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Placentation in the Opossum, *Didelphis virginiana*

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Abstract. For the first 9 days of gestation, opossum embryos float in uterine secretions, separated from maternal tissues by a shell membrane. Each embryo is part of the wall of its hollow embryonic sphere. By the 10th day of development, the embryo becomes enveloped by both the amnion and yolk-sac. The yolk-sac consits of vascular and non-vascular portions and, together with the surrounding trophectoderm (trophoblast), forms the yolk-sac placenta of the opossum: the allantois does not contribute to formation of the placenta. The vascular portion of the yolk-sac placenta establishes an intimate relationship with the uterine epithelium soon after loss of the shell membrane. The yolk-sac placenta is non-invasive. Cells of the trophoblast exhibit numerous microvilli, an apical endocytic complex and the lateral and basal cell membrane are elaborately folded. These features suggest a cell that is active in the transport of materials. Junctional complexes between cells of the trophoblast and uterine epithelium were not observed.

The uterine epithelium changes from ciliated pseudostratified columnar with few infoldings of lateral and basal cell membranes, to non-ciliated simple columnar in which these membranes show elaborate infoldings. The cells show numerous inclusions and mitochondria are polarized to the basal half of the cell. These features suggest a cell that also is active in the transport of materials.

Introduction

The North American opossum, *Didelphis virginiana*, and several Australian marsupials (brus--tailed possum, *Trichosurus vulpecula;* ring-tailed possum, *Pseudocheirus peregrinus;* Bennett's wallaby, *Protemnodon rufogrisea;* quokka, *Setonix brachycurus;* potoroo, *Potorous tridactylus*) have been described as having a well-vascularized yolk-sac placenta that establishes an intimate association with the maternal endometrium. In these species, the yolk-sac placenta consists of vascular and non-vascular portions and only a small region of true chorion is present. The latter consists of somatic mesoderm and embryonic trophectoderm [*Sharman,* 1961]. In *Didelphis,* as in many other marsupials, the allantois does not appear until relatively late in gestation and remains isolated within the folds of the yolk-sac: it never establishes an association with the chorion. The allantois does not contribute to the definitive placenta in these species, but remains an isolated, endodermally lined sac that is thought to function primarily in the storage of wastes from the mesonephric kidneys [*Krause and Cutts,* 1985a]. Three species of the family *Peramelidae* (the bandicoots) are unusual in that a true chorio-allantoic placenta, as well as a yolk-sac placenta, is formed [*Hill,* 1897; *Flynn,* 1923; *Padykula and Taylor,* 1977].

In *Didelphis,* fertilized or unfertilized oocytes reach the uterus about 24 h after ovulation [*Hartman,* 1923]. During the short transit period, each oocyte is fertilized by a single spermatozoon. Although sperm are paired in the epididymis and in the female reproductive tract [*Biggers and Delmater,* 1965; *Krause and Cutts,* 1979], the sperm pairs normally separate in the oviduct prior to fertilization [*Rodger and Bedford,* 1982a, b]. A corona radiata is not present and each oocyte initially is surrounded only by a perivitelline space and a zona pellucida [*Talbot and Di Carlantonio,* 1984]. A second layer consisting primarily of acid mucopolysaccharide is laid down around the zona pellucida after fertilization has occurred and is the secretory product of the lining epithelium of the oviduct [*McCrady,* 1938; *Rodger and Bedford,* 1982a]. The ova of the opossum and other metatherians become surrounded by a third layer, the

shell membrane [*Sharman,* 1961; *Krause and Cutts,* 1983] that is thought to be produced by secretory cells in the distal oviduct [*Hartman,* 1916; *Anderson,* 1928]. The shell membrane is said to be keratinous [*Tyndale-Biscoe,* 1975] and resistant to enzyme digestion [*Hughes,* 1977]. In most marsupials it persists as a barrier between embryo, or embryo plus forming fetal membranes, and the maternal uterine epithelium until late in gestation. The fetal and maternal tissues become intimately related only after the shell membrane is lost. Prior to this the shell membrane permits passage of large molecules to and from the surrounding uterine fluids and does not act as a barrier to most substances [*Hughes and Shorey,* 1971].

The 'morula' stage is not achieved in the opossum, which also lacks an inner cell mass [*Hartman,* 1928; *McCrady,* 1938; *Wimsatt,* 1975]. In its place a hollow, unilaminar vesicle is formed directly, from which develop all embryonic and extra-embryonic tissues [*Sharman,* 1961]. During the fourth day of gestation, the embryonic sphere of *Didelphis* consists of a single layer of flattened cells that surround a central lumen [*Selenka,* 1887; *Hartman,* 1919; *McCrady,* 1938]. Late in day 4, cells (endodermal mother cells) of this layer begin to enlarge. By day 5, the cells have migrated to the interior of the embryonic sphere to form the definitive endoderm [*McCrady,* 1938], which extends around the interior of the unilaminar sphere, converting it to a bilaminar sphere that consists of ectoderm and endoderm. By day 8, mesoderm extends beyond the forming embryo (which continues to be part of the embryonic sphere) to lie between the extra-embryonic endoderm and ectoderm (trophectoderm). The mesodermal layer is formed by a multilayered network of large stellate cells with numerous cytoplasmic processes that may course considerable distances to establish contact with adjacent mesodermal cells [*Krause and Cutts,* 1985b]. The ventral one-third of the embryonic sphere is never invaded by mesoderm and the region beyond the embryo now is termed the yolk-sac [*McCrady,* 1938].

The present study examines the development of the placenta and its relationship to the uterine epithelium in the opossum.

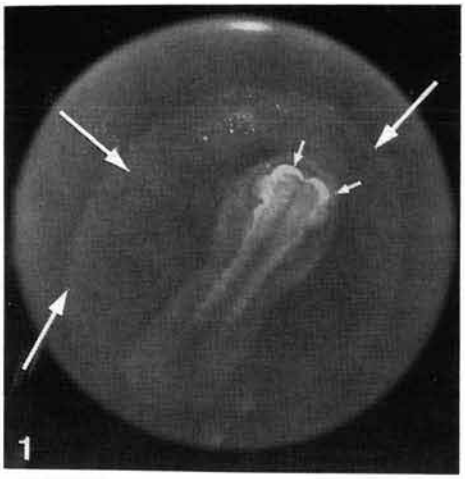
Materials and Methods

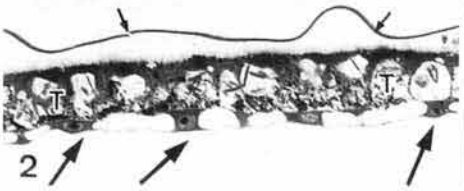
Seventy-eight embryos of *D. virginiana* were collected, the approximate ages of which were determined from timed pregnancies. Wild female opossums were stripped of any litters and placed in breeding pens with continuous access to males. A sperm-positive date was determined by examining vaginal smears each morning. At specific times after this date, embryos were collected either by killing the female by ether anesthesia and removing and opening the uteri, or by removing one uterus and allowing the mother to survive to a predetermined date when the remaining embryos were collected. Thus, a series of embryos, embryonic membranes and uteri were collected. As soon as possible after collection, the tissues were fixed in 10% neutral buffered formalin or Bouin's solution for light microscopy or in 3.0% glutaraldehyde buffered in 0.1 *M* phosphate to pH 7.4 for electron microscopy. Tissues for light microscopy were processed routinely for embedding in paraffin and sections (6 μm) were stained with hematoxylin and eosin. For scanning electron microscopy the tissues were fixed at room temperature for 4 h, transferred to 0.1 *M* phosphate buffer for 2 h, then osmicated for 2 h in 1.0% osmium tetroxide in 0.1 *M* phosphate buffer. After a brief wash in the buffer, specimens were dehydrated through graded ethanol solutions and dried by liquid CO_2 substitution in a Bomar or Ladd critical-point drier. The dried tissues were mounted on scanner stubs by means of electrical conducting tape. The surrounding shell membrane was removed from early embryos as described previously [*Krause and Cutts,* 1984]. The specimens were then coated with gold to a depth of 20 nm in a Polaron coating unit and viewed under a Joel M35 or Nanolab 2100 scanning electron microscope at 20 kV.

