Expression of CD105 (endoglin) in arteriolar endothelial cells of human endometrium throughout the menstrual cycle

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The cellular mechanisms underlying normal and pathological endometrial bleeding are not well understood, although abnormalities in the structure of endometrial blood vessels may lead to menstrual disorders. Endothelial cells in different organs are heterogeneous and differ in structure, function, antigen composition, metabolic properties and responses to growth factors. Immunostaining was performed with anti-CD105, CD31, CD34 and von Willebrand factor (vWF), and lectin binding with *Ulex europaeus* agglutinin 1 (UEA 1), *Bandeiraea simplicifolia* agglutinin 1 (BS 1), *Dolichos biflorus* agglutinin (DBA) and *Peanut* agglutinin (PNA) to characterize endothelial cells in human endometrium throughout the menstrual cycle. Serial sections fixed with formalin were stained with primary antibodies and lectins after antigen retrieval. Positive staining for CD31, CD105 and vWF was confined

to the vascular endothelium. Endothelial expression of CD31 was observed in all types of vessel, including single cells, and strong staining was found during the early proliferative and mid-secretory phases. Anti-vWF stained arterioles and veins, but there was little positive staining of capillaries. In contrast, staining for CD105 was confined to the arterioles. Although anti-CD34 strongly stained endothelial cells of small vessels and capillaries, staining was also observed on some non-endothelial stromal cells. Strong positive staining for UEA 1 was observed in endothelial cells of all types of vessel throughout the menstrual cycle. Binding of PNA, DBA and BS 1 was confined to the apical region of glandular epithelial cells. This study demonstrates that the differential binding of anti-CD31, CD34, CD105, vWF and UEA 1 distinguishes between endometrial populations of endothelial cells.

Introduction

Endothelial cells lining the inside of blood vessels are heterogeneous and differ in structure, function, antigen composition, metabolic properties and their response to growth factors. Such heterogeneity has been observed not only in different organs, but also in vessels of different calibre within a single organ (McCarthy, 1991; Bicknell, 1993). For example, endothelial cells lining microvessels, such as in brain white matter, are flattened and elongated, whereas endothelial cells lining large vessels, such as the aorta and umbilical veins, appear polygonal. Endothelial cells have distinct morphotypes in different organs so that they can adapt to different microenvironmental needs. Endothelial cells form a tight, continuous layer of cells that act as a barrier in brain, lung and muscular tissues, whereas a discontinuous layer of cells with intercellular gaps and intracellular fenestrae occurs in the endocrine glands and kidney (Augustin *et al.*, 1994). The local microenvironment has great influence on these cells. Moreover, endothelial cells have a differential response to growth factors. Microvascular endothelial cells isolated from human decidua showed no mitogenic response to several classic endothelial growth factors, such as basic fibroblast growth factor

(bFGF), epidermal growth factor (EGF) and interleukin 4 (IL-4), but were stimulated by vascular endothelial growth factor (VEGF) (Grimwood *et al.*, 1995).

CD105, also known as endoglin, is a 180 kDa homodimeric membrane protein (Gougos and Letarte, 1990), which acts as a receptor for transforming growth factor (TGF)- β 1 and - β 3 in vascular endothelial cells (Cheifetz *et al.*, 1992), and is a proliferation-associated antigen. CD105 has been located in human vascular endothelial cells of both large and small vessels. In particular, increased immunoreactivity for CD105 has been observed on endothelial cells during wound healing, in embryonic tissues and tumours (Letarte *et al.*, 1995; Thorpe and Burrows, 1995; Bodey *et al.*, 1998). These data indicate that CD105 may play a role in new blood vessel growth (Fonsatti *et al.*, 2000). CD105 knockout mice die by day 11.5 after mating because of defective vascular development. In addition, cardiac defects were observed in homozygous CD105-deficient embryos (Arthur *et al.*, 2000). Mutations in the endoglin gene lead to the hereditary haemorrhagic telangiectasia type 1 disease (HHT1 or Osler–Weber–Rendu disease). The disease is characterized by frequent nose bleeds, mucocutaneous telangiectases and arterio–venous malformation in the lung, brain and gastrointestinal tract, leading to recurrent haemorrhage and shunting (Guttmacher *et al.*, 1995). CD105 has also been found on syncytiotrophoblast, extravillous cytotrophoblasts in human placenta

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(St-Jacques *et al.*, 1994; Dagdeviren *et al.*, 1998), and in activated monocytes, tissue macrophages, and erythroid precursors in fetal and adult bone marrow (Gougos and Letarte, 1988; Bühring *et al.*, 1991; Lastres *et al.*, 1992; Robledo *et al.*, 1996).

