were vacuolised, their nuclei were pyknotic and localised apically. Sheets of epithelial cells were found in the uterine lumen. Gland lumina were less dilated than in middle metoestrus. In anoestrus, the surface epithelium was cuboidal and no crypts were present. Uterine glands were straight with narrow lumina and erythrocytes were found in the endometrial stroma.

Dogs with uterine disease showed two different histological features. In 13 dogs with pyometra, highly extended uterine glands and crypts filled with inflammatory cells, cellular debris and basophilic secretions were found. Stromal tissue was characterized by massive leukocyte infiltration, especially by plasma cells and neutrophils. The surface epithelium was composed of columnar cells with foamy cytoplasm, partially detached from the lamina propria. In 5 dogs with CEH, no inflammatory infiltration of stromal tissue was found. Endometria were characterized by a flat to cuboidal surface epithelium with only flat or no crypts. Marked glandular cysts were seen beneath non-dilated glands with columnar epithelium.

Lectin histochemistry

Lectin binding results for healthy and diseased dogs are summarized in Table 2 and 3.

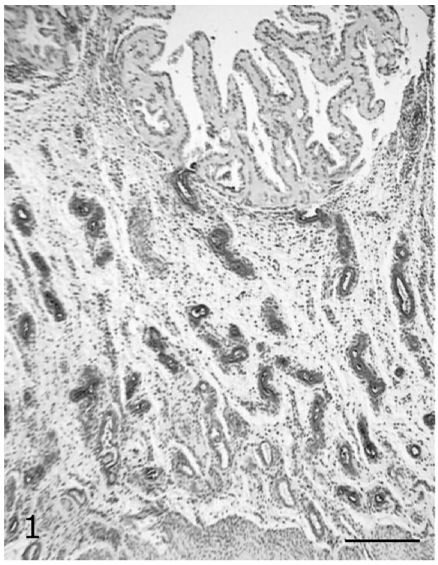
SBA, PNA, HPA

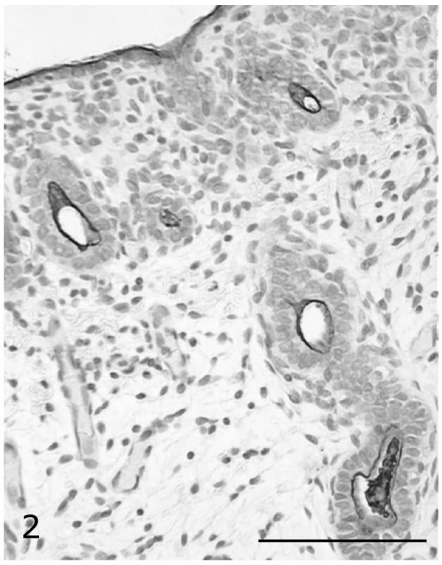
The binding pattern of SBA, PNA and HPA to galactose and galactosamine residues on epithelial surfaces was similar. With all 3 lectins, cycle-dependent changes in galactose and galactosamine expression were found. Endometria in middle metoestrous showed strong SBA, PNA and HPA binding to the glandular glycocalyx at the junction to the crypts, whereas in the intermediate zone PNA binding was irregular and missing in the basal zone. Surface epithelial cells did not show any binding. In late metoestrus, SBA, PNA and HPA clearly bound to the glandular glycocalyx in all mucosal regions (Fig. 1). Also secretions inside the glandular lumen contained galactose and galactosamine residues. The surface epithelium partly consisted of columnar epithelial cells with foamy cytoplasm but only few of them showed a positive glycocalyx. In addition, there were cuboidal luminal epithelial cells which had a strongly PNA marked glycocalyx. Sheets of desquamated epithelium in the uterine lumen did not show any SBA, PNA or HPA binding. In anoestrus, apical glandular binding intensity was strong in glandular cells near the uterine lumen and glands were filled with galactosamine-containing secretions (Fig. 2). The glycocalyx of the surface epithelium was strongly marked by SBA, PNA and HPA. In the intermediate region of glands staining intensity of the glycocalyx decreased. Strong labelling of the glandular glycocalyx was seen in the basal region and gland lumina were filled with SBA-, PNA- and HPA-binding material (Fig. 3). HPA binding was strong in all glandular regions (Fig. 4). Neuraminidase digestion generally enhanced PNA binding and led to moderate staining of the interstitial connective tissue in the muscular layer. However, binding patterns on surface or glandular epithelial cells did not change.

