Available online at www.sciencedirect.com
SCIENCE DIRECT
Micron 37 (2006) 544–550
micron
www.elsevier.com/locate/micron

Distribution of chondroitin sulfate in human endometrium

Luiz E. Nasciutti [a,*], Renato Ferrari [a], Plínio T. Berardo [a], Maísa L. S. Souza [b], Christina M. Takiya [a], Radovan Borojevic [a], Maurício S. Abrão [c], Luiz-Claudio F. Silva [b]

[a] *Departamento de Histologia e Embriologia, Instituto de Ciências Biomédicas, Universidade Federal do Rio de Janeiro, Cidade Universitária-Ilha do Fundão, 21941-590 Rio de Janeiro, RJ Brazil*

[b] *Laboratório de Tecido Conjuntivo, Hospital Universitário Clementino Fraga Filho and Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro, Brazil*

[c] *Departamento de Obstetrícia e Ginecologia, Universidade de São Paulo, Brazil*

Received 10 November 2005; received in revised form 7 December 2005; accepted 8 December 2005

Abstract

Sulfated glycosaminoglycan (GAG) composition was characterized in the human endometrium during proliferative and secretory phases of the menstrual cycle. Sulfated GAGs were analyzed in endometrium tissue using metachromatic staining, biochemical analysis including electrophoresis before and after specific enzymatic or chemical degradations, and immunostaining with an antibody against chondroitin sulfate (CS). Our results showed that CS was the main sulfated GAG species detected, accompanied by small amounts of heparan sulfate and dermatan sulfate. CS was distributed overall the connective stroma, around arteriole vessels and glands, and there was no important difference in the immunostaining between the proliferative and secretory endometrium phases. Our findings extend previous observations on the GAG composition in the human endometrium providing new information regarding the tissue distribution and location of endometrial CS.

Keywords: Chondroitin sulfate; Glycosaminoglycan; Human endometrium

1. Introduction

During the menstrual cycle, under the influence of ovarian hormones estrogen (E) and progesterone (P), deep structural changes occur in the superficial and glandular epithelium, and in the stroma endometrium. Following menstrual shedding, under E stimulation, a proliferative phase turns again the tissue to its functional thickness. After ovulation, P stimulates a secretory phase, in which changes of the cell secretion prepare a favorable environment for blastocyst implantation and subsequent fetal development. Extracellular matrix (ECM) composition and organization are also subjected to cyclic oscillations of plasma E and P levels (Cidadao et al., 1990). In the human endometrium, one of the changes in response to fluctuating steroid hormone levels is the degree of hydration of the endometrium and the appearance of edema in the stroma during the P-dominant phase of the cycle.

Glycosaminoglycan (GAGs) consist of hexosamine [D-glucosamine or D-galactosamine] and either hexuronic acid [D-glucuronic or L-iduronic acid] or galactose units that are arranged in alternating unbranched sequence, and carry sulfate substitutions in various positions. The common GAGs include the galactosaminoglycans, chondroitin sulfate (CS) and dermatan sulfate (DS) and the glucosaminoglycans, heparan sulfate (HS), heparin, keratan sulfate (KS) and hyaluronic acid (HA). DS, HS, and heparin contain both glucuronic acid and iduronic acid units, whereas CS and HA have the glucuronic acid as the only hexuronic acid. In the tissue, GAGs are covalently bound to a protein core forming a structure knowing as proteoglycan (PG). The only exception is the HA, a nonsulfated GAG, which exists as a protein-free polysaccharide on cell surfaces and in the ECM (Iozzo, 1998). The highly negatively charged GAG side chains attract osmotically active cations and an accompaniment of water-endowing PGs with the ability to contribute to tissue hydration and expansion (Laurent and Fraser, 1986). Owing to the variability in sulfate substitution, all GAGs display considerable sequence heterogeneity, and it is believed that structural differences are responsible for highly specific

* Corresponding author. Fax: +55 21 2562 6480.
E-mail address: nasciutt@ufrj.br (L.E. Nasciutti).

0968-4328/$ – see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.micron.2005.12.005

interactions of GAGs with other macromolecules (Powell et al., 2004; Coombe and Kett, 2005).

