

Lectin binding in the human endometrium in early luteal phase following controlled ovarian hyperstimulation

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Summary. A lectin histochemical study was performed to investigate the glycoconjugate saccharidic moieties on the endometrial epithelium and stroma in 12 women undergoing controlled ovarian hyperstimulation (COH) for in-vitro fertilisation for embryo transfer (IVF-ET) in early luteal phase. 7 control subjects were also evaluated. For this purpose a battery of seven horse-radish peroxidase-conjugated lectins (PNA, SBA, DBA, WGA, ConA, LTA and UEA I) was used. Cytochemical controls were performed for specificity of lectin-sugar reaction. As far as the endometrial glands and stroma are concerned, the obtained data showed no differences in the endometrial lectin binding between the subjects of the control group and the ones undergoing COH, with the exception of PNA reactivity at the level of the apical portion of the glandular cells, which was detected only in COH women. It is noteworthy that, although the endometrial dating using the Noyes's criteria showed marked dissynchronies between the stroma and the glands in COH subjects, a uniformity of lectin binding, revealing the same type and localization of terminal oligosaccharides, was observed in all the examined subjects. The uniformity in distribution of the sugar residues detected in the endometrial specimens following COH might be due to the massive FSH and/or hCG treatment which probably determines an endometrial environment almost equal in all the examined subjects. In all the treated subjects reactivity with sialidase-WGA and ConA, revealing the presence of N-acetyl-D-glucosamine and D-mannose respectively, was detected at the level of the lining epithelium.

Key words: Controlled ovarian hyperstimulation, Human endometrium, Lectins, Sugar residues

Introduction

Controlled ovarian hyperstimulation (COH) is frequently associated with morphological alterations of the endometrium (Frydman et al., 1982; Cook et al.,

1984; Graf et al., 1988; Sterzik et al., 1988; Benn-Nun et al., 1992; Seif et al., 1992; Benadiva and Metzger, 1994).

As far as the endometrial glycoconjugates are concerned, many investigations have been carried out on the glycoconjugate sugar residue content and distribution in endometria from fertile and infertile women, both in proliferative and secretive phases (Bychkov and Toto, 1986; Söderström, 1987; Aoki et al., 1989; West and Cope, 1989; Klentzeris et al., 1991; Horvat, 1993). Moreover, several reports on glycoconjugate terminal oligosaccharides in uteri of rodents showed variations following hyperstimulation with exogenous gonadotrophins (Kramer et al., 1990; Peverini and Kramer, 1995; Kramer and de Wet, 1994). To our knowledge no data are available on the oligosaccharidic distribution in human endometria during hyperstimulated cycles.

The aim of the present research was to investigate the oligosaccharidic content and distribution on endometrial epithelium and stroma in early luteal phase in women undergoing COH.

Materials and methods

Twelve women aged under 35 and affected by male factor infertility undergoing IVF at the Reproductive Unit of the Department of Gynecology and Obstetrics, Florence University, participated in this study. In these women oocytes were collected, but due to failure of fertilization embryo transfer (ET) was not made. COH was performed by individually adjusted hFSH (METRODIN 75 IU/ampoule, Serono, Rome, Italy) daily dose from 2 to 4 ampoules. To prevent an untimed luteinizing hormone (LH) release patients had been pretreated with busarelin acetate (Suprefact, Hoescht Roussel, Milan, Italy) 600 mcg/day s.c., which started to be given during the midluteal phase of the previous cycle and continued until the day of human chorionic gonadotrophin (hCG) administration. In these patients fertilization ended in failure, therefore ET was not performed. On day 16 an endometrial biopsy was carried out, after a written consent had been obtained. On the same day of the menstrual cycle, endometrial biopsies were taken from 7 control patients, after a written

consent. Endometrial specimens were taken from the anterior wall of the fundus uteri with a Novak curette, fixed in buffered formalin and paraffin embedded. Endometrial biopsies were dated by the Senior Pathologist using the standard Noyes's criteria (Noyes et al., 1950); glands and stroma were separately evaluated.

