Surface Morphology of the Human Yolk Sac: Endoderm and Mesothelium

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Summary. The yolk sac of human embryos from the 5th to 7th week of gestation was first revealed under the scanning electron microscope (SEM) with complementary observations under the transmission electron microscope (TEM). The inner and outer surfaces of the human yolk sac basically showed profiles similar to those of other mammalian yolk sacs reported by previous workers. The free surface of the endodermal cell measured 10-15 μm in diameter and was only slightly swollen in its earlier stages, possessing microvilli of 100-600 nm in length. On the other hand, the outer surface of mesothelial cells was swollen, but much smaller in size (8-10 μm). The mesothelial microvilli were much longer than those of the endoderm, measuring about 1.5 μm in length. On the endodermal surface, ruffles and various sizes of holes with short microvilli were occasionally found. The latter became larger with development, and seemed to be continuous with the endodermal tubules.

The mammalian yolk sac has lost the ability to store yolk materials as that of the lower vertebrates. However, the yolk sac remains very important as an original site of primordial germ cells and also the main site of early hemopoiesis. Furthermore, it has been indicated that the human yolk sac synthesizes certain kinds of protein (GITLIN and PERRICELLI, 1970; GITLIN et al., 1972; TIEDEMANN and MINUTH, 1980a; KING and WILSON, 1983; SHI et al., 1985).

Morphological studies of the yolk sac in some mammalian species have been made by transmission electron microscopy (TEM) (DEMPSEY, 1953; HAAR and ACKERMAN, 1971; KING, 1971; STEPHENS and EASTERBROOK, 1971; TIEDEMANN, 1976, 1977, 1979; KARIM et al., 1979; TIEDEMANN and MINUTH, 1980b; KING and WILSON, 1983; LEE et al., 1983), and by scanning electron microscopy (SEM) (KARIM et al., 1979; TIEDEMANN, 1979; TIEDEMANN and MINUTH, 1980b; KING and WILSON, 1983).

In the human, SEM investigation of the yolk sac is unavailable, except for a single report which is actually just an additional observation appended to a study of fetal protein synthesis in the human yolk sac (SHI et al., 1985), although some TEM studies have been made (HOYES, 1969; HESSELDALH and LARSEN, 1969, 1971; FUKUDA, 1973; GONZALEZ-CRUSSI and ROTH, 1976; TAKASHINA, 1981a, b). It is also important to know the surface structure of human yolk sac for a study of its function. In the present study, the surface topography of human yolk sac was studied by SEM, with some TEM observations.

MATERIALS AND METHODS

The yolk sacs used for this study were taken from human embryos from the 5th to 7th week of gestation, all being obtained by legal abortions. Six of them were from 5th week embryos, one from 6th week and two from 7th week. All materials were prefixed by 3% glutaraldehyde (0.05M cacodylate buffer pH 7.4) for 1 to 3 hrs and then fixed by 1% OsO_4 (0.05M cacodylate buffer pH 7.4) for 1 hr. Subsequently, they were washed in the same buffer. During this procedure, the yolk sacs were cut into three or four pieces respectively. They were dehydrated in a graded series of ethanol and dried by the critical point drying method using liquid CO_2 . Each piece of yolk sac was mounted either inner or outer surface up on the aluminum stub. After being coated by ion-sputter coating with gold palladium, they were examined in a JSM-50A JEOL scanning electron microscope.

For TEM, a part of the yolk sac from a 5-week-old embryo was embedded in epoxy resin after dehydration. Ultrathin sections were cut, stained with uranyl acetate and lead citrate, and observed in a Hitachi HU-12 A transmission electron microscope.

RESULTS

Constitution of the wall of the yolk sac

The wall of the yolk sac was basically constructed of three layers (Fig. 1): the internal endoderm continuous with the midgut epithelium, the external mesothelium, and the intermediate mesenchyme; these measured 100–200 μm in thickness by the 5th week, and 200–400 μm by the 7th week.

The endoderm, which was generally single layered, or partly multiple layered,

Fig. 1. Thick section of the human yolk sac (5th week) stained with toluidine blue. The wall of the yolk sac is composed of an inner endoderm layer (*En*), intermediate mesenchymal layer including blood vessels (*Bl*), and outer mesothelial layer (*Ms*). In the endoderm, the endodermal tubule is recognized (small arrow). The prominence of the mesothelium reflects the underlying blood vessels (large arrows). $\times 150$

Fig. 2. SEM image of the endodermal surface of a specimen from the 5th week. Endodermal cells generally exhibit a rounded shape and are slightly swollen at this earlier stage of development. The cell borders are clearly recognizable. $\times 1,000$