Investigations of uterine GAGs have focused their histochemical distribution during the menstrual cycle and at times of dramatic structural changes, such as during pregnancy or in association with dilatation of the cervix prior delivery. HA was observed in the human female reproductive tract, distributed in the connective tissue lamina propria (Edelstam et al., 1991). In the human endometrium, dramatic changes in HA deposition in the stromal compartment were demonstrated, which were correlated to the cyclic growth and remodeling (Salamonsen et al., 2001). In addition, two classes of polypeptide-associated KS in glandular and luminal epithelium of human endometrium were identified by immunohistochemistry and correlated to the changes in the environment of the implanting embryo (Hoadley et al., 1990). It was also shown that analysis of secretory KS in women gives an index of hormonally regulated epithelial differentiation in the peri-implantation phase (Graham et al., 1994). These glycans are hormonally regulated in endometrium, and show increased abundance in the secretory phase and may be important in the regulation of embryo attachment (Aplin et al., 1988).

Although different authors have been suggested that GAGs play an important role in wound repair, much less is known about their occurrence during human endometrial cycle changes, and HA and KS are the only GAGs reported to participate in this process. Here, we examined the sulfated GAG composition and tissue distribution in human endometrium in the proliferative/E and secretory/P phases, using biochemical and immunohistochemical analysis, and demonstrated that CS was the predominant sulfated GAG species present.

2. Materials and methods

2.1. Human endometrial tissue

We studied 18 cycling patients, between 35- and 45-year-old, 9 in the proliferative phase (days 9–13), and 9 in the secretory phase (days 19–26) of the cycle. Endometrium was obtained from women undergoing hysterectomy for benign diseases not involving the endometrium, who have not taken hormones for the last 3 months, at the Service of Gynecology of the Clementino Fraga Filho Hospital in the Federal University of Rio de Janeiro, RJ, Brazil, after approval of the Hospital Ethical Committee. After the hysterectomy, the sample was taken with a blade after the uterus had been opened from the lateral wall. A part of the sample was fixed in 4% paraformaldehyde (PFA) for immunohistochemistry, and a part was incubated with acetone for biochemistry procedures. Histopathology study was done to discard abnormal endometrium, and to date the endometrium phase using previously established criteria (Kohorn and Tchao, 1969).

2.2. Material

CS, DS, HS, twice-crystallized papain (15 U/mg protein), and deoxyribonuclease I from bovine pancreas were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Chondroitin AC lyase (EC 4.2.2.5) from *Arthrobacter aurescens*, and chondroitin ABC lyase (EC 4.2.2.4) from *Proteus vulgaris* were purchased from Seikagaku American Inc (Rockville, MD, USA). CS antibody was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.3. Metachromatic staining of sulfated GAGs

Endometrium tissues were fixed overnight in PFA, 4% in the Sorensen phosphate buffer (0.1 M, pH 7.4), at 4 °C. After fixation and washing, the tissues were dehydrated in ethanol and embedded in paraffin. Tissue sections (7 μm) were collected on polilysine-coated slides and stained with the cationic dye 1.9-dimethylmethylene blue (DMB) in 0.1 N HCl, containing 0.04 mM glycine and 0.04 mM NaCl. The sections were then examined and photographed using a light microscope (Zeiss, Axioskop 2). The positive reaction reveals GAGs as metachromatic structures (colored in purple).

2.4. Isolation of endometrial GAGs

Endometrial tissue was incubated with acetone for 24 h at room temperature and dried. The tissues were suspended in the sodium acetate buffer, pH 5.5, containing 40 mg papain in the presence of 5 mM EDTA and 5 mM cysteine, and incubated at 60 °C for 24 h. The incubation mixture was centrifuged at 2000 × *g* for 10 min at room temperature, and the supernatant, which contained the GAGs was retained. A 10% cetylpyridinium chloride solution was added to the supernatant to a final concentration of 0.5% and the mixture left to stand at room temperature for 24 h. The solution was centrifuged at 2000 × *g* for 10 min at room temperature and the pellet was washed with 10 mL 0.05% cetylpyridinium solution. This pellet, a GAG-cetylpyridinium complex, was dissolved with 3.7 mL solution of 2 M NaCl/absolute ethanol (100:15, v/v) and the GAGs were precipitated with the addition of 6 mL absolute ethanol. After 24 h at 4 °C, the precipitate was collected by centrifugation and washed twice with 10 mL 80% ethanol, followed by the same volume of absolute ethanol. The final pellet, which constitutes the total tissue GAG preparation, was dissolved in 2 mL phosphate-buffered saline containing approximately 0.5 mg deoxyribonuclease I and incubated for 12 h at 37 °C. Finally, the incubation mixture was lyophilized and dissolved in 0.2 mL distilled water (Tovar et al., 1998).

