Menstrual-cycle-dependent expression of keratan sulphate in human endometrium

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Immunochemical methods have been used to detect and characterize two classes of polypeptide-associated keratan sulphate (KS) in epithelial secretions from human endometrium. Monoclonal antibody D9B1 binds to a hormonally regulated sialylated epitope associated with KS in a high relative molecular mass (250 000–350 000) component that bands as a doublet in SDS/PAGE. These KS chain(s) are sensitive to keratanase, endo- β -galactosidase and *N*-glycanase. A second, more highly sulphated, type of KS is also present, that is resistant to all three enzymes. This can be detected using monoclonal antibody 5D4. It is present throughout the menstrual cycle and is associated principally with a component of M_r 140 000. Thus secretory KS contributes to the environment of the implanting embryo, may be used as a molecular index of endometrial function and could be important in the establishment of pregnancy.

INTRODUCTION

During the menstrual cycle, the uterine lining (endometrium) undergoes profound structural changes under the influence of ovarian hormones [1,2]. Following menstrual shedding, a proliferative phase returns the tissue to its functional thickness. After ovulation, a secretory phase ensues, in which differentiative changes prepare the endometrium to accept an implanting embryo. The secretory material released by endometrial glandular epithelial cells may play a role in creating a suitable milieu for the blastocyst [2,3]. Recent data also suggest that endometrial secretions may exert paracrine influences on the post-implantation embryo [2–6]. Thus it is important to characterize these secretions and to examine their hormonal dependence and their timing in relation to implantation.

We have reported previously a monoclonal antibody (mAb), D9B1, that binds to a menstrual-cycle-dependent epitope which is largely absent in the proliferative phase, peaks after ovulation and declines slowly during the late secretory phase [7–9]. We now report that D9B1 recognizes a sialo-oligosaccharide epitope of the sulphated lactosaminoglycan family and we investigate its relationship to other oligosaccharides of the same family [9] present in the tissue.

MATERIALS AND METHODS

Monoclonal antibodies

D9B1 was raised by immunizing mice with collagenase-dissociated glandular fragments from secretory phase endometrium [10]. 5D4 was raised against proteoglycan core protein isolated after chondroitinase ABC digestion of human articular cartilage proteoglycan monomer [11] and was obtained as a gift from Dr. B. Caterson, University of West Virginia, U.S.A., or from ICN Biomedical.

Tissue specimens

Endometrial biopsies were collected at routine dilation and curettage. In each case, one part of the biopsy was fixed in Bouin's solution for routine histopathological examination. Tissue arrived at the laboratory usually within 45 min of the biopsy being performed. It was either extracted as described below or snap-frozen in liquid N_2 for later extraction. After routine histopathological assessment had been performed independently, the results became available (approx. 1 week later) for correlation with the biochemical findings.

Western blotting

Endometrium was extracted with phosphate-buffered saline (PBS, 10 mM-sodium phosphate/1.8 mM-potassium phosphate and 0.15 M-NaCl, pH 7.4) by mincing approx. 100 mg of tissue in 1 ml of PBS at room temperature before incubating for 1 h at 4 °C. The sample was then mixed thoroughly on a rotary mixer before centrifuging at 12 000 *g* in a microfuge for 5 min. The supernatant was dialysed at 4 °C overnight against distilled water before freeze-drying and resuspending in 100 μ l of distilled water. The SDS extract (see Fig. 1, tracks b and d) was prepared by boiling approx. 100 mg of tissue in 0.05 M-Tris containing 2 % SDS. The samples, diluted 1:1 in non-reducing sample buffer [0.15 M-Tris/HCl, pH 6.7/2 % (w/v) SDS/10 % (v/v) glycerol/0.1 % Bromophenol Blue] were run on SDS/PAGE (5–15 % acrylamide gradient). The relative molecular masses of components were evaluated by using pre-stained M_r standards (range 26 000–180 000) (Sigma). The bands were transferred electrophoretically to a nitrocellulose filter (Schleicher and Schuell). This was blocked [3 % defatted milk powder in 50 mM-Tris/HCl (pH 7.4)/0.15 M-NaCl containing 0.05 % (v/v) Tween 20] to inhibit non-specific binding before placing in mAb D9B1 (1:10, v/v) or mAb 5D4 (1:40, v/v) overnight. The filter was then washed and placed in 1:1000 (v/v)

Abbreviations used: KS, keratan sulphate; PBS, phosphate-buffered saline; mAb, monoclonal antibody.

