Characterization of glycoconjugates in the endometrium of the miniature pig during different reproductive periods

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Lectin-binding glycoconjugates in the endometrium of miniature pigs were determined for sexually immature, dioestrus, oestrus and early pregnancy periods, using five horseradish peroxidase-labelled lectins. Apparent changes associated with sexual maturity were increases in binding intensity of wheat germ agglutinin (WGA), *Glycine max* agglutinin (SBA) and *Ulex europaeus* agglutinin (UEA) and the appearance of *Ricinus communis* agglutinin (RCA) binding. The binding intensity of WGA, SBA and UEA further increased at oestrus, particularly for UEA which showed a stage-specific binding on the surface of endometrial epithelium. Pregnancy-related changes included an increase in WGA binding and a decrease in UEA and RCA binding. The results indicate that stage-specific alterations in lectin binding glycoconjugates occur in the endometrium of pigs associated with sexual maturity and the reproductive cycle.

Introduction

It is well known that the receptivity of the uterus to the implanting blastocyst and the attachment of the trophoblast to the endometrium are essential for implantation. Although the molecular mechanism of trophectoderm–epithelial interaction are unclear, the cell membrane glycoconjugates probably play an important role in this process, as cell surface carbohydrate has been implicated in cellular interactions such as cell–cell recognition, adhesion, fusion and differentiation (Knudsen *et al.*, 1981; Damsky *et al.*, 1982; Brandy and Schnaar, 1986; Edelman, 1986).

Many investigations have been made on pregnancy-related changes of lectin binding glycoconjugates in the endometrium of mice (Lee *et al.*, 1983; Wu *et al.*, 1983, 1991; Chavez and Anderson, 1985), rabbits (Anderson *et al.*, 1986), cattle (Munson *et al.*, 1989), pigs (Whyte and Robson, 1984), horses (Whyte and Allen, 1985) and humans (Jansen *et al.*, 1985; Lee and Damjanov, 1985). These data indicate that the pre- and post-implantation processes are associated with changes in glycoconjugates in the endometrium. Furthermore, Lindenberg *et al.* (1988) demonstrated that a specific fucosylated pentasaccharide on the endometrial surface is involved in the initial attachment of the blastocyst to the endometrial epithelium of mice.

The expression of the glycoconjugates in the endometrium has been shown to be modulated by steroid hormones (Kimber and Lindenberg, 1990). Administration of exogenous hormones could alter the characterization of glycoconjugates in the endometrium and result in the degeneration of conceptuses (Blair *et al.*, 1991; Kramber, 1992). However, little attention has been paid to the characterization of glycoconjugates in the

endometrium associated with sexual maturity and oestrus under normal hormonal regulation.

In the present study, five lectins, labelled with horseradish peroxidase (HRP), were used to determine the glycoconjugates in the endometrium of miniature pigs at different reproductive periods to obtain a better understanding of stage-specific changes. The relationship between endometrial glycoconjugates and possible uterine receptivity was also investigated.

Materials and Methods

Tissues

Miniature pigs used in this study were sexually immature (two months), in dioestrus, in oestrus or in early pregnancy (25 days). Four pigs were selected for each period. These animals were housed in pens with concrete floors and fed formula diet based on corn and soybean meal. The average body weight was 4.6 and 34.5 kg for sexually immature and adult animals, respectively. The period of oestrus was defined by examination of the vulva for swelling and reddening together with the exhibition of a standing oestrus reflex. One week after the onset of oestrus the pigs were allocated to the dioestrous group. When sampling, the two periods were further confirmed by checking for the presence of mature follicles at oestrus and pink corpora lutea at dioestrus. Tissue samples were taken from the uterine horn and fixed with Bouin's solution. Tissues were embedded in paraplast and sectioned at 5 µm intervals.

