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The Fine Structure of the Intra-uterine Epithelium during Late Gestation in the Blue Shark, *Prionace glauca*

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Abstract The fine structure of the intra-uterine epithelium of the pregnant blue shark, *Prionace glauca*, was examined. The intra-uterine epithelium was bilaminar and the underlying epithelial cell was extremely reduced in cytoplasm. Two cytological characteristics were shown in the outer epithelial cell; open inter-cellular spaces closed in the apical portion by a junction complex and, numerous mitochondria distributed in the basal and lateral portions of the cytoplasm. Secretive characteristics were not recognized in the outer epithelial cell, although few regions composed only of mucous cells were seen. The flattened endothelium of the capillary lay closely beneath the epithelium. These structures are thought to facilitate the water-solute transport and gaseous exchange. It suggests that the intra-uterine epithelium is involved in the osmoregulation of the uterine fluid and the exchange of respiratory gases between mother and fetus.

The uterine fluid, secreted by the intra-uterine epithelium, is known to play an important role in fetal nutrition in aplacental viviparous sharks (Ranzi, 1934; Wourms, 1977). In addition, in some placental viviparous sharks the intra-uterine secretions are absorbed by the fetus as a form of nutrition, although the balance in fetal nutrition between absorption and placental transfer is unknown (Graham, 1967; Wourms, 1977). The balance is thought to depend upon the functional differentiation of the yolk sac placenta, and is one of the important basis for elucidation of placental evolution in sharks.

In a previous paper, Otake and Mizue (1985) reported that the blue shark *Prionace glauca* possessed a placenta without an egg-capsule which actively transported fetal nutrients including macromolecular materials. In the present study, the fine structure of the intra-uterine epithelium of the pregnant blue shark was observed to examine possible functions of the intra-uterine epithelium and the role of uterine fluid in fetal nutrition.

Materials and methods

The four pregnant blue shark used in this study were the same as those described in a previous report (Otake and Mizue, 1985). They were 204.0–241.0 cm in total length and carried 7–24 near full term fetuses which measured 26–38 cm in total length. They were caught with a tuna long-line from the R/V *Tansei Maru* of the Ocean Research Institute, University of Tokyo, in waters off Miyake Island between February 21–23 in 1981.

For electron microscopy, uterine tissues not participating in the placenta were cut into small pieces and fixed for 4–7 days in cold fixative containing 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), to which 10% succrose was added. They were rinsed for 2 hours in the same buffer and postfixed for 2 hours in 2% osmium tetroxide. After fixation, tissues were bloc stained overnight in 2% uranyl acetate. They were then dehydrated in graded ethanol and embedded in epon 812. Ultrathin sections were doubly stained with uranyl acetate and lead citrate and observed under a JEM-100CX electron microscope. Some thick sections of 1 μm in thickness were stained with toluidine blue for light microscopic observation.

For light microscopy, pieces of the uterine wall were fixed in 10% neutral formalin or Bouin fluid. Tissues were prepared by the usual paraffin method, sectioned to 5 μm and stained with Mayer's haematoxyline and eosin or PAS-solution.

Results

The intra-uterine epithelium was composed of thin bilaminar epithelial cells (Figs. 1, 3). The outer epithelial cells were 10–35 μm in height and contained a spherical nucleus in the center or upper portion of the cytoplasm. The underlying