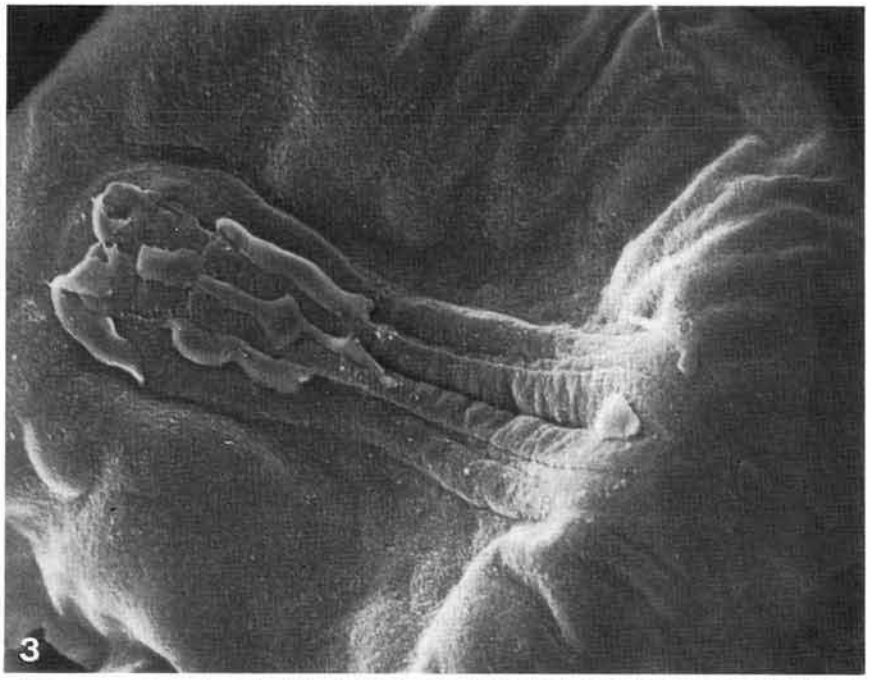
Tissues for transmission electron microscopy were fixed and dehydrated similarly, after which they were cleared in propylene oxide and infiltrated with and embedded in Epon 812. Thin sections mounted on uncoated grids were stained with uranyl acetate and lead citrate and examined under a Zeiss IIC electron microsocope operated at 60 kV. Thick sections (0.3–2.0 μm) of the Epon-embedded material were stained with toluidine blue for light-microscopic examination.

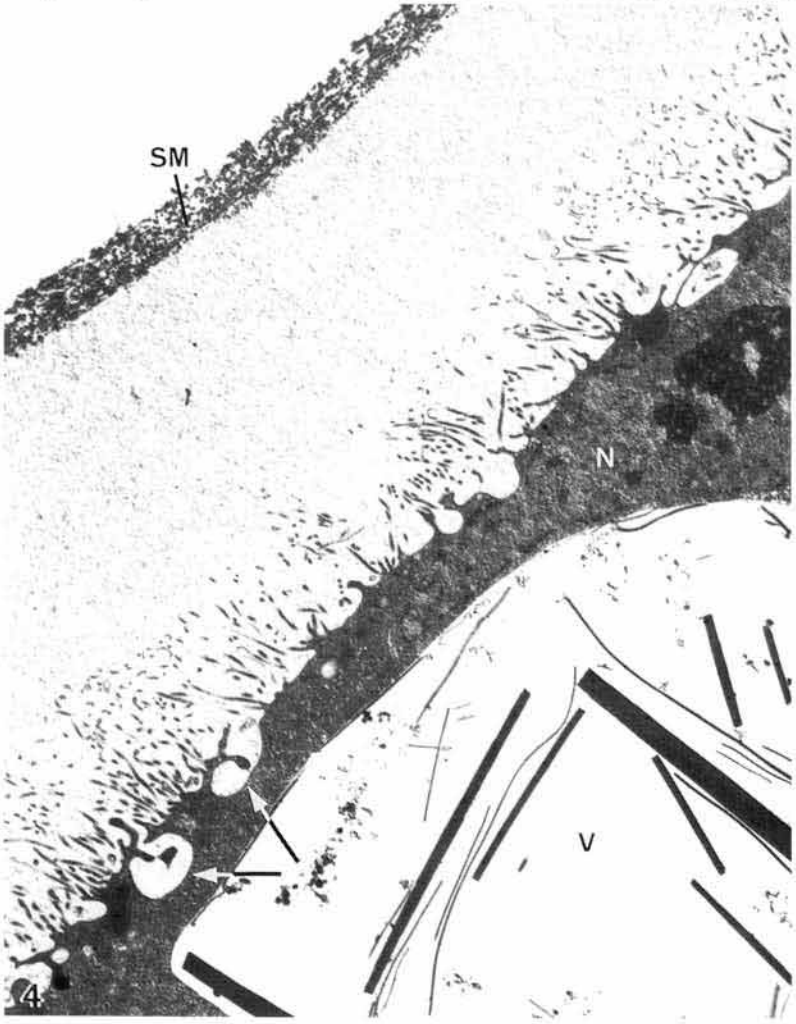
Results

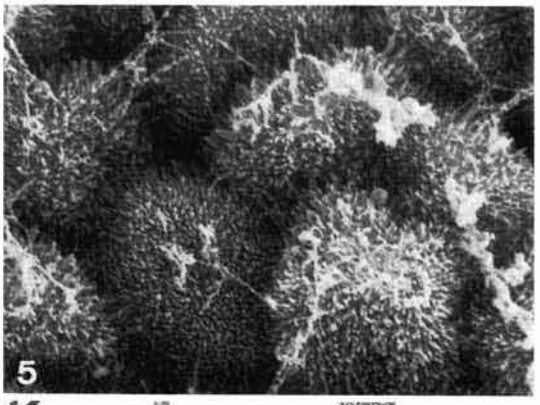
In opossum embryos, the three germ layers and the initial development of some of their derivatives are established during the first 9 days of gestation. During this period, the embryos float, unattached, in the secretions of the uterine cavity, each separated from the uterine mucosa by a transparent shell membrane (fig. 1, 2, 4). By the 9th day, the embryo occupies one pole of the embryonic sphere and structurally is part of the wall of the sphere (fig. 1, 3). The embryo proper consists of all three germ layers, while the extra-embryonic region of the sphere may show three or only two germ layers. The ectoderm of the embryo is continuous with the trophectoderm of the remainder of the embryonic sphere. The ventral one-third of the embryonic sphere is never invaded by mesoderm and represents a persisting portion of the original bilaminar sphere: it consists only of endoderm covered by trophectoderm (fig. 2, 12). It represents the bilaminar region of the embryonic sphere from which will form the non-vascular portion of the definitive yolk-sac placenta. The vascular component de-

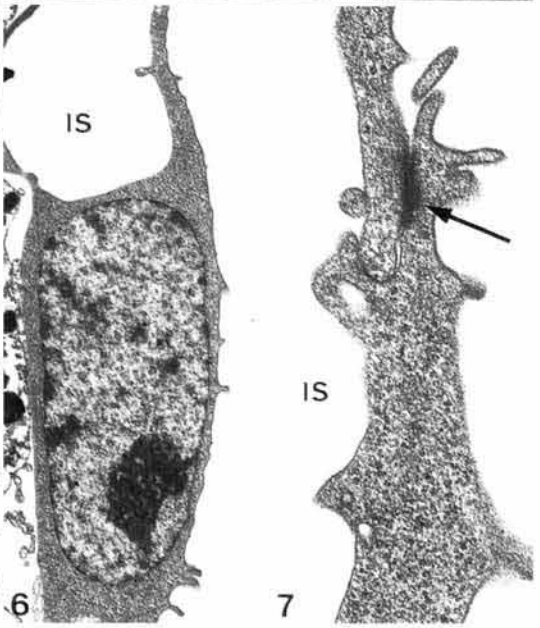












velops from the region of the embryonic sphere where extra-embryonic mesoderm lies between the endodermal layer and the trophectoderm. With continued development, small blood vessels appear within the mesodermal layer and a distinct sinus terminalis forms at the most lateral extent of the mesodermal layer. The sinus courses around the equator of the embryonic sphere (fig. 13–15).

