ULTRASTRUCTURE OF THE LUMINAL UTERINE EPITHELIUM IN THE COW

by

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INTRODUCTION

Much interest has been evident concerning changes in the uterine epithelium in recent years. In some species widely varied studies have been conducted. Some of the species differences are slight and others vary widely.

Since the uterine epithelium undergoes cyclic changes which in part are caused by fluctuating concentrations of progestins and estrogens and each cycle is supposedly priming the reproductive tract for the advent of a new embryo, an ultrastructural study of the uterine epithelium in the bovine has been conducted for two reasons. One is the cellular approach, to study the normal estrous cycle and describe the changes in cell organelles with the stages of the cycle. The other is to bridge the gap between the light microscope findings of the past and what now can be seen with the electron microscope.

It was possible to obtain specimens of uterine epithelium from ovariectomized animals providing the opportunity to see the uterine epithelium at a stage in which there are no estrogen or progesterone influences acting on these tissues.

The bovine was used because much work relating to reproduction in this animal has been done in this laboratory. The animals used had been kept in closely watched herds and the desired materials were readily available. Much attention was given to secretory mechanisms of the uterine epithelium because cell secretion seems to be the major function of this tissue.

LITERATURE REVIEW

Much work on the histology of the bovine uterine epithelium has been done by numerous workers (Asdell, 1949; Cole, 1930; Foley, 1953; Skjerven, 1956; Weeth, 1952; Larsen, 1965; Marion and Gier, 1959; and Larsen, 1968).

Uterine epithelium is part of a continuous lining of the oviduct, uterine horns, body of the uterus, uterine glands, and cervix, all of which are derived embryologically from the same tissue, so all should have similar characteristics. This epithelial layer is most homogeneous in the horns of the uterus both caruncularly and intercaruncularly and in the uterine glands.

The uterine epithelium is usually a pseudo-stratified columnar cell layer. Many studies have been done on the ultrastructure of this tissue using a variety of species, bovine, (Stinson and Weber, 1962; Marinov and Lovell, 1968; Bjorkman, 1960); human, (Wessel, 1960; Gompel, 1962; Bjorkman, 1962; Nilsson, 1962 a, b, c); rabbit, (Larsen, 1962); mink, (Enders, 1963); rat, (Warren, 1964); mouse, (Nilsson, 1958, 1959, 1962 c); guinea pig, (Burgos and Wistlocki,1958).

During the follicular phase of the bovine estrous cycle or days 17-21, the intercaruncular uterine epithelial cells have been reported to be increasing in length until they reach a maximum height of 35-40 microns in length. At this time there is a high periodic-acid-Schiff (P. A. S.) positive reaction and a low alkaline phosphatase reaction, (Marinov and Lovell, 1968; Larsen, 1965). At day 18 moderate acid phosphatase activity has been observed by Kenney (1964), but subsides by ovulation.

Within 48 hours after ovulation, the uterine epithelium has been reduced to 16-20 microns, at which time it begins to increase in height. The P. A. S. reaction is still strong, and the alkaline phosphatase activity is low since this is the transitional period between estrus and the luteal phase. By day 6 or 7, the corpus luteum is fully functional, and the uterine epithelium is in a luteal phase. The P. A. S. reaction declines from day 4 or 5 until day 12 or 13 when it begins to rise. Acid phosphatase activity reaches a peak by days 9-12 and begins to decline (Kenney, 1964). The alkaline phosphatase reaction begins to increase from day 4 or 5 until day 12 or 13 when it starts to decline.

Microvilli occur at all times on the luminal surface of the bovine uterine epithelium (Marinov and Lovell, 1968; Stinson and Weber, 1962). This is also the case of all other mammals studied ultrastructurally. The luminal epithelium has been reported to contain, on occasion, ciliated cells as in the rabbit (Larsen, 1962) and the bovine (Marinov and Lovell, 1968). After ovariectomy, the rabbit uterine epithelium loses its cilia and there is a great reduction in the number of microvilli (Larsen, 1962).

At the lateral apical side of the cells the typical junctional complex (Farquhar and Palade, 1963) is seen to exist. At the luminal side there is a tight junction (Zonula occludens), and intermediate junction (Zonula adhaerens), and then an area with many desmosomes (Macula adhaerens) situated around the cell. The term "subterminal bar" or simply "terminal bar" (an electron-dense area located slightly below the luminal surface of an epithelial

cell) is associated with either the tight junction or intermediate junction rather than with a region of continuous desmosomes as earlier assumed by light microscopists. Therefore, the region of the adjacent cell membranes which acts as a barrier to intercellular exchange between the lumen and any tissues under the uterine epithelium appears to be the tight junction (Farquhar and Palade, 1963).

All mammals studied have both ciliated and non-ciliated cells in the glandular uterine epithelium. Usually the non-ciliated cells are termed secretory cells. In the luminal epithelium, the occurrence of ciliated cells is not a constant feature, but the secretory cells are always present.

Cellular secretion appears to be the major function of uterine epithelium. Most papers dealing with more than one isolated period of cyclic changes either describe secretory, synthetic events or actual secretory accumulations either in the form of vacoules, granules or accumulations of glycogen or glycoproteins.

In the human a definite cyclic pattern of secretion was reported in the glandular epithelium (Wessel, 1960; Gompel, 1962; and Nilsson, 1962 c). During the proliferation or follicular phase, days 1-14, the Golgi is large, the endoplasmic reticulum is well developed and many mitochondria are present. Near the end of the proliferation phase, secretory granules 0.5 micron in diameter which are membrane bound, appear in the Golgi zone and move out to the apical cytoplasm (Wessel, 1960; Nilsson, 1962). These are considered to be lysosomes (Nilsson, 1962 c). At this same time glycogen appears in a system of vacoules at the

basal part of the cells as two types, a granular form with 300-900 Å particles and a homogeneous substance (Nilsson, 1962 c). By the middle of the secretory phase the glycogen vesicles have broken down to a homogeneous mass, moved around the nucleus, and out to the apical tips of the cells causing the apical membrane to extend into the lumen. This mass is thought to be either glycogen or glycoprotein (Wessel, 1960). It is assumed that the glycogen is released into the lumen of the glands either by the breaking off of the tips of the cells (apocrine) or through pores in the apical membrane (exocrine). In other species the secretory process has not been outlined in such detail by ultrastructural studies.