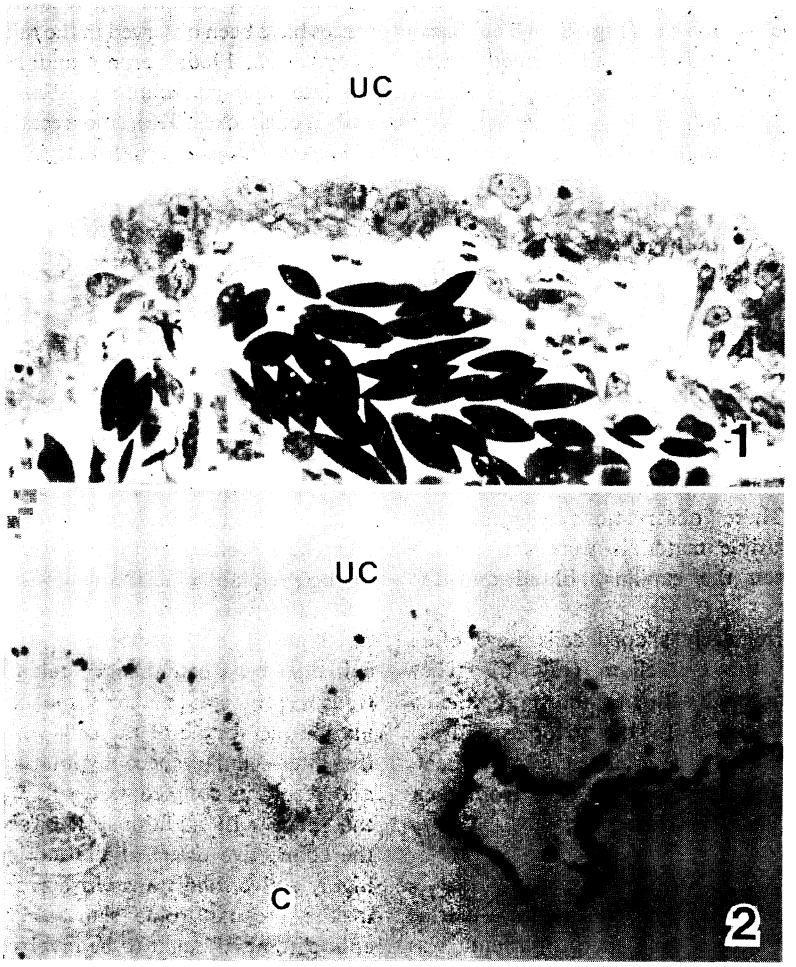


Fig. 1. The intra-uterine epithelium and the capillaries under the epithelium. Epon embedding, toluidine blue staining. UC, uterine cavity. ×500.

Fig. 2. The intra-uterine epithelium composed of mucous cells. PAS-staining. C, connective tissue; UC, uterine cavity. ×80.

epithelial cells were extremely flattened. A well developed capillary network lay beneath the epithelium. The connective tissue under the capillary network was very loose and abundant in tissue fluid. Mucous cells of 25–45 μm in height which were stained strongly with PAS-solution were scattered in the epithelium. Few outer epithelial regions composed only of mucous cells were seen in the light microscope (Fig. 2).

The free surface of the epithelial cell was covered with branched microvilli of about 1 μm in length (Figs. 3, 4). The plasmamembrane between microvilli was often invaginated and formed coated pits. The apical portion of the cytoplasm possessed many coated vesicles probably derived from invaginations of the apical cell surface (Fig. 4). Numerous mitochondria were distributed in the basal and lateral portions of the cytoplasm. But RER, SER and Golgi complex were seldom found, suggesting that the cells were not very active in secretion (Figs. 3, 6). Therefore the invaginations in the apical surface of the cell were probably involved not in secretion but in uptake of some macromolecular waste products in the uterine fluid. The outer epithelial cells were joined to adjacent cells by a junction complex in the apical portion of the cell. Desmosomes were few on the lateral surface. The inter-

cellular spaces were open and often invaginated deeply into the cytoplasm (Fig. 3). The basal and lateral surface of the cell formed many projections which greatly enlarged these surfaces (Figs. 3, 5).

Underlying epithelial cells were extremely reduced in the cytoplasm. The apical cell surface formed many projections which were not tightly interdigitated with those of the outer epithelial cells (Figs. 3, 5). The basal surface was rather smooth, although some small invaginations were present. The cell organella were very few in number. The endothelium of the capillary lay closely beneath the epithelium. A few stromal cells and collagen fibers were seen between the epithelium and the endothelium (Fig. 5). The endothelium was flattened but possessed no fenestration. These observations suggest that gases and diffusible materials were actively exchanged between the capillary blood and the epithelium.