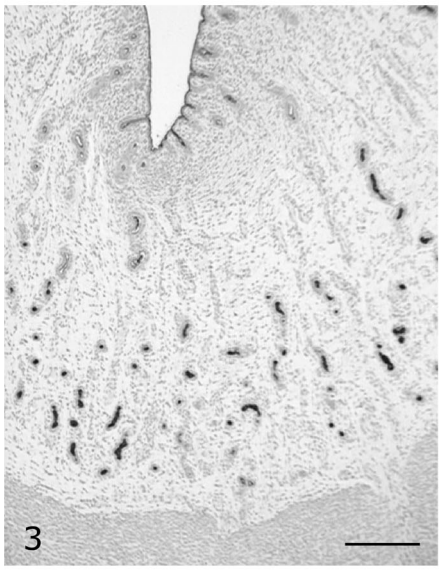
Uterine glands of dogs with pyometra mostly lacked galactosamine residues, only a few glandular epithelial cells bound PNA and SBA apically and some cells also had a positive cytoplasm. Surface epithelia were mostly negative. The same pattern was seen in dilated crypts. The more severe the pathological changes, the lower was lectin binding intensity. In 6 out of 13 animals with pyometra, moderate to strong HPA labelling of the glycocalyx from moderately dilated glands was found. The more extended the glands, the weaker was HPA binding. Uterine secretions showed the same HPA binding pattern as glands. Surface and crypt epithelia bound less HPA than the glands. The remaining 7 pyometra cases lacked HPA/SBA/PNA binding in all epithelial structures. Removal of sialic acid with neuraminidase before PNA staining demonstrated additional lectin binding on endothelia of blood vessels and on the surface of plasma cells and erythrocytes. In dogs with CEH, glands, especially in the basal zone showed a strongly labelled apical glycocalyx (Fig. 5) and positive Golgi fields in the apical cytoplasm. Glandular cysts lacked galactosamine residues in the Golgi complexes, but partly had a PNA- and SBA-labelled glycocalyx and secretions. Surface epithelia exhibited PNA and SBA binding of the apical glycocalyx but not to Golgi regions. CEH endometria carried galactosamine moieties demonstrated by HPA binding on apical surfaces and Golgi complexes of non-dilated glands (Fig. 6) in all mucosal regions. The surface epithelium showed marked HPA binding of the glycocalyx but not Golgi fields. Glandular cysts showed weak to moderate HPA labelling of the glycocalyx and secretions.

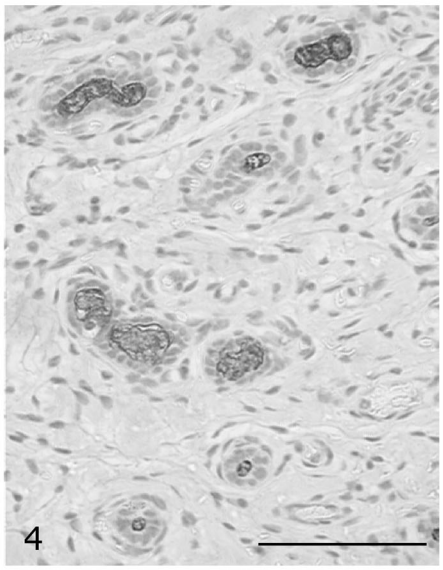
UEA I

In healthy dogs, cycle-dependent changes in fucose expression on the glycocalyx of glands and surface epithelium and in uterine secretions were demonstrated with UEA. In middle metoestrus, there was no UEA binding to glands in the basal region but glands of the middle region and closer to the luminal surface contained cells with fucosyl residues in the glycocalyx (Fig. 7). Columnar surface epithelial cells did not bind UEA. In late metoestrus marked UEA binding to the glycocalyx was found in all parts of the glands. The