In the oviduct, secretory bulges or granules have been seen in secretory cells of the cow (Bjorkman, 1962), human (Bjorkman, 1960), and rabbit (Nilsson and Rutberg, 1960). The granules seen in the rabbit epithelium during estrus changed their appearance after ovulation into one of three types.

During delayed implantation, there are times when a luminal epithelial cell has secretory bulges. In the rat (Warren and Enders, 1964), irregular extrusions of the apical cytoplasm project into the uterine lumen. These are devoid of organelles and appear to be in the process of pinching off from the surface of the cells.

Ultrastructural studies have been done on bovine uterine epithelium (Marinov and Lovell, 1968; Stinson and Weber, 1962). Evidence of apocrine secretion was observed during both early and late ovulatory cycles (Marinov and Lovell, 1968) and an apparent holocrine secretion was observed (Stinson and Weber, 1962). Apocrine secretion was seen at days 16-18 and day 1 in the glandular

epithelium and at day 5 in the luminal epithelium (Marinov and Lovell, 1968).

Cyclic variations seen in luminal epithelium appeared to be in agreement when heifers were considered, but older cows were reported to have too much variation to be considered in a general cyclic scheme (Marinov and Lovell, 1968). In general, the microvilli are short at the beginning of the cycle, mitochondria are of the filamentous type, little Golgi material is found and the endoplasmic reticulum is of the smooth type and then only seen as sparse profiles scattered throughout the cytoplasm. The above organelles develop or change during the cycle. By the end of the cycle, the microvilli are 1-2 microns long, and many become branched. The types of mitochondria change until only a few filamentous types remain, and large spherical types are predominant. The Golgi becomes prominent and has many small vesicles associated with it. The sparse, smooth endoplasmic reticulum becomes vesiculated and much rough endoplasmic reticulum is seen. All these changes point to increased synthetic or secretory activity near the end of the secretory cycle, days 20-21.

The effects of ovariectomy on animals has been studied (Nilsson, 1958; and Larsen, 1962). Both of these studies compared epithelium taken from ovariectomized animals with that taken from animals in estrus. The ovariectomized cell height is reduced at least by 50% from the estrous state. The microvilli are reduced to 0.2 micron height and their relative numbers are much lower. Mitochondria are reduced in size and number. The Golgi is small and located lateral to the nucleus. Endoplasmic reticulum

is of the smooth variety and only sparse profiles are seen. None of the synthetic activity which is present in the estrous epithelium was observed after ovariectomy.

METHODS AND MATERIALS

Tissues for this study were obtained from multiparous dairy cows that were free from any detectable pathological conditions. The cows were observed daily for signs of estrus and then palpated daily until ovulation. In the cows sampled the cycles were 20 or 21 days with day 0 being the day of ovulation. The stage of the cycle was established from recorded estrous periods, and ovarian structures being determined by rectal palpation before samples were collected. The collection period continued for 30 days. For the normal cycle specimens which came from 3 cows, the collection periods included two heat periods per cow. In the ovariectomized cows, a sample was taken before ovariectomy and then at two week intervals after ovariectomy. Six ovariectomized cows were sampled.

Samples from the normal cycling cows were taken in alternate horns for successive times, and the biopsy site was varied within the horn between subsequent biopsies. The samples were taken every 2 days for the first 7 days of the cycle, every 3 days for the second 7 days and every 2 days for the remainder of the cycle.

The biopsied tissues were rinsed in cold 0.1 N phosphate buffer (pH 7.4) and immediately placed in 1.5% glutaraldehyde (Fisher 50%) buffered to pH 7.4 at 0° C. Approximately 30 seconds elapsed between excising the tissue and putting it in fixative. The tissues were fixed 10 hours and rinsed overnight in fresh phosphate buffer. They were then post-fixed for two hours in 1% osmic acid buffered to pH 7.4 with 0.1 N phosphate which had 4.5% sucrose added to raise the osmolarity. The tissues were then

dehydrated through graded ethyl-alcohol changes and embedded in epon by the modified Luft (1961) method.

Two or three 2 micron sections were taken from each epon-embedded sample on a Spencer microtome and stained with methylene blue and azure II (Richardson, 1960). Each biopsy sample made 3 to 5 epon specimens which were initially examined for the presence of uterine epithelium. If uterine epithelium was present a 20X micrograph was taken from the thick sections of each epon block to facilitate the final trimming of the tissue block for electron microscope sections. Sections for electron microscopic exami ation were made on an LKB microtome and mounted on 200-mesh copper grids coated with formvar. These were stained for 30 minutes with an aqueous saturated uranyl acetate solution, then with lead hydroxide (Karnovsky, 1962) until it became impractical to use the lead hydroxide because of carbonate formation. The lead stain then used was lead citrate (Reynalds, 1963) for 5 minutes. The grids were stored in a desicator until observed on an RCA EMU-2D electron microscope. Pictures were taken at an initial magnification of 1200X to 16,000X and photographically enlarged 2 to 10 times.

RESULTS

Both the caruncular and intercaruncular luminal uterine epithelium consists of pseudostratified columnar cells. The cells possess microvilli but the height and complexity of these vary considerably during the cycle. Cilia were not observed on the luminal epithelium, but were numerous on gland cells. Changes in cell height were correlated with the stage of the cycle.

The Golgi apparatus undergoes considerable change and becomes well developed near the end of the estrous cycle. The endoplasmic reticulum becomes quite distended near the end of the cycle. Secretory bulges consisting of large protrusions, membrane covered and usually void of organelles occur at the beginning of the cycle and persist until day 9. Adjoining cell membranes appear to interdigitate much of the time. Terminal bars, probably associated with an intermediate junction, extend almost to the center of the cells. The basement membrane is always present and usually quite straight with slight undulations.