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biotin-conjugated goat anti-mouse antibody (Sigma) for 2 h at room temperature. After washing, the filter was placed in 1:1000 extra-avidin peroxidase (Sigma) for 2 h. It was washed again before the addition of substrate as for tissue sections. Controls were run in which the first antibody was omitted, and no staining was observed.

Enzyme treatments of blots were carried out for 5 h at 37 °C before placing in antibody. The concentrations in appropriate buffers (see below) were as follows: 10 or 50 units of sialidase/ml, 2.5 munits of endo- β -galactosidase/ml, 2 units of keratanase/ml, 2 munits of *N*-glycanase/ml.

Solid-phase binding assay

E.l.i.s.a. plates (Dynatech) were coated with 5 μ g of poly(L-lysine)/ml in 0.1 M-bicarbonate buffer, pH 8.5, for 1 h at room temperature. The plates were then coated with 1–20 μ g of corneal keratan sulphate (KS) or bovine femoral head proteoglycan/ml in PBS. After blocking with 0.1% BSA in PBS, the first antibody was added (D9B1, 1:100 or 5D4, 1:800). The second antibody was peroxidase-conjugated rabbit anti-mouse (1:500) (Dako) and the substrate was 2',2'-amino-di-3-ethylbenzthiazolin sulphonate (ABTS) plus H_2O_2 . Absorbances were measured at 405 nm using an automated plate reader. Enzyme treatments were carried out for 1 h at 37 °C before the blocking step by using the following concentrations in appropriate buffers (see below): sialidase, 10 units/ml, keratanase 0.1 unit/ml, endo- β -galactosidase, 1.25 munits/ml.

Immunohistochemistry

Indirect immunoperoxidase staining of 5 μ m sections of paraffin-wax-embedded endometrial tissue was carried out as described previously [7]. Briefly, sections were dewaxed and rehydrated before application of the first antibody [D9B1, neat culture supernatant or 5D4 (1:80)]. This was followed by addition of peroxidase-conjugated rabbit anti-mouse immunoglobulin (Dako) and then of the substrate, 3,3'-diaminobenzidine tetrahydrochloride in the presence of H_2O_2 . Negative controls (no first antibody) and positive controls were included in each experiment. A semi-quantitative method of assessment of staining was used essentially as described previously [7].

Enzyme treatment

Unless previously indicated, all enzyme treatments were carried out for 1 h at 37 °C before the application of the first antibody. The conditions were as follows: keratanase (keratan-sulphate endo-1,4- β -galactosidase; *Pseudomonas*; EC 3.2.1.103; Sigma), 10 units/ml in 0.05 M-Tris/HCl (pH 8.5)/0.15 M-NaCl; endo- β -galactosidase (*Bacteroides fragilis*; EC 3.2.1.103; provided by Dr. P. Scudder [12]), 12.5 munits/ml in 0.05 M-sodium acetate (pH 5.8) with 25 μ g of BSA/ml; sialidase (*Vibrio cholerae*; EC 3.2.1.18; Boehringer), 10 units/ml in 0.01 M-sodium acetate (pH 5.5)/1 mM- $CaCl_2$; hyaluronidase (hyaluronoglucuronidase; ovine testicular; EC 3.2.1.35; Sigma), 30 units/ml in 0.1 M-sodium phosphate (pH 5.3)/0.15 M-NaCl; *N*-glycanase (*Flavobacterium meningosepticum*; EC 3.5.1.52; Koch-Light Ltd.), 5 munits/ml in 0.2 M-sodium phosphate (pH 8.0), overnight at 37 °C. Sodium metaperiodate, 1 mM in PBS, was used at 20 °C for 30 min.

Inhibitors of antibody binding

Antibodies were incubated with various agents before applying to tissue sections in order to observe possible inhibitory effects on binding. All blocking agents were mixed at a ratio of 1:1 with the first antibody. The mixture was incubated for 1 h at room temperature before applying to sections. The following final concentrations were used: corneal KS (Sigma) 250 μ g/ml; chondroitin sulphates ABC (Sigma), 1 mg/ml; glycogen (Sigma), 10% (w/v); bovine femoral head proteoglycan (kindly provided by Dr. J. Sheehan, University of Manchester, U.K.), 100 μ g/ml.