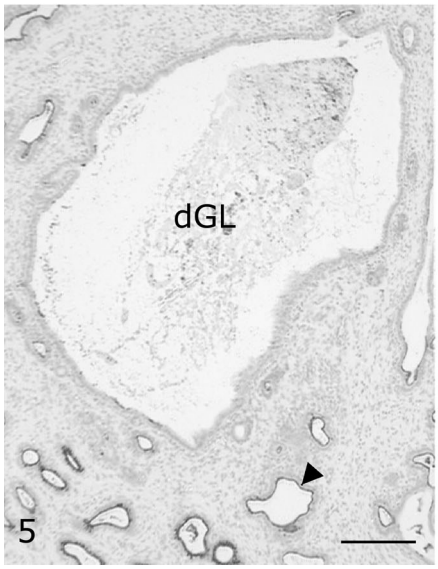


Fig. 1. SBA, late metoestrus. SBA-binding to the glandular glycocalyx and uterine secretions was strong on glands in the intermediate and crypt zone, the surface epithelium was negative. Bar: 100 μm.

Fig. 2. SBA, anoestrus. The glycocalyx of surface cells and glandular epithelial cells near the uterine lumen was strongly marked by SBA. Bar: 50μm.

Fig. 3. PNA, anoestrus. PNA regularly bound to the glandular glycocalyx in all mucosal regions. Also the surface epithelium was marked by PNA binding. Bar: 100 μm.

Fig. 4. HPA, anoestrus. The glycocalyx of all uterine glands cells contained galactose residues as demonstrated by strong HPA-binding. Bar: 50 μm.

Fig. 5. PNA, cystic endometrial hyperplasia (CEH). Moderately distended glands (arrowhead) and normal looking glands were strongly labeled by PNA, whereas cystic dilated glands (dGL) mostly lacked galactose residues. Bar: 100 μm.

Fig. 6. HPA, CEH. A prominent glycocalyx and numerous Golgi regions were marked by HPA binding in CEH samples. Bar: 50 μm.

