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Localization of the iron transport glycoprotein, uteroferrin, in the porcine endometrium and placenta by using immunocolloidal gold*

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Summary. Uteroferrin, a glycoprotein implicated in transplacental iron transport in the pig, has been localized within the porcine uterus during mid pregnancy by using immunocolloidal gold labeling of sectioned material. In the endometrium where uteroferrin is synthesized, it was located exclusively in the non-ciliated cells of the glandular epithelium where it appeared to follow a common route of secretory glycoprotein from the cell (i.e., rough endoplasmic reticulum → Golgi → condensing vacuoles → secretory granules). Uteroferrin was present in the lumen of the glands and overlying placental areolae and in the large absorptive chorionic epithelial cells of the areolae. The latter cells contained numerous small vesicles and tubules as well as large endocytotic vacuoles. All these structures contained uteroferrin and are probably involved in translocating the glycoprotein in intact form from the uterine lumen to the blood capillaries draining the placenta.

Key words: Uteroferrin – Pig placenta – Iron transport – Immunogold

Introduction

Uteroferrin, an iron-containing glycoprotein with acid phosphatase activity (Schlosnagle et al. 1974; Roberts and Bazer 1980), is secreted by the pig uterus under the control of progesterone (Chen et al. 1973; Roberts and Bazer 1980). It is synthesized maximally during midpregnancy around Day 60 (Basha et al. 1979; Ducsay et al. 1982). Uteroferrin constitutes 10 to 15% of the protein content of uterine secretions (Roberts and Bazer 1980) and is thought to play a major role in the transplacental transport of iron from the maternal endometrium to the fetus (Buhi et al. 1982; Ducsay et al. 1982; Renegar et al. 1982; Roberts and Bazer 1980).

By using immunofluorescence techniques on frozen sections (Chen et al. 1973) and the peroxidase anti-peroxidase technique on sections of paraffin embedded material (Renegar et al. 1982; Fazleabas et al. 1984) it has been demonstrated that on the maternal side of the pregnant uterus uteroferrin is localized predominantly in the uterine glandular epithelium which is presumed to be its major site of synthesis. On the fetal side, the protein was associated with the areolae. The latter are specialized regions of the chorion which develop opposite the mounths of each of the uterine glands. They consist of extensively folded regions of chorionic epithelium the cells of which are engorged with large vacuoles (Friess et al. 1981). Renegar et al. (1982) have shown that uteroferrin was present within these cells and appeared to be localized within large cytoplasmic vacuoles. Acid phosphatase activity, possibly indicative of uteroferrin, has been shown to be present in the secretory granules of the glandular epithelium of the maternal endometrium (Sinowatz and Friess 1983) and in the cavity between the mouth of the glands and the overlying areolae (Friess et al. 1978). Using autoradiographic techniques, Palludan et al. (1969) have shown that following administration of ^{59}Fe to the mother the iron accumulates in the uterine glands and within the areolae. Wislocki and Dempsey (1946), using histochemical procedures, had earlier demonstrated the localization of non-heme iron in these same regions and, like Brambell (1933), considered the areolae to be sites of transplacental nutrient transport.

Despite the extensive ultrastructural and histochemical studies that have been made of the fetal-placental unit of the pig (Bjorkman 1965; Bjorkman 1970; Bjorkman 1973; Crombie 1972; Dantzer 1982; Dantzer et al. 1981; Dempsey et al. 1955; Friess et al. 1980; Friess et al. 1981; Perry and Crombie 1982; Sinowatz and Friess 1983) and the evidence that areolae are sites of non-heme iron accumulation, little is known about the fine structural localization of uteroferrin, the proposed iron transporter. The present paper provides a detailed study on the distribution of uteroferrin in the uterine glands and placental areolae by means of immunogold staining of thin-sectioned material.