Light and irregularly shaped cells were often present within the epithelium (Fig. 6). They were never connected with neighboring epithelial cells by desmosomes. In the cytoplasm, lysosome-like electron dense granules were present. They seemed to be phagosomal cells from their morphological characteristics.

Discussion

In the pregnant blue shark the intra-uterine epithelium not participating in the placenta was quite different cytologically from the actively secreting cells found in the placenta (Otake and Mizue, 1985). The epithelium was thin and closely adjoined the endothelium of the capillary, which indicated that the epithelium facilitates gaseous exchange between maternal blood and uterine fluid. In addition, the epithelium was characterized by open inter-cellular spaces in the epithelium and numerous mitochondria distributed in the basal and lateral portions of the outer epithelial cell. Similar open lateral inter-cellular spaces have been observed in the rabbit gall bladder (Kaye *et al.*, 1966; Tormey and Diamond, 1967), the intestinal epithelium of *Xenopus* (Bonneville and Weinstock, 1970), the rectal salt glands of *Urolopus* (Doyle, 1962) and *Squalus acanthias* (Goertemiller and Ellis, 1976), and the intra-uterine epithelia of the pregnant *Salamandra* (Greven, 1977, 1980) and pregnant *Squalus acanthias*, an aplacental viviparous shark (Jollie and Jollie, 1967). All these tissues are thought to be involved in water-solute transport. According to the standing gradient osmotic flow theory of Diamond and Bossert (1967, 1968), epithelia performing solute-linked water transport possess long, narrow channels open at one end and closed at the other which may constitute the fluid transport route. Our observations revealed that the structure of the intra-uterine epithelium of the pregnant blue shark fits the above theory. The numerous mitochondria distributed in the basal and lateral portions of the outer uterine epithelial cells can then be related to the active transport of solute across the basal and lateral plasma membrane of the cell, which would make the inter-cellular space hypotonic or hypertonic and result in coupled water flow into or out of the space. In addition, plentiful tissue fluid in the connective tissue of the uterine wall suggests highly active fluid transport across the epithelium in the pregnant blue shark. The intra-uterine epithelium is thought to be involved in the regulation of the osmotic pressure of the uterine fluid.

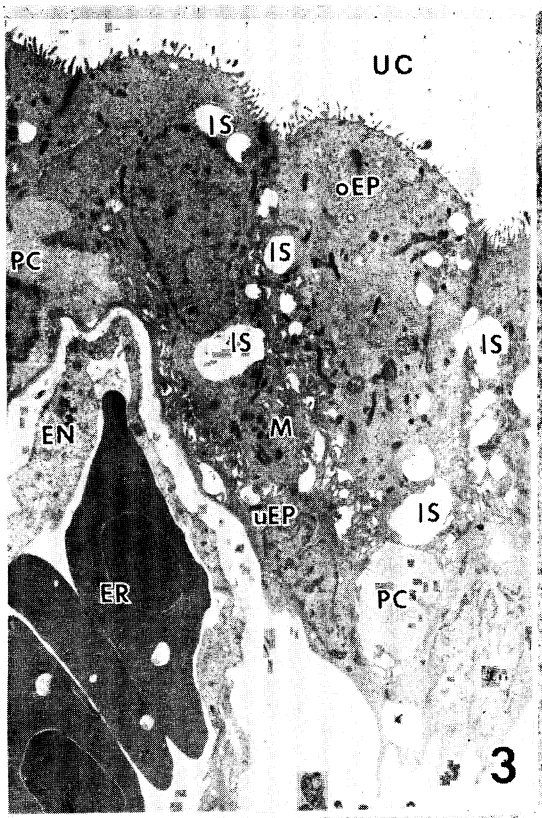
On the other hand, few mucous cells were observed in the epithelium, compared with the other placental species, *Mustelus canis* (Graham, 1967) and *Sphyrna tiburo* (Schlernitzauer and Gilbert, 1966), whose intra-uterine epithelium is covered with mucous cells that seem to play an important part in fetal nutrient supply. Moreover, secretive characteristics were not observed in the intra-uterine outer epithelial cells of the blue shark.