Lectin histochemistry

The five HRP-labelled lectins – wheat germ agglutinin (WGA), *Glycine max* agglutinin (SBA), *Ricinus communis*

Table 1. Lectins used and corresponding inhibitory sugars

Lectins	Source	Inhibitory sugars
WGA	<i>Triticum vulgare</i> (wheat germ)	N-acetyl-D-glucosamine
SBA	<i>Glycine max</i> (soybean)	N-acetyl-D-galactosamine
RCA ₁₂₀	<i>Ricinus communis</i>	D-galactose
UEA I	<i>Ulex europaeus</i>	α -L-fucose
Con A	<i>Canavalia ensiformis</i>	Methyl α -D-mannopyranoside

Table 2. Lectin binding sites on the endometrium of miniature pigs

	Endometrial epithelium		Endometrial gland	
	Surface	Cytoplasm	Surface	Cytoplasm
WGA				
Sexually immature	+	–	++	++
Dioestrus	+++	++	+++	+++
Oestrus	++++	+++	++++	+++
Early pregnancy	++++	++++	+++	++
SBA				
Sexually immature	+	–	±	–
Dioestrus	++	–	+	–
Oestrus	+++	±	+++	±
Early pregnancy	+++	++	+	±
RCA				
Sexually immature	±	–	±	–
Dioestrus	+++	–	±	–
Oestrus	+++	–	±	–
Early pregnancy	+	–	–	–
UEA				
Sexually immature	±	–	±	–
Dioestrus	±	–	++	–
Oestrus	++++	±	+++	+++
Early pregnancy	–	–	++	±
Con A				
Sexually immature	++	++	++	++
Dioestrus	++	++	++	++
Oestrus	+	+	+	+
Early pregnancy	+	+	±	±

– : negative; ± : trace; + : weak; ++ : moderate; +++ : strong; ++++ : very strong.

WGA: wheat germ agglutinin; SBA: *Glycine max* agglutinin; RCA: *Ricinus communis* agglutinin; UEA: *Ulex europaeus* agglutinin; Con A: concanavalin A.

agglutinin (RCA), *Ulex europaeus* agglutinin (UEA) and concanavalin A (Con A) – and the corresponding inhibitory sugars were purchased from Sigma Chemical Co. (St Louis, MO). Details of these lectins and sugars are presented in Table 1.

For lectin binding, tissue sections were processed through the following steps at room temperature: (1) Hydrogen peroxide (1%) in phosphate-buffered saline (PBS) (0.01 mol l^{-1} , pH 7.2; Kuhlmann, 1984) for 15 min; (2) three rinses in PBS; (3) addition of BSA for 30 min; (4) three rinses in PBS; (5) incubation with the solution of HRP-labelled lectin in a moist chamber for 1 h; (6) three rinses in PBS; (7) incubation with 0.05% diaminobenzidine (DAB) and 0.1% hydrogen peroxide in Tris buffer (0.05 mol l^{-1} , pH 7.6) to visualize the bound sites; (8) dehydration through graded ethanol, clearing

in xylene and mounting in resinous medium. Controls included (1) omitting HRP-labelled lectin, and (2) preabsorbing lectin with the appropriate inhibitory sugar (0.2 mol l^{-1}).

Results

The lectin-binding patterns in the endometrium at different reproductive periods are summarized (Table 2) and illustrated (Figs 1–4). Generally, the lectin binding sites were on the apical surface or in the cytoplasm of the endometrial epithelial cells, and on the apical surface or in the supranuclear cytoplasm of the endometrial glandular cells. In control sections, the binding of WGA, SBA, RCA, UEA and Con A could be blocked by the addition of the corresponding inhibitory sugars (listed in Table 1).

Fig. 1. Binding patterns of wheat germ agglutinin (WGA) on the endometrium in (a) sexually immature, (b) dioestrus, (c) oestrus and (d) early pregnancy. (a) There is weak binding on the endometrial epithelium and moderate binding on the endometrial gland. (b) Both endometrial epithelium and endometrial gland show strong binding. (c) The binding intensity is very strong on both endometrial epithelium and endometrial gland. (d) Note the increase in binding intensity on the cytoplasm of the endometrial epithelium. Scale bars represent 100 μm .