Ulex europaeus agglutinin 1 (UEA 1), *Bandeiraea simplicifolia* agglutinin 1 (BS 1), *Dolichos biflorus* agglutinin (DBA) and *Peanut* agglutinin (PNA) are lectins that specifically bind to sugar residues located on the cell surface. Human endothelial cells bind specifically to the lectin UEA 1 via L-fucose residues (Hormia *et al.*, 1983). BS 1 binds to α -D-galactosyl and α -D-galactopyranoside on the surface of primitive endothelial cells surface, whereas DBA binds to N-acetyl-glucosaminyl residues and PNA binds to α -D-galactosyl and D-galactosyl neuraminic acid. BS 1, DBA and PNA have been reported to label endothelial cells of some species. BS 1 is a highly reliable marker of murine and pig endothelial cells and DBA binds endothelium in different organs of pigs, including newly derived capillaries (Plendl *et al.*, 1996). PNA has a unique ability to bind murine and pig brain microvascular endothelial cells (Plendl and Sinowatz, 1995; Plendl *et al.*, 1995).

CD31, CD34 and von Willebrand factor (vWF) are classic endothelial markers and are present in a broad range of tissues. CD31, also known as platelet endothelial cell adhesion molecule (PECAM), is an endothelial cell adhesion molecule. CD34 is a cell surface antigen of approximately 110 kDa and is expressed selectively on human haematopoietic progenitor cells and vascular endothelial cells (Fina *et al.*, 1990; Traweek *et al.*, 1991). vWF is an important factor of the blood coagulation system. It facilitates adherence of blood platelets to the injured vessel wall (Sixma and deGroot, 1991). Characterization of endothelial cells in human endometrium has been undertaken previously. CD31 (Rees *et al.*, 1993; Tawia *et al.*, 1993; Nikitenko *et al.*, 2000), CD34 and vWF (Rees *et al.*, 1993; Song *et al.*, 1995; Nikitenko *et al.*, 2000) have been immunolocalized throughout the menstrual cycle. However, comparative conclusions about the distribution of the antigens on endothelial cells could not be made due to variation in the tissue preparation and staging of samples.

The aim of the present study was to characterize the endothelial cells within human endometrium and to identify differences that may have some bearing on functional differences. Endothelial cells were characterized using a panel of eight endothelial markers (anti-CD105, CD31, CD34, vWF, and lectins UEA I, BS 1, DBA and PNA) throughout the menstrual cycle.

Materials and Methods

Tissue collection and processing

Ethical committee approval for tissue collection was obtained from Addenbrooke's Hospital NHS Trust, Cambridge and written informed consent was obtained from women undergoing surgery for benign gynaecological conditions. Women with irregular menstrual cycles (25–35

days), those having received exogenous hormones within the past 3 months and those with known identified endometrial abnormalities were excluded from the study. Endometrial samples ($n = 18$) were collected by dilatation and curettage. Tissues were rinsed in PBS, fixed immediately in 10% (v/v) buffered formalin for 6–8 h and embedded in paraffin wax. Sections (4 μ m) were cut on to slides coated with 3-aminopropyltriethoxy-silane (Sigma, Poole). For frozen sections, tissues were snap-frozen in liquid nitrogen after washing with PBS. Samples were then stored at -70°C .

Staging of tissue samples

Each biopsy was dated on the basis of the histological appearance of haematoxylin–eosin-stained sections (Wells, 1996). All biopsies were evaluated twice without knowledge of the previous results. Samples from six stages were chosen in the present study: early proliferative ($n = 3$), mid-proliferative ($n = 3$), late proliferative ($n = 3$), early secretory ($n = 3$), mid-secretory ($n = 3$) and late secretory ($n = 3$).

Immunohistochemistry

Anti-CD31 (Clone JC/70A; Dako, High Wycombe), anti-CD34 (Clone QBEND10; Dako) and anti-CD105 (Clone 266; Becton Dickinson, Cowley) were monoclonal mouse anti-human antibodies. Anti-vWF was a rabbit anti-human antibody (F8/86; Dako) (Table 1). Lectins UEA 1 (Vector Laboratories, Peterborough), BS 1 (Sigma), DBA (Sigma) and PNA (Sigma) were biotin-labelled (Table 1). Preliminary experiments were carried out to determine the optimum concentration of primary antibodies that resulted in clear staining with low background.

