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Uterine Glands of the Pig During Pregnancy

An Ultrastructural and Cytochemical Study

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Summary. The ultrastructure of the porcine uterine glands is described from material taken from 11 pregnant pigs at exactly known stages of gestation (day 30; 58; 80; 100; 110). Fixation was performed by perfusion via a branch of the uterine artery and the tissue was routinely processed for electron microscopy. Additionally, cytochemical studies (phosphotungstic acid reaction for glycoproteins, according to Rambourg 1967; acid phosphatase reaction; ultrastructural localization of cellular iron, according to Parmley et al. 1978) were performed.

On day 30 of pregnancy the uterine glands are coiled, simple tubular glands with a narrow lumen. The epithelial lining is simple columnar and consists basically of two cell types, ciliated cells and secretory cells. The secretory activity of the glandular epithelium is low; only a few secretory granules are present in the supranuclear cytoplasm.

At midpregnancy the ultrastructure of the glands has significantly changed and the cells now show all the characteristics of high secretory activity: numerous parallel cisternae of rough endoplasmic reticulum, an extensively developed Golgi apparatus and many secretory granules which give a positive reaction for acid phosphatase and glycoproteins. The lumina of the glands are significantly enlarged and filled with a great amount of a granular, acid phosphatase-positive material.

In the last third of pregnancy, only minor changes in the ultrastructure of the uterine glands are observed. The secretory activity is still high. The amount of rough endoplasmic reticulum has further increased and parallel arrays of cisternae occupy a considerable part of the supranuclear cytoplasm. The importance of the uterine secretion for embryonic nutrition and development is only partly understood. One of the secreted glycoproteins, uteroferrin, is believed to play an important role in the iron transfer from mother to fetus.

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From midpregnancy onward, a special cell type, the "granule laden cell" is found scattered between normal secretory cells of the uterine glands. Contrary to the opinion of Perry and Cromby (1982), we could demonstrate that these cells frequently extend to the lumen of the gland; hence the term "basal cell" seems inappropriate for this cell type.

Key words: Uterine glands – Pig – Ultrastructure – Cytochemistry

Introduction

In the mature, nonpregnant pig, the uterine glands are simple, slightly branched tubular glands (Amoroso 1952) which show characteristic changes of their morphology during different phases of the sexual cycle (Fabian 1960; Kuschma 1980). Using morphological and histochemical criteria, three distinct parts of the uterine glands can be distinguished (Kuschma 1980): the neck, which possesses a columnar ciliated epithelium similar to the uterine surface epithelium; a middle part, which comprises most of the gland and where the cyclic changes are pronounced, and a basal part which is adjacent to the myometrium.

During metestrus the uterine glands become gradually more coiled. The nuclei of the glandular epithelial cells are situated in the basal area of the cytoplasm. In the early days of diestrus, the neck and the middle part of the uterine glands start to secrete and at the end of diestrus a marked secretory activity can also be observed in the distal part of the glands. The onset of secretory activity in the glandular epithelium is accompanied by an apical shift of the nuclei (Kuschma 1980). In preestrus, with declining secretory processes the nuclei return to their basal position.

The height of the glandular epithelium reaches its maximum during metestrus and gradually decreases towards preestrus. From preestrus to metestrus the glandular epithelium gradually increases again. Contrary to numerous physiological and biochemical studies dealing with various aspects of the secretions of uterine glands (literature reviewed by Bazer 1975) and several communications describing some histological and histochemical features of the uterine glands (Brambel 1933; Wislocki and Dempsey 1946; Amoroso 1952; Christie 1968; Kuschma 1980), only one ultrastructural investigation using immersion fixation (Perry and Crombie 1982) has been performed. Preserving the structure of biological tissue by immersion in chemical fixatives gives satisfactory results in many instances. However, the method also has some inherent limitations; for example, delayed fixation and distortion of the normal structure to give the appearance of "apocrine secretion" (Nicander et al. 1974; Björkman et al. 1981). Due to the size of the porcine uterus it is difficult to properly exploit the benefits of perfusion fixation (Björkman et al. 1981). Recently EM-studies of the porcine placenta using local vascular perfusion (Friess et al. 1980, 1981) and the technique of perfusion fixation of the porcine placenta were described in great detail by Björkman et al. (1981). The aim of our study was to investigate the morphological changes in the fine structure of the uterine glands

at exactly known stages of pregnancy. Further it was sought, using cytochemical techniques, we wanted to correlate biochemical data on uterine secretions (Bazer 1975) with the morphological and cytochemical changes of the uterine glands during gestation.

Material and Methods

The material used in this investigation comprised the placentae of 11 sows (German landrace) removed on day 30 (two animals), day 58 (two animals), day 80 (two animals), day 100 (three animals) and day 110 (two animals). For fixation the uterus was extirpated as in Caesarian section under deep thiopental anesthesia. Fixation was then performed as described in a previous paper (Friess et al. 1980). The fixation solution consisted of 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 and 0.2 M Dextran 40. After perfusion-fixation strips of tissue were cut from different sites of the uterus and postfixed for two h in a fixation solution of the same composition. The specimens were washed overnight in 0.1 M cacodylate buffer (pH 7.4) and embedded in glycol-methacrylate (GMA) according to Leduc and Bernhard (1967), or postfixed in 1% cacodylate buffered osmium tetroxide (pH 7.4), dehydrated in a graded series of ethanol and embedded in an Epon/Araldite muxture (Texas).