Materials and methods

Tissue preparation

Uterine endometrium with apposed chorioallantois from interareolar and areolar regions was excised from pregnant gilts on Days 60 and 68, respectively. Tissues were cut into 1–2 mm^3 pieces and fixed for 2 h at 25° C by immersion in 1.5% (v/v) glutaraldehyde buffered in 0.1 M sodium ca-

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codylate, pH 7.4 or 4% (w/v) paraformaldehyde and 0.5% (v/v) glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.4 for 2 h at 25° C followed by 4% (w/v) paraformaldehyde in 0.1 M sodium phosphate buffer, pH 10.4 overnight at 4° C (Eldred et al. 1983). Samples were washed, dehydrated through a graded series of ethanol with two changes in acetone, and embedded in Epon 812 (Fisher Sci., lot # 721281, prepared by Shell Oil Co.). Poly/Bed (Polysciences, Inc) 812 was unsuitable since it consistently resulted in excessive background labeling. Sections approximately 70 nm thick were cut with a diamond knife, mounted on 300 mesh nickel grids, and processed for immunocytochemistry.

Antiserum

Purification of uteroferrin is described by Roberts and Bazer (1980). Immunization of rabbits and immunoaffinity purification of the antiserum by using uteroferrin coupled to CNBr-activated Sepharose CL-4B is described by Baumbach et al. (1984). Both whole antiserum, diluted 1:100, and immunoaffinity-purified anti-uteroferrin immunoglobulin, diluted to 50 μg/ml, were used for the immunolocalization of uteroferrin in sectioned material.

Control experiments included the use of preimmune serum diluted 1:100 instead of specific antiserum. Using the immunoperoxidase technique, Fazleabas et al. (1984) further demonstrated specificity by preabsorbing the antiserum with an excess amount of purified uteroferrin.

Preparation of protein A-gold complex

Colloidal gold approximately 16 nm in diameter was prepared by sodium citrate reduction according to Frens (1973). After adjusting the colloid to pH 7 with 0.1 M K_2CO_3, the amount of protein A required for stabilization of the gold particles was determined by the method of Horisberger et al. (1975) with modifications by Roth and Binder (1978). After centrifugation at 27,000 × g for 30 min, protein A-gold complex was washed once and resuspended at one tenth the original volume in 0.02 M phosphate buffer, pH 7.2 containing 0.5 mg/ml polyethylene glycol (MW 20,000) and 0.02% (w/v) sodium azide and stored at 4° C.

Immunocytochemical labelling

Mounted sections were treated by floating the grids on drops of solutions at 25° C. Sections were incubated in saturated aqueous sodium meta-periodate (Bendayan and Zollinger 1982) for 60 min, rinsed in several changes of distilled water, and preincubated for 30 min in phosphate buffered saline (PBS; 0.14 M NaCl, 1.5 mM KH_2PO_4; 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, pH 7.4), containing 10% normal sheep serum (NSS). Without prior rinses, sections were incubated for 60 min in a 1:100 dilution of anti-uteroferrin antiserum or preimmune serum in PBS/NSS. Following several changes in PBS and PBS/NSS, sections were incubated for 60 min in a 1:10 dilution of protein A-gold in PBS, rinsed by a stream of distilled water, poststained with uranyl acetate and lead citrate (Reynolds 1963), and examined and photographed with a JEOL JEM-100CX electron microscope operated at 80 kV.

Results

The uterine gland

The fine structure of the glandular epithelial cells was well preserved with 1.5% (w/v) glutaraldehyde as a fixative. Numerous uterine glands displaying a morphology typical of mid pregnancy (Sinowatz and Friess 1983) were located within sections through Day 60 endometrial tissue (Fig. 1). Uteroferrin, as detected by the presence of protein A-gold conjugates following exposure of the sections to anti-uteroferrin, was confined to the columnar, non-ciliated epithelial

Fig. 1. Porcine maternal-fetal placental complex, day 60 of pregnancy. Light micrograph of a thick section (1 μm) showing choirioallantois (*CA*), uterine epithelium (*UE*), maternal capillaries (*C*), and uterine glands (*gl*) with material-filled lumina (*L*). Bar = 2 μm

Figs. 2–6. Porcine uterine gland, day 60 of pregnancy. Thin sections through non-ciliated glandular epithelial cells that were treated with anti-uteroferrin antiserum followed by protein A-gold conjugates. Gold particles indicate the localization of immunoreactive uteroferrin

Fig. 2. Actively secreting, columnar epithelial cell containing whorled, rough endoplasmic reticulum (*rER*), a centrally located nucleus (*N*), and extensive Golgi complex (*G*). Note microvilli (*mv*) projecting into the lumen (*L*). Bar = 5 μm