2.5. Identification of the endometrial sulfated GAGs

Sulfated GAGs were characterized by agarose gel electrophoresis, digestion with chondroitin lyases and deaminative cleavage with nitrous acid as described below (Rocha et al., 2000).

Agarose gel electrophoresis was carried out as previously described. Approximately, 10 μg of endometrial sulfated GAGs, estimated by a metachromatic staining procedure with the cationic dye 1.9-dimethylmethylene blue (Farndale et al., 1986), before and after chondroitin lyase digestion or deaminative

cleavage with nitrous acid (see below), as well as a mixture of standard CS, DS, HS (10 μg of each) were applied to 0.5% agarose gels in 0.05 M 1,3-diaminopropane: acetate (pH 9.0). After electrophoresis, sulfated GAGs were fixed in the gel with 0.1% *N*-acetyl-*N*,*N*,*N*-trimethylammonium bromide in water, and stained with 0.1% toluidine blue in acetic acid:ethanol:water (0.1:5:5, v/v).

Digestions with chondroitin AC or ABC lyases were carried out according to Saito et al. (1968). Approximately, 10 μg of endometrial sulfated GAGs were incubated with 0.3 units of chondroitin AC lyase or chondroitin ABC lyase for 8 h at 37 °C in 100 μL 50 mM Tris–HCl (pH 8.0) containing 5 mM EDTA and 15 mM sodium acetate.

Deamination by nitrous acid at pH 1.5 was performed as previously described (Shively and Conrad, 1976). Briefly, approximately 10 μg endometrial sulfated GAGs were incubated with 200 μL freshly generated HNO_2 at room temperature for 10 min. The reaction mixture was then neutralized with 1.0 M Na_2CO_3.

2.6. Immunodetection of CS

Endometrium tissues were prepared as described above for histochemistry. The endogenous peroxidase activity was blocked with 3% hydrogen peroxide. The slices were then incubated with goat normal serum and 1% bovine serum albumin (BSA) to reduce nonspecific antibody binding. After washing in 0.01 M phosphate buffer saline (PBS) pH 7.5, the sections were incubated overnight at 4 °C with monoclonal anti-CS antibody (mouse IgM isotype) (Sigma) at 1:200 dilution. The reaction was revealed with StreptAB Complex/HRP Duet Kit (Dako Corporation, Carpinteria, CA, USA) and 3,3′ diaminobenzidine (DAB) tablets. Counterstaining was carried out with Harris's haematoxylin. Negative controls were