Fig. 3. Enlargement of a part of Figure 2. The cell surface is covered with various lengths of microvilli. The microvilli appear to be branched. $\times 8,000$

Fig. 4. Ruffles on the surface of the endoderm at late 5th week. Among the microvilli, a number of long and thin fibers are present (arrows). $\times 6,000$

Fig. 5. A pit found on the surface of the endoderm of a specimen from the 7th week. The pit is edged by several endodermal cells. Within the pit, round and small droplets are found. At this later stage of development, microvilli on the endodermal surface become extremely shortened. $\times 1,000$

was comprised of large cuboidal or columnar cells ($20\text{--}40\ \mu\text{m}$). The nucleus of the cell was large and round, containing one or two distinct nucleoli. In the endoderm, various sizes of lumens, or so-called 'endodermal tubules' (HESSELD AHL and LARSEN, 1969; TAKASHINA, 1981a), were observed (Fig. 1). The endodermal tubules developed and increased in size and number with the progress of development.

The mesothelium was composed of simple squamous or cuboidal cells which were much smaller than the endodermal cells, measuring about $10\ \mu\text{m}$ in size. These two layers mentioned above showed epithelial properties in that they had microvilli on each free surface and a basement membrane at the basal portion of the cell. The space between the endoderm and mesothelium was occupied by mesenchymal cells and blood vascular tissues.

Inner surface of the yolk sac

In the 5th week of development, the inner surface of the yolk sac, consisting of endodermal cells, looked rather flat at low magnifications compared with the outer mesothelial surface. The shape of the free surface of the endodermal cell was usually round or oval, and sometimes polygonal (Fig. 2). The free surface of each cell was slightly swollen and measured about $10\text{--}15\ \mu\text{m}$ in diameter. The borders of the cells were easily recognizable.

At higher magnifications, many microvilli were observed to cover the free surface of the cell (Fig. 3). The diameter of the microvilli measured about $100\ \text{nm}$ and their lengths were variable. Specifically, the longer microvilli were about $0.6\ \mu\text{m}$, but shorter ones were only $0.1\ \mu\text{m}$ or less. The microvilli at latter developmental stages tended to be shortened. They were frequently observed to be branched. At the peripheral edges

Fig. 6. Enlargement of a part of the pit shown in Figure 5. The wall of the endodermal hole possesses the same microvilli as seen on the endodermal surface. $\times 3,000$

Fig. 7. TEM image of the apical part of the endodermal cells (5th week). The apical edge of the cell is somewhat raised and has microvilli. At the cell neck, junctional complexes are observed (arrowheads). $\times 6,000$

of the cells there were somewhat fewer microvilli, so that the border of each cell was distinct.

In some specimens, ruffles were observed on the endodermal surface (Fig. 4). These structures resembled the secreting feature of the goblet cell in the respiratory tract (ANDREWS, 1979), or absorbing feature observed in the thyroid gland (KETELBANT-BALASSE, 1980).

At times, long fibers more slender than the microvilli were observed. It would seem that these fibers resemble the collagen fibers reported in the monkey by KING and WILSON (1983).

In latter stages, various sizes of round holes were found at the endodermal surface (Fig. 5), ranging from 30 to 60 μm in diameter. The surface of the cells lining these holes also had short microvilli. Moreover, several droplet-like structures were found within the holes (Fig. 6). With the advance of development, the swelling of the cell surface was reduced and became flattened.

In TEM (Fig. 7), the apical edge of the endodermal cell exhibited a slightly raised appearance, with microvilli. The length of the microvilli measured 1.5–2 μm . Just beneath the apical cell membrane a bundle of microfilaments was observed. Each cell was connected with its neighboring cell at the cell apex, where a junctional complex could be observed.

Outer surface of the yolk sac

The outer surface of the yolk sac, which was composed of the mesothelial epithelium, exhibited a conspicuously rugged profile at low magnifications, especially compared

Fig. 8. SEM image of the mesothelium of a specimen from the 5th week. Mesothelial cells show a warty appearance, due to the conspicuous swelling of each cell. Various sizes of aggregations of the mesothelial cells are found (arrows). Beneath these aggregations, blood islands are present. $\times 300$

Fig. 9. Mesothelial surface of an older specimen (7th week). The ramified blood vessels are distinctly in relief (arrows). $\times 300$

Fig. 10. Higher magnification of the mesothelial surface. Mesothelial microvilli are much longer than the endodermal ones. A majority of these is oriented randomly except those of the upper left cell, which has radially oriented microvilli. $\times 3,000$

Fig. 11. This image also shows the mesothelial surface (7th week). Note the long and slender fibers among microvilli (arrows). These fibers seem to be collagen fibers. $\times 6,000$

Fig. 12. TEM image of the mesothelial cell (5th week). Microvilli are much longer than those in the endoderm, and most of them are branched. Just beneath the surface, large numbers of vesicles are found. $\times 5,000$

with the endodermal surface (Fig. 8). This appearance is attributed to the prominence of the aggregation of mesothelial cells. In the 5-week-old specimens, these mesothelial prominences measured about $13\text{--}20\ \mu\text{m}$. They represented the blood islands and primitive blood vessels developing in the underlying mesoderm (Fig. 1). The rugged structures on the surface became increasingly prominent with maturation. Particularly by the 7th week of gestation was the arborizing relief of underlying blood vessels clearly recognized (Fig. 9).