Staining of the Sections

Ultrathin sections of the tissue embedded in Epon/Araldite were routinely stained with uranyl acetate and lead citrate (Reynolds 1963).

Rambourg Technique for Glycoproteins (Rambourg 1967)

Silver sections from glycol-methacrylate embedded uterine material were collected on nickel or gold grids and floated on a solution of 1% phosphotungstic acid (PTA) in 10% chromic acid (CrA), pH 0.3 for 1–5 min. Following staining, the sections were rinsed briefly in double-distilled water. For further characterization of the PTA-positive material, sections were also stained in PTA-solutions adjusted with 1 N NaOH to various pH-values (pH 0.5; 1.0; 1.5; 2.0; 2.5). Sections of GMA-embedded material were also floated on 1% PTA in 1 N hydrochloric acid for 1 min or less (Flechon 1974). The sections were then either washed with 1.25 N hydrochloric acid to avoid a rise in pH or immediately dried with the aid of a filter paper. Additionally, the following enzymatic digestion experiments were performed on thin sections of GMA-embedded material:

Neuraminidase (α neuraminidase ex Cl. perfringens, Sigma type V); 0.5% solution in double-distilled water at pH 5.0 for 3 h at 37° C.

Pepsin (Serva, 30 Anson units/mg); 0.1% solution in 0.2 M HCl. Incubation: 60 s at 37° C.

Pronase P (Serva, ca. 45,000 PUK-E/g); 0.5% solution in double-distilled water, pH 7.4; Incubation: 60 s at 37° C.

Trypsin (Seromed, 1:250); Incubation: 60 s at 37° C, pH 8.0.

After thoroughly rinsing with double-distilled water, sections were stained with 1% PTA in 10% CrA at pH 0.3 for 2 min.

For controls of the enzymatic digestion experiments alternate sections were floated for the same length of time on distilled water at the appropriate pH. Ultrastructural localization of nonheme iron with ferrocyanide (according to Parmley et al. 1978).

After fixation and rinsing, 40 µm thick sections were cut using a Lancer vibratome. These sections were incubated for 30 min at room temperature in fresh Perls solution (Perls 1867) prepared by dissolving 500 mg of potassium ferrocyanide in 49.5 ml distilled water and immediately adding 0.5 ml of concentrated HCl. After staining, the specimens were rinsed in 0.1 M cacodylate buffer (pH 7.4) containing 7% sucrose. All specimens were then dehydrated in a graded series of ethanol and propylene oxide and embedded in an Araldite/Epon mixture.

Acid Phosphatase

Acid phosphatase was demonstrated according to Barka and Anderson (1962). 40 µm sections were cut with a Lancer vibratome and were incubated in a medium containing 1.25% β-glycerophosphate, 0.2% lead nitrate in 0.1 M Tris maleate buffer pH 5.0. To inhibit alkaline phosphatase, 1 mM levamisol was added to the medium. Incubation was performed at 37° C. After incubation the 40 µm sections were postfixed in 1% osmium tetroxide (in 0.1 M cacodylate buffer, pH 7.4) for 30 min, dehydrated in a graded series of ethanol and embedded in Araldite.

Electron micrographs were taken using a Zeiss EM 10 A electron microscope.

Results

On day 30 of pregnancy the uterine glands are coiled, simple tubular glands with a narrow lumen. Branching is only occasionally observed.

The epithelial lining is simple columnar and consists basically of two cell types: ciliated cells and nonciliated secretory cells. The gland can be subdivided into three morphological by different parts; in accordance with topography. The neck of the gland, where the glandular epithelium merges with the general lining of the uterine surface epithelium, contains a high number of ciliated cells which show no significant changes of their morphology during the different stages of pregnancy. In the middle part of the gland the epithelium is also high columnar but ciliated cells are only rarely seen. In the distal part of the gland which is adjacent to the myometrium, epithelial height appears somewhat reduced. The glandular epithelium is isoprismatic or low columnar. Ciliated cells are only occasionally seen. As in the glandular neck, the cyclic changes of cell morphology during pregnancy are not so pronounced.

Fine Structure of the Ciliated Cells

The ciliated cells are wedge-shaped with a tapering basal part. The apical border carries cilia of typical structure, comprising of $9 \times 2 \times 2$ microtubules, a ciliary shaft, and a basal body with cross-striated rootlets. Slender microvilli are interspersed between the cilia. The lateral cell membranes run comparatively straight and are closely apposed at their luminal end to those of adjacent cells by means of extensive junctional complexes. The large oval nucleus which is rich in euchromatin, is situated in the basal half of the cell (Fig. 1). Occasionally it contains a single nucleolus. Oval or elongated mitochondria are usually concentrated in the apical cytoplasm adjacent to the ciliary rootlets. The Golgi-apparatus is situated supranuclearly and consists of several dictyosomes.