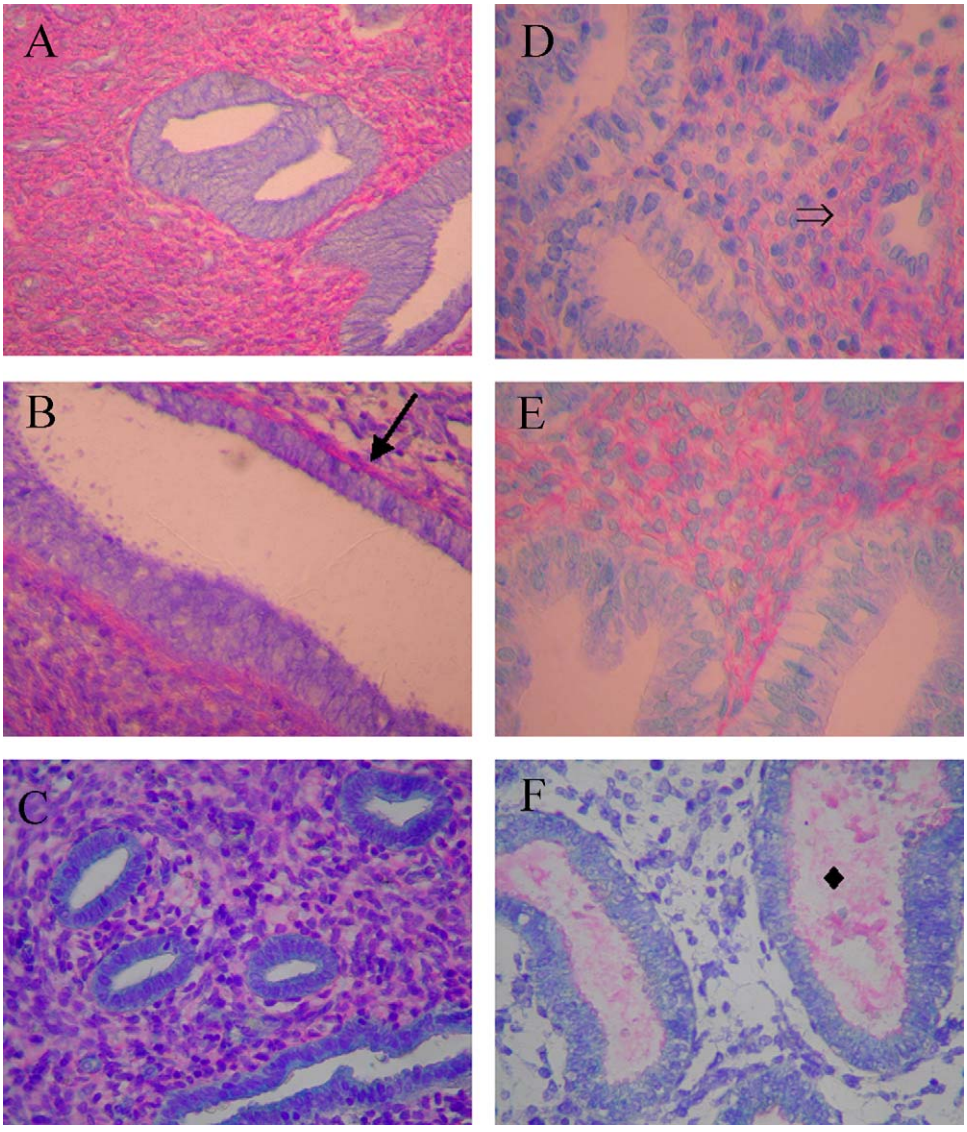

Fig. 1. Fotomicrographies of human endometrium in proliferative (A–C) and secretory (D–F) phases of menstrual cycle, stained with DMB. Observe abundant purple metachromatic material distributed all over the stroma, concentrated around blood vessels (⇒), endometrial glands and in the basement membrane (→). Note that the metachromasia is more diffuse in the secretory phase. In some glands, the staining is present inside the lumen at the secretory product (◆). (A–F) 400×.

obtained omitting the primary antibody (incubating the sections in 0.01 M phosphate buffer saline pH 7.5).

3. Results

Histological findings confirmed the morphological characteristics of normal human endometrium during proliferative and secretory phases (data not shown). In the proliferative phase, the tubular glands were straight and lined by quite regular, tall, columnar cells. Mitotic figures were numerous, associated with epithelial pseudoestratification, and there was no evidence of mucus secretion or vacuolation. The endometrial stroma was composed of thickly compacted spindle cells (fibroblasts) that had a scant cytoplasm but abundant mitotic activity. After ovulation, the secretory endometrium was marked by basal secretory vacuoles beneath the nuclei in the glandular epithelium and the tortuosity of the glands. In some cases, we observed rupture of columnar epithelial cells and release of secretions into the gland lumen. The stroma between the glands became edematous, and the spiral arterioles more prominent. Scattered neutrophils and occasional lymphocytes were also noted.

3.1. Metachromatic staining of endometrial sulfated GAGs

The endometrium stained with cationic dye DMB showed abundant metachromatic (purple) material, distributed all over the stroma. In the proliferative phase (Fig. 1A–C), metachromatic staining was intense and homogeneous between the connective cells in stroma, and more concentrated on the basal layer close to the myometrium. In some sub-epithelial regions of superficial or glandular epithelia, the basal membrane region was evident. Medium staining intensity was found around arterioles, but not around venules. In the secretory endometrium (Fig. 1D–F), the metachromatic distribution was similar. As expected, in this phase the stroma is edematous and the purple material was more diffuse. In some sections, metachromatic reaction was observed in the apical surface of epithelial cells and inside the lumen of the glands.