Fig. 3. Uteroferrin is localized over cisternae of the rough endoplasmic reticulum (*arrows*). Bar = 0.5 μm

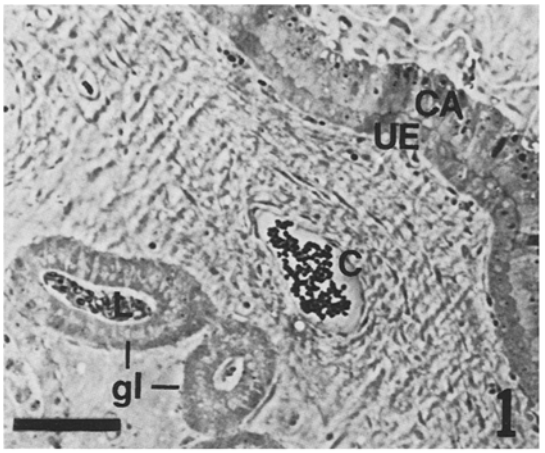
Fig. 4. Uteroferrin is specifically associated with dense material appearing in condensing vacuoles (*arrows*) that are peripheral to the Golgi cisternae (*Gc*). Bar = 1 μm

Fig. 5. Within the supranuclear cytoplasm are numerous secretory granules that contain uteroferrin. *L* lumen; *PM* plasma membrane. Bar = 1 μm

Fig. 6. These secretory granules (*SG*) appear to discharge their contents into the glandular lumen (*L*) or are occasionally present intact within the lumen (*arrow*). *mv* microvilli. Bar = 1 μm

Fig. 7. Section through the Golgi complex of a day 60 uterine glandular epithelial cell treated with preimmune serum and protein A-gold conjugates. Bar = 1 μm