At the 9th day of gestation, cells of the trophectoderm show numerous, elongated microvilli and a well-developed endocytic complex that occupies the apical cytoplasm (fig. 4, 5). Large, intracellular vacuoles that contain electron-dense crystalline structures or a flocculent amorphous material also characterize the cells of the trophectoderm. The vacuoles often develop to such an extent that the nuclei are displaced to the apical region of the cell and the cytoplasm is compressed to a narrow peripheral rim. Cells of the trophectoderm also show numerous free ribosomes, profiles of granular endoplasmic reticulum, scattered mitochondria, round electron-dense granules and occasional small Golgi complexes. Nuclei contain abundant euchromatin, with scattered clumps of heterochromatin along the nuclear envelope. The apices of adjacent cells are united by tight junctions and the lateral cell membranes may show elaborate infoldings that interdigitate with those of neighbouring cells of the trophectoderm. The basal cell-membrane is often smooth and without much infolding.

In contrast, endodermal cells lack the large intracellular vacuoles, the endocytic complex and the heavy concentration of elongate microvilli (fig. 2, 6). They form a single layer of squamous to cuboidal cells, often bearing elongated processes that attach, by junctional complexes, to similar processes of adjacent endodermal cells (fig. 2, 6, 7). The large intercellular space enclosed by these processes is usually devoid of any material. The nuclei of endodermal cells also contain abundant euchromatin and frequently show one or more prominent nucleoli. The cytoplasm contains numerous free ribosomes, occasional mitochondria and profiles of granular endoplasmic reticulum. Mitotic figures are frequently seen in the endodermal layer.

Scattered ciliated cells are present within the uterine epithelium during the 9th day of gestation and often appear to be nearly equidistant from one another (fig. 8). The apices of adjacent, non-ciliated cells are characterized by numerous elongated microvilli (fig. 9, 10). The uterine epithelium is pseudostratified at this time and lies on a distinct basal lamina (fig. 10, 11). The lamina propria contains abundant ground substance with only occasional connective tissue cells and collagenous fibers. The lateral and basal cell membranes of the uterine epithelial cells are relatively smooth and show few infoldings (fig. 11). Cell apices are united by tight junctions, scattered desmosomes occur between adjacent, lateral cell membranes, and the cytoplasm contains organelles typical of other epithelia. Mitochondria are localized in the basal cytoplasm (fig. 10, 11). Inclusions are abundant, some being electron-dense and irregular in shape while others (the more numerous) are less dense, fairly uniform in shape and often show a central region of greater electron density (fig. 10, 11). The prominent nuclei mainly consist of euchromatin and usually contain one or more nucleoli.

As development continues into the 10th day, the embryonic mesoderm and the extra-embryonic mesoderm immediately at its borders, split into splanchnic and somatic layers. All blood vessels that form within the extra-embryonic mesoderm lie within the splanchnic mesoderm only: the extra-embryonic mesoderm does *not* invade the region of the proamnion in *Didelphis*

Fig. 1. The 9-day opossum embryo floats freely in uterine secretions, separated from maternal tissues by a transparent shell membrane. The lateral extent of the mesoderm is visible in the embryonic sphere (large arrows). Note the initial formation of the proamniotic folds (small arrows) around the head region of the embryo. ×20.

Fig. 2. A section from the embryonic sphere on the side oppostite the embryo shows the enveloping shell membrane (small arrows). The wall of the embryonic sphere consists of trophectoderm (T) and endoderm (large arrows) only. 9-day opossum embryo. Toluidine blue. ×250.

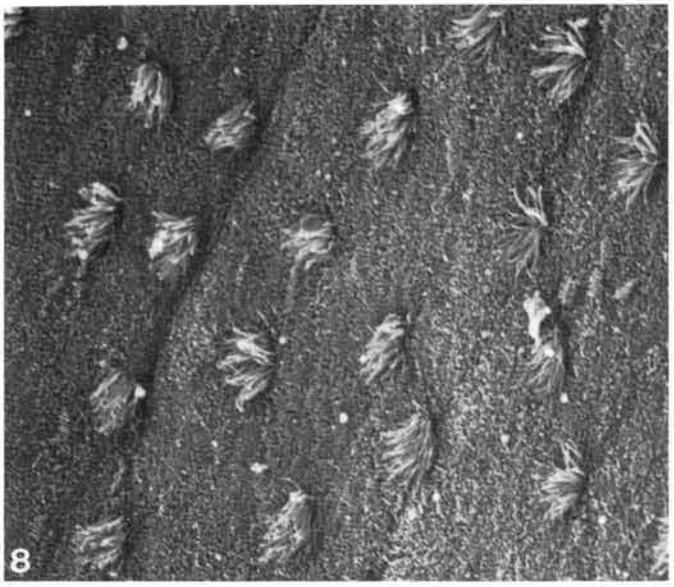
Fig. 3. Scanning electron microscopy shows the 9-day opossum embryo to be continuous with and part of an embryonic sphere. ×35.

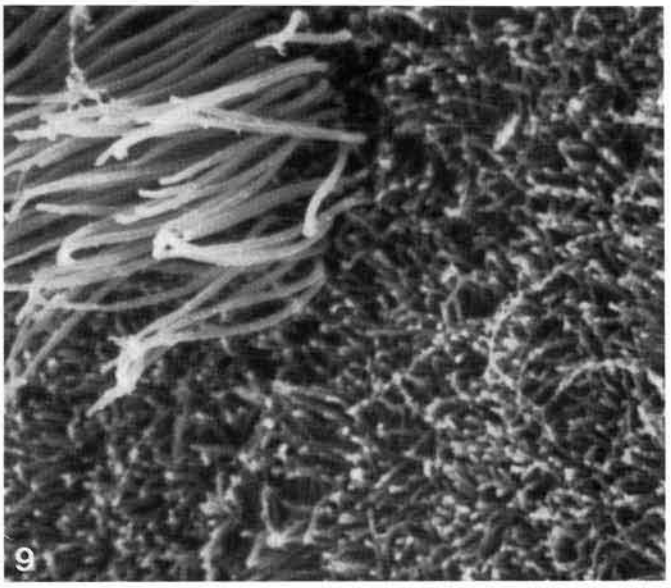
Fig. 4. Cells of the trophectoderm show a well-developed system of invaginations from the apical cell membrane (arrows). A large central vacuole (V) is usually present and may contain electron-dense crystals or flocculent amorphous material. The nucleus (N) occupies an apical position. The shell membrane (SM) is at the upper left of the micrograph. 9th day of gestation. ×12,500.

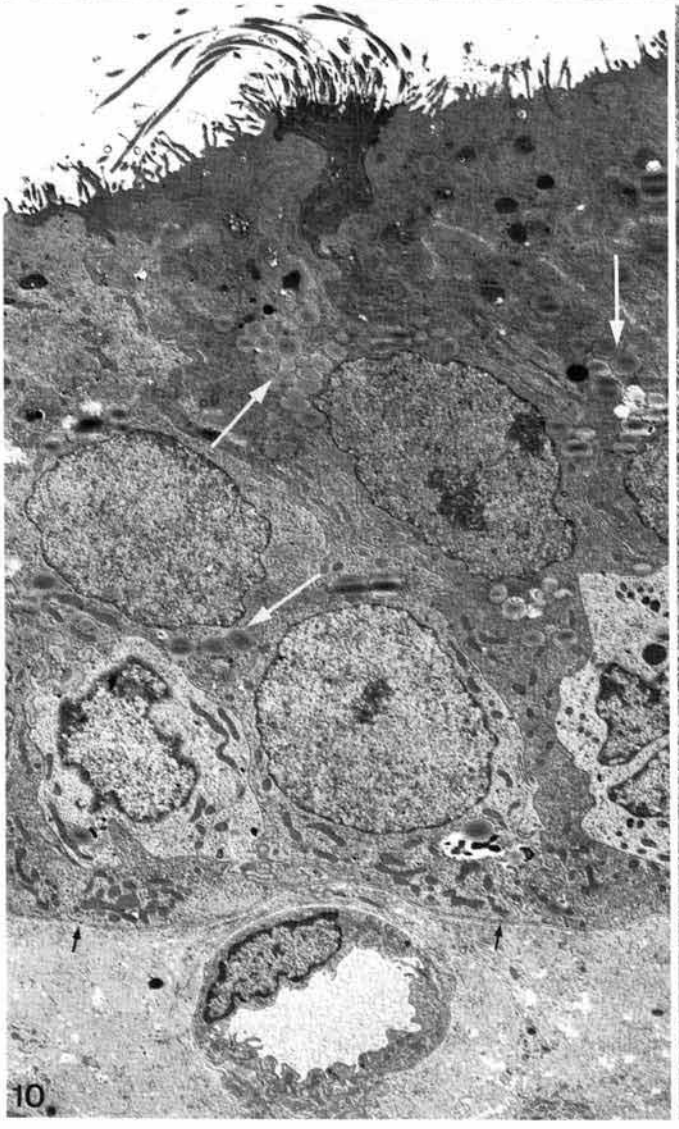
Fig. 5. The external (apical) surfaces of cells of the trophectoderm show elongate microvilli. Compare this illustration with figure 4. 9th day of gestation. ×2,000.