3.2. Characterization of endometrium sulfated GAGs

The GAGs were characterized upon their migration on agarose gel and digestion with specific lyases or deamination with nitrous acid. The GAG preponderant in the human endometrium, both in proliferative and secretory phases, migrated on agarose gel as the CS standard, and disappeared totally from the gel after chondroitin AC lyase digestion. Two other GAGs, found in minor amounts, migrated as HS and DS standards, which disappeared totally from the gel after deaminative cleavage with nitrous acid and digestion by chondroitin ABC lyase, respectively. These experiments characterized these sulfated GAGs as CS, HS and DS, respectively, and revealed a great dominance of CS in comparison with HS which was the second most present sulfated GAG, and DS the third one (Fig. 2). With these

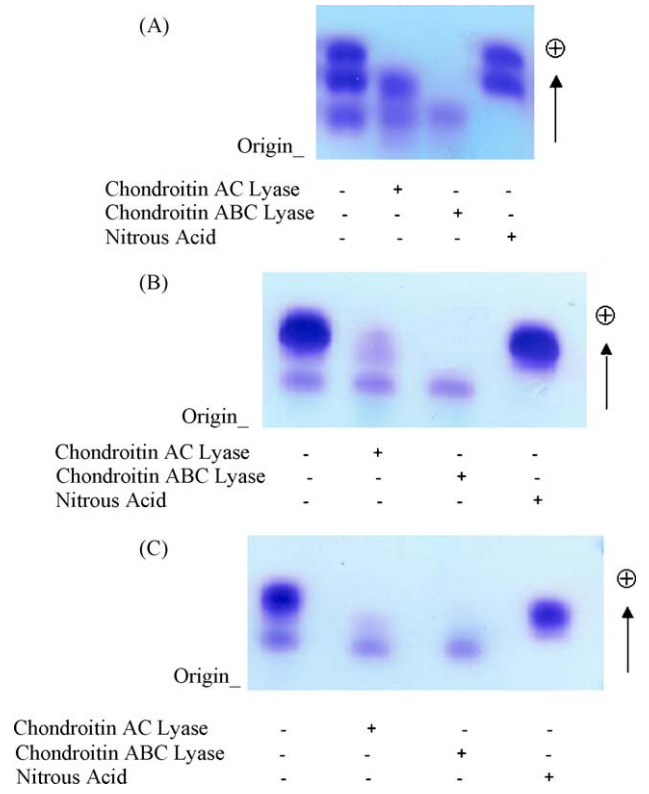

Fig. 2. Representative electrophoretograms of sulfated GAGs from proliferative (B) and secretory (C) human endometrium, before (−) and after enzymatic degradation with chondroitin AC and ABC lyases (+) or deaminative cleavage by nitrous acid (+). The agarose gel electrophoresis was performed as described in Section 2. Sulfated GAGs were detected on gels by staining with toluidine blue. In (A) a mixture of standard GAGs containing heparan sulfate (HS), dermatan sulfate (DS) and chondroitin sulfate (CS) was analyzed by agarose gel electrophoresis as described above. Note that chondroitin AC lyase degrades CS standard, while chondroitin ABC lyase degrades both CS and DS standards. Treatment with nitrous acid specifically degrades HS standard.

procedures, we had no evidence for the presence of KS in the endometrium. However, we cannot rule out the possibility that endometrial KS, detected immunohistochemically by other authors previously, if present in the tissue of our patients, might not either be large enough or present in a concentration insufficient to be precipitated, and was not detected in these studies.

3.3. Tissue distribution of endometrial CS

As revealed by the biochemistry analysis, CS was very abundant in human endometrium. Using a monoclonal antibody against CS GAG chains, we decided to investigate the localization of these molecules in the proliferative and secretory phases of endometrium. CS was observed in the same regions as the DMB metachromatic staining. As expected, the peroxidase deposit was observed in both endometrial phases, distributed overall the connective stroma, concentrated around the arteriole vessels and the glands, and in the epithelial basal membranes. In the secretory phase, the peroxidase product was present concentrated around the edematous regions, and also in the lumen of some glands (Fig. 3).