At all stages of the cycle, electron-dense cells are present. During the normal cycle, these appear to be modified epithelial cells, but in the ovariectomized animal they appear to be either leucocytes or modified fibroblasts.

The junctional complex is similar to that of most columnar epithelial cells. This consists of a tight junction nearest the lumen, an intermediate junction, then a desmosome. In this report the intermediate junction will be associated with the term "subterminal bar" which describes the dense fibrous area present from the lateral cell membrane to the center of the cell (Fig. 9).

Cyclic Changes In Intercaruncular Epithelium

Day 1 and 2. At this stage the hormonal influences shift from an estrogen dominance to a progesterone influence. The height of the uterine epithelium may change from 40 microns two days previous, to 15-20 microns at this stage with width changing from 3 to 4-5 microns.

The microvilli are about 0.5 micron long and appear to have a small sub-base which may be the remains of a previously distended microvillus. The luminal surface of all cells is covered with microvilli (Fig. 1). Many small mitochondria about 0.25 micron in diameter are present at the apical or luminal end of the cells. In deeper parts of the cells there are a few profiles of long filamentous mitochondria. The Golgi apparatus is located in a lateral mid-nuclear position (Fig. 1) and consists of 5-6 parallel membranes that extend in any one cut for about one micron. Little synthetic activity is seen at this stage. Short profiles of granular endoplasmic reticulum are seen throughout the cell, but no accumulation of material is seen except in large clear or lightly opaque vesicles near the base of the cells.

Large secretory protrusions, which are cell membrane bound, occur on some of the cells (Fig. 2) and appear to be secretions that push the normal cell membrane into the lumen. Some of these are quite small (1.5 microns wide and 3 microns long) while others are 5-6 microns in diameter. They appear to be located laterally at the apical end of the cell rather than in the middle and are relatively free of cell organelles, except for a few pieces of endoplasmic reticulum and possibly ribosomes. Small

particles located in these processes fit the appearance and size of glycogen particles.

The basement membrane is approximately 1000 Å thick and lies directly beneath the basal cell membrane. A few half-desomosomes are found on the basal cell membrane. Fibroblasts are separated from the basement membrane by a cell-free area 2-3 microns wide that has a high concentration of collagen fibers.

Day 3. The uterine epithelium is approximately 18 microns high and each cell is 4 microns wide. The terminal bars are quite dense and extend completely across the cells. Mitochondria are mostly circular and are extremely sensitive to osmotic shock, tending to swell during fixation. No filamentous mitochondria are seen. The Golgi in some cells is in the same location as at day 1-2, but in others it has taken a position 1-2 microns above the nucleus. Some vesicles, 0.3 to 0.6 micron in diameter are associated with the Golgi. The smaller ones are incorporated in the ends of Golgi lamellae, while the larger ones are located in the cytoplasm near the center of the curved portion of the aggregates of lamellae.

In some regions of individual cells, the endoplasmic reticulum is vesiculated and lined by ribosomes. There is an extensive system of smooth endoplasmic reticulum that is also dilated in some isolated areas. An undulating basement membrane is present under all uterine epithelium cells with an interval of 1-2 microns separating it from the main cell body of fibroblasts. In some areas, however, the processes of the fibroblasts come within 0.1 micron of the basement membrane.

A few electron-dense cells are seen at this stage (Fig. 5). The one seen in the above figure is 18 microns long by 2 microns wide. It appears to be reduced to vesiculated endoplasmic reticulum and mitochondria. It has pseudopodia-like structures along the lateral borders that may extend for 1-2 microns along the cell. Its basal end seems to be shaped like a pedestle and in some cases may be spread over the basement membrane for 7-8 microns.

Secretory vesicles that incorporate the entire apical end of a cell are seen bulging out into the lumen from a few of the cells (Fig. 4). There are no cell organelles other than possibly a few ribosomes in these secretory processes. It appears that a system of microtubules that will be pointed out later, extend across the apical end of the secretory cell and retain the major cell organelles.

Day 5. Cells have elongated to 20 microns and are 4.5 microns wide. The microvilli are 0.6 micron long and 300 Å wide. Mitochondria are mainly filamentous and unbranched. Golgi material is located in a supranuclear position and appears to be horseshoe shaped with small vesicles 0.2 micron in diameter at the ends of the lamellae. Two types of cells are apparent at this stage. The first is a cell that has many microvilli and undilated endoplasmic reticulum. The other has few microvilli and relatively dilated endoplasmic reticulum.

Day 6. Cell length is 23 microns and the average width is 3 microns. The lateral cell margins are less complicated, especially near the apical end (Fig. 6).

Branched mitochondria appear in some of the large concentrations of mitochondria that occur apically. The Golgi is becoming more extensive with lamellae spread out 3 microns long above the nucleus. Endoplasmic reticulum is mostly undilated with many small patches of granular endoplasmic reticulum incorporated into quite extensive arrays of smooth endoplasmic reticulum. Again there are two types of cells, lighter and darker, with the above description fitting the darker cells. The light cells have less extensive arrays of endoplasmic reticulum than the darker cells. Secretory processes appear essentially the same as they did on days 1-5. They contain no organelles, small granules (presumably glycogen), and are covered by the cell membrane. The organelles of the cells are limited apically by the terminal bar (Fig. 6). Some of the secretory vesicles appear to have remnants of microvilli at their lateral margins. The lighter cells are more likely to have a secretory process at their apical ends.

Day 12. Cells of the uterine epithelium are 26 microns long and 3.2 microns wide. Microvilli are about 1.2 microns long and about 0.1 micron wide.

At the apical end of the cells the usual concentration of mitochondria is present with an increasing number of filamentous mitochondria comprising the total.

The Golgi is located 2-3 microns above the nucleus with 5-6 lamellar sheets seen in partial section on two opposite sides of a cell (Fig. 7). In some areas, opaque granules, membrane bound and 0.6 micron in diameter are located on the inside curvature of the Golgi lamellae. Although these bodies are located near

the Golgi, there is no evidence they are products of the Golgi. All cells contain such opaque granules but they may occur anywhere, basally to apically within the endoplasmic reticulum cisternae. The endoplasmic reticulum is composed of extensive arrays of smooth membranes with ribosomes attached in areas of dilation. Few polysomes are seen at this time.