RESULTS

D9B1 is an IgM that binds to a polypeptide-associated glycan in endometrial epithelium. The menstrual cycle dependency of this glycoconjugate has been clearly demonstrated and correlated with the luteinizing-hormone (LH) peak [7–9]. It is largely absent in the proliferative phase, peaks after ovulation and declines during the late secretory phase. MAb 5D4 is an IgG that binds to both corneal and cartilage KS [11,12].

The macromolecules recognized by mAbs D9B1 and 5D4 were characterized by immunoblotting of endometrial extracts and solid-phase assay of binding to KS species from cornea and cartilage. These assays were combined with selective enzymic degradation and inhibition of binding by related glycans.

Immunoblotting of endometrial tissue extracts

On blots made from longer gels (overnight electrophoresis), mAb D9B1 stained two major bands at high M_r (250 000–350 000) in secretory-phase endometrium

Fig. 1. SDS/PAGE and immunoblotting with antibody D9B1 of extracts of secretory-phase endometrial tissue

Coomassie Blue-stained gel (lanes a–c) and immunoblot (lanes d and e) of aqueous buffer extract (lanes a,e) or SDS extract (lanes b,d). The top of the resolving gel is indicated (5–15% acrylamide, run time 17 h). Note the presence of (interface) antibody reactivity at the gel interface and in two bands that do not stain with Coomassie Blue. Pre-stained M_r standards (26 000–180 000) are shown in lane c.

Fig. 2. Effect of reduction on D9B1 immunoblot

Lane a, aqueous extract of endometrial secretory tissue; b, the same extract after reduction with 2-mercaptoethanol (100 °C, 5 min); c, M_r markers (5–15% acrylamide gradient; run time 2 h).

Fig. 3. Immunoblotting of proliferative phase endometrial tissue extracts with antibodies D9B1 (lanes a–d) or 5D4 (lanes a'–d')

Lanes a–d and a'–d' show aqueous extracts of biopsy specimens. Each panel is matched so that extracts b and b' etc. are the same. The prominent M_r -140 000 component that binds 5D4 but not D9B1 is indicated. Note the presence of variable amounts of lower- M_r species, many of which carry both epitopes, and the absence of high- M_r components that stain with either antibody. Lanes e and e' show M_r markers.

(Fig. 1, lanes d and e). The bands were present in tissue extracts made both with aqueous buffer and with denaturing detergent (SDS) and were not stained by Coomassie dye (Fig. 1, lanes a and b). As aqueous and detergent extraction gave essentially the same results, subsequent gels used aqueous extracts only. Staining also sometimes occurred at the stacking–resolving gel interface which, along with the streaking sometimes observed in the D9B1-binding bands, suggests self-aggregation. Variability occurred in bands of lower M_r which were not always present (compare Fig. 1, lane d, with Fig. 4, lane a). These either appeared or increased in intensity with storage of extracts in the freezer and therefore probably arise from breakdown by endogenous enzymes.

When electrophoresis was conducted on minigels for 2 h, the two major bands were not resolved from one another (see Figs. 2, 4 and 5). Material running at the gel

Fig. 4. Immunoblotting of secretory phase endometrial extracts with D9B1 (lanes a,b) or 5D4 (c,d) before (a,c) and after (b,d) treatment with sialidase

Blots were incubated with the enzyme (10 units/ml; as described in the Materials and methods section) before staining with antibody. The major 140 000- M_r species stained with 5D4 is indicated. Note also some 5D4 reactivity near the top of the gel. The high- M_r D9B1-positive complex is sialidase-sensitive, but some staining is retained at the gel interface. 5D4 staining is not affected by the enzyme, and the lower- M_r D9B1-positive bands are also insensitive.

Fig. 5. Section from an immunoblot of a secretory phase endometrial extract with antibody D9B1 showing sensitivity to glycosidases of the 250 000–350 000- M_r component

Enzymes: lane a, endo- β -galactosidase; b, sialidase (50 units/ml); c, keratanase; d, N-glycanase; e, untreated control.

interface again usually stained. Some increase in the rate of migration was detected after reduction of disulphide bonds, and some low- M_r species appeared in the blot (Fig. 2), suggesting that these may be associated covalently with the high- M_r components.