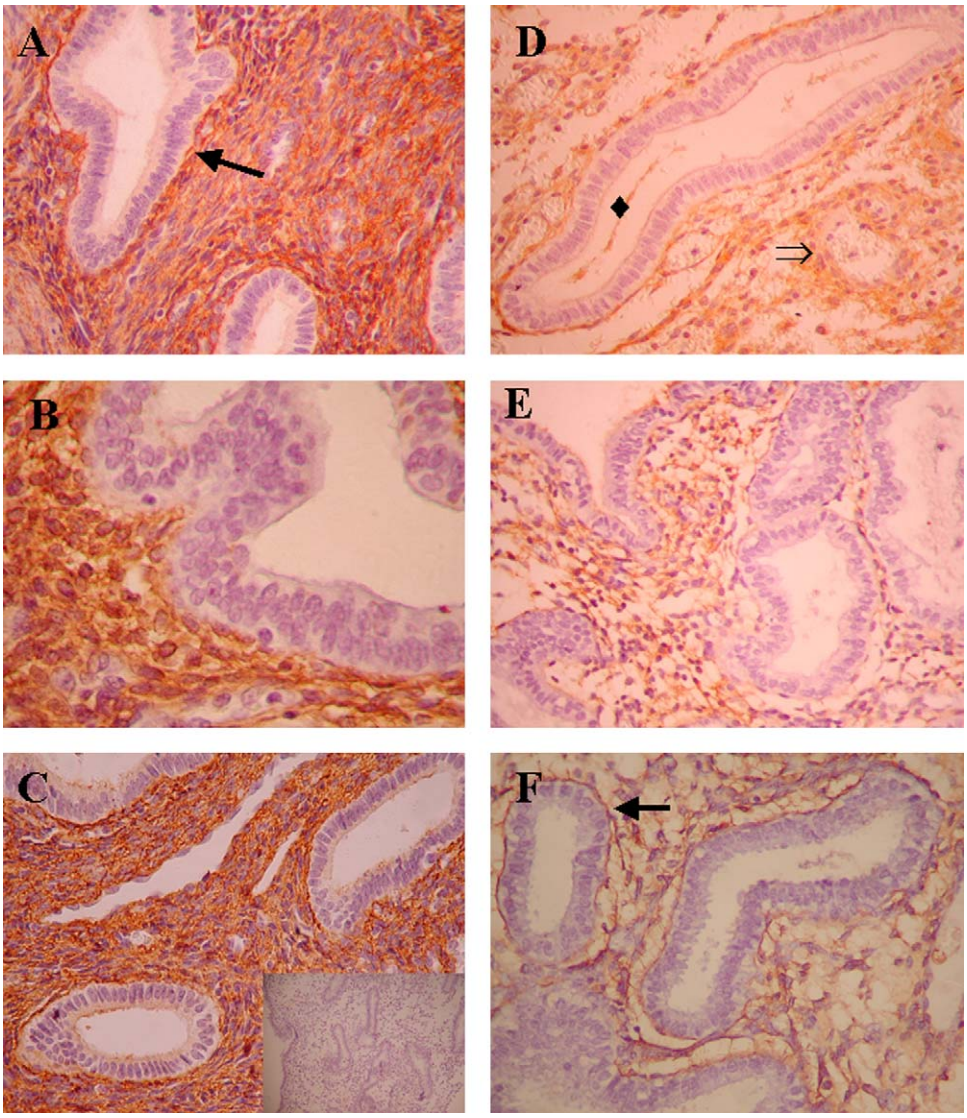

Fig. 3. Fotomicrographies of human endometrium in proliferative (A–C) and secretory (D–F) phases of menstrual cycle, immunostained with an antibody against CS. The peroxidase product is distributed overall the stroma, concentrated around blood vessels (⇒), endometrial glands and in the basal membrane (→). In the lumen of some glands, the secretory product is also immunostained (◆). Observe that the edema regions in the secretory endometrium stroma are delimited by CS deposit. Insert in C: negative control. (A–F) 400×; insert 100×.

4. Discussion

Many studies have demonstrated the importance of ECM components in tissue behavior and organization. In particular, GAGs and PGs have important roles during normal and pathological events, related to organogenesis in embryonic development, cell recognition, cell division, adhesion, migration, regulation of growth factor action, lipid metabolism, wound repair and infection (Lindahl et al., 1998; Sugahara and Kitagawa, 2000; Sugahara et al., 2003).

