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The Fine Structure of the Placenta of the Blue Shark, *Prionace glauca*

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Abstract The fine structure of the placenta of the blue shark, *Prionace glauca*, was observed. The placenta was divided into distal and proximal portions. In the distal portion, an egg-capsule was not present, and maternal and fetal epithelia lay side by side. In the distal portion, the maternal epithelial cells contained many granules, lipid droplets, mitochondria, and developed RER and Golgi complexes. Fetal epithelial cells were covered with microvilli and possessed many small tubular structures and coated vesicles in the apical region. The space between maternal and fetal epithelia was filled with high electron dense and PAS-positive materials which seemed to be derived from maternal epithelium. In the proximal portion, the epithelium was characterized by expanded intracellular spaces. Many mitochondria were scattered in the basolateral portion of epithelial cells. From these morphological observations, it is thought that the fetus obtains its nutrients including macromolecular materials through the placental tissue and through the exchange of water-solute and gases between the uterine fluid and the epithelium of the proximal portion of the placenta or the gill, at least at full-term. Ovoid cells which seemed to be macrophage cells were observed in the intracellular spaces of the fetal placenta.

The reproductive pattern of sharks includes a placental form of viviparity. Morphological studies on the placenta have been carried out on *Scoliodon palasorrah*, *S. surrakowah* (Mahadevan, 1940), *S. laticaudus* (Teshima *et al.*, 1978), *Carcharhinus falciformis* (Schlernitzauer and Gilbert, 1966), *C. dussmieri* (Teshima and Mizue, 1972), *Sphyrna tiburo* (Gilbert and Schlernitzauer, 1966), *Mustelus canis* (TeWinkel, 1963; Graham, 1967), and *M. griseus* (Teshima, 1975). The placentae are all of yolk-sac type and contain an egg-capsule between the maternal and fetal tissues except in the case of *Scoliodon*. The placental tissue which intervenes between the maternal and fetal blood systems is composed of the following five cell layers; (1) maternal endothelium, (2) maternal epithelium, (3) egg-capsule, (4) fetal epithelium, (5) fetal endothelium, although the thickness of these layers or the intimacy of maternal-fetal tissue contact varies among the species (Wourms, 1977). Despite these studies, there have been few physiological studies on the function of the placentae except for Graham's (1967) study on *M. canis* using H^3-glucose as a tracer.

The blue shark, *Prionace glauca*, one of the most common pelagic sharks, is also known to have a placenta (Calzoni, 1935; Tucker and Newnham, 1957). But there have been no reports on the detailed structure and function of the placenta. In the present study, the fine structure of the placenta was observed and possible functions of the placenta are discussed.

Materials and methods

The four pregnant specimens of blue shark used in this study (204.0–241.0 cm in total length) were caught with a tuna long line from the R/V *Tansei-Maru*, a research vessel of the Ocean Research Institute, University of Tokyo, in the waters off Miyake Island on Feb. 21–23 in 1981.

These specimens carried 7–24 fetuses which measured 26–38 cm in total length. The number of fetuses was few, compared with the data of Suda (1953), Gubanov and Grigor'yev (1975) and Pratt (1979). This was because premature partrition occurred when the sharks were lifted on deck. Suda (1953) and Pratt (1979) estimated that the size at birth for the blue shark was between 30 and 45 cm in folk length. Therefore, the fetuses collected in this study seemed to be near full term.