Fig. 3. The intra-uterine epithelium and the capillaries under the epithelium. Open inter-cellular spaces (IS) can be observed. Many mitochondria (M) are distributed in the basal and lateral portions of the outer epithelial cell. EN, endothelium of capillary; ER, erythrocyto; oEP, outer epithelial cell; PC, phagosomal cell; UC, uterine cavity; uEP, underlying epithelial cell. ×2790.

Fig. 4. The free surface of the epithelium. The plasma membrane between microvilli (MV) often invaginate (arrows). CV, coated vescicle; UC, uterine cavity. ×25,740.

Fig. 5. The basal portion of the epithelium. Abbreviations as in Fig. 1. ×26,550.

Fig. 6. Light, phagosomal cell (PC) in the epithelium. Lysosome-like granules (L) are found in the cytoplasm. Other abbreviations as in Fig. 3. ×4230.


UC
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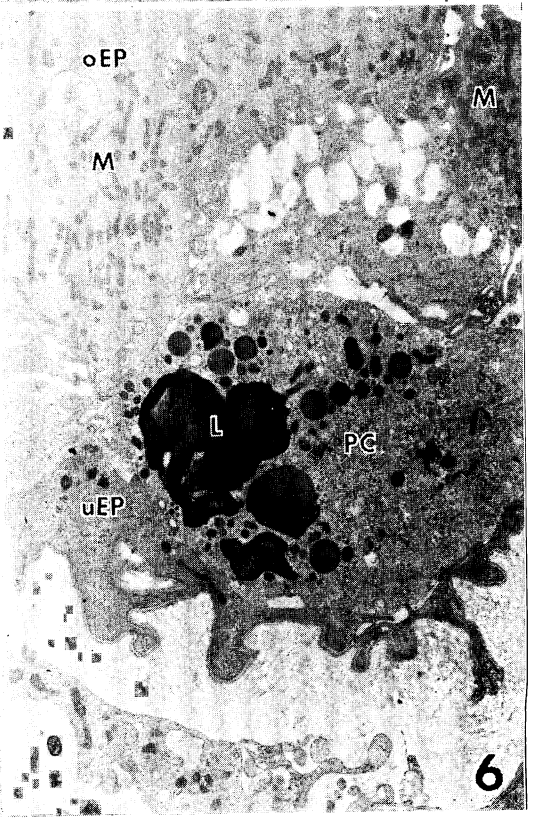
oEP
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6

These observations suggest that the uterine fluid of the blue shark contributes to fetal nutrition to a lesser extent than in *Mustelus* and *Sphyrna* at least in late gestation. That is to say, the balance in fetal nutrition between absorption of uterine fluid and placental transport leans toward the latter in the blue shark. If this balance reflects the process of evolution of placental viviparity, the reproductive system in the blue shark can be said to be more advanced.

In *Mustelus canis* fetal absorption of uterine secretions dramatically increased after the establishment of placenta (Graham, 1967). The function of the intra-uterine epithelium seems to change in consequence with the establishment of placenta. The functional change of the intra-uterine epithelium during gestation should be examined in relation to placental establishment and fetal development.

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妊娠後期ヨシキリザメの子宮内壁の微細構造

大竹二雄・水江一弘

妊娠後期のヨシキリザメの子宮内壁の微細構造を観察検討した結果，次のことが明らかとなった．子宮内壁上皮は2層の細胞からなっていた．上層の上皮細胞の細胞間隙は極めて拡張しており，多数のミトコンドリアが細胞質の基底部から側部にかけて分布していた．下層の上皮細胞は著しく扁平化していた．上皮中に粘液細胞が並ぶ部分がわずかに見られたが，その他の部分には粘液細胞は少数しか認められなかった．また上皮細胞には分泌像が認められなかった．上皮下の毛細血管の内皮は薄く，上皮に密着していた．これらの形態は上皮を通じての水

分・電解質の輸送およびガス交換に有利なものと考えられる．以上のことからヨシキリザメの子宮内壁は胎仔の栄養物質の分泌よりもむしろ子宮内液の浸透圧調節および母体-胎仔間のガス交換に関与しているものと推察された．

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