In humans, the endometrial tissue undergoes cyclic morphological and functional modifications, stimulated by estrogen and progesterone, and involving rearrangement of extracellular matrix components. Unfortunately, studies regarding GAGs distribution during the human endometrial cycle are scarce. An analysis of the HA distribution was performed in biopsy specimens from the female reproductive tract, using a biotinylated hyaluronan binding protein (HABP) as a histochemical probe (Edelstam et al., 1991). In normal endometrium, the staining was confined to the vessels wall and to a weak and patchy distribution in the connective tissue, and no cyclic changes in HA content were observed. On the other hand, histochemical analysis has shown peaks of HA deposition in the stromal compartment during the mid-proliferative (days 5–10) and the mid-secretory phases (days 19–23) of the human endometrium cycle. In contrast, the HA staining around blood vessels was constant throughout the cycle (Salamonsen et al., 2001). Besides HA, KS was the other GAG molecule detected in human endometrium. Using immunohistochemistry, KS was observed associated with glandular epithelium throughout the normal human endometrium cycle, increasing in the secretory phase of the cycle, inside the epithelial cells and secretions, and

probably correlated to the regulation of embryo attachment (Graham et al., 1994; Aplin et al., 1988).

In our study, we showed by biochemical methods that CS was the major sulfated GAG species, followed by HS and DS, present in proliferative and secretory phases of human endometrial cycle. KS might be present in trace amounts, which escaped from our biochemical methods and could be only detected by immunohistochemical methods such as those performed by other authors (Graham et al., 1994; Aplin et al., 1988). These observations confirmed the occurrence of an abundant sulfated material distributed all over endometrial phases, as suggested by DMB metachromatic staining. In the ovine endometrium, it was shown significant differences between the GAG content during estrogen- and progesterone-dominant phases of the estrous cycle, with estrogen-dominant tissue containing significantly more total GAG. Characterization of endogenous GAGs showed the presence of CS, HA, HS and KS, with CS and HA contributing over 80% of total GAG (Tellbach et al., 2003).

When compared to HA distribution observed by other authors (Salamonsen et al., 2001), the tissue localization of CS was similar: it was present overall the stroma and concentrated around blood vessels and glands. In addition, we also observed CS deposition in the epithelial basal membrane regions, suggesting its participation in basal membrane composition and function.

In the secretory phase, which is concomitant to the implantation of the conceptus in a fertile cycle, CS was present at the epithelial surface and inside the lumen of glands. This localization was very similar to that observed for KS in endometrium (Graham et al., 1994; Aplin et al., 1988). Interestingly, we also observed a CS concentration around the edematous regions, suggesting its participation in the physical control of these formations and in the degree of hydration of the endometrium. The presence of CS in the gland secretions raises questions regarding endometrial glandular maturation in the normal cycle, the influence of progesterone in the control of this localization, and the CS participation in embryo attachment. The important distribution of CS in the whole endometrium tissue could be also related to the growth factors storage and the modulation of the cyclic angiogenesis processes. Interestingly, very recently Yamaguchi et al. (2006) reported a homogeneous immunoreactivity for chondroitin sulfate proteoglycan 2 (CSPG2) distributed all over epithelium, stroma and endothelium of proliferative and secretory human endometrium. CSPG2 is a PG that binds to selectin L using its specific GAG chains (Kawashima et al., 2000). The immunoreactivity for CSPG2 was membranous, cytoplasmatic, and homogeneous in the epithelium. The immunostaining intensity in the stroma was equivalent to that in the epithelium which elevated from the proliferative phase to the secretory phase (Yamaguchi et al., 2006). The authors concluded that their findings support the idea that the interaction between selectin L and selectin L ligands, as the endometrial CSPG2, functions in the postovulatory selective recruitment of CD16(−) natural killer cells from peripheral blood into the endometrium.

In conclusion, our results with the histochemical, biochemical and immunohistochemical procedures showed a predominance of CS as the main sulfated GAG present in human endometrium. The present study extends previous observations on the GAG composition in human endometrium providing new information regarding the tissue distribution and location of endometrial CS. These observations may be useful for future investigations aiming to evaluate the possible implication of these GAGs in normal and pathologic human endometrial cycle.

Acknowledgements

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq: PADCT and PRONEX), Fundação Universitária José Bonifácio (FUJB) and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

References

Aplin, J.D., Charlton, A.K., Ayad, S., 1988. An immunohistochemical study of human endometrial extracellular matrix during the menstrual cycle and first trimester of pregnancy. Cell Tissue Res. 253, 231–240.

Cidadao, A.J., Thorsteinsdóttir, S., David-Ferreira, J.F., 1990. Immunocytochemical study of tissue distribution and hormonal control of chondroitin-, dermatan, and keratan sulfates from rodent uterus. Eur. J. Cell Biol. 52, 105–116.