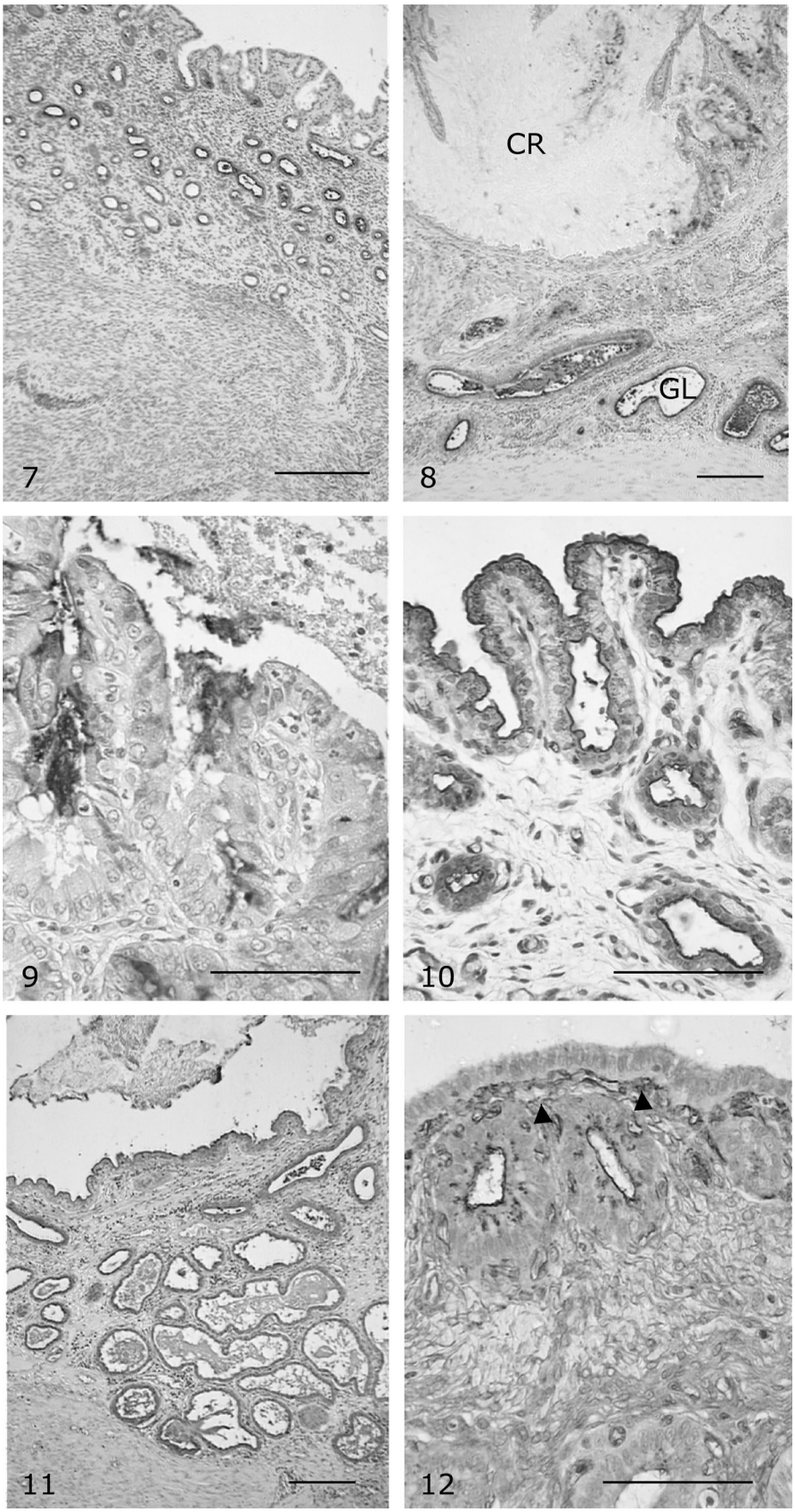


Fig. 7. UEA I, metoestrus. Only parts of glands located in the intermediate zone of the endometrium were marked by UEA I-binding, whereas glandular parts in the basal region were negative. Bar: 100 μm.

Figs. 8, 9. UEA I, pyometra. Dilated glands (GL), secretions and cellular debris bound UEA I in pyometra samples. Distended crypts (CR) demonstrated an irregular binding pattern. A glycoclayx was only labelled in individual cells. (8) Bar: 100 μm, (9) Bar: 50 μm.

Fig. 10. WGA, metoestrus. WGA intensely labeled the apical cell membrane and Golgi regions in surface and crypt epithelial cells. In addition, the glycocalyx of uterine glands showed remarkable WGA-binding. Bar: 50 μm.

Fig. 11. WGA, pyometra. Uterine glands, secretions and the surface epithelium were moderately marked by WGA. Bar: 100 μm.

Fig. 12. LCA, CEH. Non-dilated glands showed a strongly labelled glycocalyx and Golgi regions. LCA also bound to the uterine stroma (arrowhead). Bar : 50 μm.

surface epithelium consisted partly of cuboidal cells with fucosyl residues in the apical glycocalyx, whereas columnar cells with foamy cytoplasm did not bind UEA I. In anoestrus, no fucose expression was detectable in uterine glands or surface epithelium.

The same 7 pyometra endometria that lacked galactose and galactosamine residues also did not bind UEA. In the 6 remaining dogs, the glycocalyx of dilated glands was strongly marked. Extended and normal glands had fucosyl moieties in the apical glycocalyx, cytoplasm and uterine secretions (Figs. 8, 9). The surface epithelium and extended uterine crypts mostly lacked fucose expression, only a few crypt cells showing UEA binding to the apical glycocalyx (Fig. 9) and Golgi complexes. Secretions in the crypts lumina were mostly negative. Columnar surface epithelial cells and non-dilated crypts had irregularly distributed UEA binding patterns and cuboidal surface epithelia showed no fucosyl expression. UEA binding to CEH endometria (glycocalyx of surface and glandular epithelial cells, glandular Golgi complexes, secretions) were moderate to strong.

