0013-7227/02/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 87(6):2954–2959

Uterine Glands Provide Histiotrophic Nutrition for the Human Fetus during the First Trimester of Pregnancy

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Providing adequate nutrition to the fetus is key to a successful pregnancy. The interstitial form of implantation displayed by the human blastocyst is generally associated with early onset of maternal blood flow to the developing placenta, and hence hemotrophic exchange. However, the recent finding that the maternal intraplacental circulation is not fully established until the third month of gestation suggests that human fetal nutrition may be initially histiotrophic. We therefore investigated activity of the uterine glands during early pregnancy. We demonstrate here that these glands remain active until at least wk 10 of pregnancy, and that their secretions are delivered freely into the placental intervillous space. We also demonstrate phagocytic uptake by the placental syncytiotrophoblast of two glycoproteins, the mucin MUC-1 and glycodelin A, synthesized in the maternal glands. Glycodelin was also detected within the epithelium of the secondary yolk sac lining the exocoelomic cavity, indicating that the yolk sac may play an important role in nutrient exchange before vascularisation of the chorionic villi. Our findings demonstrate that the uterine glands are an important source of nutrients during organogenesis, when metabolism is essentially anaerobic. (*J Clin Endocrinol Metab* 87: 2954–2959, 2002)

AMONG EUTHERIAN MAMMALS, two principal pathways have evolved to transfer nutrients from the mother to her fetus. These are termed histiotrophic and hemotrophic, respectively (1). Histiotroph is an extracellular material derived from the endometrium and the uterine glands that accumulates in the space between the maternal and fetal tissues. It is phagocytosed initially by the trophectoderm of the blastocyst, and later by the trophoblast of the placenta or the endoderm of the yolk sac. By contrast, hemotrophic nutrition is the exchange of blood-borne materials between the maternal and fetal circulations. This is facilitated by the extensive and intimate apposition of the maternal and fetal tissues that occurs within the placenta. Before implantation, nutrition of the mammalian conceptus is therefore essentially histiotrophic. Once the placenta is established, hemotrophic nutrition becomes predominant, although the two pathways may coexist for much of gestation in certain species (1).

In the human, the period of histiotrophic nutrition is thought to be particularly brief for two principal reasons. Firstly, the human blastocyst implants at an early stage, and the invasive nature of this process results in the conceptus being completely embedded within the superficial layer of the endometrium by d 10 post fertilization. In this situation it is thought that the glandular secretions in the uterine lumen are no longer accessible to the conceptus (2). Secondly, during invasion the trophoblast rapidly erodes into the vascular network of the endometrium, and the dogma has been that maternal blood flows through the developing placenta from d 17–20 onwards (3), thus establishing the hemotrophic pathway.

These assertions were, however, based on morphological observations alone, and more recent integrative data have revealed that the second is no longer valid. Using a variety of techniques, Hustin and colleagues (4, 5) were unable to detect significant maternal blood flow within the placenta before 12 wk of gestation. This finding, which has since been confirmed by others (6, 7), has been attributed to the presence of aggregates of cytotrophoblast cells that effectively plug the distal portions of the endometrial spiral arteries during the first trimester (4, 8). Only when the plugs loosen at the start of the second trimester is significant flow observed ultrasonographically. Measurements of the oxygen tension within the placenta and extraembryonic cavities (9–11) *in vivo* at different gestational ages have provided further physiological support for this claim. Between 10–12 wk, the oxygen tension in the placenta rises from $<$20 mm Hg to $>$50 mm Hg, and is associated with increases in the activity of the principal antioxidant enzymes in placental tissues confirming changes in oxygenation at the cellular level.

This new interpretation casts doubt upon the ability of the hemotrophic pathway to meet the energy and elemental requirements of the fetus during the first trimester of pregnancy. The aim of the present study was to determine whether other pathways, in particular histiotrophic nutrition via the uterine glands, might contribute to fetal development during this period. Use was made of an archival histological collection of early placenta-*in situ* material in which the normal topographical relationships between the glands and the placenta had been preserved. Observations based on this material were supported with immunohistochemical techniques, and with electron microscopic studies of fetal and maternal tissues obtained from first trimester pregnancies.