The components of high M_r were detected in extracts taken throughout the secretory phase, although the concentrations varied (results not shown).

Proliferative-phase tissue, though lacking significant amounts of the D9B1-binding species of high M_r , contained variable amounts of lower- M_r products carrying the D9B1 epitope (Fig. 3, lanes a–d).

Fig. 6. Section from an immunoblot of a secretory-phase endometrial extract with antibody 5D4 showing lack of sensitivity to glycosidases of epitope associated with the 140 000- M_r component

Lane a, control; b, *N*-glycanase; c, sialidase (10 units/ml); d, keratanase; e, endo- β -galactosidase.

The high M_r and lack of Coomassie staining of the macromolecule carrying the D9B1 epitope is suggestive of a mucin or proteoglycan. The similarity of immunostaining of secretory phase endometrial tissue observed with D9B1 and the anti-KS mAb 5D4 has been reported previously [9]. Thus binding of the anti-KS mAb 5D4 to immunoblots was compared with binding of D9B1.

The major component observed in immunoblots of endometrial extracts developed with mAb 5D4 was a band of apparent M_r of approx. 140 000. This component is present in both the proliferative and the secretory phases of the cycle (Fig. 3, lanes a'-d' and Fig. 4, lanes c and d) and is not seen in blots developed with D9B1. In secretory-phase extracts, 5D4 stains both the interface band (Fig. 4, lanes c and d) and a second band with an M_r of approx. 400 000, but this staining is weak and does not always occur. Numerous variable minor bands stained by 5D4 were identical with those stained by D9B1 both in proliferative and secretory phases (Figs. 3 and 4).

Binding of D9B1 to the 250 000-350 000- M_r band was sensitive to sialidase (Figs. 4 and 5) and to keratanase, endo- β -galactosidase and *N*-glycanase (Fig. 5). Material running at the gel interface was resistant to sialidase, as were the minor bands at lower M_r (Fig. 4). The 5D4 epitope associated with the major band at 140 000- M_r was resistant to sialidase, endo- β -galactosidase, keratanase and *N*-glycanase (Fig. 6).

Solid-phase assay of antibody binding to keratan sulphates

In solid phase (microtitre plate) assays, D9B1 did not bind to corneal KS but did bind to cartilage proteoglycan and so this was used as a control for enzyme treatments (Fig. 7a). Binding could be eliminated by sialidase or keratanase and was drastically decreased by endo- β -galactosidase (Fig. 7a). Sialidase also eliminated binding in solid-phase assays of secretory phase endometrial extracts (results not shown).

In solid-phase assays, 5D4 bound strongly to corneal KS as reported previously [13], and this binding was used subsequently as a control for enzyme treatments (Fig. 7b). Binding could be decreased by treatment of the substrate with either keratanase or endo- β -galactosidase (Fig. 7b). As expected 5D4 bound strongly to cartilage proteoglycan (Fig. 7b).

Fig. 7. Solid-phase assay of the binding of antibodies D9B1 (a) and 5D4 (b) to cartilage proteoglycan and corneal KS, and the effects of glycosidases

Abbreviations: K, keratanase (0.1 unit/ml); E, endo- β -galactosidase (1.25 units/ml); S, sialidase (10 units/ml); C, control; PG, proteoglycan (20 μ g/ml); CKS, corneal KS (20 μ g/ml).

Immunoperoxidase histochemistry of endometrium

mAbs D9B1 and 5D4 showed similar but not identical staining patterns on endometrial tissue sections from biopsies collected during the secretory phase of the menstrual cycle [9]. The staining was confined to cells in glandular and luminal epithelium and their related secretions, there being no detectable stromal reactivity in either case (Figs. 8a and 9a). Study of serial sections demonstrated that secretory material in the lumen of a given gland frequently carried both epitopes [9]. A similar pattern of binding was also observed when another anti-KS antibody, 1B4 [11], was used (results not shown).

Antibody D9B1 did not bind appreciably to endometrial tissue from the proliferative phase of the cycle (Fig. 8f; [7,8]), and production of the corresponding epitope is therefore characteristic of the secretory phase. In contrast, antibody 5D4 did bind to proliferative phase epithelium (Fig. 9d).