The mesothelial cells were generally round or oval in shape with a hemispherical appearance, measuring $8\text{--}10\ \mu\text{m}$ in diameter (Fig. 10). On the surface, numerous microvilli were present. The mesothelial microvilli were much longer than those of the endodermal cells, measuring about $1.5\ \mu\text{m}$ in length. They were branched and complex in structure. In other cases, however, they were extended radially on the hemispherical surface. Among the microvilli, long and thin fibers were often found (Fig. 11). These would seem to be the collagen fibers mentioned by KING and WILSON (1983). The borders of each cell were clearly discerned in the surface view in spite of their long microvilli.

The surface of the stalk portion continuous with the embryo proper showed the

same profile as the outer surface of the yolk sac, although deep foldings were present parallel to the long axis of the stalk.

The sizes of the microvilli were measured more exactly by TEM. Their lengths were 3–3.5 μm , almost double those of the endoderm (Fig. 12). Branching microvilli were also found. In addition, many vacuoles were present in the apical cytoplasm.

DISCUSSION

In the present study, the yolk sac of the human embryo was revealed under the SEM, and the detailed morphology of its internal and external surface described. The results were almost same as those from other mammals but for certain respects (bat: KARIM et al., 1979; monkey: KING and WILSON, 1983; cat: TIEDEMANN, 1979; pig: TIEDEMANN and MINUTH, 1980). Although most of those references did not describe the size of the microvilli, it was reported that pig endodermal microvilli measured 0.8 μm , while cat mesothelial microvilli measured 2.5 μm . Generally, the microvilli of the mesothelium are much longer than those of the endoderm. In the human yolk sac, in fact, the mesothelial microvilli were almost twice as long as those of endoderm.

Recently, SHI et al. (1985) have presented some SEM photographs of the human yolk sac in addition to a study on the synthesis of fetal proteins. However, they did not refer to its detailed structure.

In specimens from the late 5th week, the ruffles were found on the endodermal surface. These ruffles may be characteristic in the endoderm of the human yolk sac. Similar structures have been observed in the thyroid gland (KETELBANT-BALASSE, 1980) and uterus (ENDERS and NELSON, 1973), and considered to be related to absorption. The ruffles found in this study, however, rather resemble the secreting figures of goblet cells (ANDREWS, 1979). As it has been reported that the yolk sac excretes some kinds of proteins in the human and pig (GITLIN and PERRICELLI, 1970; GITLIN et al., 1972; TIEDEMANN and MINUTH, 1980a; SHI et al., 1985), it may be thought that endodermal ruffles are related to secretory processes.

Another particular structure observed in this study was the holes on the endodermal surface found at latter stages. SHI et al. (1985) also observed the same holes in the yolk sac of a 7-week-old embryo. The lining of these holes had short microvilli as well. Since the endodermal tubules also have similar short microvilli (HESSELDahl and LARSEN, 1969; TAKASHINA, 1981b), it is presumed that these holes continue to the endodermal tubules. The droplet-like structures found in the endodermal holes may represent the excretory figures. This, however, remains to be ascertained by TEM.

KING and WILSON (1983) observed much longer and thinner fibers than the microvilli on the mesothelial surface and assumed them to be collagen fibers. We have also observed similar fibers on the mesothelial surface. In our observation, however, these fibers were found on the endodermal surface as well.

The sizes of the microvilli observed in the SEM was different from those in the TEM. This difference is thought to be due to several reasons, one being that the measurement of microvilli in SEM was not done from the side view but their oblique appearance. Another reason is probably the shrinkage of the tissues during the preparation procedures for SEM.

As the samples for TEM in this study were insufficient in number, further study into this problem by TEM is deemed necessary.