Abbreviations: IVS, Intervillous space; PAS, periodic acid-Schiff.

Materials and Methods

Archival material

The Boyd Collection was assembled by J. D. Boyd as Professor of Anatomy at the University of Cambridge, and it formed the basis of classic descriptions of placental development. It includes several placentas-*in situ* of various gestational ages as assessed by the crown-rump length of the fetus, and only those specimens with no recorded history of pathology were reviewed. Ten specimens met these criteria, and gestational age (from the last menstrual period) was estimated from their crown-rump length. This ranged from 43 to 87 d. Extensive runs of serial paraffin sections of either the whole uterus or of blocks excised from the uterine wall were available. The sections had been prepared using a variety of stains, but principally with hematoxylin and eosin, periodic acid-Schiff reagent (PAS), or Masson's trichrome.

Occasional unstained sections had been left and two from the earliest specimen available (H710) were dewaxed and rehydrated. These were immunolabeled for two antigens; the epithelial mucin MUC-1 using the monoclonal antibody HMFG-1 (clone 1.10.F3, Coulter) and a fluorescently labeled secondary, and glycodelin (previously known as placental protein 14) using a fluorescently labeled lectin from *Wisteria floribunda* (Vector) (12).

First trimester material

Placental, secondary yolk sac and decidual tissue was collected with informed written consent from healthy women undergoing surgical termination of pregnancy at University College Hospital, London, under general anesthesia for psychosocial reasons at 8–16 wk of gestation (from last menstrual period). The collection of these samples was approved by the University College London Hospitals Committee on the Ethics of Human Research.

Samples of five yolk sacs from 8–12 wk gestational age (from last menstrual period) were fixed for immunohistochemistry at the light microscope level in 4% formaldehyde, and embedded in paraffin wax. Sections were subjected to antigen retrieval by microwaving at 640 W for 2 × 5 min in citrate buffer. They were then immunolabeled using a mouse monoclonal antibody raised against glycodelin (gift from Prof. M. Seppälä) at a concentration of 1:60, and a fluorescently labeled secondary. Negative controls were performed by replacing the primary antibody with nonimmune mouse serum at the same concentration. Images were captured with a Leica Corp. (Heidleburg, Germany) true confocal scanner-spectrophotometer detector-multiphoton confocal microscope.

Villous and decidual tissue from 6–10 wk gestational age (from last menstrual period) was fixed for ultrastructural study by transmission electron microscopy in 2% glutaraldehyde in 0.1 M piperazine-N,N′-bis(2-ethanesulfonic acid) buffer. Following secondary fixation in 1% osmium tetroxide for 1 h at room temperature, the tissue was embedded in Araldite epoxy resin. Ultra-thin sections (150 nm) were counterstained with uranyl acetate followed by lead citrate and viewed using a Philips (Eindhoven, The Netherlands) CM100 microscope.

Villous tissue was also prepared for immunoelectron microscopy of ultrathin, thawed, cryo-sections. Tissue was fixed in 4% formaldehyde in 0.1 M piperazine-N,N′-bis(2-ethanesulfonic acid) buffer for 2 h, encapsulated in gelatin, cryoprotected in 1.9 M sucrose and 10% polyvinyl pyrrolidone and frozen in liquid nitrogen. Ultrathin sections were mounted on carbon and Formvar-coated nickel grids and labeled using the secondary antibody technique. They were embedded in methyl cellulose and uranyl acetate before examination in a Philips CM 100 transmission electron microscope.