Lectin histochemistry

5- μ m thick paraffin sections were obtained for lectin histochemistry. After hydration, sections were treated with 0.3% hydrogen peroxide for 10 min (to inhibit the endogenous peroxidase), rinsed in distilled water and washed with 1% bovine serum albumin (BSA) (Murata et al., 1983) in 0.1M phosphate-buffered saline (PBS) pH 7.2. The sections were then incubated for 30 min at room temperature in horseradish peroxidase-conjugated lectins (HRP-conjugated lectin) dissolved in phosphate-buffered saline (0.1M PBS pH 7.2, 0.1M each of NaCl, 0.1 mM CaCl₂, MgCl₂ and MnCl₂) and then rinsed three times in PBS. The optimal concentration for each lectin (Sigma Chemical Co., St. Louis, MO) which allowed maximum staining with minimum background was as follows: PNA (*Arachis hypogaea*, binding specificity D-Gal (β 1 \rightarrow 3)-D-GalNAc) 25 μ g/ml; SBA (*Glycine max* binding specificity α / β -D-GalNAc) 20 μ g/ml; DBA (*Dolichos biflorus*, binding specificity α -D-GalNAc) 25 μ g/ml; WGA (*Triticum vulgare* binding specificity (α -D-GlcNAc)_n and sialic acid) 20 μ g/ml; ConA (*Canavalia ensiformis* binding specificity α -D-Man) 50 μ g/ml; LTA (*Lotus tetragonolobus* binding specificity α -L-fucose) 25 μ g/ml; and UEA I (*Ulex europaeus* binding specificity α -L-fucose) 25 μ g/ml. Staining of the sites containing bound lectin-HRP was obtained by incubating the slides with PBS (pH 7.0), containing 3,3'-diaminobenzidine (DAB) (25 mg/100 ml) and 0.003% hydrogen peroxide, for 10 min at room temperature. Specimens were rinsed in distilled water; dehydrated using graded ethanol solutions, cleared in xylene and mounted in Permount. Controls for lectin staining included: 1) substitution of unconjugated lectins for lectin-HRP conjugates; 2) exposure to HRP and substrate medium without lectin; 3) oxidation with 1% periodic acid for 10 min prior to lectin staining; and 4) exposure of sections to 10-20 μ g/ml of each lectin-HRP conjugate containing 0.1 M D-galactose, D-glucose, D-mannose, L-fucose, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine and methyl- α -mannopyranoside.

Sialidase digestion

In some experiments sialic acid was removed by pre-treating the sections for 18 hr at 37 °C in a solution of sodium acetate buffer 0.25M, pH 5.5, containing 0.1 unit/ml sialidase (neuraminidase Type X from *Clostridium perfringens* (Sigma Chemical. Co., St. Louis, MO)), 5.0 mM CaCl₂ and 154 mM NaCl, prior to staining with lectin-HRP conjugates. Controls containing

the sialidase buffer without the enzyme were also prepared.

Results

Endometrial dating is shown in Table 1.

Lectin histochemistry (Table 2)

If not otherwise specified the following results are common to all the examined specimens (hyperstimulated and control endometria).

PNA-HRP

In women undergoing COH, apical reactivity was seen in some glandular cells (Fig. 1). Reactivity at this site was never observed in the women of the control group. At the lining epithelium the cilia and the basal corpuscle of the cilia of the ciliated cells showed a strong reactivity (Fig. 2).

Following sialidase digestion no increase in PNA binding was observed.

SBA-HRP

Strong SBA reactivity was observed at the basal corpuscle of the cilia of the ciliated cells (Fig. 3). In 2 cases of patients undergoing COH the cilia of very few ciliated cells weakly reacted with SBA; in 3 cases, weak reactivity was detected at the Golgi region of the glandular cells

Following sialidase digestion no increase in SBA binding was observed.

DBA-HRP

In all the examined specimens DBA gave negative results.

Following sialidase digestion no DBA binding was observed.

Table 1. Endometrial dating for glands and stroma in COH women.

No.	NAME	AGE	16th - ENDOMETRIAL DATING	
			Glands	Stroma
1	D.A.	32	17	17
2	S.A.	35	20	22
3	P.D.	35	23	26
4	C.S.	27	20	24
5	G.E.	30	18	25
6	B.L.	32	21	22
7	B.S.	34	17	22
8	D.B.C.	33	20	25
9	F.R.	34	22	25
10	V.E.	35	17	23
11	M.G.	35	17	22
12	B.G.	29	16	16

Lectin binding in the human endometrium following COH

WGA-HRP

In women undergoing COH, the apical portion of the cells of the lining epithelium strongly reacted. Reactivity at this site was never observed in the women of the control group. In the cells of the glandular epithelium, the apical portion and the Golgi region showed strong reactivity. Within the glandular lumen reactive material was constantly observed. The surface of the stromal cells showed WGA reactivity (Fig. 4). This reactivity was focal in the control group.

Following sialidase digestion no decrease in WGA binding was observed.

ConA-HRP

In women undergoing COH, the cells of the lining epithelium showed strong reactivity at the level of cytoplasmic granules. Reactivity at this site was never

observed in the women of the control group. The cells of the glandular epithelium showed reactive cytoplasmic material. Within the glandular lumen reactive material was observed (Fig. 5). Reactivity at this site was never observed in the women of the control group. The stromal cells reacted with ConA. This reactivity was focal in the control group.