For electron microscopy, placental tissues were cut into small pieces and fixed for 4–7 days in a cold fixative containing 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.4, with 10% succrose added. They were then rinsed for 2 hours in

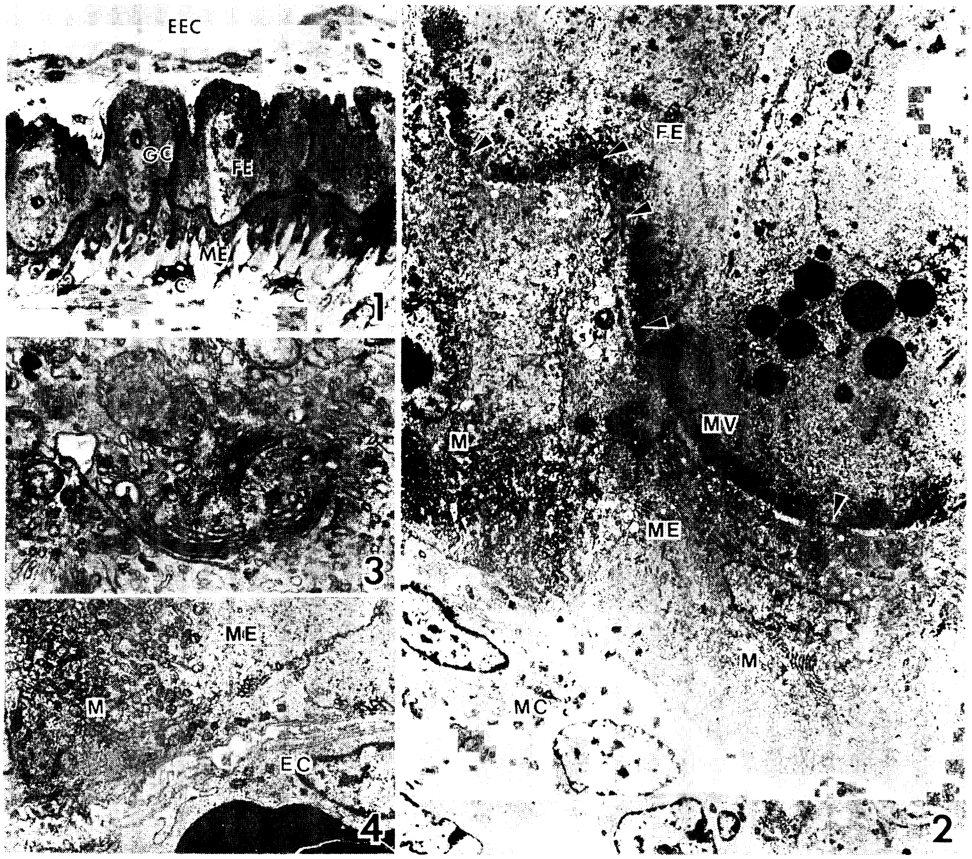

Fig. 1. Distal portion of the placenta. FE, fetal epithelium; ME, maternal epithelium; C, capillary; EEC, extra-embryonic coelm; GC, giant cell. Epon embedding, toluidine blue staining. ×210.

Fig. 2. Distal portion of the placenta showing maternal epithelium (ME) and fetal epithelium (FE). Numerous mitochondria (M) are observed in the basal region of the cytoplasm of the maternal epithelium. Fetal epithelium is covered by microvilli (MV). Arrows show the portion where maternal and fetal epithelia are in close contact. MC, maternal connective tissue. ×2,660.

Fig. 3. Golgi complex in the epithelial cell of the maternal placenta. ×14,000.

Fig. 4. Basal portion of the maternal epithelium (ME) and the endothelium of the capillary (EC). ×4,620.

the same buffer. Then they were postfixed for 2 hours in 2% osmium tetroxide. After fixation, blocks of tissue were bloc stained overnight in 2% uranyl acetate. They were dehydrated in graded ethanol and embedded in epon 812. Ultrathin sections were cut on a LKB-ultratome, stained doubly with uranyl acetate and lead citrate and examined with a JEM-100CX electron microscope. Some thick sections, which were 1.0 μm thick, were stained with toluidine blue for light microscopic observations.

For light microscopy, whole placentae 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 placenta was divided into two distinct portions; (1) a distal portion which contacted and intimately interdigitated with the uterine wall, and (2) a proximal portion exposed to the uterine cavity.

Distal portion of the placenta.