Fig. 8. Control section as in Fig. 7 showing secretory granules (*SG*) within the apical portion of the cytoplasm. Note the glandular lumen (*L*) containing cross-sections through cilia. Bar = 1 μm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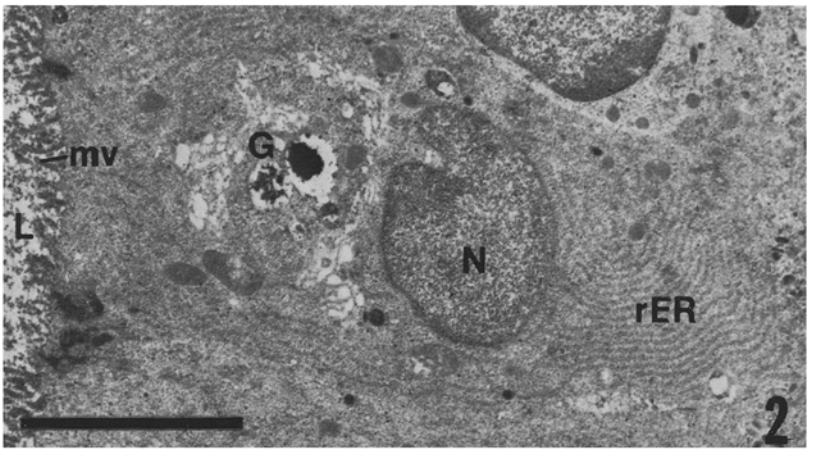
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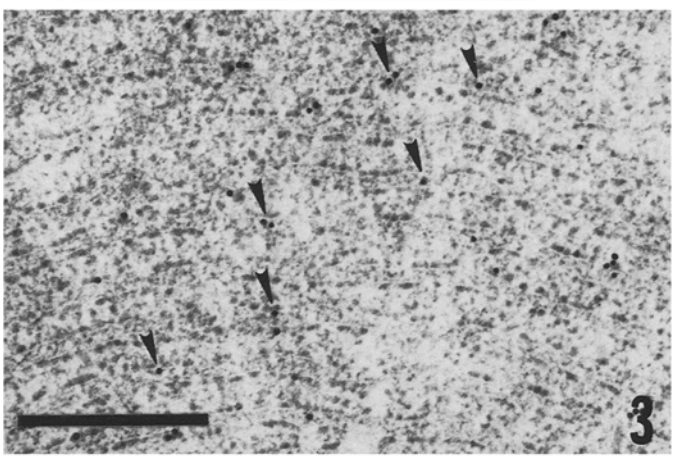
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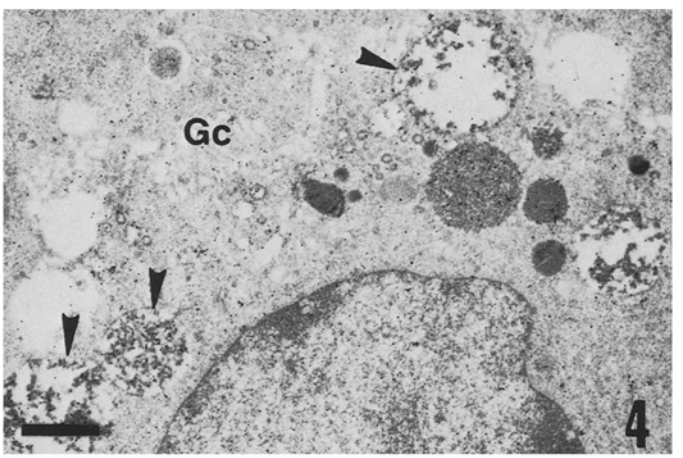