Results

Placenta-in situ specimens

The earliest placenta-*in situ* specimen available (H710) was associated with a 4-mm embryo, and contemporary dating techniques place this at 43 d from the last menstrual period. The conceptus had embedded within the superficial layer of the endometrium, and the uterine glands were conspicuous beneath the developing placenta (Fig. 1a). The glands were lined by a columnar epithelium and their lumens were filled with precipitated secretions that stained bright pink with the PAS technique and blue with Alcian Blue. This staining pattern, confirming the high carbohydrate content of the secretions, was in marked contrast to that of the plasma within the maternal endometrial arteries. Interspersed among the precipitate were numerous small globular secretions that stained purple/red with the Neutral Red counterstain, suggesting these were lipid droplets.

At several points along the materno-fetal interface the uterine glands could be observed opening through the cytotrophoblastic shell directly into the intervillous space (IVS) (Fig. 1a), and their secretions could be seen dispersed between the villi. It was notable that on sections stained by the PAS technique many villi displayed crimson arcs around that portion of their surfaces orientated toward the cytotrophoblastic shell (Fig. 1b). The staining was most intense for those villi closest to the shell, but the same pattern was observed in villi further removed. At higher power it could be seen that the PAS-positive material was contained within the syncytiotrophoblast covering of the villi rather than merely being adherent to the surface (Fig. 1c). This pattern was repeatedly observed in sections taken across the placenta, and so could not be considered a sectioning artifact.

To trace the passage of the secretions, immunolabeling for two specific products of the uterine glands, the epithelial mucin MUC-1 and glycodelin, was carried out on unstained sections from this specimen. The uterine glands showed an intense and specific reaction for both these products, and there was no reactivity within the myometrium or maternal blood vessels (Fig. 1d). The adjoining IVS gave a strong positive signal, which served to outline the placental villi. In the syncytiotrophoblast close to the shell punctate sites of intense fluorescence scattered throughout the trophoblast suggested vesicular uptake of MUC-1 (Fig. 1e). Labeling for glycodelin demonstrated a more uniform fluorescence throughout the syncytiotrophoblast with less punctate staining (Fig. 1f), but the macrophages, or Hofbauer cells, within the villous core also reacted strongly. Negative controls omitting the primary antibody or lectin showed no labeling or autofluoresence (data not shown).

In the older specimens available for study, the endometrium beneath the implantation site was considerably thinner and the uterine glands were less prolific. Expansion of the conceptus had caused many of the glands to be displaced to the margins of the site, but their lumens were still full of secretions indicating continuing activity. Direct communications between the glands and the IVS could be observed up to 10 wk gestational age (Fig. 2a), but not in later specimens. However, it should be noted that in the later specimens the materno-fetal interface became more complex topographically, making tracing connections through serial sections considerably more difficult.

Transmission electron microscopy

Uterine glands were prominent in the decidua basalis at 6 wk gestational age. The epithelial cells were of irregular height, some being columnar and others cuboidal (Fig. 2b). The apical surfaces of the columnar cells bulged into the gland lumen, and while some areas possessed microvilli