Samples from one particular stage were chosen for analysis using frozen sections in addition to formalin fixation to determine whether tissue preparation and fixation influenced the sensitivity of immunostaining.

Tissue sections fixed in formalin were deparaffinized in xylene three times, each for 10 min, followed by rehydration through a graded series of ethanol and dH_2O . For all eight markers, slides were pressure cooked for 1 min in 0.01 mol sodium citrate buffer I^{-1} (pH 6.0) to unmask the antigens. For frozen sections, the slides were fixed in cold acetone for 10 min. All sections were then washed in three changes of PBS and the slides were treated with 3% (v/v) H_2O_2 in methanol for 10 min to quench endogenous peroxidase. After three washes in PBS for 30 min, the sections were ready for primary antibody labelling.

For immunostaining of anti-CD31, CD34, CD105 and vWF, the sections were blocked by 20% (v/v) goat serum (Sigma) in 1% (w/v) BSA–PBS and incubated with primary antibody at 37°C in a humidified chamber for 60 min. The concentrations used for each antibody were as follows: CD31 (0.41 mg ml^{-1}), CD34 (0.05 mg ml^{-1}), CD105 (0.5 mg ml^{-1}) and vWF (5.7 mg ml^{-1}). Slides were washed in PBS for 30 min (three changes) before application of the biotinylated secondary goat anti-rabbit antibody (for vWF; Zymed Laboratories, Cambridge Biosciences, Cambridge)

Table 1. Antibodies and lectins used in this study

Antigens or lectins	Clone	Stock concentration (mg ml ⁻¹)	Dilution	Supplier
CD31	JC/70A	0.41	1:20	Dako
CD34	QBEnd10	0.05	1:25	Dako
CD105	Clone 266	0.5	1:100	Phar-Inter
von Willebrand factor (vWF)	F8/86	5.7	1:200	Dako
<i>Ulex europaeus</i> agglutinin I (UEA 1)-biotinylated	NA	1.0	1:100	Vector Laboratories
<i>Bandeiraea simplicifolia</i> agglutinin 1 (BS 1)-biotinylated	NA	1.0	1:100	Sigma
<i>Dolichos biflorus</i> agglutinin (horse gram) (DBA)-biotinylated	NA	1.0	1:100	Sigma
<i>Arachis hypogaea</i> (peanut) (PNA)-biotinylated	NA	1.0	1:100	Sigma

NA: not applicable.

or goat anti-mouse antibody (for anti-CD31, CD34 and CD105; Zymed) at 1:100 dilution in PBS for 10 min at room temperature. Sections were then washed in PBS three times (10 min each) and antigen detected by the streptavidin-conjugate-horseradish peroxidase complex (Zymed). After further washing, antibody binding was visualized by the liquid DAB-plus substrate kit (Zymed). Slides were counter-stained using Meyer's haematoxylin.

For detection of lectin binding, sections were incubated with biotinylated UEA 1, BS 1, DBA and PNA (1 mg ml⁻¹) at 1:100 dilution in a humidified chamber for 60 min (37°C). After washing with PBS, lectin binding was detected using a streptavidin-conjugate-horseradish peroxidase complex (Zymed), following the staining by liquid DAB-plus substrate kit (Zymed).

Negative controls for the immunostaining were carried out as above, except that the primary antibodies were replaced with normal mouse or normal rabbit immunoglobulins. Controls for lectins were performed by adding the competing sugars at 200 mmol l⁻¹ simultaneously with the lectins.

Subjective assessment of immunostaining intensity was based on a four point scale as absent, weak, moderate and strong.