WGA

Cycle-dependent changes in WGA binding were not pronounced, but in late metoestrus and anoestrus binding intensity to the glandular epithelium declined. In the endometrial stroma and in endothelia of blood vessels glucosamine residues were found with WGA independent from the stage of reproductive cycle. After removal of sialic acid by neuraminidase treatment, WGA binding to the stroma and the endothelia of blood vessels was eliminated. In middle metoestrus, endometria showed strong WGA binding to the glandular glycocalyx in all regions and to secretions in the lumen. Surface epithelial glycocalyx and Golgi regions were strongly marked (Fig. 10). Endometria in late metoestrus and anoestrus showed strong WGA labelling of the glandular glycocalyx and uterine secretions in basal regions. Closer to the uterine lumen, the glycocalyx of the glands and secretions bound weakly. Surface epithelial cells had an intensely marked apical glycocalyx.

WGA binding in disordered dogs was comparable to healthy ones in late metoestrus and anoestrus with a decrease in lectin binding intensity. Uteri with pyometra showed a moderately marked glandular glycocalyx and some groups of glands contained glucosamine residues in the cytoplasm (Fig. 11). Uterine secretions contained inflammatory cells and were moderately WGA marked. Apical structures of the surface and crypt epithelium exhibited glucosamine moieties with lower intensity than the glands. Secretions were mostly negative or showed weak WGA binding. Glandular cysts and non-dilated uterine glands of CEH bitches showed moderate amounts of glucosamine residues on the glycocalyx and in Golgi complexes.

As described for the healthy dogs, neuraminidase digestion eliminated WGA binding of stromal cells and endothelial cells. WGA binding to the surface epithelium was reduced, indicating the presence of sialic acid.

LCA

No cyle-dependent changes were detected by LCA binding. All endometria bound LCA to connective tissue and serosa. Middle metoestrous endometria showed no LCA binding to any epithelial structure, only uterine secretions containing mannose residues. In endometria of late metoestrus and anoestrus, some glandular epithelial cells had a weakly to moderately stained glycocalyx and uterine secretions in the basal regions. Glands next to the uterine lumen did not bind LCA in any epithelial structure and surface epithelial cells mostly lacked mannose.

In dogs with pyometra, uterine glands and dilated crypts showed no lectin binding to the glycocalyx. Surface epithelial cells were mostly negative, only single cells bound LCA to the glycocalyx and, in some cases, Golgi complexes, and cytoplasm. Mucosal connective tissue had mannose/glucose moieties in all altered endometria but LCA binding intensity varied. In CEH endometria, some non-dilated glands had strongly LCA labelled Golgi regions and glycocalyx (Fig. 12). Glandular cysts varied in LCA binding. Some epithelial cells had a non-labelled glycocalyx while others showed variable amounts of mannose and glucose residues. Secretions inside the cysts and mucosal connective tissue varied in binding intensity from negative to moderate.

Discussion

Glycoconjugates on the surface of the uterine epithelium and in uterine secretions fulfill essential functions during reproductive processes. The uterine glands provide a microenvironment that supports survival of the gametes and the early embryo. The glycocalyx is important for gamete and embryo recognition and implantation. Using lectin histochemistry, we could demonstrate cycle-dependent changes in glycoconjugate expression of the healthy canine endometrium. Expression of galactosamine and fucosyl residues started in glandular regions near the uterine lumen in early metoestrus and spread into deeper parts during late metoestrus. In anoestrus, glands of all regions showed galactosyl residues on epithelia and in uterine secretions, whereas fucosyl moieties were not present. Cycle-dependent changes in endometrial lectin binding and localization of binding sites, predominantly on the apical surface or in the cytoplasm of glandular or surface epithelial cells, are in agreement with observations in pigs (Zhou et al., 1994; Walter and Bavdek, 1997). It has been suggested that oestrogens stimulate carbohydrate synthesis in the endometrium (Zhou et al., 1994) and that progesterone downregulates galactosamine expression in the baboon endometrium

(Jones et al., 1998). These findings are in agreement with our results where binding of HPA, PNA and SBA demonstrating galactose and galactosamine residues was low under progesterone influence. In contrast, in humans different lectin binding patterns were reported for DBA, SBA and PNA binding which was absent during the proliferative and early secretory phase but increased thereafter in uterine glands and surface epithelia (Jones et al., 1998). Other studies in humans (Gheri et al., 1998; Sivridis et al., 2000) and horses (Walter et al., 2001) showed no changes in lectin binding during the oestrous cycle. Endometrial carbohydrate expression thus differs significantly between species. Knowing the specific lectin binding patterns in the healthy canine endometrium enabled us to evaluate changes in pathologically altered endometria.