UEAI-HRP

Moderate reactivity was detected in the endothelial cells. In 3 cases of COH women, the cilia of some ciliated cells of the lining epithelium reacted with this lectin; in 2 cases the apical portion of some glandular cells showed weak reactivity (Fig. 6).

LTA-HRP

In all the examined specimens LTA gave negative

Table 2. Lectin reactivity at the endometrium (16th day) in COH for IVF-ET women and in the control group.

	SURFACE EPITHELIUM		GLANDULAR EPITHELIUM		GLANDULAR SECRETION		STROMAL CELLS		ENDOTHELIAL CELLS	
	COH	Control	COH	Control	COH	Control	COH	Control	COH	Control
PNA	3cl3bc 12/12	3cl3bc 7/7	1a 12/12	-	-	-	-	-	-	-
SBA	3bc(1cl) 12/12	3bc 7/7	1Go 3/12	-	-	-	-	-	-	-
WGA	3a2Go 12/12	-	3a3Go 12/12	3a3Go 7/7	2 12/12	2 7/7	2s 12/12	2s* 7/7	-	-
ConA	3gr 12/12	-	2gr 12/12	2gr 7/7	2 12/12	-	2s 12/12	2s* 7/7	-	-
UEAI	1cl 3/12	-	2a 2/12	-	-	-	-	-	2 12/12	2 7/7
LTA	-	-	-	-	-	-	-	-	-	-
DBA	-	-	-	-	-	-	-	-	-	-

(): reaction observed in few ciliated cells (only in 2 specimens); cl: cilia; a: apical portion of the cell; cb: basal corpuscle of the cilia; Go: Golgi region; gr: cytoplasmic granules; *: focal positive cells only. Numer indicates the intensity of the reaction: -, negative; 1, weak reaction; 2, moderate reaction; 3, strong reaction.



Fig. 1. PNA-HRP. B.S., 34 y.o. Reactivity is observable at the apical surface of the superficial and glandular cells. x 90



Fig. 2. PNA-HRP. B.S., 34 y.o. A higher magnification shows the reactivity at the basal corpuscle and at the cilia of the ciliated cells more clearly. x 900

results.

Cytochemical controls

When the sections were stained with lectins in the presence of the haptenic sugars pertinent to each lectin, the positive reactions described above completely disappeared or were strongly reduced. No staining was evident in the sections which had been exposed to only substrate medium or to the unconjugated lectins. Pretreatment of sections with periodic acid abolished the affinity of the histological sites to the lectins.

Discussion

Our data on lectin binding at the endometrial lining and glandular epithelium and stroma in women undergoing COH for IVF-ET in the early luteal phase,

showed, with very few exceptions, the same type and localization of terminal oligosaccharides in all subjects. The same behaviour was previously observed in postmenopausal atrophic and hyperplastic endometria where no significant differences in lectin binding were detected (Gheri et al., 1996).

We were surprised by the uniformity of the type and distribution of the detected sugar residues in endometria of women undergoing COH for IVF-ET for two reasons: 1) in these women marked differences of hormonal levels have been registered (Noci et al., unpublished data), but these hormonal differences did not correspond to variations in lectin binding at the various endometrial components. With regard to this other authors (Bychkov and Toto, 1986; Söderström, 1987; Aoki et al., 1989; West and Cope, 1989) claimed that differences in sera values of various hormones during the proliferative and secretive phases of the menstrual cycle correspond to

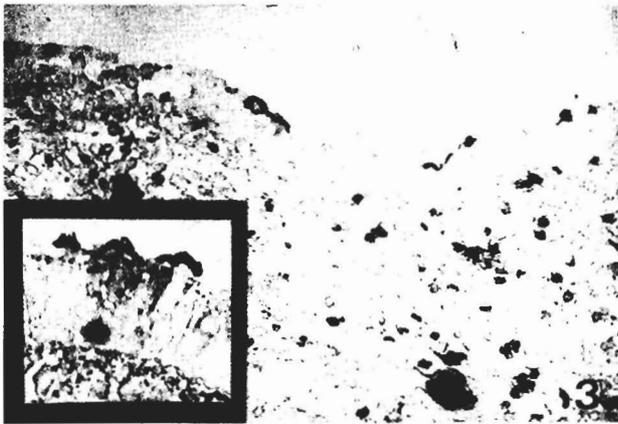


Fig. 3. SBA-HRP. P.D., 35 y.o. At the lining epithelium, the basal corpuscle of some ciliated cells reacts with this lectin. Within the stroma hemorrhagic suffusion is observable. x 40. The insert shows a particular of the lining epithelium. x 900



Fig. 4. WGA-HRP. M.C., 35 y.o. The apical portion of lining cells, the glandular epithelium and the secretion within the glandular lumina are strongly reactive. Reactivity is also observable at the stromal cells. x 180