Results

Distribution of CD31, CD34, CD105 and vWF in human endometrium throughout the menstrual cycle

Positive staining for CD31, CD34, CD105 and vWF was confined to vascular endothelium and showed an overlapping, but non-identical, staining pattern. Endothelial expression of CD31 was observed in all types of vessel (arteries, veins and capillaries) (Fig. 1a–d). Arteries were identified by being surrounded by whorls of smooth muscle cells and having a smooth profile; veins were identified by the paucity of surrounding smooth muscle cells and as having a large calibre and an irregular profile; and capil-

laries were of small calibre. Strong staining was found during both the early proliferative (Fig. 1a,b) and mid-secretory phases of the menstrual cycle (Fig. 1c,d). Although endothelial cells were immunostained for anti-CD34 in the arteries, veins and capillaries (Fig. 1e), strong staining was associated with small vessels and, in particular, capillaries during both the early proliferative (Fig. 1e,f), and early and mid-secretory phases (Fig. 1g,h). Staining for anti-CD34 was also detected around the glands (Fig. 1f), particularly at the early and mid-proliferative phases. Strikingly, CD105 was distributed predominantly on arteries (Fig. 1k). There was only weak positive staining for anti-CD105 on veins (Fig. 1l). In contrast, anti-vWF tended to stain large vessels, and there was weak or even absent staining of capillaries (Fig. 1n,o). Medium-sized vessels located close to the glands always stained positive for anti-CD31 (Fig. 1b), anti-CD34 (Fig. 1f) and anti-vWF (Fig. 1o).

These results are summarized (Table 2). Frozen tissues from the early secretory phase (when strong staining was observed in the material embedded in paraffin wax) were chosen to assess the effect of different tissue preparation and fixation on the sensitivity of immunohistochemistry. Immunohistochemistry for anti-CD34, anti-CD105 and vWF showed similar sensitivity on paraffin wax and frozen sections, whereas immunohistochemistry with anti-CD31 showed good staining on frozen sections (data not shown).

No staining was observed for all relevant negative controls (Fig. 1p). No glandular epithelial staining for these four antibodies was detected at any stage of the menstrual cycle.

Binding of UEA 1, BS 1, DBA and PNA in human endometrium during the menstrual cycle

Good staining with lectins was observed on sections embedded in paraffin wax and sections fixed in formalin, whereas there was high background in frozen sections.

Endothelial cells in arteries, veins and capillaries from all

Fig. 1. Reactivity of endothelial cells with anti-CD31, CD34, CD105 and von Willebrand factor (vWF) in human endometrium tissue fixed in formalin and embedded in paraffin wax in (a,b,e,f,i,j,m-p) the early proliferative phase and (c,d,g,h,k,l) the mid-secretory phase of the menstrual cycle. Positive staining for (a-d) CD31, (e-h) CD34, (i-k) CD105 and (n-o) vWF was confined to endothelial cells. (a-d) Anti-CD31 stained all

Table 2. Reactivity of endothelial cells in human endometrium with antibodies and lectins

Menstrual phase	Antibodies				Lectins			
	Anti-CD31	Anti-CD34	Anti-CD105	vWF	UEA 1	PNA	DBA	BSI
Early proliferative (n = 3)	A ++	A +++	A ++	A +	A +++	EC –	EC –	EC –
	V ++	V +++	V +	V +	V +++	G +	G ++	G ++
	C +	C ++	C –	C –	C +++			
Mid-proliferative (n = 3)	A +	A +	A +	A +	A +++	EC –	EC –	EC –
	V +	V +	V –	V +	V +++	G +	G ++	G ++
	C +	C +	C –	C –	C +++			Single stromal cell ++?
Late proliferative (n = 3)	A +	A +	A +	A +	A +++	EC –	EC –	EC –
	V +	V +	V –	V +	V +++	G +	G ++	G ++
	C +	C +	C –	C –	C +++			
Early secretory (n = 3)	A ++	A +++	A +++	A ++	A +++	EC –	EC –	EC –
	V +++	V +++	V +	V ++	V +++	G +	G ++	G +++
	C +++	C +++	C –	C +	C +++			
Mid-secretory (n = 3)	A +++	A ++	A +	A +	A +++	EC –	EC –	EC –
	V +++	V ++	V –	V +	V +++	G +	G ++	G +++
	C +++	C ++	C –	C –	C +++			
Late secretory (n = 3)	A +	A ++	A +	A +	A +++	EC –	EC –	EC –
	V ++	V ++	V –	V +	V +++	G +	G ++	G ++
	C +	C +	C –	C –	C +++			
Frozen section (early secretory)	A +++	A +++	A ++	HB	HB	EC –	EC –	EC –
	V +++	V +++	V +			G +	G +	G +
	C +++	C +++	C –			HB	HB	HB

(–): no staining; (+): weak staining; (++) : moderate staining; (+++) : intensive staining.