Secretory Cells

The nonciliated glandular cells (Fig. 1) which predominate in the middle and distal part of the glands are columnar and often pyramidal in shape. Along the luminal surface closely packed microvilli extend into the narrow glandular lumen which contains only little secretory material. In the apical two-thirds of the cells the lateral plasma membranes are comparatively straight. Adjacent cells are apically attached by extensive junctional com-

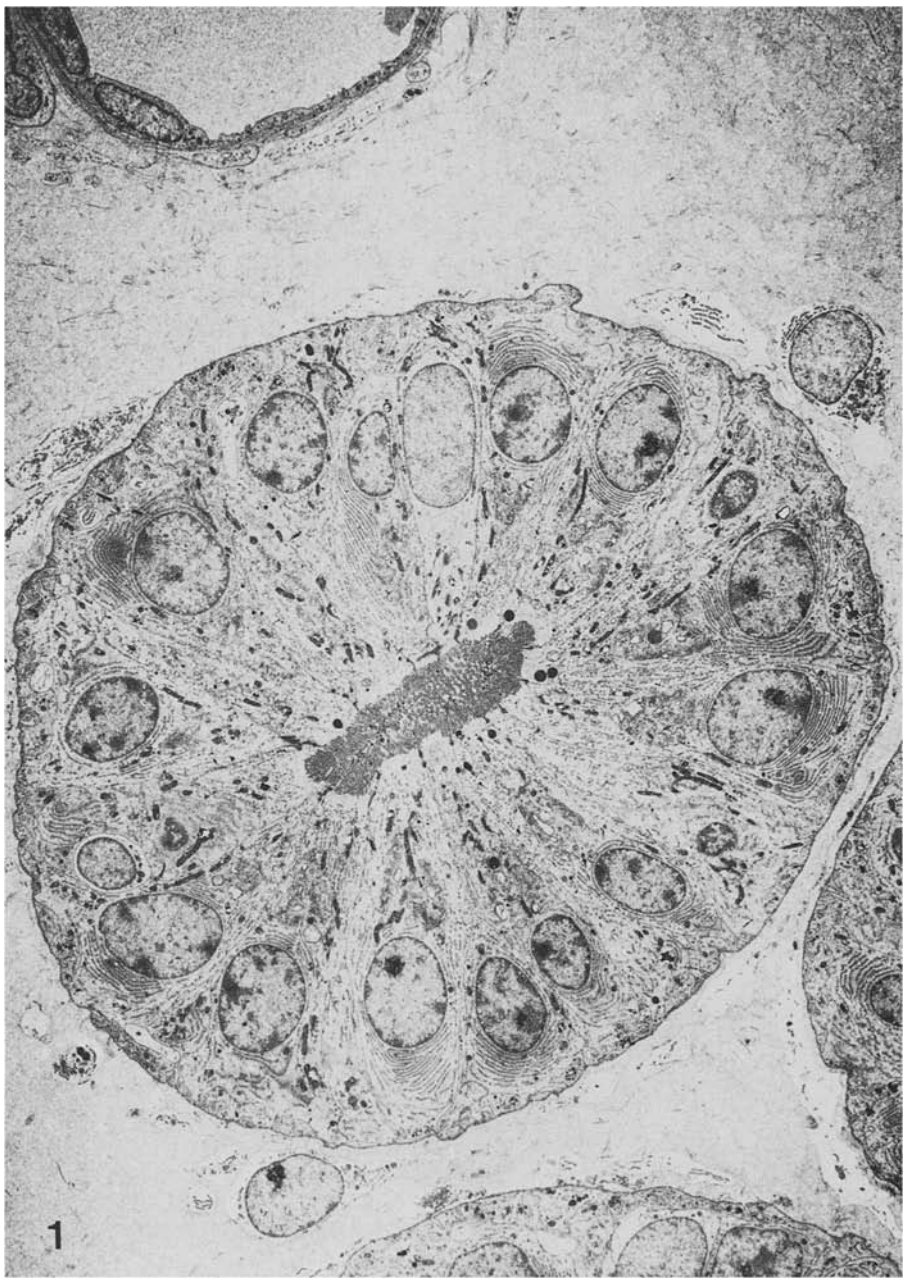


Fig. 1. Porcine uterine gland, day 30 of pregnancy. ×2,800

plexes and more basally by interspaced desmosomes. The basal third of the lateral plasma membrane as well as the basal cell membrane exhibit a high degree of infolding. In the apical part of the cells, just beneath the microvilli, a terminal web region containing assorted vesicles can be observed. An extensive Golgi-complex is found supranuclearly; it is composed of several stacks of cisternae and numerous surrounding vesicles. A few secretory granules are seen adjacent to the Golgi complex. The spherical nucleus is located basally. It sometimes contains one or two nucleoli and is comparatively rich in heterochromatin. Elongated and sometimes branching mitochondria of the cristae type are scattered throughout the cytoplasma. Around the nucleus several long and concentrically arranged cisternae of rough endoplasmic reticulum occur. A great number of cisternae are also seen in the cytoplasm adjacent to the lateral cell membranes.