Day 17. Cells have expanded to 35 microns long and 4 microns in diameter. The number of microvilli has increased so all cells have a continuous covering of microvilli of which many are branched, with the branch occurring in the basal one-third. Terminal bars are present but do not extend medially into the cells. Most mitochondria are large and oval, with few branched forms occurring.

The Golgi is composed of 5 or 6 lamellar sheets 2-3 microns in length located 4-5 microns above the nucleus. Based on longitudinal and cross sections, the Golgi apparatus appears to be composed of two long C-shaped lamellar sheets of membranes parallel to the longitudinal axis of the cells.

The endoplasmic reticulum is partially dilated. In areas of considerable dilation, finger-like projections of endoplasmic reticulum, which are completely covered with ribosomes, extend back into the dilated cisternae (Fig. 8). This is in contrast to the rest of the endoplasmic reticulum which has only scattered areas of attached ribosomes.

Fewer electron-dense cells are seen at this stage. Those seen are of the same height as other uterine epithelial cells but have been reduced in width. The nucleus and all cytoplasmic organelles are stained very dark, with only mitochondria and endoplasmic reticular channels visible.

Secretory cells are few in number. The ones seen are similar to those at days 3-6.

Day 20. The uterine epithelium has expanded to about 40 microns, its greatest height of the cycle, with the average cell 3 microns in width. All cells are covered with microvilli about 1.6 microns in length, which appear similar to those at day 17. Microvillus branching has reached its maximum at this time. The substructure of the microvilli in longitudinal section consists of fibers oriented parallel to the long axis of the microvilli (Fig. 9). In cross-section, the fibers appear as 5 to 10 dots. Except for being parallel to the microvilli, these fibers have no definite orientation such as the tubules found in cilia (Fig. 3).

Terminal bars have taken a position about 0.2 micron from the apical end of the cells. They are composed of dense bundles of fibers running into the center of the cells (Fig. 9). The types of mitochondria are mixed, but the filamentous form is the predominant type. The Golgi is located about 6 microns above the nucleus, and consists of 5-6 longitudinal lamellar sheets 4-5 microns long arranged either in a cylinder or partial cylinder (Fig. 10).

The endoplasmic reticulum is considerably dilated, but in few cases is any material seen in the cisternae. This may be due to leaching out of materials present prior to fixation. The finger-like projections into the cisternae are still present and covered by ribosomes.

Post Ovariectomized Cows

19 days post ovariectomy. The luminal epithelial cells are about 15 microns high and 3.5 microns wide. The cells appear disorganized because of the extensive interdigitation and difficulty in getting longitudinal sections of a complete cell. Microvilli are similar to those of days 1-3 in intact animals. Mitochondria are mainly unbranched and ovoid. The Golgi consists of 5 lamellar sheets reduced to a small region either immediately above the nucleus or slightly lateral (Fig. 11).

1 year post ovariectomized cows. Cell height is 17 microns and the width is 3.2 microns. Microvilli are unbranched and approximately 0.6 micron long. A few have the sub-base structure found at day 1 of the estrous cycle. The fibrous interior is still present. Terminal bars are associated with the tight and intermediate junctions. Fibers that run into the center of the cells are still present.

Most mitochondria are short and ovoid, but a few branched forms occur. The Golgi appears similar to that of day 1, with 5 lamellar sheets, and is located either immediately above the nucleus or slightly lateral (Fig. 11). Sparse profiles of undilated endoplasmic reticulum, mostly of the granular type, are seen in the apical cytoplasm.

Electron-dense cells are present, but their appearance is not of the electron-dense cells seen from days 1 through 17 in the intact animals. They appear similar to leucocytes seen below the basement membrane (Fig. 12).

DISCUSSION

The most obvious change of uterine epithelium during the bovine ovulatory cycle occurs in the luminal epithelium, which at ovulation, is 35-40 microns high and two days later is 15 microns high. With this decrease there are distinct changes in the cell organelles. The microvilli are short and reduced in number. The types of mitochondria are different and the endoplasmic reticulum is reduced to sparse undilated profiles. The Golgi has been reduced from 4-5 microns long to short lamellae 1 micron long. All evidence of synthetic activity is diminished. The secretory bulges which appear on many of the cells are of great interest. These are located on the luminal border which is intensely stained by the P. A. S. reaction at this time (Larsen, 1965; Marinov and Lovell, 1968). The bulges are a direct continuation of the apical cell membrane which appears to be distended by internal pressure. The microvilli have disappeared except for a few at the base of the bulges. There were no observations of the bulges breaking free from the apical end of the cells but they appeared to be in the process of pinching off. The secretory bulges contain glycogen according to the critera of Revel et al. (1960). The only organelles observed in these bulges were ribosomes.

The secretory bulges were observed from day 1 until day 17 but the most intense activity occurred from days 1-9. They resembled those seen in oviduct epithelium (Bjorkman, 1960). Similar bulges incorporating the entire apical end of a cell were observed by Wessel (1960) in the human gland cells and acinar cells (Clyman, 1963).

Many people have described secretory extrusions from the apical cell membrane, but these are only small cytoplasmic processes (Warren and Enders, 1963; Nilsson, 1959; Marinov and Lovell, 1968). Whether these extrusions are only the beginning of a large protrusion incorporating the whole apical part of a cell or only small secretory processes can not be determined.

No cell organelles, i. e. mitochondria or endoplasmic reticulum, were found beyond the terminal bars, as was also observed by Stinson and Weber (1962). It appears that a system of fibers or microtubules restricts organelles from going beyond the terminal bar, but allows glycogen and other secretory material to go through the terminal bar boundary. This secretory material distends the apical end of the cell until either the bulge with intact membrane pinches off from the end of the cell or the membrane breaks allowing the secretory mass to drain out. In either case, the major organelles remain intact, and the broken membrane either coalesces or drops back to preserve the integrity of the cell. The secreted P. A. S. positive material must be retained in the uterine lumen to be beneficial to an anticipated blastocyst.