A: artery; V: vein; C: capillary; EC: endothelial cells; G: gland; HB: high background; vWF: von Willebrand factor; UEA 1: *Ulex europaeus* agglutinin 1; PNA: *Peanut* agglutinin; DBA: *Dolichos biflorus* agglutinin; BSI: *Bandeiraea simplicifolia* agglutinin.

stages of the menstrual cycle stained intensely with UEA 1 (Fig. 2b,d,e,f). In contrast, binding of BS 1, DBA and PNA was restricted to the apical region of glandular epithelial cells (Fig 2g–i). No endothelial staining with these three lectins was observed in human endometrium. Relevant negative controls resulted in no staining of any type of cell (Fig. 2j).

Discussion

The present study demonstrated that the distribution of CD105, CD31, CD34, vWF and UEA 1 binding sites differs among populations of endometrial endothelial cells, and, in contrast to that in other species, binding of BS 1, DBA and PNA in humans was restricted to the glandular epithelial cells.

The most marked observation in the present study was that staining for CD105 was confined to arterial endothelial

cells. CD105 is a receptor for TGFβ1 and -β3 (Cheifetz *et al.*, 1992). It does not show signalling activity by itself but functions as a regulator to modulate the access of TGFβ to the signalling complex (Wrana *et al.*, 1994). TGFβ is chemotactic for monocytes and macrophages, and stimulates angiogenesis in the rabbit cornea (Eisenstein, 1991; Klagsbrun and D'Amore, 1991). It enhances VEGF-induced tube formation of endothelial cells on a collagen gel at low concentrations, but inhibits tube formation at higher concentrations (Pepper *et al.*, 1993). Immunoreactivity of TGFβ1, -β2 and -β3 has been located in glands and stroma in normal human endometrium (Gold *et al.*, 1994) and TGFβ increases matrix metalloproteinase concentrations in endometrial stroma (Bruner *et al.*, 1995). TGFβ mRNA content is higher in the secretory phase than in the proliferative phase of the menstrual cycle (Kauma *et al.*, 1990). In the early proliferative phase, spiral arteries have thin walls and are

types of vessel. (b,d,f) Higher magnification of the boxed sections in (a,c,e, respectively). (f) The arrowhead indicates that positive staining was also around the gland. (g,h) Intensive staining for CD34 was associated with small vessels and capillaries. (h) Higher magnification shows capillaries (indicated by arrows) stained with anti-CD34. Endothelial cell expression of CD105 was predominantly in arteries (as shown in (j) and (k)), with weak staining on veins (as shown in (i)). (l) Haematoxylin–eosin staining of a nearby slide of (k) reveals the vessel structure. (n) Immunostaining of vWF shows positive staining on the large vessels, whereas staining was absent from capillaries (indicated by arrows), which stained positively for CD31, as indicated by arrows in (a). (o) Higher magnification of the boxed section in (n). (m) Haematoxylin–eosin staining of a nearby section of (a,e,i,n). (p) Negative control for (a). The negative controls for CD34, CD105 and vWF were similar to (p), data not shown. Scale bars represent (a,c,e,g,i,m,n,p) 50 μm and (b,d,f,h,j,k,l,o) 12.5 μm. G: glands; A: arteries; V: veins.

Fig. 2. Reactivity of endothelial cells with *Ulex europaeus* agglutinin 1 (UEA 1), *Peanut* agglutinin (PNA), *Dolichos biflorus* agglutinin (DBA) and *Bandeiraea simplicifolia* agglutinin 1 (BS 1) in human endometrium tissue in the (a–f) early proliferative phase and the (g–j) mid-secretory phase of the menstrual cycle. (a,c) Haematoxylin–eosin staining. (b,d) Serial sections corresponding to (a) and (c) stained with UEA 1. (e,f) Low and high magnification of UEA 1 binding. UEA 1 shows

relatively straight; however, during the secretory phase they become coiled and the thickness of the walls increases. As this remodelling occurs, it is possible that the TGF β system plays a part in the interaction between the endothelial cells and the surrounding vascular smooth muscle cells. Endothelial cell proliferation is significantly increased in the functional layer in comparison with the basal layer (Rogers *et al.*, 1998). Therefore, the presence of CD105 on the functional layer vessels may antagonize the inhibitory effects of TGF β on endothelial cells. In fact, antisense inhibition of CD105 translation enhances the ability of TGF β 1 to suppress growth and migration of cultured endothelial cells and reduces the length of capillary-like structures (Li *et al.*, 2000). Spiral arteries, which supply the functional layer of the endometrium, play a key role in the initiation and control of menstrual bleeding. During normal menstrual cycles, vascular contraction increases as a result of decreasing steroid concentrations. Subsequently, ischaemia and necrosis of the functional layer and sloughing occur. Therefore, the TGF β -CD105 system could be one of the factors involved in the development of spiral arteries, which is important in endometrial physiology.