WGA binding

In the sexually immature animals, WGA binding was weak on the surface of the endometrial epithelium and moderate on the surface and in the cytoplasm of endometrial glands (Fig. 1a). At dioestrus, the binding on the surface of both endometrial epithelium and gland was strong, and the cytoplasm of endometrial epithelium also showed moderate WGA binding (Fig. 1b). The binding at oestrus increased to very strong on the surface and in the cytoplasm of both endometrial epithelium and gland (Fig. 1c). Compared with oestrus, stronger binding on the endometrial epithelium but weaker

binding on the endometrial gland were found in early pregnancy (Fig. 1d).

SBA binding

SBA-binding sites in the sexually immature animals were present only on the surface of both the endometrial epithelium and gland, and the intensity was greater on the former and weaker on the latter (Fig. 2a). During dioestrus the binding intensity on the surface of endometrial epithelium and gland was moderate and weak, respectively (Fig. 2b). The binding intensity on the surface of both endometrial epithelium and

Fig. 2. Binding patterns of *Glycine max* agglutinin (SBA) on the endometrium in (a) sexually immature, (b) dioestrus, (c) oestrus and (d) early pregnancy. (a) The endometrial epithelium and gland show weak and trace binding respectively. (b) The binding intensity is moderate on the endometrial epithelium and weak on the endometrial gland. (c) Both endometrial epithelium and epithelial gland show strong binding. (d) An increase in binding intensity is seen on the cytoplasm of the endometrial epithelium. Scale bars represent 100 μm .

gland increased to strong at oestrus (Fig. 2c). In early pregnancy the endometrial epithelium had strong SBA binding on the surface and also showed moderate binding in the cytoplasm, while the binding on endometrial gland was lower (Fig. 2d).

RCA binding

The surface of both endometrial epithelium and gland showed very weak RCA binding in sexually immature animals (Fig. 3a). During the dioestrus period the binding on the surface of endometrial epithelium increased to strong, while the

endometrial gland had very weak binding (Fig. 3b). The binding pattern at oestrus (Fig. 3c) was similar to that at dioestrus. In early pregnancy the RCA binding on the surface of endometrial epithelium was weak, and there was no binding on the endometrial glands (Fig. 3d).

UEA binding

In sexually immature animals, UEA binding was very weak and limited to the surface of endometrial epithelium and gland (Fig. 4a). At dioestrus the binding on the surface of the endometrial gland, but not on the epithelium, increased to

Fig. 3. Binding patterns of *Ricinus communis* agglutinin (RCA) on the endometrium in (a) sexually immature, (b) dioestrus, (c) oestrus and (d) early pregnancy. (a) Both endometrial epithelium and endometrial gland are almost unreactive. (b) The binding intensity is strong on the endometrial epithelium but very weak on the endometrial gland. (c) There is no stage-specific change. (d) Both the endometrial epithelium and endometrial gland show a decrease in binding intensity. Scale bars represent 100 µm.

moderate or strong (Fig. 4b). At oestrus, the binding increased to very strong on the surface of both the endometrial epithelium and gland, and the cytoplasm of some of the endometrial glands was also intensively stained (Fig. 4c). However, in early pregnancy there was no UEA binding on the surface of endometrial epithelium and that on the endometrial gland was also markedly decreased (Fig. 4d).

Concanavalin A binding

Both endometrial epithelium and gland had moderate Con A binding in sexually immature animals. No apparent changes

were observed with sexual maturity, although at oestrus and in early pregnancy, the binding intensity showed a gradual decrease, especially on the endometrial gland.

Discussion

Although the pregnancy-related changes in lectin binding patterns on the endometrium have been demonstrated, little is known about the alterations associated with sexual maturity and during the oestrous cycle. Results presented here provide evidence of stage-specific changes of lectin binding on the

Fig. 4. Binding patterns of *Ulex europaeus* agglutinin (UEA) on the endometrium in (a) sexually immature, (b) dioestrus, (c) oestrus and (d) early pregnancy. (a) Only trace binding is seen. (b) The binding intensity increased to moderate on the endometrial gland. (c) Note the appearance of strong binding on the endometrial epithelium. (d) There is no binding on the endometrial epithelium. Scale bars represent 100 μm .

endometrium associated with sexual maturity and oestrus, as well as with pregnancy.