1. Maternal placenta. This portion was composed of a simple columner epithelium and

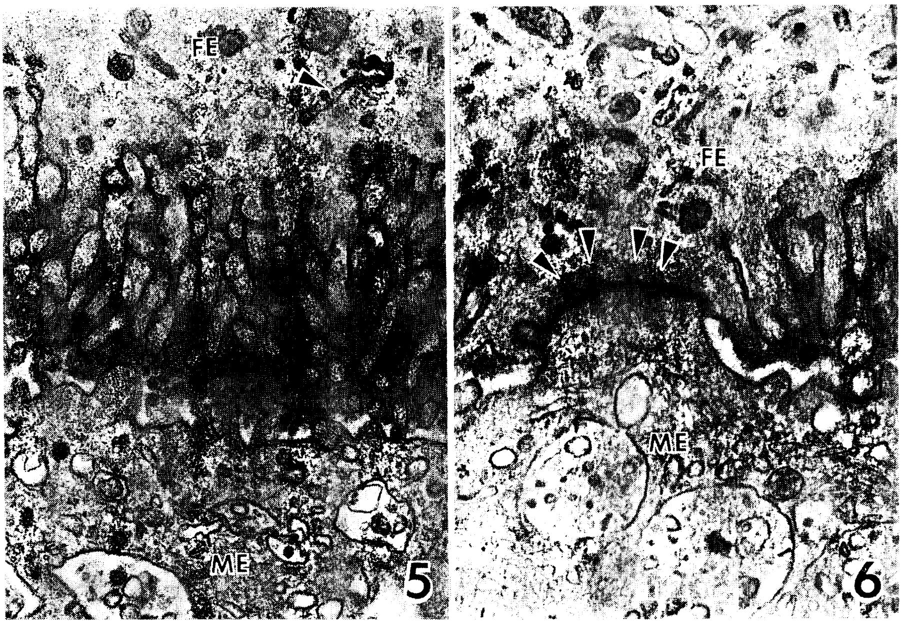

Fig. 5. The space between maternal (ME) and fetal (FE) epithelia. Arrow shows apical tubular structures in the fetal epithelial cell. ×1,800.

Fig. 6. The region where maternal (ME) and fetal (FE) epithelia are in close contact. Arrows show electron dense parts. ×22,860.

capillary network which was underlain by loose connective tissue of the uterine wall (Figs. 1, 2).

The epithelial cells varied in size. They ranged from 5 to 50 μm in height. The nucleus with one or two nucleoli was elongated and located centrally. The apical portion of the cell possessed numerous PAS-positive or negative granules and lipid droplets. Each granule was enclosed by a membrane and contained numerous particles which varied in size and in electron density (Figs. 2, 6). The inclusions of some granules were observed to be discharged by reverse pinocytosis (Fig. 5). Numerous mitochondria with several mitochondrial granules were scattered in the basal region of the cytoplasm. RER were distributed mostly under the nucleus and many Golgi complexes were present in the supranuclear part of the cell (Figs. 2, 4). In the Golgi zone, many vesicles, which appeared to be derived from the Golgi stack, were present (Fig. 3). Some of them were coated with bristles and some contained high electron dense material. All these structures are characteristic of secretive cells (Fujita, 1975). Judging from these observations, the secretory level of the cell was at a high level. The apical parts of the cells were joined on their lateral surfaces by junctional complexes. The lateral surface was smooth, although it displayed some interdigitations with adjacent cells in the basal portion. The basal surface was smooth and was underlain by basal lamella (Figs. 2, 4). The capillaries were lined up closely under the basal lamella of the epithelium. There was no stromal cell or pericapillary cell between the epithelium and the endothelium of the capillary. All of these features seem to provide an advantage for the transport of materials between the epithelium and blood in the capillaries.