In agreement with reports by Rees *et al.* (1993) and Tawia *et al.* (1993), the findings from the present study indicate that endothelial cells in all types of vessel are immunostained for CD31. Staining for CD34 was found on most endothelial cells. In particular, strong staining was observed on small vessels and capillaries, which is in agreement with reports by Rees *et al.* (1993) and Song *et al.* (1995). Particularly strong staining was found around glands, which may be due to a close association of a network of capillaries around the glands; however, this contention is difficult to determine precisely, as the histological appearance of such vessels is compromised due to tissue shrinkage during fixation (this is approximately 40% in material fixed in formalin). Another possibility is that the antibody is staining non-endothelial cells in the stroma around the glands. It has been suggested that non-vascular CD34-positive cells are able to form basement membrane and play a supportive role in the maturation or proliferation of adjacent stem cells (Weiss and Nickoloff, 1993; Suster and Fisher, 1997). In human endometrium, some basal cells do express CD34 (Kohnen *et al.*, 2000).

Endothelial cells were positively stained for anti-vWF within large vessels, but staining was weak or even absent in capillaries. Song *et al.* (1995) reported that in women with normal menstrual cycles, the number of CD34-positive vessels was significantly higher than the number of vWF-positive vessels. This difference is probably because these two antibodies stain different populations of endothelial cells, possibly reflecting differences in the behaviour of these cells.

Vessels located close to the glands were always stained

strongly with anti-CD31, CD34, vWF and UEA 1. Endometrial epithelial cells are capable of producing a variety of growth factors, some of which stimulate endothelial cell growth or migration. Vascular endothelial growth factor A (VEGF-A) mRNA is abundant in glandular epithelial cells in the luteal phase (Charnock-Jones *et al.*, 1993, Sharkey *et al.*, 2000), whereas its receptors, flt1 KDR, are located on endothelial cells (Millauer *et al.*, 1993). The close association between epithelial and endothelial cells may be due to the secretion of this, or similar factors, and these capillaries may be required to support the active metabolism and secretory activity of these cells.

BS 1, DBA and PNA bind specifically to endothelial cells in some species (Plendl and Sinowatz, 1995; Plendl *et al.*, 1995, 1996). In humans, only a few studies have described the binding of these lectins. BS 1 has been demonstrated to react with endothelium in human placenta (Lang *et al.*, 1994; Zhang *et al.*, in press). DBA reacted with glandular epithelial cells of endocervical adenocarcinoma, but not those of endometrial adenocarcinoma (Toda *et al.*, 1998), although PNA reactivity was lost in endometrial adenocarcinomas (Sivridis and Agnantis, 1996). In the present study, no endothelial binding of BS 1, DBA and PNA was detected in human endometrium at any stage of the menstrual cycle. In contrast, these lectins strongly stained glandular epithelial cells and, thus, they are of little use as endothelial markers in human endometrium.

In conclusion, binding of anti-CD105, CD31, CD34, vWF and UEA 1 distinguishes between different populations of endometrial endothelial cells. Anti-CD31, anti-CD34 and UEA 1 are reliable markers for endothelial cells in human endometrium, although the precise populations stained at any one time differ. vWF is somewhat selective for the endothelial cells within larger vessels and anti-CD105 binds specifically to endothelial cells within endometrial arterioles. The lectins BS 1, DBA and PNA do not bind to endometrial endothelial cells but do bind to glandular epithelial cells; this finding is in contrast to that in other species.

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positive staining in endothelial cells of all types of vessel, including (b,d) large vessels and (e,f) capillaries. Binding of (g) DBA, (h) PNA and (i) BS 1 was confined to the apical region of glandular epithelial cells. (j) The negative control for UEA 1. The negative controls for DBA, PNA and BS 1 were similar to that of (j), data not shown. Scale bars represent (a,b,e,g,h,i,j) 50 μ m and (c,d,f) 12.5 μ m. G: gland; V: vessel.

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