By day 5, two types of cells are observed. One type has branched mitochondria, rather long microvilli, many small patches of granular endoplasmic reticulum continuous with extensive arrays of smooth endoplasmic reticulum, Golgi with lamellae spread out about 3 microns above the nucleus, and dense cytoplasm. The other is a lighter cell with few microvilli, smaller Golgi and few branched mitochondria. The lighter cells appear to be the ones that are getting ready to secrete, while the darker cells

have already undergone secretion and are continuing through a recuperative part of the cycle. All the cells with secretory processes are lightly staining and lay behind the dark cells in terms of beginning another build up for synthetic activity.

By day 12 most cells are at the same stage of synthetic activity. The previous differences between cells have disappeared. The cells have elongated to 24-30 microns and all have the same density and distribution of organelles. The microvilli have increased to 1.2 microns, mitochondria are undergoing much branching, and the endoplasmic reticulum is composed of both smooth and rough types. Moderate dilation of the endoplasmic reticulum occurs throughout the cells. The Golgi has increased in size and can be seen on opposite sides of the nucleus. Slightly opaque round granules 0.6 microns in diameter and membrane bound are seen near the Golgi, but there is no indication of the Golgi forming the granules. These granules are seen throughout the cells from day 12-17 with no indication of being secreted from the cells. The branching of the mitochondria appears to be a step in mitochondrial proliferation by elongating and then pinching off.

By days 17-20, the epithelium has reached its maximum synthetic activity. Many mitochondria, both branched and ovoid, are scattered throughout the cells. The endoplasmic reticulum is distended considerably with many areas lined with ribosomes. In some dilations slight remains of a lightly staining material is present. It is assumed that the dilations were filled with some material that was leached out during fixation. The Golgi is at

its maximum size although there is no evidence as to what it is processing or producing at this time.

It appears that the luminal epithelium produces its secretory material, either glycogen or glycoprotein, during the follicular phase, reaching a maximum before the time of ovulation until a few days after estrus (Marinov and Lovell, 1968; Larsen, 1965). The synthetic activity, as evidenced by the build up of cell organelles, reaches a peak before ovulation and decreases by the time of ovulation. By one day after ovulation, the secretory material is distributed throughout the apical cytoplasm and is not confined to the endoplasmic reticulum. One of the methods by which the newly synthesized material is released is by the secretory protrusions. Since there is a decline in the amount of glycogen in epithelial cells at days 4-5, and few secretory bulges are seen after day 6, the occurrence of the secretory bulge correlates with histochemical findings. It appears that the reduction in cell height after ovulation is accompanied by an increase in cell width which would allow the cell volume to remain approximately the same.

Although histochemical studies show maximal P. A. S. positive material in the uterine epithelium at estrus, it appears the P. A. S. positive material is secreted beginning at day 1 and continues until days 6-9, with the number of secretory protrusions reaching a maximum at day 6. This would be at the time a blastocyst would reach the uterine horn. Since no luminal secretion is seen at estrus, the medium for sperm passage might come from the oviducts since similar secretory protrusions have been seen in the bovine oviducts (Bjorkman and Fredricsson, 1960).

After ovariectomy, the epithelium loses all signs of cyclic activity. At 3 weeks post ovariectomy, the epithelial cells appear to be in a state of reorganization to establish a simple columnar epithelium. The cells are reduced to 15 microns high, microvilli are short, Golgi is reduced, endoplasmic reticulum is seen only as sparse, undilated profiles and mitochondria are small. It appears the cells regress to a state in which they only maintain themselves and are not concerned with any extra synthetic activity. At one year post ovariectomy, the individual cell appearance is the same although the cells seem to be well organized into a simple columnar epithelium.

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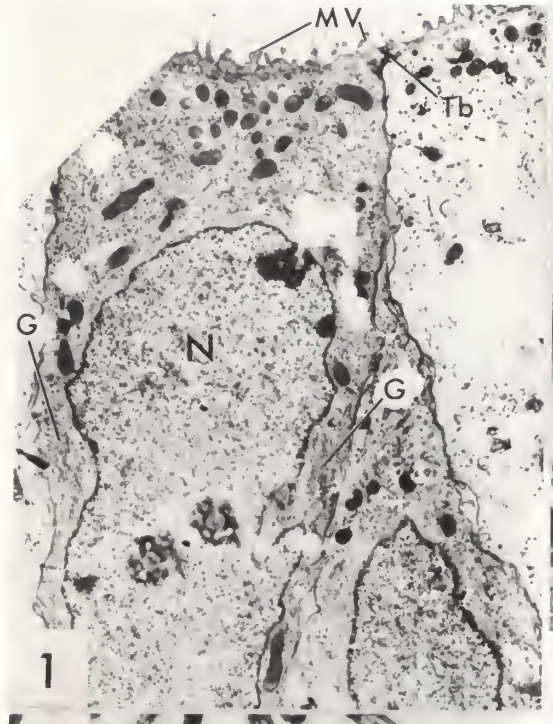
EXPLANATION OF FIGURES

Fig. 1. Bovine uterine epithelium from an intercaruncular position at day 1 of the estrous cycle. Microvilli (MV) are short, terminal bar (Tb) near the luminal surface, and Golgi (G) lateral to the nucleus (N). x 8,700.

Fig. 2. Secretory protrusion (P) on epithelium at day 1 of the estrous cycle. (Dark line through protrusion is a wrinkle in the section). x 25,000.

Fig. 3. Epithelium from a uterine gland at day 1 of the estrous cycle. Cells have both cilia (C) and microvilli (MV). x 19,000.

Fig. 4. Secretory protrusion (P) on uterine epithelial cell at day 3 of the estrous cycle. System of fibers (F) or microtubules hold cell organelles within the cell as secretory protrusion is extruded. x 11,200.