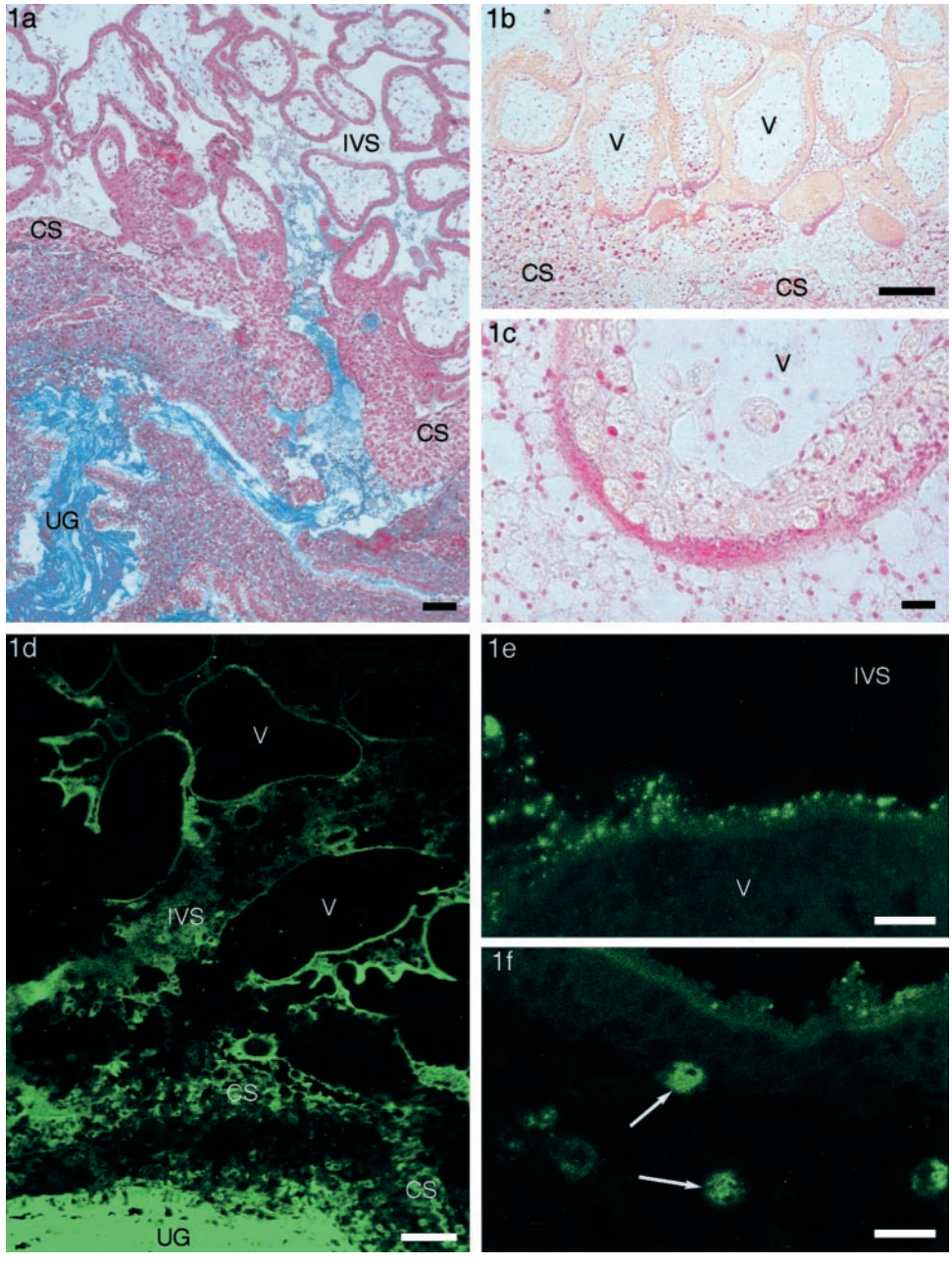

FIG. 1. a, Photomicrograph of a specimen (H710) at 43 d gestation showing secretions from a uterine gland (UG) entering the IVS and dispersing between the villi. The cytotrophoblastic shell (CS) at the materno-fetal interface is clearly visible. Alcian *blue/neutral red* stain. *Scale bar*, 100 μm. b, Photomicrograph from H710 illustrating arcs of PAS positive material within the syncytiotrophoblast of each villus (V) orientated toward the cytotrophoblastic shell (CS) and underlying endometrium. The intensity of staining is greatest near the endometrium but the arcs are still visible on more distant villi. PAS stain. *Scale bar*, 100 μm. c, Higher power photomicrograph of a villus (V) confirming that the PAS positive material is contained within the apical portion of the syncytiotrophoblast. The reactivity was lost after diastase treatment, indicating the material was glycogen. PAS stain. *Scale bar*, 10 μm. d, Confocal immunofluorescent image of a slide from H710 reacted with a fluorescently labeled lectin from *Wisteria floribunda* to detect glycodelin, a product of the uterine glands. The epithelium and lumen of the glands (UG) gives an intense signal, as does the cytotrophoblastic shell (CS). The secretions can be seen dispersing throughout the IVS where the fluorescence serves to outline the villi (V). *Scale bar*, 100 μm. e, Confocal immunofluorescent image of a slide from H710 reacted with a monoclonal antibody against MUC-1. Punctate fluorescence within the syncytiotrophoblast covering a villus (V) suggests vesicular uptake of this maternal glycoprotein from the IVS. *Scale bar*, 20 μm. f, Confocal image of a slide from H710 reacted with a fluorescently labeled lectin from *Wisteria floribunda* to detect glycodelin. The fluorescence is more uniform throughout the syncytiotrophoblast than for MUC-1, and the macrophages (*arrowed*) within the villous stromal core also show a strong signal. *Scale bar*, 20 μm.