REFERENCES

- Andrews, P. M.:** The respiratory system. In: (ed. by) G. M. Hodges and R. C. Hallows: Biomedical research applications of scanning electron microscopy, Vol. 1. Academic Press, New York, 1979 (p. 177-202).
- Dempsey, E. W.:** Electron microscopy of the visceral yolk sac epithelium of the guinea pig. *Amer. J. Anat.* 93: 331-364 (1953).
- Enders, A. C. and D. M. Nelson:** Pinocytotic activity of the uterus of the rat. *Amer. J. Anat.* 138: 277-300 (1973).
- Fukuda, T.:** Fetal hemopoiesis. I. Electron microscopic studies on human yolk sac hemopoiesis. *Virchows Arch. Abt. B Zellpathol.* 14: 197-213 (1973).
- Gitlin, D. and A. Perricelli:** Synthesis of serum albumin, prealbumin, α -fetoprotein, α_1 -antitrypsin and transferrin by the human yolk sac. *Nature* 228: 995-997 (1970).
- Gitlin, D., A. Perricelli and G. M. Gitlin:** Synthesis of α -fetoprotein by liver, yolk sac, and gastrointestinal tract of the human conceptus. *Cancer Res.* 32: 979-982 (1972).
- Gonzalez-Crussi, F. and L. M. Roth:** The human yolk sac and yolk sac carcinoma. An ultrastructural study. *Human Pathol.* 7: 675-691 (1976).
- Haar, J. L. and A. Ackerman:** Ultrastructural changes in mouse yolk sac associated with the initiation of vitelline circulation. *Anat. Rec.* 170: 437-456 (1971).
- Hesseldahl, H. and J. F. Larsen:** Ultrastructure of human yolk sac: endoderm, mesenchyme, tubules and mesothelium. *J. Anat.* 126: 315-336 (1969).
- : Hemopoiesis and blood vessels in human yolk sac. *Acta anat.* 78: 274-294 (1971).
- Hoyes, A. D.:** The human fetal yolk sac. An ultrastructural study of four specimens. *Z. Zellforsch.* 99: 469-490 (1969).
- Karim, K. B., W. A. Wimsatt, A. C. Enders and A. Gopalakrishna:** Electron microscopic observations on the yolk sac of the Indian fruit bat, *Rousettus leschenaulti*. *Anat. Rec.* 195: 493-510 (1979).
- Ketelbant-Balasse, P.:** The endocrine system. In: (ed by) G. M. Hodges and R. C. Hallows: Biomedical research applications of scanning electron microscopy, Vol. 2. Academic Press, New York, 1980 (p. 197-218).
- King, B. F.:** Differentiation of parietal endoderm cells of the guinea pig yolk sac, with particular reference to the development of endoplasmic reticulum. *Devel. Biol.* 26: 547-559 (1971).
- King, B. F. and J. M. Wilson:** A fine structural and cytochemical study of the rhesus monkey yolk sac: endoderm and mesothelium. *Anat. Rec.* 205: 143-158 (1983).
- Lee, S. Y., J. W. Anderson, G. L. Scott and H. W. Mossman:** Ultrastructure of the placenta and fetal membranes of the dog: II. The yolk sac. *Amer. J. Anat.* 166: 313-327 (1983).
- Shi, W-K., B. Hopkins, S. Thompson, J. K. Heath, B. M. Luke and C. F. Graham:** Synthesis of apolipoproteins, alphafoetoprotein, albumin, and transferrin by the human foetal yolk sac and other foetal organs. *J. Embryol. exp. Morphol.* 85: 191-206 (1985).
- Stephens, R. J. and N. Easterbrook:** Ultrastructural differentiation of the endodermal cells of the yolk sac of the bat, *Tadarida brasiliensis cynocephala*. *Anat. Rec.* 169: 207-242 (1971).
- Takashina, T.:** The significance of human yolk sac (In Japanese). *Sunfujinka Chiryō* 42: 735-744 (1981a).
- : An ultrastructural study of human ovarian yolk sac tumor.—An investigation of its histogenesis compared to that of human yolk sac (In Japanese). *Acta obst. gynaec. jap.* 33: 916-924 (1981b).
- Tiedemann, K.:** On the yolk sac of the cat. Endoderm and mesothelium. *Cell Tiss. Res.* 173: 109-127 (1976).
- : On the yolk sac of the cat. II. Erythropoietic phases, ultrastructure of aging primitive erythroblasts, and blood vessels. *Cell Tiss. Res.* 183: 71-89 (1977).
- : The amniotic, allantoic and yolk sac epithelia of the cat: SEM and TEM studies. *Anat. Embryol.* 158: 75-94 (1979).

Tiedemann, K. and W. W. Minuth: Synthesis of serum proteins by the posthaematopoietic feline yolk sac. *Histochemistry* 67: 155-167 (1980a).

———: The pig yolk sac I. Fine structure of the posthaematopoietic organ. *Histochemistry* 68: 133-146 (1980b).

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