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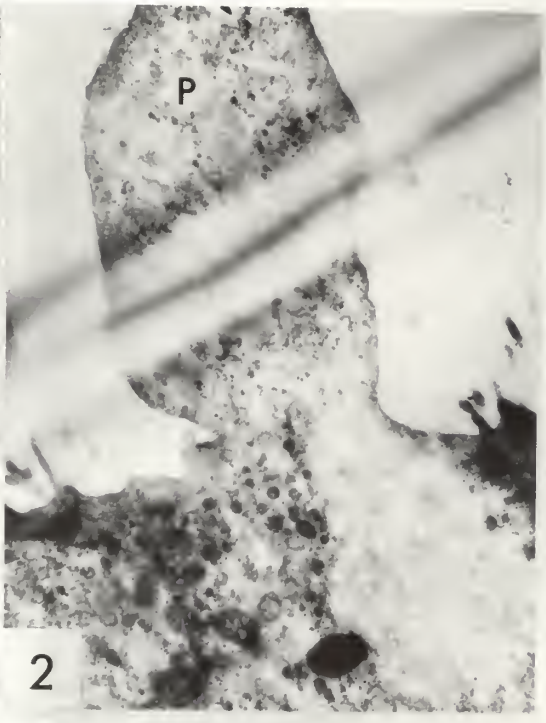

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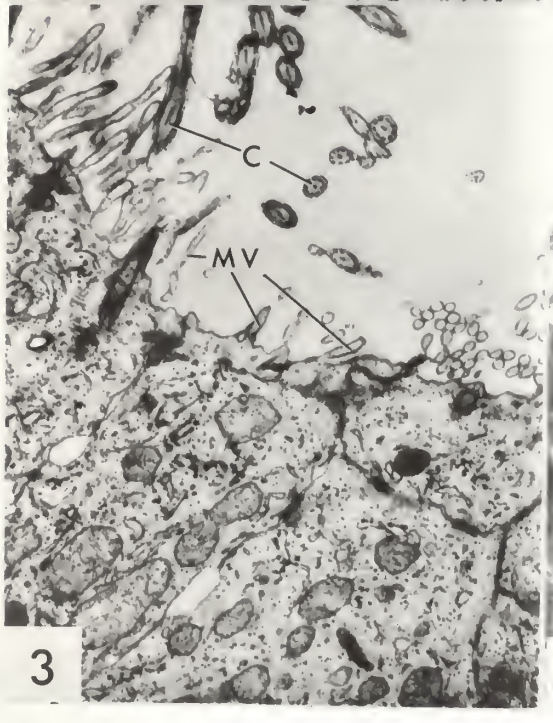

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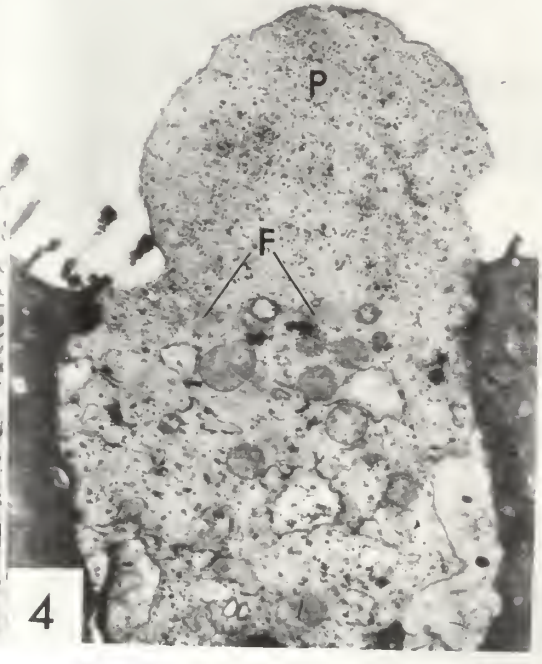

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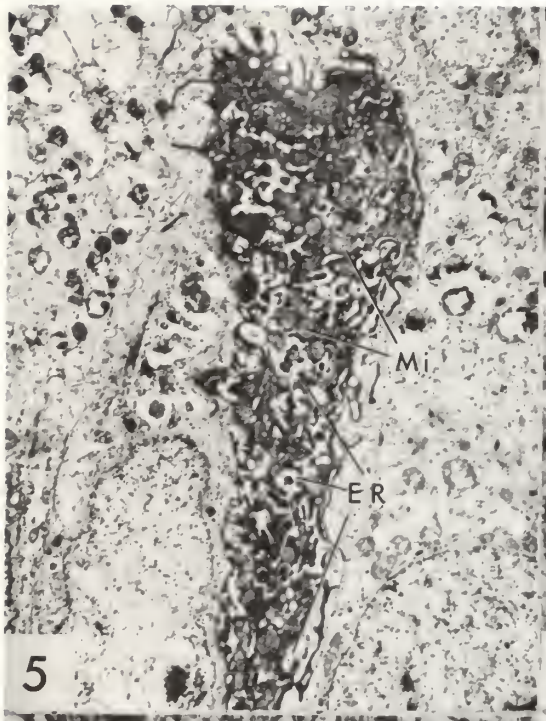
EXPLANATION OF FIGURES

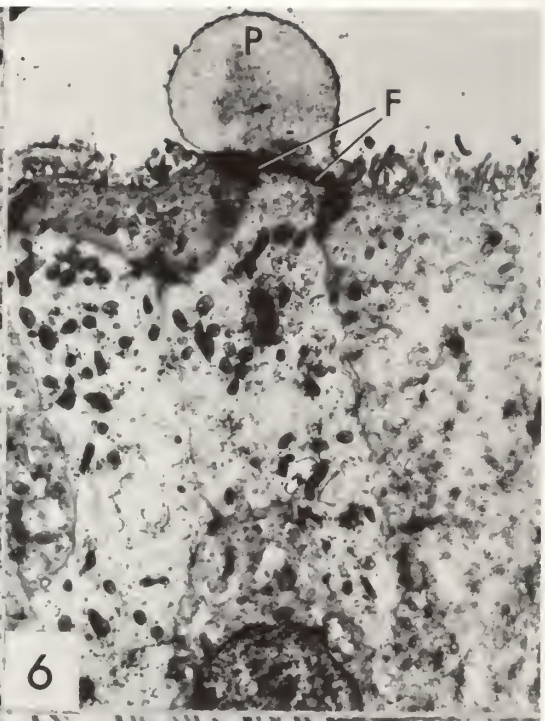
Fig. 5. Electron-dense cell and light cells in luminal epitheliu at day 3 of the estrous cycle. The electron-dense cell appears to be an epithelial cell reduced to mitochondria (Mi) and endoplasmic reticular channels (ER). x 8,700.