other areas were smooth. Large quantities of glycogen were present within the cytoplasm of most epithelial cells, particularly in the apical region. Otherwise the gland cells contained considerable quantities of rough endoplasmic reticulum, Golgi bodies and numerous mitochondria.

Proteinaceous deposits filled the lumens of the glands, and dispersed among these were free glycogen particles, amorphous globular droplets and occasional cell debris.

Placental villi from the corresponding pregnancies displayed a profuse covering of microvilli on the apical membrane. The arching of microvilli over scallop-shaped depressions on the apical surface suggested phagocytosis from the IVS, and numerous vacuoles containing an amorphous material were present within the apical cytoplasm. These vacuoles were of similar size to the punctate sites of MUC-1 immunofluorescence (Fig. 1e). Glycogen deposits were conspicuous within the syncytioplasm at 6 wk but became less prominent in villi of 8 wk gestational age (Fig. 2c). Immunocytochemistry for MUC-1 on ultrathin, thawed, cryosections revealed specific gold-labeling in smaller vesicles within the syncytioplasm (Fig. 2d), but no labeling could be detected for glycodelin using either the lectin or the monoclonal antibody (data not shown).

Secondary yolk sacs

Immunolabeling for glycodelin was observed in the yolk sac at 8 wk gestational age. The most intense labeling was present within the thin mesothelial layer lining the exocoelom, where it was uniformly distributed throughout the cy-