In dogs with CEH, lectin binding was generally strong, demonstrating glycoconjugate residues on the uterine glandular glycocalyx, in Golgi regions, and secretions. Endometria from dogs with CEH are characterized by hyperplastic and dilated glands and distended crypts filled with secretions. We suggest that the intense lectin binding to CEH endometria is a sign of glandular hyperstimulation. Although glycoconjugates are strongly expressed on epithelial structures in endometria with CEH, we assume that later the glands become exhausted and cease to express carbohydrates on apical surfaces. Dogs with pyometra differed in lectin binding from healthy dogs and dogs with CEH. In pyometra bitches there was an evident decrease in expression of galactose, glucose, and fucose residues in uterine glands, crypts and surface epithelium depending on the severity of mainly inflammatory alterations. The more these structures were affected, the lower was the binding intensity. It is not clear yet, why lectin binding did not decline in all cases, but it is suggested that this was caused by different stages of pathological changes. Reduced lectin binding in pyometra samples could also be a consequence of bacterial occupation of lectin receptors. Summarizing, a sequence of alterations starting with CEH, characterized by an excessive glycoconjugate production, could lead to pyometra by providing a supportive environment for bacterial adhesion and growth.

Bacteria and endotoxin production in the uterus play an important role in pyometra development. The most important and frequently found bacteria in pyometra are *E. coli* (Grindlay et al., 1973; Sandholm et al., 1975; Vandeplassche et al., 1991; Wadas et al., 1996; Fransson et al., 1997; Dhaliwal et al., 1998). Bacterial adherence depends on the presence of certain carbohydrates. Uterine defence mechanisms also depend on the stage of the reproductive cycle. When rat uteri were inoculated during metoestrus with E. coli strains from canine pyometra cases, endometritis was induced, whereas E. coli binding was reduced during oestrus (Nishikawa et al., 1984). The mechanisms by which oestrus decreases E. coli binding to the endometrium remain to be determined. Oestrogens might modify receptiveness of the cell surface to bacteria by inducing alterations in the microenvironment in such a way that colonisation with pathogens is prevented (Nishikawa and Baba, 1985). Glycolipids change during cellular differentiation and oestrogens cause various cell responses, including epithelial cell proliferation (Karlson, 1976). It has also been suggested that progesterone slows the migration of extravasated leukocytes during the luteal phase which may give bacteria more time for multiplication (Hawk et al., 1957). In our study carbohydrate expression in healthy endometria was limited to luminal glandular regions during the early luteal phase, but when progesterone levels declined during late metoestrus and anoestrus, all glandular regions exhibited glycoconjugates. This indicates an inhibitory effect of progesterone on endometrial carbohydrate expression or a stimulating effect of oestrogen, respectively. However, as glycoconjugates are clearly altered in diseased dogs although steroid hormone levels are within physiological range, other modulating factors such as inflammation have to be considered as well. The described alterations in endometrial glycoconjugate expression in dogs with CEH or pyometra are likely to lead to alterations in the uterine microenvironment. It has been suggested that bacterial infections can be prevented by blocking the adherence of the pathogens to mucosal surfaces with receptor analogues such as galactosamine- or mannose-containing sugars (Beachey, 1981). In horses, uterine lavages with specific sugar solutions have been discussed as a treatment to displace pathogenic bacteria from cellular receptors (King et al., 2000). Associations between uterine carbohydrates as potential pathogen adherence mechanisms and canine pyometra need to be investigated.

Acknowledgements. The authors gratefully thank Magdalena Helmreich, Sonja Kirpicsenko, Doris Rosenfellner and Silvia Kluger for their excellent technical support.

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Accepted March 18, 2003