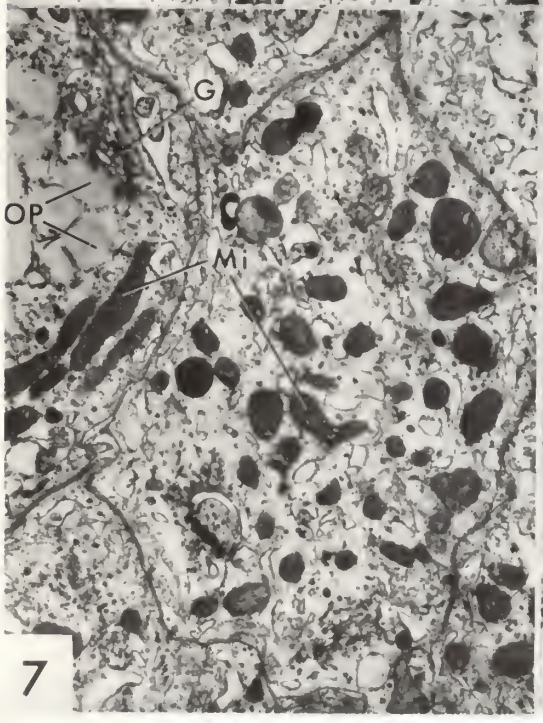
Fig. 6. Secretory protrusion (P) on luminal epithelium at day 6 of the estrous cycle. A dense band of fibers (F) retain cell organelles. x 8,100.

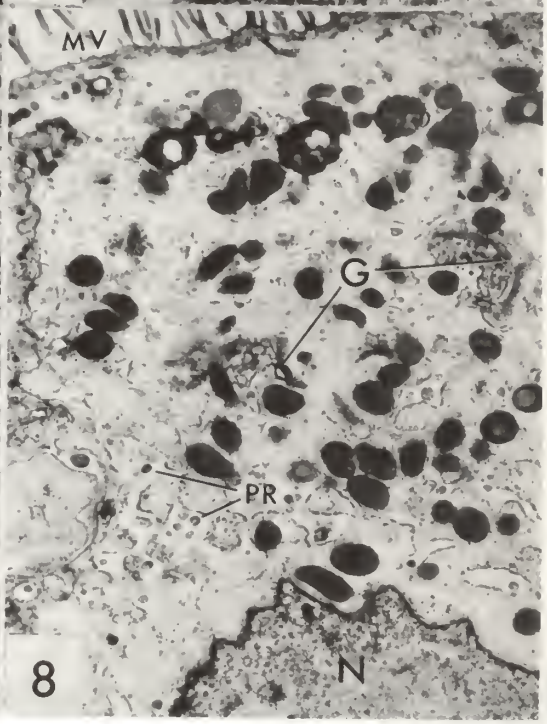
Fig. 7. Cross section of luminal epithelium at day 12 of the estrous cycle. Opaque granules (OP) are located near the Golgi (G) and filamentous mitochondria (Mi) are present. x 14,500.

Fig. 8. Luminal epithelial cell at day 17 of the estrous cycle. Cells have branched microvilli (MV), finger-like projections (PR) extending into the endoplasmic reticulum cisternae and Golgi (G) that occupies a considerable area above the nucleus (N). x 10,000.


Mi
ER
5


P
F
6


G
OP
Mi
7


MV
G
PR
N
8

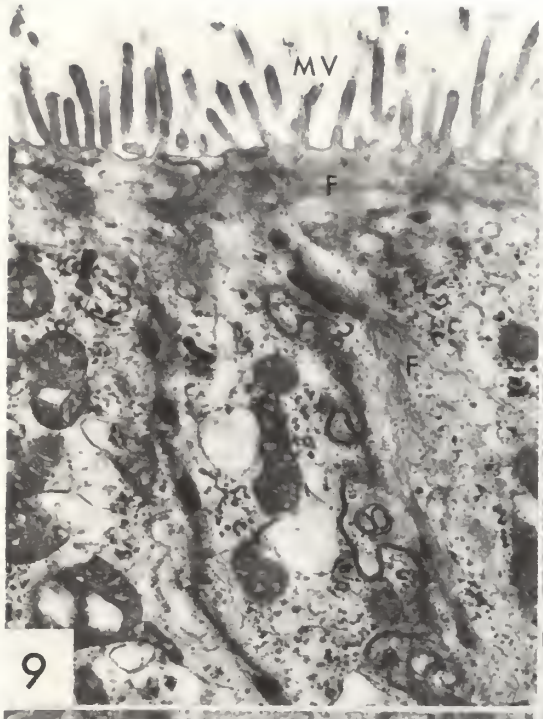
EXPLANATION OF FIGURES

Fig. 9. Luminal epithelium at day 20 of the estrous cycle. Fibers (F) or microtubules run across the apical tips of the cells and also away from the terminal bars. Branching of microvilli (MV) is at a maximum. x 17,500.

Fig. 10. Luminal epithelium at day 20 of the estrous cycle. The endoplasmic reticulum (ER) is considerably dilated. Both branched and ovoid mitochondria are present. The Golgi (G) is located 4 microns above the nucleus (N). x 8,700.

Fig. 11. Luminal epithelium at 19 days post ovariectomy. The Golgi (G) is located lateral and immediately apical to the nucleus (N). x 15,200.

Fig. 12. Luminal epithelium 1 year post ovariectomy. Cells have been reduced to a simple columnar epithelium with short microvilli (MV), sparse endoplasmic reticulum and few mitochondria. Electron-dense leucocytes (L) are seen between epithelial cells. x 8,800.