Day 58 of Pregnancy

On day 58 (Fig. 2) the glandular lumen appears significantly enlarged. The lumen in the neck region and in the middle part of the gland is filled with a great amount of moderately electron-dense material; the basal part contains less secretory material. Whereas the number and ultrastructure of the ciliated cells is similar to the situation on day 30, several marked changes can be observed in the nonciliated secretory cells in the later stages of pregnancy. In the supranuclear and apical cytoplasm a varying number of secretory granules in different stages of condensation can be observed. They are derived from immature granules containing an electrolucent, flocculent material. The extensive Golgi complex consists of several stacks of dilated cisternae and associated vesicles. The infranuclear cytoplasm is mainly occupied by numerous cisternae of rough endoplasmic reticulum which show a parallel or concentric arrangement. Near to the basal lamina lysosome-like granules occur in varying numbers. Due to the great amount of rough endoplasmic reticulum, the nuclei of the glandular cells on day 58 assume a more luminal position compared to day 30 and are usually situated in the middle of the cells. They are light and rich in euchromatin. They frequently posses one or two pronounced nucleoli. The oval or elongated mitochondria of the cristae-type are more or less equally distributed within the cytoplasm. In some cells clusters of mitochondria are seen in the supranuclear cytoplasm.

The apical cytoplasm also contains small coated and uncoated vesicles interspersed among short cisternae of smooth endoplasmic reticulum. Quite frequently, parallel arrays of rough endoplasmic reticulum are seen in the apical portion of the cell.

Day 110 of Pregnancy

In the last third of pregnancy only minor changes in the ultrastructure of the uterine gland cells can be observed (Figs. 3 and 4). The amount

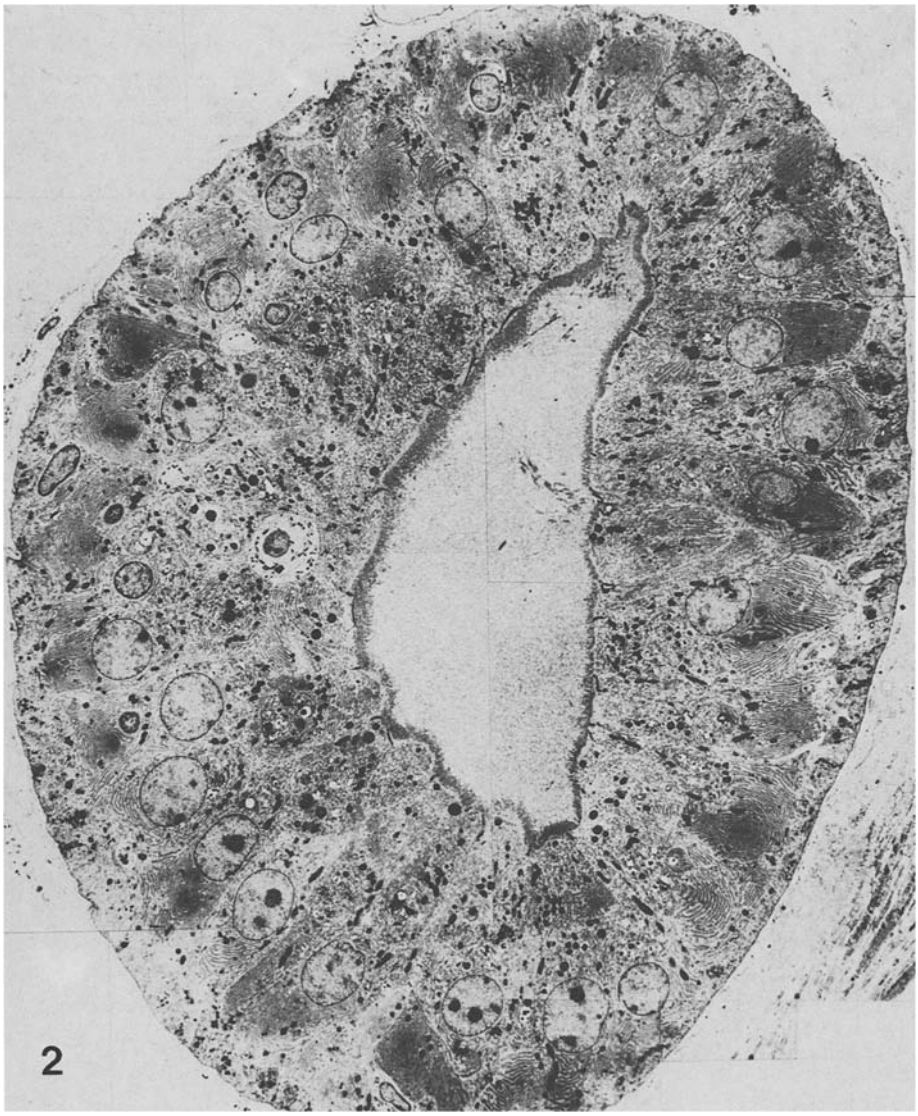


Fig. 2. Porcine uterine gland, day 58 of pregnancy. On day 58 the glandular lumen has significantly enlarged and is filled with a moderately electron dense material. ×1,400

of rough endoplasmic reticulum has further increased. It now also occupies a large proportion of the supranuclear cytoplasm. This causes a luminal shift of the Golgi complex, which is of similar complexity as on day 58. The number of secretory granules in the apical cytoplasm is also comparable to day 58. Sometimes gland cells which are almost filled with secretory granules (Fig. 5) are found between normal epithelial cells.