Changes in lectin binding pattern on the endometrium, associated with sexual maturity, have been demonstrated in the present study, including increases in reactivity of WGA, SBA and UEA and the appearance of RCA binding. These results indicate that both the quantity and pattern of glycoconjugates in the endometrium change relative to sexual maturity. These changes probably result from stimulation by sex hormones, as previous reports have shown that oestrogen stimulates the synthesis of glycoconjugates in the endometrium of some mammals (Kuivanen and DeSombre, 1985; Teng *et al.*, 1986; Pentecost and Teng, 1987; Kimber and Lindenberg, 1990).

Among the five lectins used in the present study, the binding patterns of WGA, SBA and UEA showed apparent stage-specific changes. The binding intensities of the three lectins were stronger in both endometrial epithelium and endometrial gland at oestrus compared with dioestrus, particularly in the cytoplasm. The increased cytoplasmic binding may reflect active secretion by the endometrium. Alternatively, the binding of UEA to the surface of endometrial epithelium was specific for the stage of the oestrous cycle. Because glycoconjugates in the endometrial epithelium have been shown to be involved in the initial adhesion of the blastocyst to the endometrial epithelium (Jansen *et al.*, 1985; Lindenberg *et al.*, 1988), the appearance of UEA on the endometrial epithelial surface at oestrus suggests that the UEA binding

glycoconjugate may be related to preparation of the uterine endometrium before implantation.

The pregnancy-associated alterations in lectin binding patterns observed in the present study include mainly increased WGA binding and decreased binding of RCA and UEA on the endometrial epithelium. The increase of WGA binding to the endometrial epithelium was similar to that reported for most other animal species (Lee *et al.*, 1983; Whyte and Robson, 1984; Whyte and Allen, 1985; Anderson *et al.*, 1986; Munson *et al.*, 1989; Wu *et al.*, 1991), although WGA binding did not change in the endometrium of pregnant women (Lee and Damjanov, 1985). The decrease of UEA binding also agrees with findings in mice (Lee *et al.*, 1983; Wu *et al.*, 1991), cattle (Munson *et al.*, 1989) and women (Lee and Damjanov, 1985), but this result is contrary to the report for the horse (Whyte and Allen, 1985). Our observations on the pregnancy-related change of RCA binding are not in agreement with reports of increased binding in mice (Lee *et al.*, 1983; Chavez and Anderson, 1985; Wu *et al.*, 1991; Horvat, 1993) and rabbits (Anderson *et al.*, 1986), and of no change in binding in cows (Munson *et al.*, 1989) and humans (Lee and Damjanov, 1985). In the present study, lectin binding to the endometrial gland during pregnancy was weaker than during oestrus, but stronger than during dioestrus. This finding is in agreement with the finding of increased lectin binding on the endometrial gland in pregnant versus nonpregnant cattle (Munson *et al.*, 1989).

Differences in lectin binding between the endometrial epithelium and endometrial gland were found in the study reported here. The limited binding of RCA to the surface of endometrial epithelium indicates that the corresponding glycoconjugate is a structural component of the endometrial epithelium, but is not a secretory component. This result differs from the positive staining of RCA in the endometrial glands of cows (Munson *et al.*, 1989) and women (Lee and Damjanov, 1985). Contrary to RCA, UEA was mainly bound to the endometrial gland in miniature pigs, whereas UEA binding was totally absent in cows (Munson *et al.*, 1989) and women (Lee and Damjanov, 1985; Yen *et al.*, 1986). The discrepancy in UEA binding on the endometrial glands in different species may reflect a species difference in the composition of uterine secretions. The binding patterns of WGA, SBA and Con A to the endometrial epithelium and endometrial glands noted in this study are similar to those in other species (Lee and Damjanov, 1985; Yen *et al.*, 1986; Munson *et al.*, 1989).

In summary, the present study shows that the binding patterns of lectins on the endometrial epithelium and gland of miniature pigs change during the reproductive cycle. These changes suggest that the glycoconjugates in the endometrium are related to receptivity of the uterus for the implanting blastocyst. On the basis of these data, future studies will include the isolation of glycoproteins from the endometrium, using lectin affinity chromatography, to determine their significance in the interaction between the endometrium and trophoblast.

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