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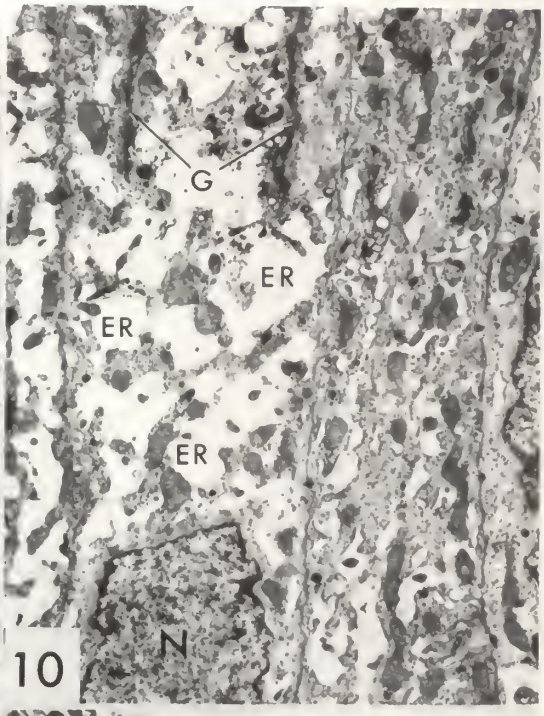

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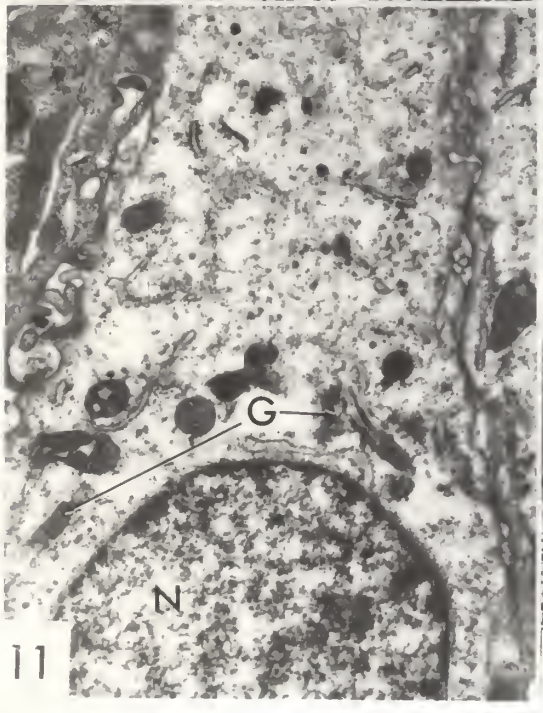

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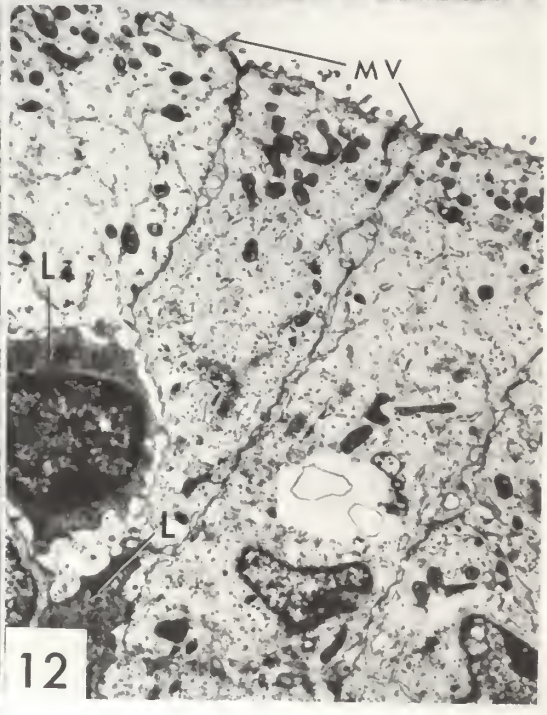

12

ULTRASTRUCTURE OF THE LUMINAL UTERINE EPITHELIUM IN THE COW

by

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B.S., Kansas State University, 1966

AN ABSTRACT OF A MASTER'S THESIS

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MASTER OF SCIENCE

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1969

The uterine epithelium from biopsy samples of 3 normal cycling dairy cows and 6 ovariectomized dairy cows was studied by electron microscopy. Samples from the normal cows were obtained at known stages of the estrous cycle. The cows were observed for estrus twice daily. Before biopsy samples were taken, a rectal palpation was performed, and the sizes and conditions of ovarian and uterine structures were recorded.

A definite secretory cycle was observed in the luminal uterine epithelium. At one day after ovulation, the cell height was 15 microns. All cell organelles were reduced either in number or size. All signs of synthetic activity were low. From days 1-9 secretory protrusions consisting of the apical cell membrane distended by glycogen or glycoprotein were present, apparently in a process of pinching off. The integrity of the cells was maintained by fibers or microtubules which kept organelles from being lost from the cells. By mid-cycle the cells had lengthened and there were signs of increased synthetic activity, moderate dilation of the endoplasmic reticulum, increased size of the Golgi, and more mitochondria.

A maximum of synthetic activity was reached 17-20 days after estrus. Cell height was increased to 35 microns with branched microvilli 1.2 microns long. Many mitochondria, both branched and ovoid, were present. The endoplasmic reticulum was distended and lined by ribosomes in many areas. The Golgi was at a maximum of 5 microns in length and cylindrical.

Electron-dense cells were seen in all stages of the cycle. These appeared to be modified epithelial cells, possibly degenerative, with dilated endoplasmic reticulum and numerous mitochondria.

After ovariectomy, the epithelium was reduced to 15 microns in thickness, and all signs of cyclic activity were lost. Three weeks after ovariectomy, cells were in a state of reorganization, but by 80 days the cells were organized into a simple columnar epithelium without any sign of secretory activity.

This study has served to establish a definite secretory cycle of the uterine epithelium in the cow. One method of cell secretion was observed which would be classified as exocrine. Further investigations should be conducted to compare the epithelium of the uterine glands and oviduct at known stages of the estrous cycle. This information would help in understanding the environments which are produced in the uterus at both the times of sperm passage and early embryonic arrival.