2. The space between maternal and fetal placenta. In the placenta of the blue shark, the maternal epithelium adjoined directly to the fetal epithelium without an egg-capsule intervening between these two tissues (Figs. 2, 5, 6). The space was stained strongly by PAS solution. At the electron microscopic level, the space was filled with high electron dense material in which numerous small particles were present (Fig. 5). In some areas the space was very narrow (Figs. 2, 6). In these areas, both the surface of the maternal and fetal epithelial cells were smooth and electron dense parts were

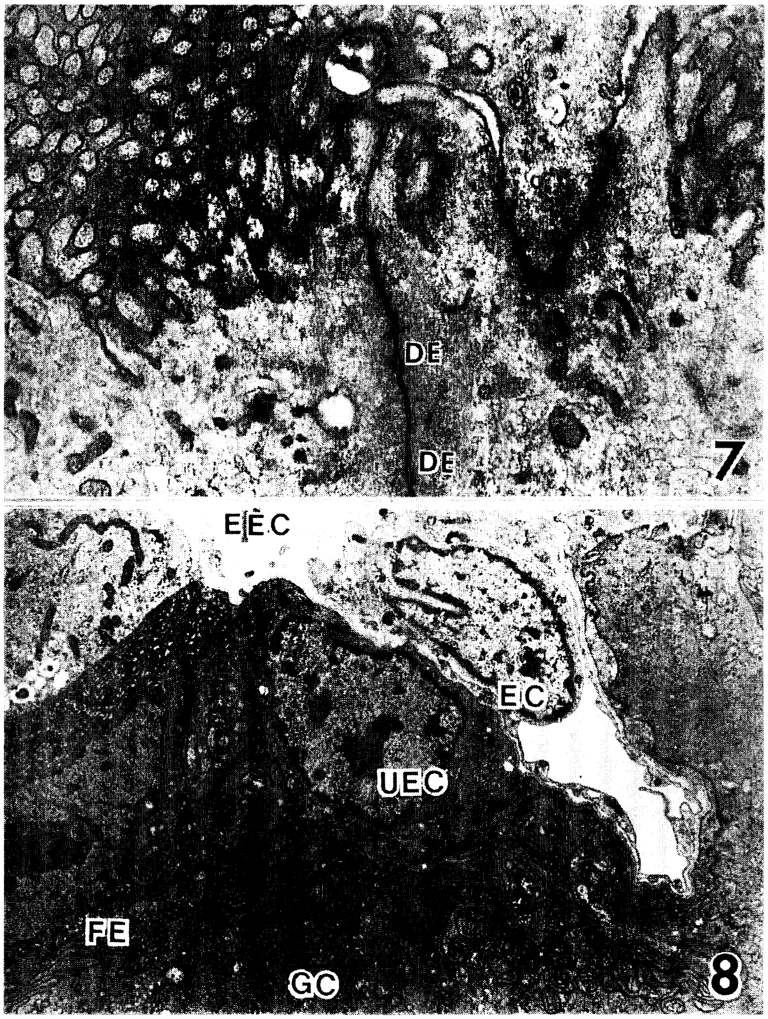

Fig. 7. The apical part of the fetal epithelium. The lateral intracellular space was of high electron density. DE, desmosomes. ×20,500.

Fig. 8. The basal portion of the fetal epithelium (FE) and the endothelium of the capillary (EC). Underlying epithelial cells (UEC) are degenerate and intimately interdigitate with the giant cell (GC). EEC, extraembryonic coelom. ×4,400.

observed lined up on the inner side of the plasmamembrane in the cytoplasm (Fig. 6). This suggests the presence of some structure connecting these two tissues.

3. Fetal placenta. This portion was composed of four tissues as follows; an epithelium, a capillary network, loose connective tissue and an endothelium lined the extra-embryonic coelom which was connected to the intestinal lumen by a vitello-intestinal duct that ran through the umbilical stalk.