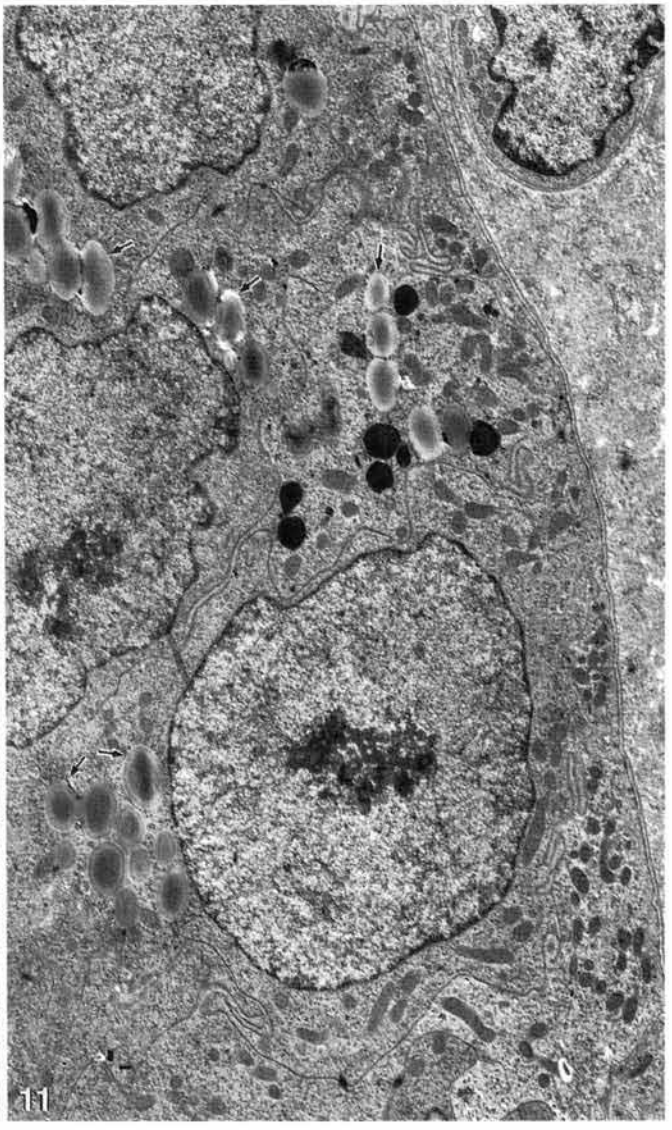
Fig. 6. A portion of an endodermal cell from the embryonic sphere on the side oppostie the embryo. Note the large intercellular space (IS). Compare with the endodermal cells shown in figure 2. 9th day of gestation. ×4,000.

Fig. 7. A junctional complex (arrow) between two cytoplasmic processes of adjacent endodermal cells. Such narrow processes (also shown in figure 2) unite with those of adjacent cells to bound the intercellular spaces (IS). ×12,000.









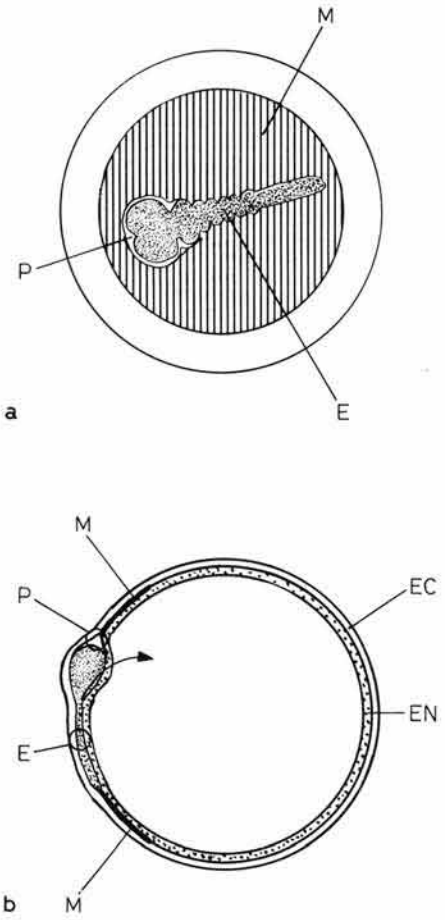


Fig. 12. **a** The extent of the extra-embryonic mesoderm (M) beyond the embryo (E) is shown diagrammatically in an embryonic sphere at 9 days of gestation. **b** The proamnion (P) lacks mesoderm and consists only of ectoderm (EC) and endoderm (EN). The head region of the embryo will extend ventrally into the embryonic sphere as a result of the cervical flexure (arrow).

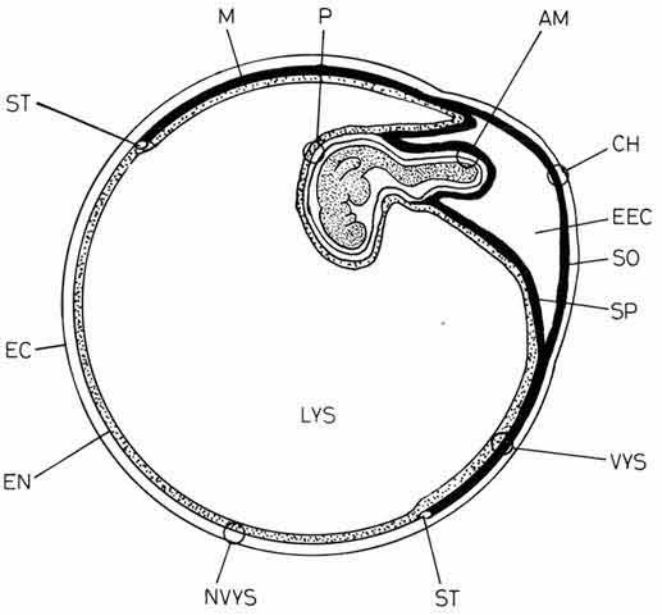


Fig. 13. After the extra-embryonic mesoderm separates into splanchnic (SP) and somatic (SO) layers, the 10-day embryo becomes enveloped by the amnion as well as the yolk-sac placenta. The amnion – proamnion (P)–that surrounds the cranial half of the embryo consists of ectoderm and endoderm only, whereas the caudal half of the amnion (AM) consists of ectoderm and somatic mesoderm. The extent of the extra-embryonic mesoderm (M), sinus terminalis (ST), extra-embryonic coelom (EEC), chorion (CH), vascular yolk-sac placenta (VYS), non-vascular yolk-sac placenta (NVYS), ectoderm (EEC; trophectoderm), endoderm (EN), and the lumen of the yolk-sac (LYS) also are shown.