D9B1 binding was reduced greatly by pretreatment of tissue sections with sodium metaperiodate (Fig. 10), confirming the presence of a glycan epitope. Sections were pretreated with endo- β -galactosidase or keratanase, both of which cleave lactosaminoglycan-related chains at unsulphated galactose residues. Antibody binding was abolished by keratanase and drastically reduced by endo- β -galactosidase (Figs. 8 and 10). The staining was not

Fig. 8. Immunoperoxidase histochemistry of human endometrium using mAb D9B1: distribution of the epitope and its sensitivity to glycosidases and blocking agents

(a) Positive secretory phase control showing black peroxidase reaction product in glands and in association with the luminal epithelium. Secretory material in the gland lumens is strongly stained. Pretreatment of the secretory phase tissue sections with sialidase (b) or keratanase (c) abolishes the staining. Pretreatment with endo- β -galactosidase (d) or co-incubation of the tissue with antibody and cartilage proteoglycan (e) gives significantly reduced staining. (f) Proliferative-phase tissue shows no immunoreactivity in glands. Sections were counterstained with haematoxylin. Magnifications: (a)–(e), $\times 58$; (f), $\times 291$.

detectably affected by hyaluronidase or *N*-glycanase, but it was completely abolished by pretreatment of the tissue with sialidase (Figs. 8 and 10). Binding of D9B1 to tissue sections could not be blocked by *N*-acetylneuraminic acid (5 mM), corneal KS, chondroitin sulphate or glycogen, but bovine femoral head proteoglycan (which contains KS) did reduce binding (Figs. 8 and 10). Collectively, these results therefore suggested that antibody D9B1 was binding to a sialic-acid-containing epitope located on a sulphated lactosaminoglycan chain in which not all galactose residues were sulphated.

5D4 binding to tissue sections was not affected by any of the above enzymes but was completely blocked by corneal KS (Figs. 9 and 10). Chondroitin sulphate did not block binding (Fig. 10).

DISCUSSION

The characteristics of the epitope recognized by mAb D9B1 [7–9] are (1) it is present in endometrial secretions, where its expression is menstrual cycle-dependent, (2) it is present in bovine femoral cartilage and we have also detected it immunohistochemically in human cartilage

(M. W. Seif, unpublished work), (3) it is associated with a sulphated lactosaminoglycan as judged by the sensitivity of antibody binding to treatment of the antigen with endo- β -galactosidase or keratanase, (4) it is not abundant in corneal KS, (5) sialic acid contributes to the epitope as judged by its sensitivity to sialidase in both tissue sections and solid-phase binding assays. These data are consistent with the presence of sialylated KS chains in cartilage proteoglycan; relatively few such moieties are present in corneal KS [13–15]. The inability of *N*-acetylneuraminic acid to inhibit D9B1 binding suggests either that a different sialic acid is present or that other sugar residues also contribute to the epitope, or both. It is not possible to conclude from the present data that other KS determinants contribute directly to the epitope.

Antibody 5D4 was raised to cartilage proteoglycan [11] and has been shown [16] to bind to the epitope:

Fig. 9. Endometrium staining using mAb 5D4: sensitivity to glycosidases and blocking agents

(a) Positive secretory phase control. Note the black reaction product in the gland lumens and associated with the glandular epithelial cells. There is no detectable stromal epitope. (b) Secretory phase section pretreated with keratanase. Immunodeposits are still evident in the glands; the pronounced heterogeneity between glands is also characteristic of the control tissue. (c) Staining of secretory-phase tissue using a mixture of mAb 5D4 and corneal KS. No antibody binding is detected. (d) Proliferative phase tissue showing some glands with 5D4-positive material. All sections were counterstained using Haematoxylin. Magnification, $\times 58$.

and other related highly sulphated keratans. Our evidence that this antibody binds to endometrial secretions therefore demonstrated the presence of highly sulphated lactosaminoglycan chains. This is confirmed by the insensitivity of the endometrial 5D4-binding epitope to keratanase or endo- β -galactosidase, both of which hydrolyse glycosidic linkages involving C-1 of non-sulphated galactose residues [12,17]. In contrast, both enzymes are effective in the hydrolysis of corneal KS, and this is consistent with previous evidence [12,17]. As expected, binding of 5D4 is unaffected by sialidase.