The epithelium was composed of a giant cell and an underlying, extremely flattened cell. The giant cell was from 50 to 70 μm in height and from 75 to 100 μm in width. It had one or two large nuclei which were irregular in outline. The nucleus contained one or two nucleoli and was located centrally (Fig. 1). The upper portion of the cell possessed numerous granules which were weakly stained with PAS solution. They were round in shape and varied in size (0.9–2.8 μm in diameter). The free surface was covered with numerous microvilli, except for the smooth portion closely faced to the maternal epithelum

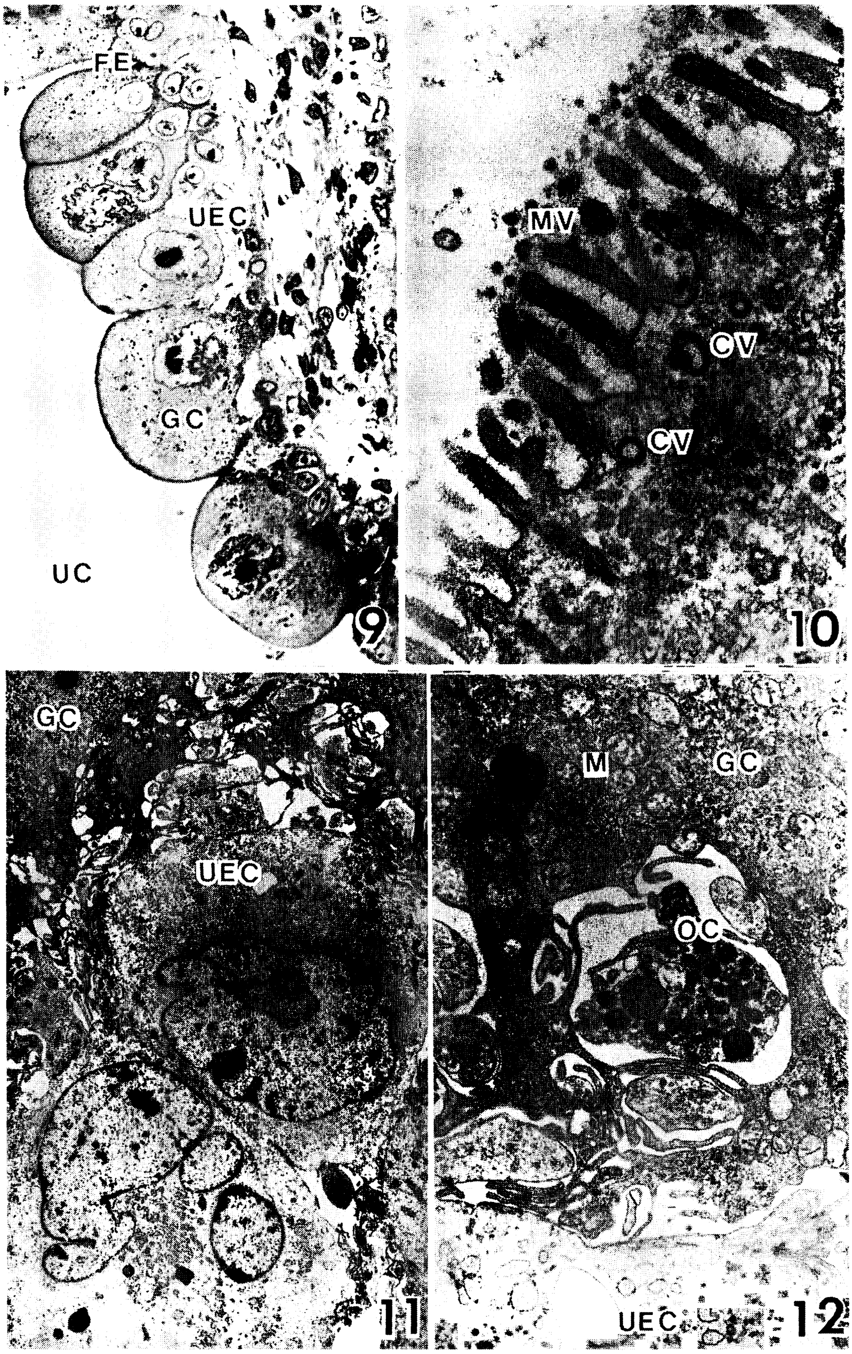

Fig. 9. Proximal portion of the placenta. GC, giant cell; UEC, underlying epithelial cell; UC, uterine cavity. Epon embedding toluidine blue staining. ×270.