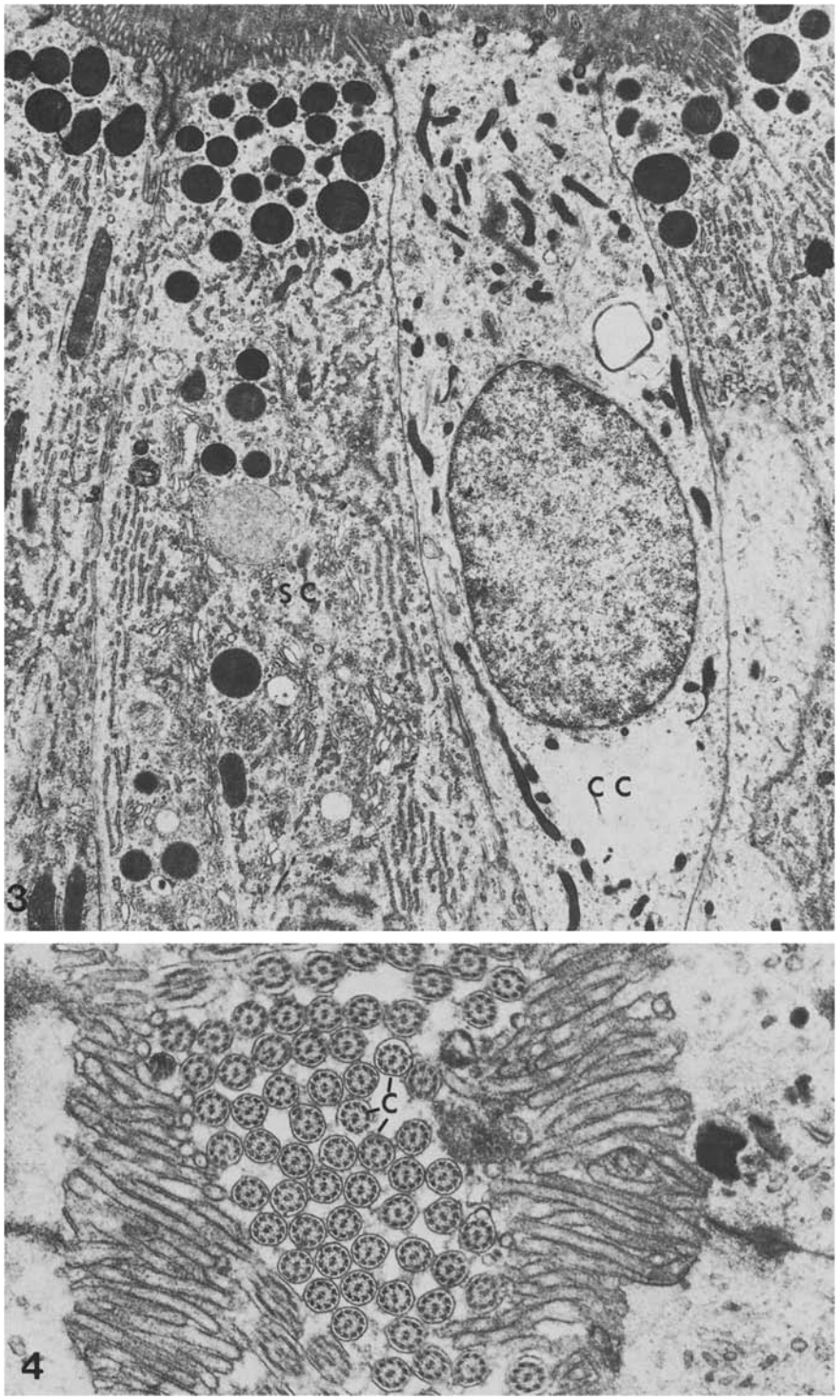


Fig. 3. Porcine uterine gland, day 110 of pregnancy; *SC* secretory cell; *CC* ciliated cell; × 6,500

Fig. 4. Porcine uterine gland, day 110 of pregnancy; *C* cilia; *MV* microvilli of the secretory cells; × 22,500

Ultrastructure of "Granule Laden Cells"

During all stages of pregnancy, cells laden with electron-dense granules are found irregularly distributed between the glandular epithelial cells of the middle and distal parts of the glands (Fig. 5). Their number appears to increase somewhat during gestation. Serial sectioning of the material showed that many but not all of these cells extend from the basal lamina to the glandular lumen. The luminal plasma membrane is invested with microvilli similar to those of normal secretory cells. The most characteristic feature is an abundance of comparatively electron-dense granules which fill the supranuclear cytoplasm. An oval nucleus, usually containing a well developed nucleolus, is situated in the basal part of the cell. Supranuclearly a distinct Golgi-apparatus is seen. Beneath the nucleus a great amount of rough endoplasmic reticulum and some lysosomal granules occur.

Glycoprotein staining using the PTA-technique of Rambourg (1967): With PTA-staining at low pH (Fig. 8) a marked staining of the glycocalix of the microvilli, the supranuclear secretory granules and the luminal content is obtained in all stages of pregnancy. Also the lysosomal bodies an the basis of the glandular epithelium are PTA-positive. The most advantageous staining with PTA is obtained with staining solutions of extremely low pH (0.3). The staining appears only moderately reduced when the pH of the staining solution is 0.5 or 1.0. With PTA-solutions of higher pH-values ($\geqq 1.5$), unspecific staining of the nuclear chromatin occurs. The secretory granules are still PTA-positive but not PTA-staining of the glycoproteins of the microvilli is observed.