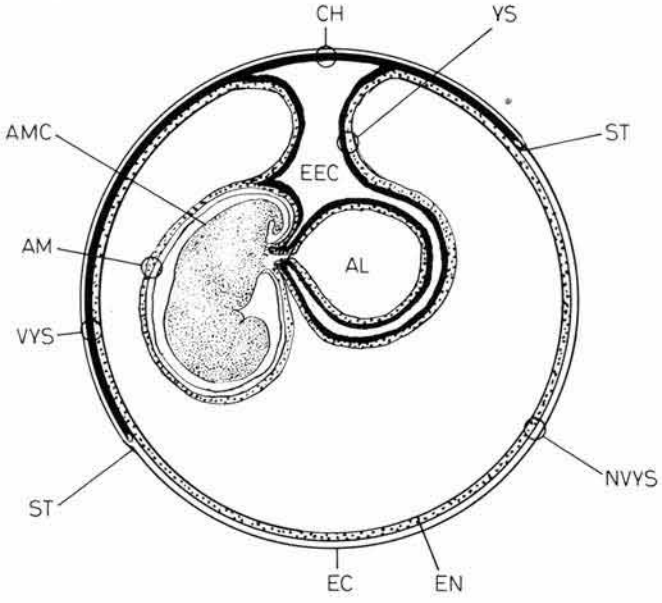


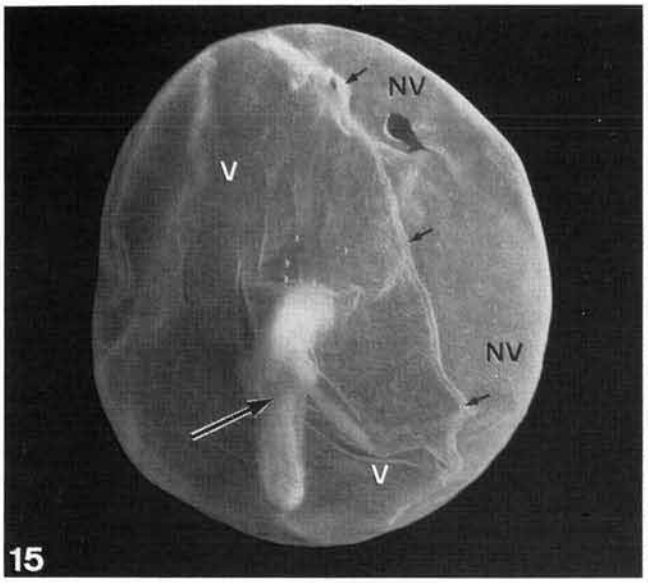
Fig. 14. By the 11th day of gestation the allantois (AL) is well established and lies within the extra-embyonic coelom (EEC), separated by folds of the yolk-sac (YS) from the adjacent chorion (CH). Also shown are the: sinus terminalis (ST), vascular yolk-sac placenta (VYS), non-vascular yolk-sac placenta (NVYS), endoderm (EN), ectoderm (EC), anmion (AM), and amnionic cavity (AMC).

Fig. 8. During the 9th day of gestation, the surface of the uterine epithelium shows numerous ciliated cells spaced nearly equidistant from one another. ×600.

Fig. 9. A higher magnification of the uterine epithelium shows that the non-ciliated cells have numerous elongate microvilli on their apical surface. ×3,400.

Fig. 10. The uterine epithelilum is pseudostratified columnar during the 9th day of gestation and lies on a distinct basal lamina (small arrows). Numerous electron-dense and electron-lucent granules are present. The latter often exhibit a slightly more electron-dense core (large arrows). Mitochondria appear in greater numbers in the basal cytoplasm. The lateral and basal plasmalemmae are relatively smooth with few infoldings. ×5,000.

Fig. 11. Greater details of the electron-dense and electron-lucent granules (arrows) are shown. Note the concentration of mitochondria near the basal plasmalemma of the uterine epithelial cells. ×12,5000.



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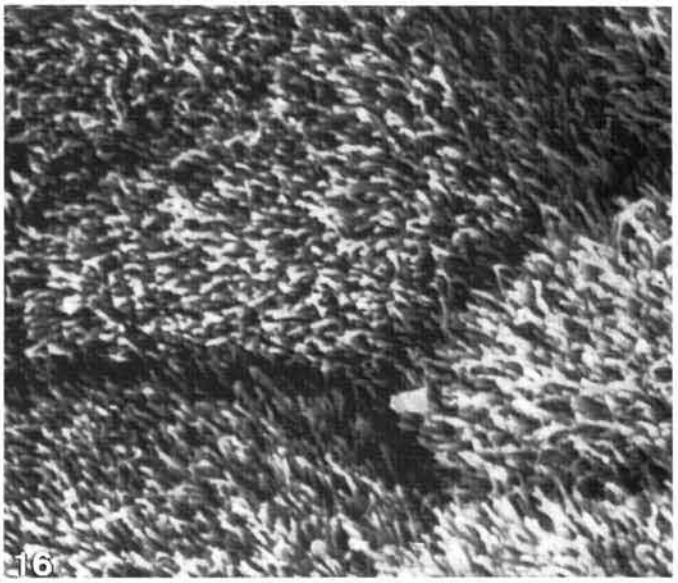
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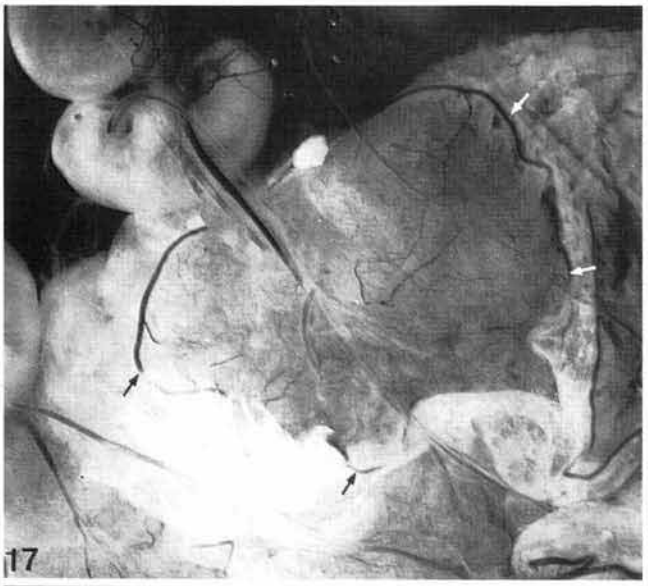
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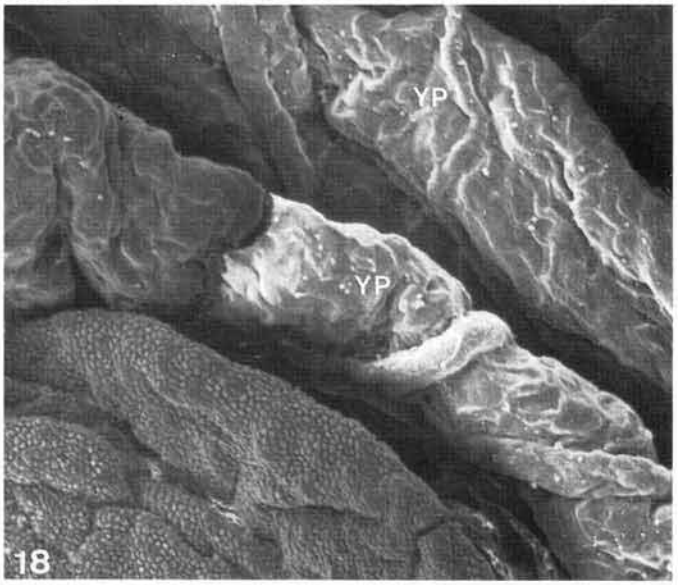