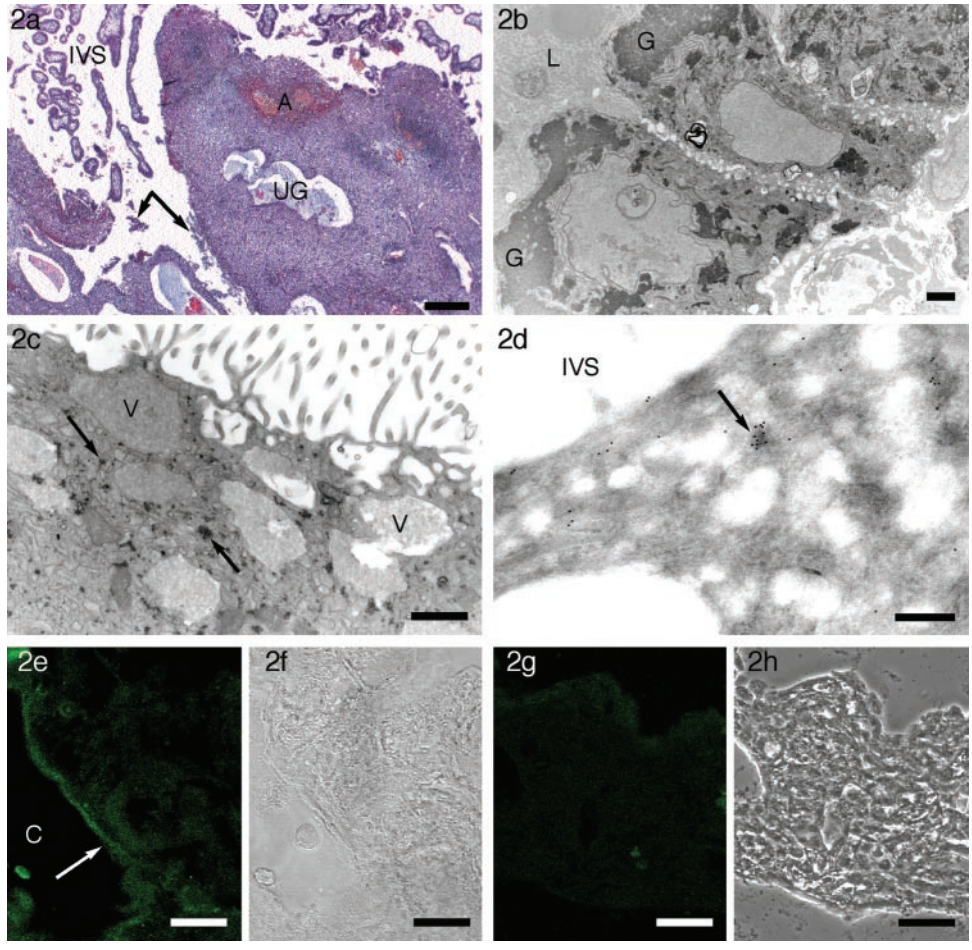

FIG. 2. a, Photomicrograph from H630 at 69 d gestational age. The uterine glands (UG) in the decidua basalis are less prominent than in H710, and are displaced to the margins of the implantation site. Their lumens still contain precipitated secretions, and similar material can be seen in the IVS close to the opening of a gland (*arrow*). Note that the distal portion of the nearby spiral artery (A) is undergoing conversion, but that the lumen is largely occluded by invading trophoblast cells. The full maternal circulation to the IVS has yet to be established in this placenta therefore. Masson's trichrome stain. *Scale bar*, 250 μm. b, Electron micrograph of uterine glandular epithelial cells at 6 wk of gestation. The cells contain large deposits of glycogen (G) in the apical cytoplasm. Glycogen particles are also present in the secretions within the lumen (L), along with lipid droplets and proteinaceous precipitates. *Scale bar*, 5 μm. c, Electron micrograph of the apical portion of the syncytiotrophoblast at 6 wk gestational age. Numerous vacuoles (V) containing an amorphous material are present, and the appearances suggest phagocytosis from the IVS. Free glycogen particles (*arrowed*) are conspicuous throughout the syncytioplasm. *Scale bar*, 10 μm. d, Electron micrograph of an ultrathin cryosection of a 6-wk placental villus reacted with a monoclonal antibody for MUC-1. Specific gold labeling over a vesicular structure (*arrowed*) confirms uptake of this maternal glycoprotein from the IVS by the syncytiotrophoblast. *Scale bar*, 250 nm. e, Confocal image of the yolk sac wall from an 8-wk pregnancy after reaction with a monoclonal antibody for glycodelin. There is strong and uniform fluorescence throughout the mesothelial layer (*arrowed*) lining the coelom (C). *Scale bar*, 50 μm. f, Phase-contrast image of the section shown in 2e. g, Confocal image of the negative control for the same yolk sac shown in 2e after replacement of the primary antibody with nonimmune serum. *Scale bar*, 40 μm. h, Phase-contrast image of the section shown in 2 g.

toplasm (Fig. 2, e and f). In contrast, the 12-wk samples displayed a more punctate immunolabeling within the epithelium (data not shown). No fluorescence was observed in controls where the primary antibody was replaced with nonimmune serum (Fig. 2, g and h), although autofluorescence was observed from the erythrocytes.

Discussion

The recent realization that the maternal circulation to the placenta is not fully established until 10–12 wk of gestation has necessitated a radical reappraisal of the nutrition and metabolism of the early human fetus. Although based on a small number of samples as placenta-*in situ* samples during early pregnancy are extremely rare, our results indicate that all the elements required for histiotrophic nutrition are in place until at least 10 wk of pregnancy. They therefore raise the possibility that the uterine glands may be a major source of nutrients for the fetus during the period of organogenesis. Such a recognition would realign the human with the majority of eutherian mammals.