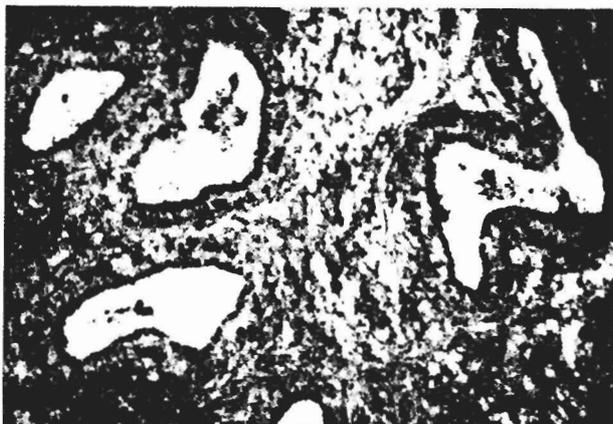


Fig. 5. ConA-HRP. G.E., 30 y.o. Reactivity is seen at the apical and basal portion of glandular cells and at the secretion within glandular lumina. x 180

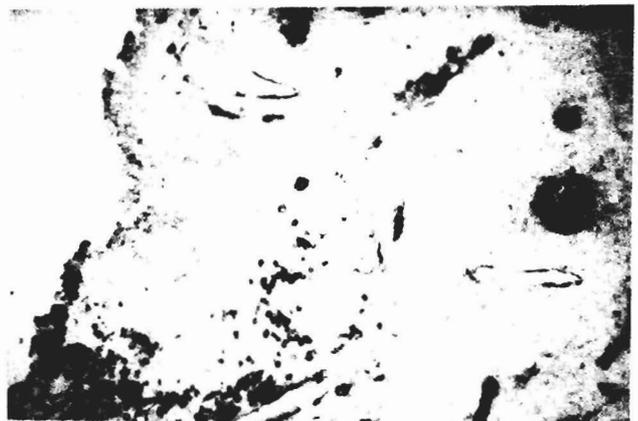


Fig. 6. UEAI-HRP. M. C., 35 y.o. In this subject moderate reactivity is observable at the apical surface of glandular cells. The endothelial cells show reactivity. x 90

differences in lectin reactivity.

2) the datation of the endometrial biopsies in COH women using the standard Noyes's criteria (Table 1) has shown differences between the glands and the stroma. Sometimes the dissynchrony between the two endometrial components was remarkable. In general more advanced stromal maturity was detected. Furthermore it is to be noted that COH women, who show, from a morphological point of view (see cases 1 and 12), a normal endometrial environment, revealed a lectin binding pattern not at all similar to those women in which the Noyes criteria showed a more advanced endometrial environment, characterised by a marked dissynchrony between glands and stroma.

The uniformity in distribution of the sugar residues detected in the endometrial specimens following COH might be due to the massive FSH and/or hCG treatment which probably determines an endometrial environment almost equal in all the examined subjects.

Comparing the results obtained in COH women with those of the control group some differences in lectin binding can be noted:

- 1) N-acetyl-D-glucosamine and α -D-mannose are present at the surface epithelium in COH women and are absent in the women of the control group
- 2) D-galactose-(β 1 \rightarrow 3)-N-acetyl-D-galactosamine was detected at the apical surface of the glandular epithelium of the COH women and was absent in those of the control group
- 3) Glandular secretion rich in D-mannosyl residues was detected only in COH women

Although massive treatment with hCG is performed with the purpose of inducing ovulation, this treatment determines some important modifications at the level of uterine components. In fact, it is well known that hCG and LH are structurally and functionally homologous gonadotropins (Pierce and Parsons, 1981; Lin et al., 1994) and they bind to the same transmembrane glycoprotein receptors, which are present at the level of the uterine components. The receptors are more abundant at the glandular and luminal epithelium than those present at the stromal and vascular cells (Reshef et al., 1990). Furthermore the receptors for hCG and LH are more numerous in the secretive rather than in the proliferative phase (Reshef et al., 1990).

Taking into account these data, the above mentioned differences between COH women and those of the control group in lectin binding at the epithelial uterine components could be explained.

The role played by massive hCG treatment in determining the appearance of WGA and ConA reactivity at the surface epithelium and the appearance of PNA reactivity at the glandular epithelial cells is very difficult to explain. A role played by hCG treatment in activating epithelial cell functions via cAMP mediation or in increasing the local synthesis of hormonal and nonhormonal proteins (Reshef et al., 1990) is known. The hCG treatment could influence the synthesis and/or activation of specific glycosyltransferases which could

add new terminal sugar residues to the glandular and surface epithelial glycoconjugates.

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