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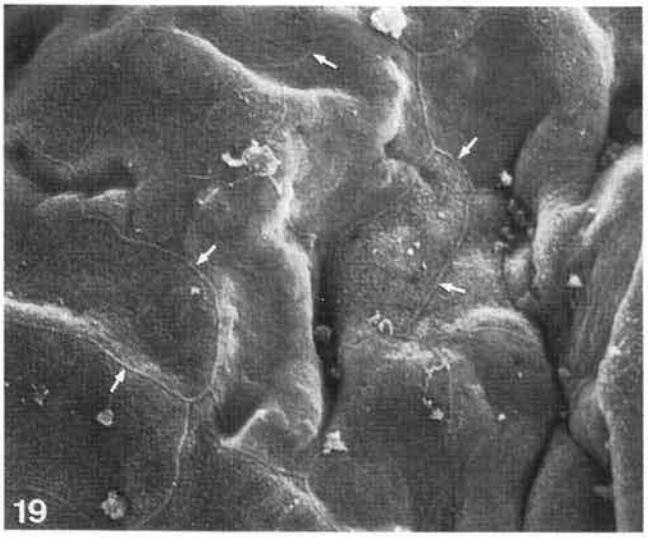
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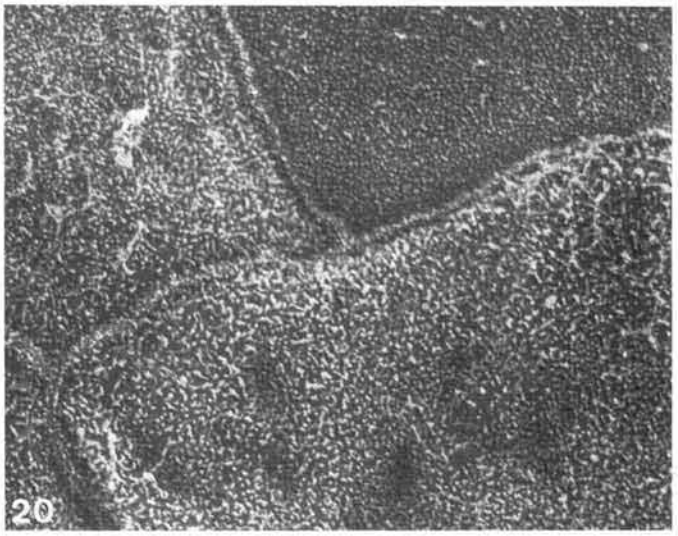
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(fig. 12). At this time, the head region of the embryo elongates and, as a result of the flexure in the region of the midbrain, the head region extends ventrally into the proamnion and thus begins to enter the lumen of the embryonic sphere (fig. 12). The amnion that surrounds the cranial half of the embryo consists only of the ectoderm and endoderm of the original proamnion. The remainder of the amnion of the opossum develops by formation of folds. The caudal and lateral folds of the forming amnion arise from extra-embryonic ectoderm and somatic mesoderm. The amniotic folds meet and fuse dorsal to the embryo during the 10th prenatal day, resulting in the formation of the amniotic sac and a small, avascular region of the true chorion. The latter consists of ectoderm and avascular somatic mesoderm only. A short stalk may unite the amnion and serosal chorion for a brief period. As a result of the formation of the amnion in this manner, the opossum embryo descends into and becomes enveloped by, its own yolk-sac (fig. 13, 14). With the appearance of the vasculature within the extra-embryonic splanchnic mesoderm, the forming placenta can be subdivided into the chorion, consisting of ectoderm and somatic mesoderm, a vascular yolk-sac placenta formed of trophectoderm, mesoderm, and endoderm, and a non-vascular yolk-sac placenta that consists of trophectoderm and endoderm only. The latter represents that region of the original embryonic sphere never invaded by mesoderm: the sinus terminalis separates it from the vascular yolk-sac placenta (fig. 13–15). In both regions, the cells of the overlying trophectoderm continue to show numerous elongated microvilli on their apical surfaces (fig. 16).

Near the end of the 10th prenatal day, the surrounding shell membrane breaks down, the yolk-sac placenta expands and the vascular portion of the yolk-sac placenta is soon closely apposed to the uterine epithelium. The yolk-sac placenta continues to expand and, by the 11th day of gestation, overlies the elaborate folds and crypts of the endometrium (fig. 17, 18): the sinus terminalis continues to define the boundaries of the vascular yolk-sac placenta. Once established, the relationship between the vascular yolk-sac placenta and the uterine mucosa remains relatively unaltered until term.

The allantois now evaginates into the extra-embryonic coelom and continues to increase in size as development progresses. During later stages, the allantois becomes quite large and contains a light-amber fluid. Throughout the prenatal period, the allantois remains trapped within the extra-embryonic coelom and never forms a firm relationship either with the serosal chorion or with the yolk-sac placenta (fig. 14).

The endoderm of the vascular yolk-sac placenta is lined by large, flat, irregularly shaped cells that show distinct cell boundaries (fig. 19). Numerous short microvilli are present on the surface that faces the lumen of the yolk-sac (fig. 20). Ultrastructurally, these endodermal cells are characterized by fewer free ribosomes than are present in earlier stages and the cells now contain more mitochondria and profiles of rough endoplasmic reticulum (fig. 24). Bundles of cytoplasmic filaments are also present. Nuclei are more irregular in shape and show an increased content of heterochromatin.

Endodermal cells of the non-vascular region of the yolk-sac placenta tend to be less attenuated and make desmosomal contacts with overlying cells of the trophectoderm (fig. 30). Although the vascular yolk-sac placenta and the uterine epithelium establish an intimate association with each other early in day 11, the two can be separated by gentle teasing, even late in gestation (fig. 21). In such a preparation, the surface of the trophectoderm associated with the uterine epithelium can be seen to contain honey-comb-shaped impressions (fig. 21). In life, these cavities are associated with and fit over the apices of cells of the uterine epithelium which, for the most part, lacks cilia at the 11th day of gestation

Fig. 15. The opossum embryo is enveloped by fetal membranes late in the 10th prenatal day. A distinct sinus terminalis (small arrows) divides the yolk-sac placenta into vascular (V) and non-vascular (NV) regions. The closing amniotic pore (large arrow) also can be seen. ×8

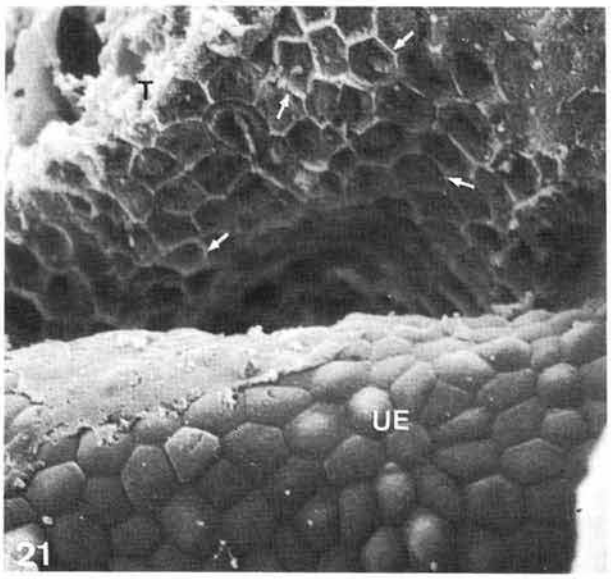
Fig. 16. In both regions of the trophoblast, the external surfaces of cells continue to show numerous, elongate microvilli. 10-day opossum embryo. ×6,500.

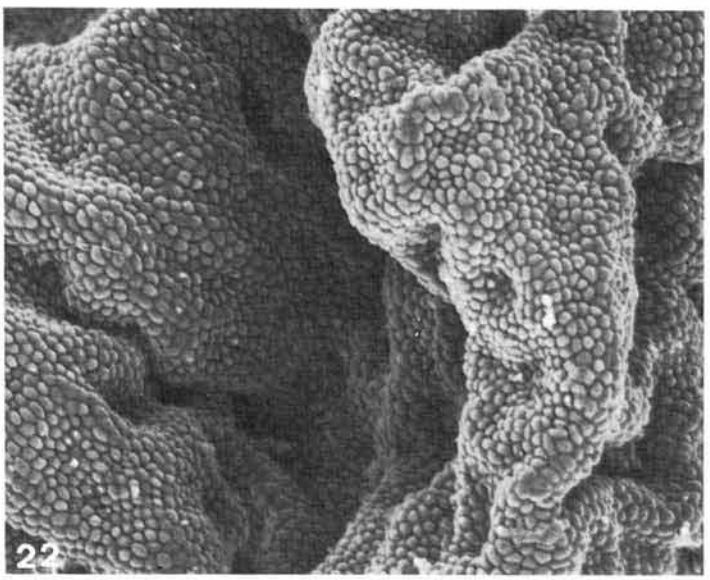
Fig. 17. A region of the endometrial surface showing its relationship to the vascular portion of the yolk-sac placenta at the 12th day of gestation. Note that the sinus terminalis (arrows) continues to define the limits of the vascular yolk-sac placenta. Each placental unit is united to a single embryo by two vitelline veins and a vitelline artery. ×4.