The morphology of the endometrial glands has been studied extensively during the various phases of the menstrual cycle (13), and the accumulation of glycogen appears to be a prominent marker of secretory activity. Three days after the LH surge, glycogen begins to accumulate within the cytoplasm, initially in a subnuclear location but by 6 d large aggregations are seen in the apical regions of the cell. The mechanism of release of glycogen and the formation of the classical secretions

is not yet clear (14), but by d 23 of the normal cycle, the deposits begin to disperse and by d 24 only small isolated cytoplasmic aggregates are visible. Our findings indicate that for at least the first 6 wk of pregnancy the uterine glands resemble those during the luteal phase of the cycle, when progesterone concentrations are high. Glycogen particles are clearly present in the uterine secretions at this stage, and undoubtedly contribute to the PAS reactivity of the secretions in the archival sections.

Proteins are also a major component of the glandular secretions. Changes in the pattern of secretion during the normal menstrual cycle have been investigated by short-term incubation of tissue explants (15), or on the basis of acrylamide gel electrophoresis of minute quantities of *in vivo* secretions (16). Both the number and intensity of the bands increases during the postovulatory period, with the most dramatic changes occurring in the molecular weight range of 14K-18K. During early pregnancy, the secretory pattern appears to be an extension of that seen in the late luteal phase. Quantitatively the major protein is a dimeric glycoprotein referred to by various synonyms in the past, most commonly PP14 or α_2-PEG, but is now termed glycodelin A. This protein was first isolated from amniotic fluid, where concentrations peak at wk 10 of gestation, but *in situ* hybridization studies have confirmed the absence of the mRNA from fetal and placental tissues, localizing it instead to the endometrial glandular epithelium (17). Indeed, it was recognition of the maternal origin of this protein that led to the adoption of the name glycodelin in preference to the earlier synonym of Placental Protein 14. The gene encoding for glycodelin contains three putative progesterone regulatory elements, but interestingly secretion of the homolog in the baboon also appears to be responsive to chorionic gonadotrophin, raising the possibility of dual maternal and fetal control (17, 18). The function of the protein is still not clear, but its close sequence homology to β-lactoglobulins of various species suggests that it may act as a carrier protein for small hydrophobic molecules such as retinol or RA. However, no specific binding properties have been detected (12). MUC-1 is a large glycoprotein whose expression in the glandular epithelium is also progesterone dependent. In the normal cycle, secretion begins 3–4 d after the LH surge and continues into the late secretory phase (19). The immunofluorescent data presented here show that MUC-1 continues to be secreted during the first 6 wk of pregnancy.

Our results also show that glycogen, glycodelin A, and MUC-1 are all present within the IVS and the cytoplasm of the villous syncytiotrophoblast during the first trimester. Trophoblast is a highly phagocytic tissue capable of engulfing particles as large as maternal erythrocytes (20), and endocytic uptake of proteins is well recognized (21). Tracer studies have demonstrated that proteins absorbed nonspecifically in this manner pass into multivesicular bodies where they are presumably degraded to their constituent amino acids (22). These multivesicular bodies are of comparable size to the apical vacuoles and fluorescently labeled accumulations of MUC-1 observed within the syncytiotrophoblast in the present study. Breakdown of large glycoproteins such as MUC-1 would provide the developing feto-placental unit with a rich source of elements and amino acids to meet its synthetic requirements while obviating the need for energy-dependent specific transporter mechanisms. Such a mechanism would be functionally equivalent to that in the visceral yolk sac of the rat, where endocytic uptake of maternal proteins accounts for around 95% of amino acids incorporated into the embryo during organogenesis (23).