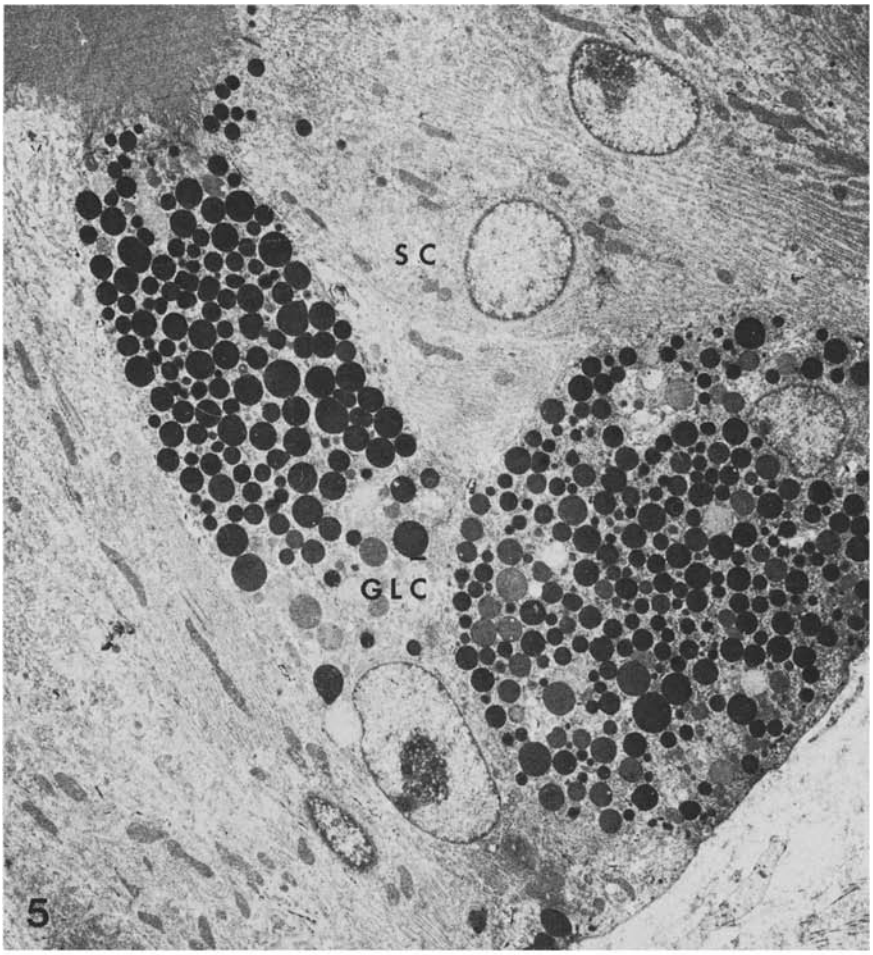
Previous acetylation of the sections followed by PTA-staining results in a marked reduction of the PTA-staining intensity of the microvilli and that of the secretory granules, whereas desamination only slightly reduces the subsequent PTA reaction. Enzymatic pretreatment of the sections with pepsin or trypsin causes only a slight reduction of the PTA staining. No diminution of the PTA-staining in the secretory granules and of the luminal material is observed when α-neuraminidase digestion precedes the staining reaction. Obviously the glycoproteins of the secretory granules and of the luminal content do not posses a considerable amount of neuraminic acid whereas neuraminic acid-containing glycoproteins play a significant role in the composition of the glycocalix of the microvilli.

Ultrastructural localization of the nonheme cellular iron with ferrocyanide (according to Parmley et al. 1978).

Lysosomal bodies containing ferric ions were identified in the infranuclear area of the uterine glands (Fig. 6). The positive reaction was mainly restricted to the periphery of the granules. No iron could be identified by this technique in the supranuclear secretory granules or in the luminal content of the uterine glands.

Cytochemical Localization of Acid Phosphatase

With the cytochemical reaction for acid phosphatase according to Barka and Anderson (1962), a marked enzyme activity could be observed in many



