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Tracking nutrient transfer at the human maternofetal interface from 4 weeks to term

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ABSTRACT

Introduction: In this study we have tracked glycogen and glycoprotein flux associated with nutrient uptake into trophoblast in early deciduochorial and later haemochorial placenta.
Methods: α-amylase, glycogen synthase and glycogen phosphorylase were immunohistochemically localised in 6–14 week and term placenta and first trimester decidua. Placentae of 4–18 weeks' gestation and term were also stained with 22 biotinylated lectins.
Results: Histochemical data were consistent with glycogenolysis in decidual gland epithelium and placental cyto- and syncytiotrophoblast; α-amylase was present in decidual secretions but absent in placenta. Glycogen and glycogen synthase were both apparent in villous cytotrophoblast cells and columns. Profound changes were observed in placental glycosylation during gestation. Syncytial microvilli were richly glycosylated as were first trimester vacuoles but, by term, syncytiotrophoblast showed little lectin binding except in microvillous and basal membranes. Cytotrophoblast Golgi bodies were active in the first trimester; at term the cells were generally more glycosylated than syncytiotrophoblast.
Discussion: We deduce that decidual cell glycogen is broken down for transport into the placenta where the products may be reassembled into glycogen or used for metabolic processes. First trimester histiotrophe is internalised by syncytiotrophoblast, then broken down in apical vacuoles containing lysosomal markers. This process declines after haemotrophic nutrition commences. Transition from histiotrophic to haemotrophic nutrition involves reduced amounts of uterine secretory derivatives reaching the placenta, and reduction in internalisation of glycoprotein by syncytiotrophoblast, presumably reflecting the shift to low molecular weight nutrients. Glycogen accumulates in cytotrophoblast from early pregnancy and is mobilised for utilisation by fetoplacental tissues.

1. Introduction

The placenta is the conduit through which nutrients are passed to the developing embryo, initially by means of the secretions produced by uterine glands (glycoprotein-rich histiotrophe) but then after about 11 weeks of pregnancy via the maternal blood which surrounds the placental villi. Direct functional approaches to observing these events are unavailable in human and little is known about how glycogen is transported out of decidual cells to nourish the embryo, the mechanism of uptake of histiotrophe or how the transition from histio-to haemotrophic nutrition is reflected in changes to the morphology and biochemistry of syncytiotrophoblast. We have previously examined the glycan composition of histiotrophe in first trimester human decidua and noted changes compared to endometrial glands in the non-pregnant state [1] which facilitate absorption by the placenta. However the nature of the ingested material within the placenta and whether it changes over the course of pregnancy has not been fully investigated.

To date, there have been a few histochemical studies of glycosylation in normal human placenta [2,3] and in placentae from pregnancies with complications [4–6] but none have tracked changes over the course of pregnancy. Some early studies examined first trimester tissue with a restricted panel of lectins [7–9] and a few studies have compared first trimester tissue with term [2,10] but in general little detail was given of subcellular localisation and resolution of cryostat or wax sections was generally poor. Recently, the N-glycome of human placental tissue from first and

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third trimester was investigated, but the tissue was homogenised so no localisation to tissue layers or components was possible [11]. Here we use immuno- and lectin histochemical approaches to examine macromolecular traffic (glycogen and secretory glycoprotein) between decidual gland cells and trophoblast, illuminating the changing nature of maternofetal nutrient exchange and concomitant changes in trophoblast morphology. Following an earlier study of the glycoprotein component of gland-derived histiotrophe [1], we have now localised glycogen with the lectin *Bandeiraea simplicifolia*-II and mapped key enzymes in the biosynthesis (glycogen synthase) and breakdown (glycogen phosphorylase, α-amylase) of glycogen in the first trimester decidua and placenta.

2. Materials and methods

2.1. Immunohistochemistry

Specimens of placenta with gestational ages of 6 (2), 8 (1), 9 (1), 10 (2), 12 (2), 14 (1) weeks and term (10) were obtained from women attending St Mary's Hospital, Manchester; informed consent was gained from all participants (NRES 08/H1010/55). Samples were fixed in buffered formalin and processed into wax. First trimester specimens of decidua (5) were also obtained and treated in a similar manner. Tissue sections 5 μm thick were immunostained as described previously [14]. Briefly, after incubation in a non-immune block, primary antibodies to α-amylase (1:1600) and glycogen synthase (1:100, both from Cell Signaling Technology, Danvers, MA, USA) and glycogen phosphorylase (1:750, Sigma Aldrich, Poole, UK) in non-immune block were applied to the sections overnight at 4 °C. Biotinylated secondary antibodies (2.5 μg/ml) diluted in non-immune block were added to the sections for 30 min at room temperature, followed by 5 μg/ml of avidin-peroxidase in TBS after washing. Sites of antibody binding were revealed with diaminobenzidine/hydrogen peroxide (SIGMAFAST™ Sigma Aldrich, Poole UK), counterstained with Harris' haematoxylin and assessed using a semiquantitative ranking system in which staining intensity was allocated a grade from 0 (negative) to 4 (intense) staining. Sections of human pancreas were used as positive controls for α-amylase and human liver for the glycogen synthase and phosphorylase antibodies. Substitution of appropriate concentrations of non-immunised rabbit IgG for the antibody was used as a negative control.