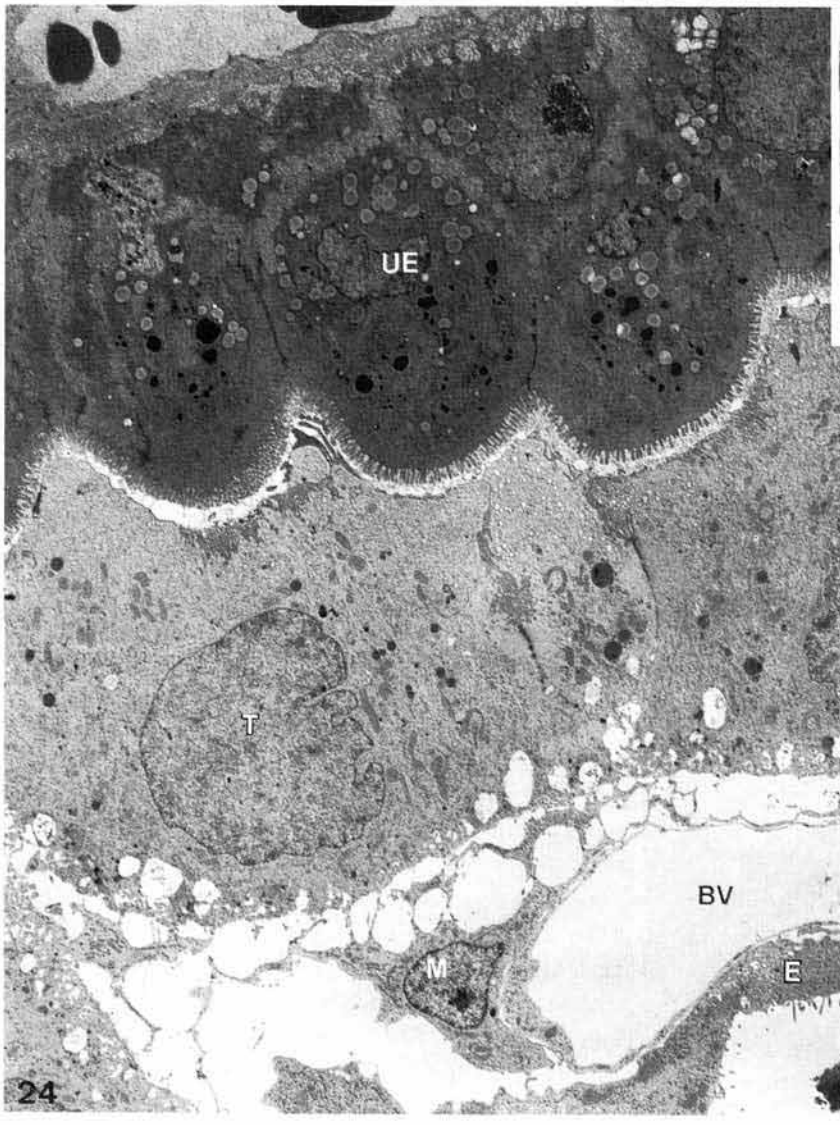
Fig. 18. A scanning electron micrograph illustrates the large folds of the uterine mucosa during the 12th day of gestation. Cilia are absent on the surface of the uterine epithelium (lower left). The remainder of the uterine mucosa is covered by yolk-sac placenta (YP). ×54.

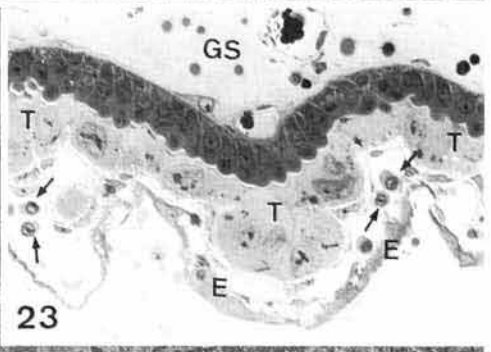
Fig. 19. The lumen of the yolk-sac is lined by large squamous to cuboidal cells. Distinct cell boundaries are evident (arrows). 12-day opossum embryo. ×440.

Fig. 20. Increased magnification of portions of four endodermal cells illustrates in greater detail the cell boundaries and the nature of microvilli on their luminal surface. Yolk-sac. 12-day opossum embryo. ×2,600.









(fig. 22). From the 11th day until term, the cells of the trophectoderm (vascular region of the yolk-sac placenta) are closely associated with the uterine epithelium but are never directly attached to it by junctional complexes (fig. 24). The luminal surface of trophoblastic cells may show regions where microvilli are abundant, adjacent to areas that are relatively smooth and devoid of microvilli (fig. 25, 27, 28). Apices are united by junctional complexes and desmosomes are occasionally found between lateral cell membranes. Both the basal and lateral membranes may form complex infoldings that interdigitate with those of adjacent cells (fig. 26). The apical cytoplasm shows an active endocytic complex and numerous vacuoles of varying size and electron density. Crystalline structures are scattered throughout the cytoplasm and inclusions; crystalline structures similar to those in cells of the uterine epithelium are also seen occasionally (fig. 26–28). Numerous invaginations occur in the cell membranes between microvilli and in areas that lack microvilli. The invaginations often contain material of an electron density similar to that of the material seen in the uterine lumen. Mitochondria appear to be distributed fairly evenly in the cells of the trophectoderm and scattered profiles of granular endoplasmic reticulum and small Golgi complexes are present (fig. 25, 26).

From day 11 until term, cells of the uterine epithelium differ markedly from those seen during the 9th day of gestation. Cilia are lacking, the lateral and basal cell membranes show elaborate infoldings that interdigitate with those of neighbouring epithelial cells; mitochondria appear to be polarized to the basal region of the cells and inclusions are increased in number (fig. 29, 30). The apices of the cells continue to be united by junctional complexes.

As earlier in gestation, the inclusions consist of an electron-dense variety and an electron-lucent form that sometimes shows a central region of greater electron density. The first type is often surrounded by material of lesser electron density and the entire inclusion is limited by a membrane. The more electron-lucent variety lacks a limiting membrane, suggesting that it is a form of lipid or lipid complex. The uterine epithelium continues to lie on a distinct basal lamina and a rich network of capillaries. Venules lie beneath the surface epithelium.

Discussion

Eutherian mammals usually give birth to large, well-developed young after a long gestation period that interrupts the oestrous cycle. The corpus luteum of pregnancy commonly remains functional beyond the normal oestrous cycle and progesterone levels remain high until birth. Marsupials, on the other hand, have a relatively short gestation period and give birth to extremely small, embryonic young. In most marsupials the period of gestation is shorter than the oestrous cycle which, in the opossum, occurs monthly from January to July, regardless of the breeding status of the female or whether lactational anoestrus is prevented as by forcible removal of all pouch young [*Hartman,* 1923; *Jurgelski and Porter,* 1974]. After removal of the pouch young, ovulation occurs in 5–7 days [*Renfree,* 1974].

In *Didelphis,* endocrine recognition of pregnancy does not occur since the blood concentrations of oestradiol and progesterone are not significantly different during the 12.5-day gestational period from those of equivalent days of the oestrous cycle [*Harder and Fleming,* 1981]. Thus, it is not surprising that the ovaries in *Didelphis* are not necessary for gestation to be maintained, but are needed for parturition to occur [*Hartman,* 1925; *Renfree,* 1974]. In *Didelphis,* as well as in the Australian marsupial, *T. vulpecula,* the increase in weight of the uterus and its secretions after ovulation are similar in pregnant and non-pregnant females [*Tyndale-Biscoe* et al., 1974; *Renfree,* 1975; *Fleming and Harder,* 1981a, b].