Fig. 10. Free surface of the giant cell of the distal portion. MV, microvilli; CV, coated vesicle. ×28,620.

Fig. 11. Underlying epithelial cell (UEC). GC, giant cell. ×2,430.

Fig. 12. Ovoid cell (OC) observed in the intracellular space of the epithelium of the proximal portion. GC, giant cell; UEC, underlying epithelial cell; M, mitochondria. ×10,620.

(Figs. 2, 5, 6, 7). The microvilli varied in length and was branchial. The apical portion of the cell possessed numerous small tubular structures and spherical coated vesicles (Figs. 5, 6, 7). The tubular structures contained electron dense material that filled the space between maternal and fetal tissues and often connected with the plasmamembrane between the microvilli (Fig. 5). These tubular structures suggest that the cell is involved in the uptake of exogenous macromolecular material (Mayahara, 1976; Seader, 1969). Mitochondria with several mitochondrial granules were more abundant in the basal region of the cell. RER and SER were scattered throughout the cytoplasm. The lateral surface of the cell was rather smooth and came into close contact with adjacent cells (Fig. 7). The narrow intracellular space was highly electron dense. Several desmosomes were distributed along the lateral plasmamembrane. The basal surface displayed many projections and intimately interdigitated with the underlying cells (Fig. 8). Desmosomes were lined up along the basal surface.

The underlying flattened cell contained mitochondria with several mitochondrial granules, whose matrix was of higher electron density than that of the giant cell (Fig. 8). RER and SER were present all around the cytoplasm. The basal portion was irregular and rugged but few invaginations were observed (Fig. 8).

The endothelium of the capillary lay immediately beneath the epithelium, and only the basal lamina of both cells intervene between (Fig. 8). The endothelium extremely reduced the cytoplasm and several fenestrations were identified. These observations suggest that materials were actively transported in this portion.

Proximal portion of the placenta.

The structure of this portion was fundamentally the same as the fetal placenta of the distal portion. The epithelium consisted of a giant cell and one or two layers of underlying cell followed by capillaries and loose connective tissue. Under the connective tissue, one or two layers of endotherial cells lined the extra-embryonic coelom (Fig. 9). The giant cell was smaller than that of the distal portion, from 50 to 70 μm in height and from 40 to 100 μm in width. The nucleus with a nucleorus was large and irregular in shape. The free surface of the cell was covered with microvilli which measured 0.7 μm in length (Fig. 10). They were arranged more sparsely than those of the giant cell in the distal portion. The plasmamembrane between microvilli was invaginated and formed coated pits. The apical portion of the cell possessed some coated vesicles which appeared to be derived from invaginations. These observations suggest that macromolecular materials were uptaken in this area. Mitochondria with several mitochondrial granules were scattered mostly in the basolateral portion of the cell (Fig. 12). RER and SER were distributed throughout the cytoplasm. The lateral and basal surface of the cell was covered with long-branchial projections. The projections did not interdigitate with the underlying cell and the intracellar spaces were open, which suggests the epithelium is involved in water-solute transport (Diamond and Bossert, 1967, 1968; Greven, 1980). Water-solute is probably exchanged between the epithelium of this portion and uterine fluid.

The underlying cells, unlike in the distal portion, were not reduced and possessed an irregular central ovoid nucleus that contained one or two nucleoli. Many RER, SER, and mitochondria were scattered throughout the cytoplasm (Fig. 11).