2.2. Lectin histochemistry

Specimens of placenta with gestational ages of 4, 6, 7, 8, 14 and 18 weeks, and four at term were collected from women as above. Small pieces were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.3 for 4 h and processed into epoxy resin (Taab Laboratories Equipment, Aldermaston, UK). Semithin sections 0.75 μm were cut and stained with a panel of 22 lectins as previously described [12]. Two specimens of late secretory phase endometrium and four first trimester decidua from previous studies [1,13] were stained with BSA-II, the latter also with AHA. Sections stained with AHA, ECA, SBA, SNA-1, MAA, PAA and WGA were treated with 0.1 units/ml neuraminidase (0.1 units/ml, type VI from *Clostridium perfringens*, Sigma) for 2 h at 37 °C before incubation in the lectin to cleave off terminal sialic acid. The trypsinisation step was omitted with sections stained with BSA-II. Comments on the results of pretreatment with neuraminidase are restricted to those which showed a clear effect. Selected sections were counterstained in Harris' haematoxylin for 30 s before washing and mounting. Staining was graded as above. Controls were carried out as previously described [12] and as a control for glycogen, paired sections treated with BSA-II were pre-digested with 1% amylase (Sigma) in distilled water at 37 °C for 30 min, and then washed under running water before incubation in the lectin. For lectin binding specificities, readers are referred to [12].

For the purposes of this study, only glandular epithelium of endometrium and decidua and placental trophoblast are described.

3. Results

3.1. Glycogen localisation (BSA-II)

Sections of *secretory phase endometrium* showed strong staining with BSA-II in the gland epithelium (Fig. 1A). Staining was eliminated by pre-treatment with α-amylase (Fig. 1B). Binding was seen in *decidual glands* in all specimens, most markedly in columnar cells with prominent apices (Fig. 1C); flattened cells showed less stain. Glycogen was not detected in gland secretions.

In the *placenta*, little BSA-II staining was evident on the microvillous brush border or syncytiotrophoblast at any stage of pregnancy (Fig. 1D, F), apart from syncytial surface staining of one specimen at term and some very occasional weak reactivity in the syncytium in early pregnancy. The cytotrophoblast, in contrast, showed intense staining in the first trimester (Fig. 1D), and this was frequently maintained to term (Fig. 1F). Pre-digestion with α-amylase completely removed BSA-II stain (Fig. 1E, G).

3.2. Glycogen biosynthesis (glycogen synthase)

Table 1 provides an overview of the staining expression in each case examined. In the *decidua*, the luminal surface and cytoplasm of gland epithelium were well stained (Fig. 2A) with a variable

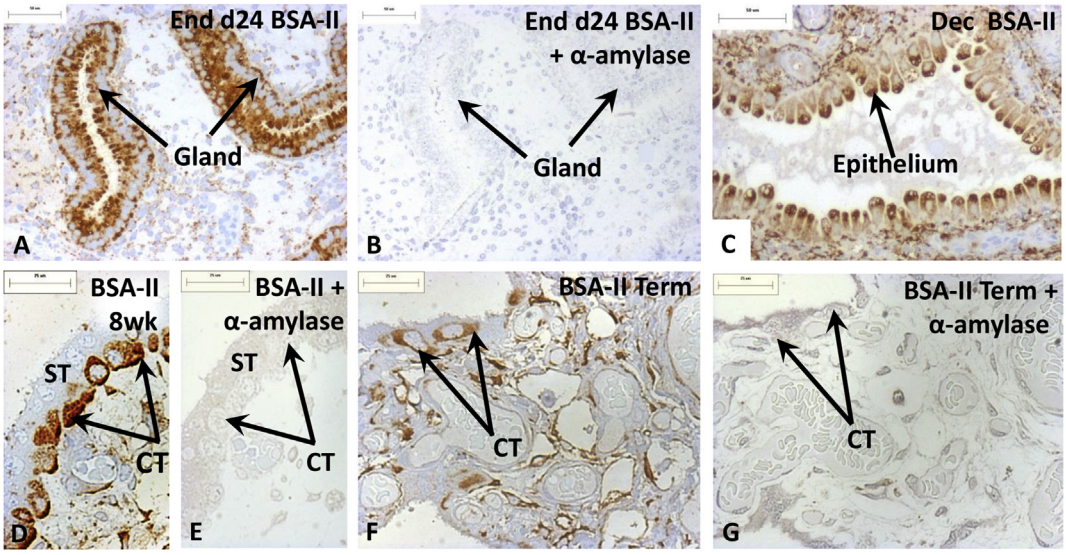

Fig. 1. BSA-II staining of endometrium, decidua and placenta. A) Late secretory phase endometrium (day 24) stained with BSA-II showing strong binding by gland epithelium, especially in the apical regions of the cells. B) After α-amylase pretreatment, BSA-II staining is lost, C) First trimester decidua stained with BSA-II with apical areas of domed epithelial cells showing strong binding of the lectin. D) At 8 weeks, BSA-II binds to cytotrophoblast cells (CT) with only weak binding to the syncytiotrophoblast (ST). E) After α-amylase pretreatment, this staining has disappeared. F) At term, cytotrophoblast cells still show binding of BSA-II. G) BSA-II staining disappears after α-amylase pretreatment of an adjoining section.