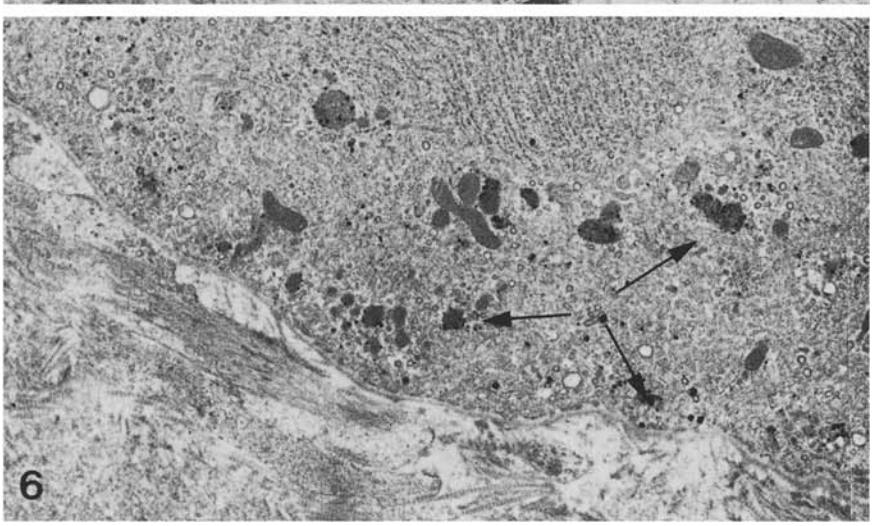
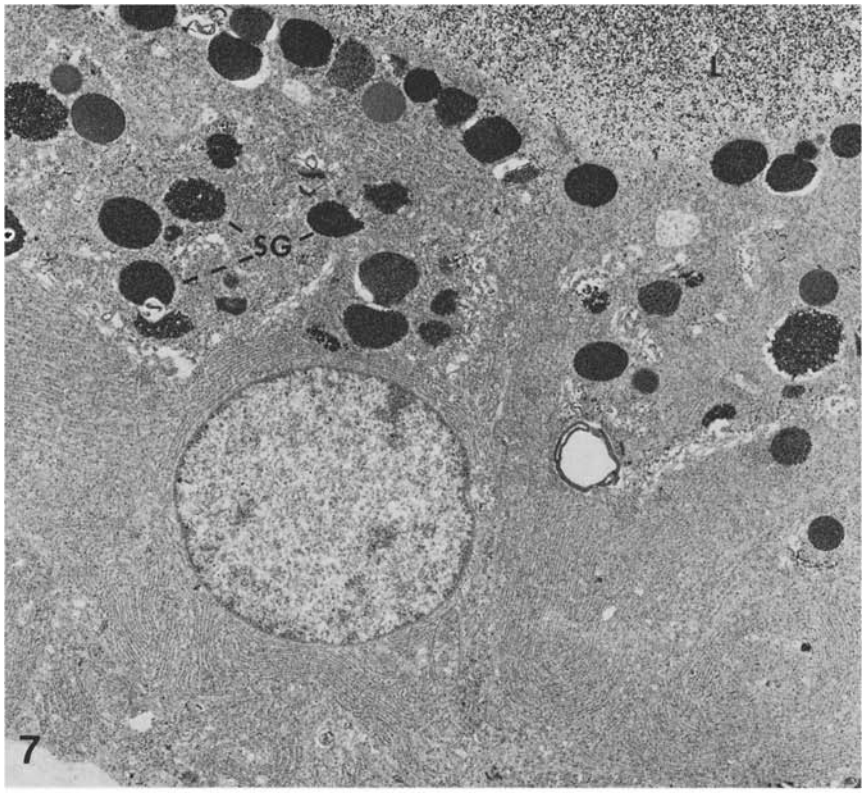


Fig. 5. Porcine uterine gland, day 110 of pregnancy. During all stages of pregnancy cells loaden with electron dense granules ("granule laden cells" = *GLC*) are found irregularly distributed between glandular epithelial cells (*SC* secretory cell). × 3,400

Fig. 6. Porcine uterine gland, day 58 of pregnancy. Ultrastructural localization of nonheme cellular iron with ferrocyanid. Granules containing ferric ions were identified in the infranuclear area of the uterine glands (*arrows*); × 10,000



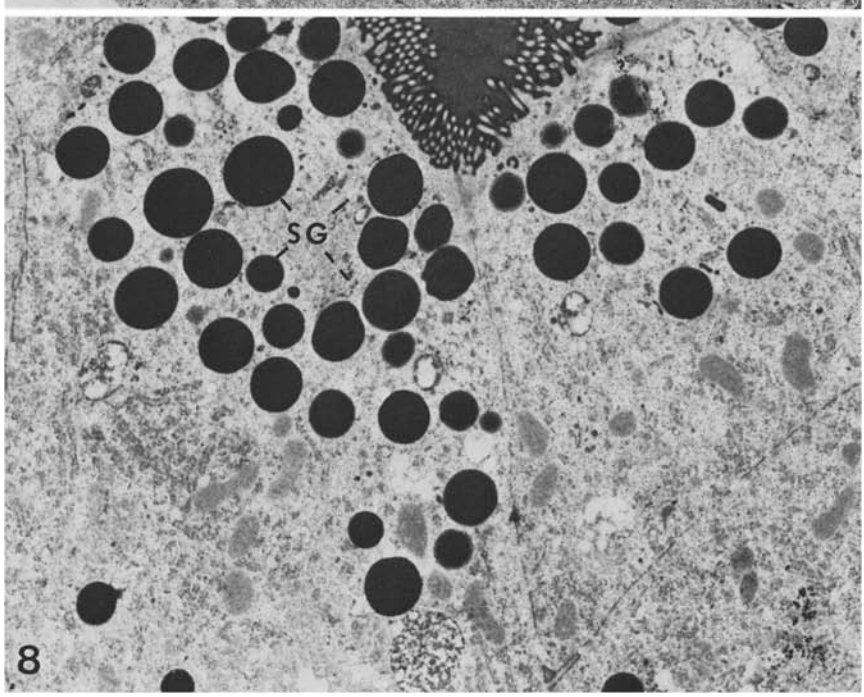


Fig. 7. Porcine uterine gland, day 58 of pregnancy. Cytochemical localization of acid phosphatase. A strong activity of acid phosphatase was observed in the secretory granules (*SG*) and in the glandular lumen (*L*); × 6,500