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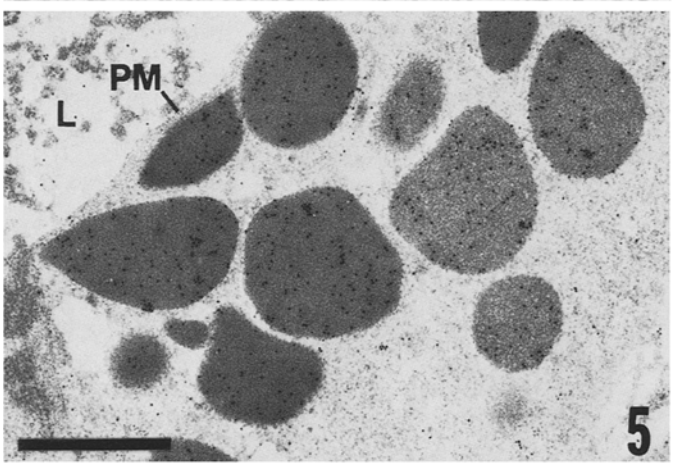
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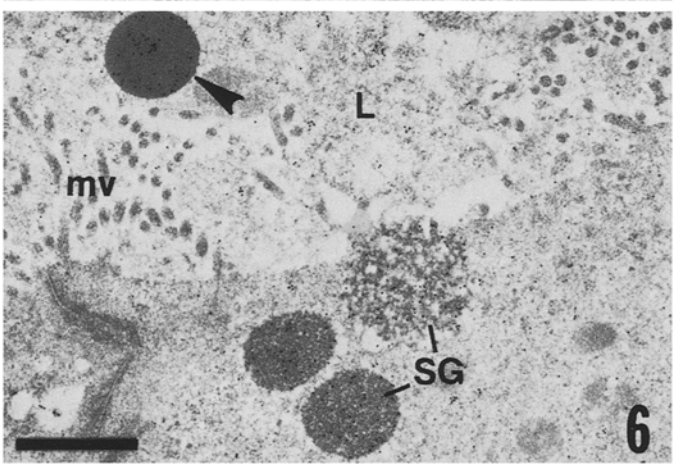
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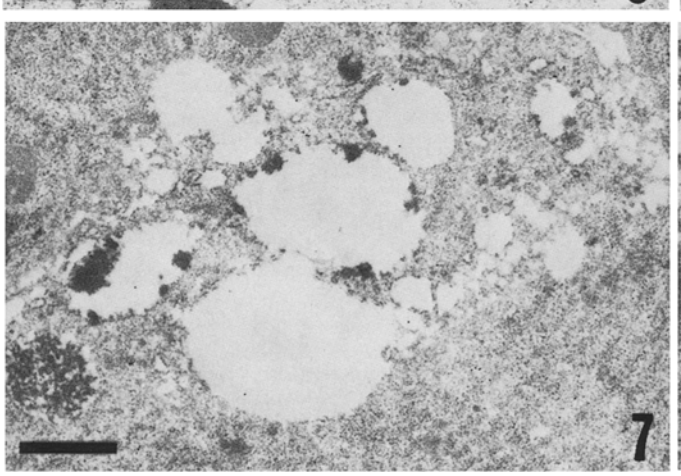
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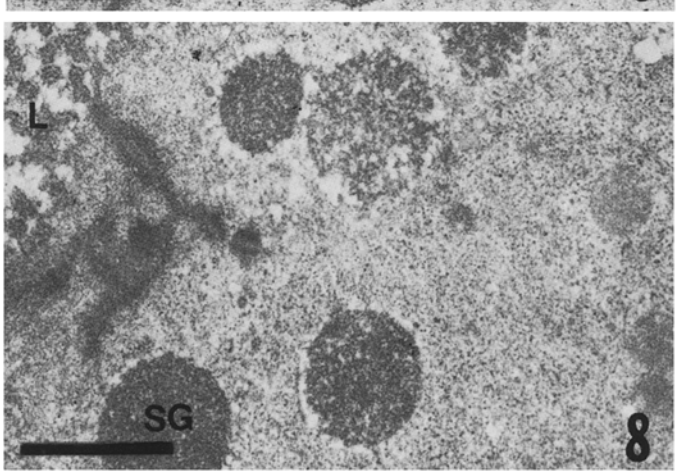
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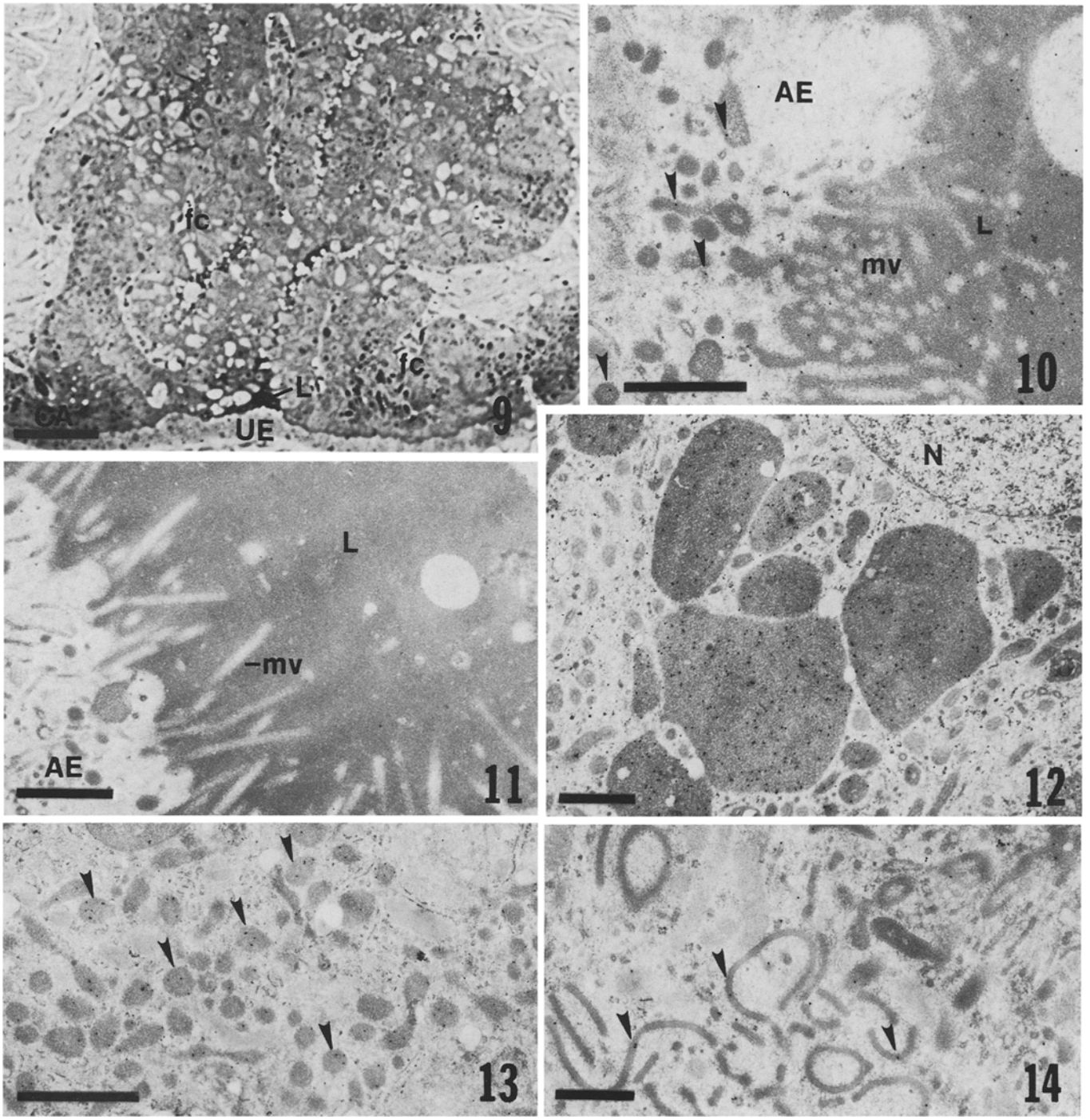