Often ovoid cells, which were different from other epithelial cells, were observed in the intracellular spaces of the epithelium (Fig. 12). They displayed some projections which were not attached to adjacent cells by desmosomes. Furthermore two of them were not seen to be adjacent to one another. They were characterized by many round electron dense, lysosome-like inclusions and rather large spherical membrane-bounded granules which varied in size and in electron density. These observations of structure indicate that the cells were some kind of macrophage cells.

Discussion

All the placentae of sharks, which have been studied (TeWinkel, 1963; Gilbert and Schlernitzauer, 1966; Schlernitzauer and Gilbert, 1966; Graham, 1967; Teshima, 1975), have very degenerate maternal and fetal epithelia, except for that of *Scoliodon* which establishes a characteristic trophonematous cup (Mahadevan, 1940).

In these placentae, maternal and fetal blood streams are in close contact, although the lining of the egg-capsule, which is about 1 μm thick, intervenes between them (Graham, 1967). According to Graham (1967), low molecular weight organic materials are filtered across the placental tissues and passed up the yolk-duct into the spiral valve in the full-term fetus of *Mustelus canis*. However, the rate of exchange of materials between mother and fetus is thought to be low, because the egg-capsule is a barrier to materials passing through. So only low molecular weight materials, water-solute and gases would be exchanged through the placenta.

In contrast, in the placenta of the blue shark, an egg-capsule was not present, and both maternal and fetal epithelia were not degenerate. In the distal portion, maternal and fetal epithelia were in an active state of secreting or uptaking materials, including macromolecular materials. Furthermore, PAS-positive and electron dense materials, which seemed to be derived from maternal epithelial cells, had accumulated in the space between maternal and fetal tissues. These observations suggest that fetal nutrients, including macromolecular materials, are transported through the placental tissues. Unlike the placentae of other species, gaseous exchange did not seem to occur in placenta, because a large epithelia intervened between maternal and fetal blood systems. On the other hand, in the proximal portion the epithelium was characterized by water-solute transport rather than uptake of materials. Furthermore, the intrauterine wall was mostly covered with a reduced cell layer (Otake, in preparation). The intrauterine wall was like that of *Squalus acanthias* which is thought to be involved in with gaseous exchange and water-solute transport (Jollie and Jollie, 1963). The uterine fluid of the blue shark does not provide fetal nutrients but is involved in osmoregulation and gaseous exchange with the fetus. In the blue shark, the fetus obtains its nutrients through the placenta and the exchange of water-solute and gases occur between the uterine fluid and the fetal gill or the epithelium of the proximal portion of the placenta, at least at full-term.

Generally, the placenta is known to be an important endocrine organ that controls gestation. In the present morphological study, hormonal producing cells were not observed in the placental tissue of the blue shark. But it could not be denied that some hormonal connection between mother and fetus occurred, because macromolecular materials were transported through the placental tissues.

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ヨシキリザメ胎盤の微細構造

大竹二雄・水江一弘

ヨシキリザメの胎盤の微細構造を検討した．胎盤内には卵殻が認められず，母体部と胎仔部の両上皮が直に接していた．胎盤遠位部（接合部）の母体部上皮の細胞質内には多数の大小顆粒，脂肪滴，発達したRER，ゴルジ体，ミトコンドリアが認められ，活発な分泌活動が示唆された．母体部—胎仔部両上皮間には母体部上皮に由来する電子密度の高い，PAS 陽性の物質が充満していた．胎仔部上皮の自由表面は微絨毛で被われ，細胞質表層部には高分子物質の吸収を示す小管状構造や被覆小胞が多数認められた．一方，胎盤近位部（非接合部）の上皮では，細胞間隙の拡張が認められた．またミトコンドリアが細胞質の側部から基底部に多数分布し，胎盤近位部が水分・電解質の輸送に関与していることが示唆された．胎盤胎仔部の上皮中に大食細胞と考えられる細胞が認められた．

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