Our data therefore suggest the presence of at least two classes of KS chain in endometrial secretions: one carries a menstrual-cycle-dependent sialylated epitope and contains at least one unsulphated galactose residue, whereas the other, which lacks the sialylated epitope, contains

Fig. 10. Semi-quantitative analysis of the binding of antibodies D9B1 (a) and 5D4 (b) to endometrial tissue sections as assessed by immunoperoxidase histochemistry

Sections were preincubated with enzymes or periodate, or were stained with antibody in the presence of glycan inhibitors, as follows: C, control; K, keratanase (10 units/ml); E, endo- β -galactosidase (12.5 units/ml); H, hyaluronidase (30 units/ml); KS, corneal KS (250 μ g/ml); CS, chondroitin sulphate ABC (1 mg/ml); S, sialidase (10 units/ml); G, glycogen; PG, bovine cartilage proteoglycan (100 μ g/ml); P, sodium meta-periodate (1 mM).

sulphated galactose residues and is less sensitive to the hormonal milieu (i.e. it is present throughout the endometrial cycle). The presence of KS in a wide range of tissues has already been described [18] but to our knowledge, this is the first indication that it occurs in endometrium.

Further information on the macromolecular carriers of the endometrial keratan sulphates was obtained by immunoblotting using the two antibody probes. This indicates the presence in both the proliferative and secretory phases of the menstrual cycle of a glycoprotein of 140000- M_r bearing the 5D4 epitope. The enzyme-sensitivity profile of the 140000- M_r -associated 5D4 epitope confirms the above structural predictions and suggests that this species is primarily responsible for the

binding of 5D4 to endometrial tissue. It forms a discrete band in SDS/PAGE, indicating a relatively limited degree of microheterogeneity, but its failure to bind Coomassie Blue protein stain suggests that it may be highly glycosylated. It does not carry the sialylated structure recognized by mAb D9B1. The failure of *N*-glycanase to release KS even after denaturation with SDS suggests that this glycan component of the M_r -140 000 species is attached to polypeptide via an *O*-glycosidic linkage at serine or threonine.

The major carrier of the D9B1 epitope bands as a doublet near the top of 5–15% polyacrylamide gels and has some characteristics of a mucin or proteoglycan. It does not bind Coomassie dye, it appears to self-aggregate as judged by streaking and the presence of residual antigen at the top of the resolving gel, and it exhibits an apparent approx. M_r in the range 250 000–350 000. Some increase in mobility in polyacrylamide gels is observed after reduction of disulphide bonds, but the major components retain their high M_r . Binding of D9B1 to these bands is drastically decreased by sialidase, endo- β -galactosidase and keratanase. This is consistent with the solid phase binding and with immunohistochemical data. The observation that *N*-glycanase significantly decreases binding of D9B1 on blots suggests that KS chains in the high- M_r components are attached to polypeptide via an *N*-glycosidic linkage at asparagine. The failure of *N*-glycanase to affect staining in tissue sections and of sialidase to eliminate completely antibody binding to plots in the gel interface region may both be due to aggregation leading to restriction of enzyme access. Taken together, this evidence supports the notion that the high- M_r species are responsible for the observed binding and distribution of D9B1 on endometrial tissue.

Tissue extracts from either proliferative or secretory phase biopsy specimens contain variable quantities of a series of bands of M_r less than 85 000. These were observed either to appear or to increase in intensity with storage. They seem to represent breakdown products, and their presence in some freshly made extracts suggest a process of physiological lysis that may occur after the secretory products arrive in glandular lumens. The observation that most of these species contain both the D9B1 and the 5D4 epitopes suggest that they must derive from breakdown of components of high M_r . We are unable at present to explain why sialidase fails to abolish binding of D9B1 to these species, but this observation confirms the idea that they do not contribute to staining observed in tissue sections (which is completely abolished by sialidase). One possible reason for this is that smaller secretory components may be lost during tissue processing for immunohistochemistry.

The observation of at least one class of menstrual-cycle-dependent oligosaccharide structures raises questions regarding the stage of biosynthesis at which hormonal control may be exerted. At present we have no information as to the variation of expression of the presumed high- M_r core protein(s) during the menstrual cycle. Thus it is not clear whether the level of the D9B1

epitope is controlled by variable glycosylation of a constantly expressed core polypeptide, or whether the polypeptide is itself under hormonal control. Measurement of components whose concentrations vary in response to the hormonal environment is valuable in the assessment of endometrial responses in infertile women [19]. Further work is required to identify hormone-sensitive steps in this biosynthetic pathway.

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