Fig. 9. Light micrograph of a thick section (1 μm) giving a general view of an areola, day 68 of pregnancy, in cross section. Organization is complex due to infolding of the chorioallantoic epithelium (*CA*). Shown are the uterine epithelium (*UE*), areolar lumen (*L*) containing an electron-dense histotroph, and fetal capillaries (*fc*). Bar = 20 μm

Figs. 10–14. Porcine areola, day 68 of pregnancy. Thin sections were stained for uteroferrin using anti-uteroferrin antiserum followed by protein A-gold conjugates

Fig. 10. High magnification of the apical portion of an areolar epithelial cell (*AE*). Note the histotroph in the areolar lumen (*L*) and in numerous vesicles within the cytoplasm (*arrows*) that contain uteroferrin. Bar = 1 μm

Fig. 11. Control section through an areolar epithelial cell (*AE*) as in Fig. 10 that has been treated with preimmune serum prior to protein A-gold conjugates. *mv* microvilli; *L* lumen. Bar = 1 μm

Fig. 12. Large endocytotic vacuoles of varying sizes are located within the supranuclear cytoplasm of areolar epithelial cells. These are filled with an electron-dense material that contains uteroferrin. *N* nucleus. Bar = 1 μM

Fig. 13. Distributed throughout the cytoplasm of areolar epithelial cells are small, smooth-membraned vesicles (*arrows*) that contain uteroferrin. Bar = 1 μm

Fig. 14. Also within the areolar epithelial cells is a system of smooth-membraned tubules (*arrows*) that contain uteroferrin-positive, electron-dense material. These tubules are present within the supranuclear cytoplasm. Bar = 1 μm

cells lining the glandular lumen (Fig. 2). These cells possessed an organization typical of cells active in secretion (Reviewed in Farquhar and Palade 1981). Extensive regions of rough endoplasmic reticulum, a well-developed, perinuclear Golgi complex and large secretory granules were evident.

Gold particles were specifically associated with the rough endoplasmic reticulum within the infranuclear cytoplasm (Fig. 3). Lysosome-like bodies in this region did not contain immunoreactive uteroferrin. Although the Golgi cisternae were not well preserved and slightly inflated, gold particles were associated with these structures (Fig. 4). The condensing vacuoles associated with the Golgi complex also contained uteroferrin. These vacuoles varied considerably in their content of electron-dense material (Fig. 4).

Most of the large granules in the apical portion of the cytoplasm were heavily labeled (Figs. 5, 6). In some instances, granules either in the process of disgorging their contents or themselves being released from the cells were evident. Occasionally whole granules apparently free in the lumen of the gland, were noted (Fig. 6).

Control sections treated with preimmune rabbit serum (Figs. 7, 8, 11) contained only a few gold particles that were randomly and evenly scattered over the sections.

The areola

Immunocytochemical localization of uteroferrin was performed on thin sections through a Day 68 areola as shown in Fig. 9. The areolae at these stages of pregnancy are more developed that at Day 30 (Friess et al. 1981), and have an extensive surface area owing to the development of numerous folds. They have a complex convoluted appearance in cross-section. Immunoreactive uteroferrin was confined to the epithelial cells lining the areola and was not observed over chorionic epithelial cells lateral to the areola.

The lumen of the areola, which has an electron-dense appearance, contained uteroferrin (Fig. 10). The cells of the areola possessed large endocytic vacuoles, (Fig. 12), numerous small vesicles (Fig. 13), and long tubular structures (Fig. 14). Each of these were filled with material of an electron density similar to that of the areolar lumen. All these structures were labeled with gold particles. The large vacuoles and tubules were localized predominantly in a supranuclear region while the more basal regions were more densely occupied by the vesicles.