Fig. 8. Porcine uterine gland, day 58 of pregnancy. With PTA-staining at low pH a marked staining of the glycocalix of the microvilli, of the secretory granules (*SG*) and of the luminal content was observed. × 8,300

but not all of the secretory granules and in the lumina of the glands (Fig. 7). As the number of secretory granules significantly increases from day 30 to day 58 of pregnancy, there is also a parallel increase in acid phosphatase activity in the lumina of the glands which then remains roughly at the same level till the end of pregnancy.

Discussion

The porcine placenta is of an epitheliochorial type. At no stage of gestation does the trophoblast erode the uterine epithelium. Therefore the conceptus relies extensively upon the nutrients from the uterine glands and the uterine surface epithelium. The uterine secretions might be regarded therefore as comprising a specialized culture medium designed for support of the embryo. Recent investigations have concentrated on the biochemical composition of the uterine secretions (Bazer 1975; Roberts et al. 1976; Baska et al. 1979; Mullins et al. 1980). Among the uterine secretory material, especially proteins appear to have a potential for intervention in embryonic development due to the time at which they are secreted and due to the fact that major qualitative changes of protein secretion are observed during pregnancy. In the rabbit (Krishnan and Daniel 1967; Beier 1974; 1976; 1979) and the pig (Murray et al. 1972; Squire et al. 1972) proteins of low molecular weight are secreted by the uterus under the influence of progesterone. In both species a marked change in uterine secretion coincides with the time of implantation. The differences in the secretory activity of the uterus glands find their morphological equivalent in marked ultrastructural changes of the epithelium during pregnancy. On day 30 the lumina of the glands are narrow and contain only a small amount of secretory material. The secretory activity of the glandular epithelium is low and only a few secretory granules are present in the supranuclear cytoplasm. At midpregnancy the ultrastructure of the glands has significantly changes and the cells now show all the characteristics of a high secretory activity: The Golgi complex is extensively developed. Numerous secretory granules in different stages of maturation are seen in the apical and supranuclear cytoplasm. The amount of rough endoplasmic reticulum has considerably increased and numerous parallel cisternae occupy the infranuclear part of the glandular epithelium. The lumina of the glands are dilated and filled with a great amount of moderately electron-dense material. These changes are particularly marked in the middle part of the gland, whereas in the distal part, adjacent to the myometrium, the secretory activity appears to be less pronounced.

Our cytochemical investigations permit some conclusions about the chemical composition of the secretory material. With the Rambourg-technique for glycoproteins (1967) at low pH, a marked PTA-positive staining was observed in the secretory granules and in the secretory material in the glandular lumina. Pretreatment of the sections with α-neuraminidase nearly completely reduced the PTA-staining of the microvilli but did not change the PTA-reaction of the secretory material; it can therefore be concluded that the secretory glycoproteins are devoid of a sialic acid component. The glycoproteins also show a distinct acid phosphatase activity. Roberts

and Bazer (1976) have demonstrated that one of the uterine secretory proteins, a purple iron-containing basic glycoprotein, has the ability to act as an acid phosphatase. Their results indicate that the glycoprotein which is now called uteroferrin is maximally synthesized around day 60, and it was estimated that as much as 1 g/day may be produced at that time. The biological role of uteroferrin is not completely understood. Baska et al. (1979) suggest that the protein plays a major role in placental iron transport. This hypothesis is supported by autoradiographic studies of Palludan et al. (1970) who demonstrated the localization of iron in the uterine glands and in the areolae after intravenous administration of the isotope. Immunofluorescent studies (Chen et al. 1975) further demonstrate that uteroferrin is synthesized and secreted by the uterine glands as well as by the surface epithelium and also verify the resorption of uteroferrin by the epithelium of the areolae. As it was suggested (Chen et al. 1975) that uteroferrin may transport ferric ions to the fetus, we tried to localize iron cytochemically. Applying the same technique also used in this study, Parmley et al. (1978) could demonstrate ferric ions in ferritin, hemosiderin and some less well defined nonheme proteins containing iron. We failed to find iron in the secretory granules but we were able to observe small-iron-containing bodies, obviously lysosomes, in the infranuclear area of the glandular epithelium. In the secretory granules the iron content of uteroferrin, which is 1 iron ion per molecule uteroferrin, probably too small for cytochemical demonstration.

During all stages of pregnancy cells laden with electron-dense granules are irregularly distributed between the glandular epithelium cells. They were first described by Perry and Cromby (1982) and termed "basal cells", these authors claiming that this cell type is confined to the basal area of the epithelium. Careful serial sectioning of the material showed that the "granule laden cells" quite frequently reach the lumen of the gland. We therefore consider that the name "basal cell" is inappropriate for this cell type. The functional significance and the origin of these cells is not clear at the moment. In our opinion the granule laden cells is an autochtonic cell type of the uterine glandular epithelium and is not derived from the supporting connective tissue.

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