Coombe, D.R., Kett, W.C., 2005. Heparan sulfate–protein interactions: therapeutic potential through structure-function insights. Cell. Mol. Life Sci. 62, 410–424.

Edelstam, G.A., Lundkvist, E.O., Wells, F.A., Laurent, C.T., 1991. Localization of Hyaluronan in regions of the human female reproductive tract. J. Histochem. Cytochem. 39, 1131–1135.

Farndale, R.W., Buttle, D.J., Barret, A.J., 1986. Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. Biochim. Biophys. Acta 883, 173–177.

Graham, R.A., Li, T.C., Cooke, I.D., Aplin, J.D., 1994. Keratan sulphate as a secretory product of human endometrium: cyclic expression in normal women. Hum. Reprod. 9, 926–930.

Hoadley, M.E., Seif, M.W., Aplin, J.D., 1990. Menstrual-cycle-dependent expression of keratan sulphate in human endometrium. Biochem. J. 266, 757–763.

Iozzo, R.V., 1998. Matrix proteoglycans: from molecular design to cellular function. Annu. Rev. Biochem. 67, 609–652.

Kawashima, H., Hirose, M., Hirose, J., Nagakubo, D., Plaas, A.H.K., Miyasaka, M., 2000. Binding of a large chondroitin sulphate/dermatan sulphate, versican, to delectin L, P-selectin and CD44. J. Biol. Chem. 275, 35448–35456.

Kohorn, E., Tchao, R., 1969. Conversion of proliferative endometrium to secretory endometrium by progesterone in organ culture. J. Endocrinol. 45, 401–405.

Laurent, T.C., Fraser, J.R., 1986. The properties and turnover of hyaluronan. Ciba Found. Symp. 124, 9–29.

Lindahl, U., Kusche-Gulberg, M., Kjellen, L., 1998. Regulated diversity of heparan sulfate. J. Biol. Chem. 273, 24979–24982.

Powell, A.K., Yates, E.A., Fernig, D.G., Turnbull, J.E., 2004. Interactions of heparin/heparin sulfate with proteins: appraisal of structural factors and experimental approaches. Glycobiology 14, 17R–30R.

Rocha, L.A.G., Martins, R.C.L., Werneck, C.C., Feres-Filho, E.J., Silva, L.C.F., 2000. Human gingival glycosaminoglycans in cyclosporin-induced overgrowth. J. Periodontal Res. 235, 158–164.

Saito, N., Yamagata, T., Suzuki, S., 1968. Enzymatic methods for the determination of small quantities of isomeric chondroitin sulfates. J. Biol. Chem. 243, 1536–1544.

Salamonsen, L.A., Shuster, S., Stern, R., 2001. Distribution of hyaluran in human endometrium across the menstrual cycle. Implications for implantation and menstruation. Cell Tissue Res. 306, 335–340.

Shively, J.E., Conrad, H.E., 1976. Formation of anhydrosugars in the chemical depolymerization of heparin. Biochemistry 15, 3932–3942.

Sugahara, K., Kitagawa, H., 2000. Recent advances in the study of the biosynthesis and functions of sulfated glycosaminoglycans. Curr. Opin. Struct. Biol. 10, 518–527.

Sugahara, K., Mikami, T., Uyama, T., Mizuguchi, S., Nomura, K., Kitagawa, H., 2003. Recent advances in the structural biology of chondroitin sulfate and dermatan sulfate. Curr. Opin. Struct. Biol. 13, 612–620.

Tellbach, M., Salamonsen, L.A., Van Damme, M.P., 2003. The influence of ovarian steroids on ovine endometrial glycosaminoglycans. Glycoconjug. J. 19, 385–394.

Tovar, A.M.F., Cesar, D.C.F., Leta, G.C., Mourão, P.A.S., 1998. Age-related changes in populations of aortic glycosaminoglycans: species with low affinity for plasma low-density lipoproteins, and not species with high affinity, are preferentially affected. Arterioscler. Thomb. Vasc. Biol. 18, 604–614.

Yamaguchi, T., Kitaya, K., Daikoku, N., Yasuo, T., Fushiki, S., Honjo, H., 2006. Potential selectin L ligands involved in selective recruitment of peripheral blood CD16(−) natural killer cells into human endometrium. Biol. Reprod. 74, 35–40.