An extensive, fenestrated capillary bed is found beneath the basal lamina of the chorionic epithelium. Gold label was not observed over the endothelial cells lining these capillaries.

Discussion

Our results agree with earlier immunohistochemical studies (Chen et al. 1975; Renegar et al. 1982) and suggest that the primary sites of uteroferrin synthesis in the porcine uterus are the non-ciliated epithelial cells lining the uterine glands. The surface epithelium does not appear to be involved in uteroferrin production. Within the glandular epithelium, label was associated with the rough endoplasmic reticulum, with the Golgi complex and its associated condensing vacuoles, and with large secretory granules in the supranuclear cytoplasm. The results are consistent with the view that uteroferrin production follows an endomembrane sequence typical of secretions from other exocrine cells (e.g., those of the pancreas and parotid glands) namely ER → Golgi → secretory vesicles. The so-called transfer tubules noted in uterine epithelial cells by Dantzer (1982) were not obviously labeled and may not be involved in the secretion process. Whether secretion is a continuous process or is triggered intermittently is not known. Recent results have suggested that estrogens released by the conceptus may promote the release of uterine secretions from secretory granules during early pregnancy (Geisert et al. 1982). Whether estrogens trigger secretory activity during the later stages of pregnancy is not known.

Uteroferrin is clearly a part of the electron-dense luminal material or histotroph which has been described earlier in glands and areolae of pregnant animals by Sinowatz and Friess (1983). These secretions have also been shown to have acid phosphatase activity (Friess et al. 1978; Sinowatz and Friess 1983) and to contain non-heme iron (Friess et al. 1981).

The porcine placenta is of the diffuse, epitheliochorial type in which there is no close contact betwen the chorion and the maternal blood supply (Amoroso 1952). Indeed, the uterine epithelium is not eroded throughout pregnancy. Areolae have been proposed as the major sites for macromolecular transfer of nutrients from the mother to the conceptus (see Amoroso 1952; Brambell 1933). The present study reinforces the hypothesis proposed by others (Chen et al. 1975; Friess et al. 1981; Renegar et al. 1982) that these structures are involved in the uptake of uteroferrin released by the uterine glands. Uteroferrin is the major iron-containing protein of uterine secretions of the pig (Roberts and Bazer 1980) and at least until Day 75 of pregnancy is produced in sufficient quantities to provide for the iron requirement of the developing conceptus (Buhi et al. 1982; Ducsay et al. 1982). Renegar et al. (1982) have detected uteroferrin in the fetal placental drainage, and uptake and metabolism of uteroferrin by the fetal liver and the rapid incorporation of its iron into fetal hemoglobin has been demonstrated (Buhi et al. 1982). Our studies reinforce the view that the cells of the areolae are the major sites of uteroferrin transport across the placenta and that the accumulation of iron in the areolae (Wislocki and Dempsey 1946; Palludan et al. 1969) is most likely due to the presence of this protein. Uteroferrin was associated with vacuoles within the supranuclear cytoplasm. These presumably resulted from endocytosis of material from the areolar lumen. An elaborate tubular system found in these specialized epithelial chorionic cells also contained uteroferrin. Dantzer (1982) has proposed that tubules are involved in the direct transfer of maternal secretions across this cell layer. Similar structures are found in the yolk sac endoderm of several animal species (King and Enders 1970; Moxon et al. 1976; Mobbs and McMillan 1981) and have been implicated in the transfer of IgG across the human syncytial trophoblast (Lin 1980). The results presented in this paper are consistent with such a hypothesis.

However, the route by which these cells transfer their contents to the underlying capillaries is unclear. Label was not observed over the capillary endothelial cells, and we assume, therefore, that the uteroferrin did not pass through these structures. Since the capillaries beneath the chorion are fenestrated it seems more likely that uteroferrin, like hepatocyte secretory product, is released directly into the capillary lumen. Renegar et al. (1982) have detected utero-

ferrin in the umbilical vein of the placenta. Much of the protein is then cleared by the fetal liver and kidney (Renegar et al. 1982) and its iron used in erythropoiesis (Buhi et al. 1982).

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