It is clear, therefore, that all the elements necessary for histiotrophic nutrition from the uterine glands are present in the human during the first trimester of pregnancy. The glandular secretions are delivered into the IVS, where it is possible they may be supplemented by secretions from the endometrial stromal cells or by a plasma filtrate emanating from the endometrial vasculature. Further biochemical analyses are required to confirm the composition of the intervillous fluid during the first trimester, but direct observations *in vivo* using a hysteroscope revealed it to be a clear liquid with no evidence of blood discoloration (5). This is consistent with our measurements *in vivo* demonstrating that the oxygen tension within the placenta is low during this period (9, 10), and that metabolism within the fetal and placental tissues is largely anaerobic (11). Experiments with villous explants *in vitro* have confirmed the importance of anaerobic pathways in placental metabolism during the first 9 wk (24), and the accumulations of glycogen within the syncytiotrophoblast illustrated here are consistent with this view. The first trimester of pregnancy coincides with the major phase of organogenesis in the human embryo. Many mammalian species rely on anaerobic glycolysis to meet their metabolic demands during this phase of development (25), and our results indicate that the human is no exception. This may confer benefits on the embryo by minimizing the risk of damage to DNA (26) or disruption of signaling pathways by reactive oxygen species generated during aerobic metabolism. These species are so reactive that many of their interactions are diffusion limited. Hence physiological levels of antioxidants cannot provide complete protection. Limiting production of these species by restricting the oxygen supply and providing nutrients via the histiotrophic pathway may provide the optimal environment for correct cell differentiation (27–29).

Although breakdown of maternal glycoproteins may occur in the syncytiotrophoblast the constituent amino acids and sugars still have to be transported to the fetus. The initial isolation of glycodelin from amniotic fluid indicates that some maternal products cross the trophoblast into the villous core, from where they may diffuse freely through the extracellular fluid into the exocoelomic and amniotic cavities. By contrast with most mammalian species, in primates, and in humans in particular, the secondary yolk sac floats within the exocoelomic cavity that lies between the placenta and the amniotic cavity (30). The secondary yolk sac is directly connected to the embryonic gut, and possesses a rich vascular plexus at an earlier stage of pregnancy than do the placental villi. Its absorptive role has been recently demonstrated, confirming that the exocoelomic cavity and the secondary yolk sac are a transfer interface between the extra-embryonic and embryonic compartments of the human gestational sac (31). Our findings demonstrate that the mesothelial epithelium can take up maternal glycodelin, and so lend support to the theory that the yolk sac has a nutritive role during early pregnancy. By 10 wk of gestation, however, it shows signs of

cellular degeneration (30). This may explain the change in pattern of immunolabeling for glycodelin observed, and may reflect the increasing importance of hemotrophic nutrition as the maternal circulation is established.

Uterine secretions may also perform an important role in feto-placental development besides acting as a source of nutrients. There is increasing evidence that they contain a number of cytokines, such as TNF-α (32), uteroglobin (33), epidermal growth factor, colony stimulating factor, and vascular endothelial growth factor. These are powerful regulators of trophoblast proliferation and migration *in vitro* (34, 35), and so may exert a profound influence on remodelling the uteroplacental arteries and in defining the ultimate form and extent of the villous tree. In addition, glycodelin is known to have many immunosuppressive properties and so could be an important factor in the maternal tolerance of the placenta (17, 36). Low concentrations of glycodelin in uterine flushings have been reported in cases of recurrent miscarriage (37), although it is not known whether the association is causal.

In conclusion, it is clear that the opportunity for hemotrophic nutrition of the fetus during the first trimester of pregnancy is limited due to the absence of significant blood flow through the IVS. The results presented here suggest that histiotrophic nutrition via the uterine glands is an alternative pathway. The glands also release cytokines that may exert a powerful influence on placental development. Uterine glands therefore play a more major role in early human pregnancy than previously recognized, and their malfunction could be factor in early pregnancy loss.

Acknowledgments

We are grateful to Prof. M. Seppälä, University of Helsinki, for the gift of the monoclonal antibody to glycodelin, and to Ms. Janet Powell for expert technical assistance.

Received December 5, 2001. Accepted February 21, 2001.

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This work was supported by grants from the Medical Research Council (G9701485) and WellBeing, London. The confocal imaging was performed in the MultiImaging Center of the School of Biological Sciences, which has been supported with grants from the Wellcome Trust (039398/Z/93/Z & 055203/Z98/Z/ST/RC).

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