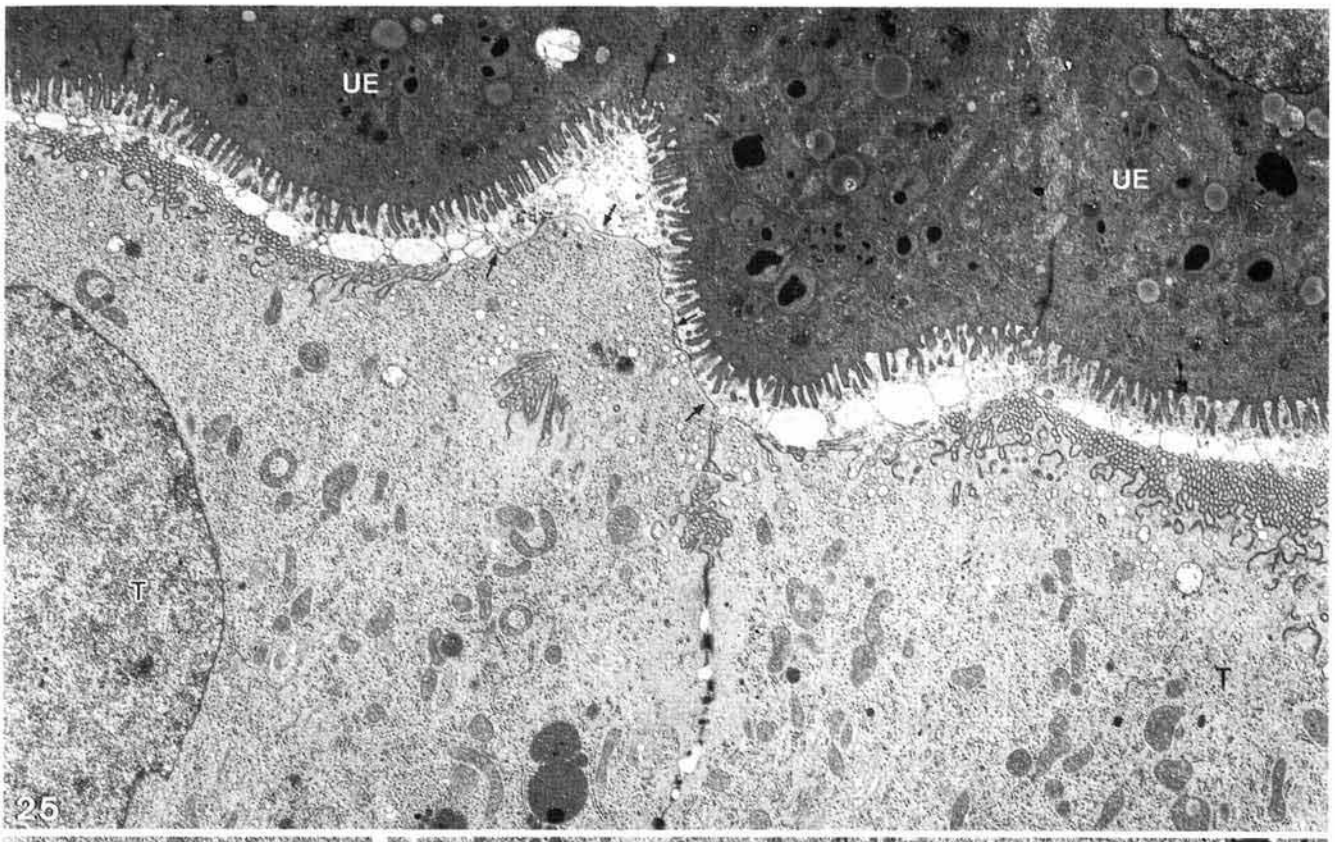
Placentation in eutherian mammals is based largely upon allantoic vascularization of the trophoblast. In most metatherian forms, however, placentation is based upon yolk-sac vascularization of the trophoblast. *Didel-*

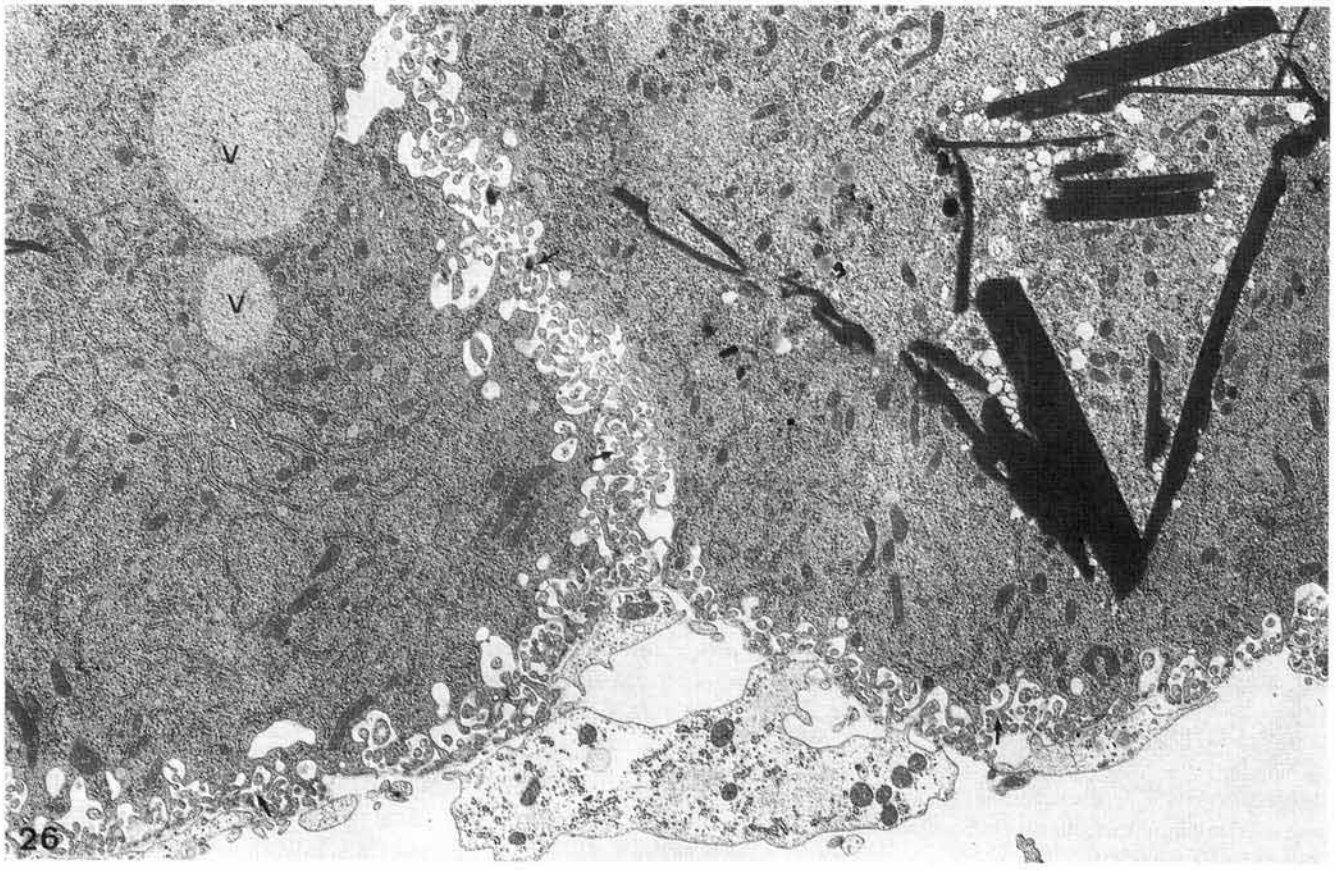
Fig. 21. A region of the uterine mucosa where the vascular yolk-sac placenta has been teased away from the uterine epithelilum (UE). Note the honey-combed appearance (arrows) of the luminal surface of the trophoblast (T). 12th day of gestation. ×480.

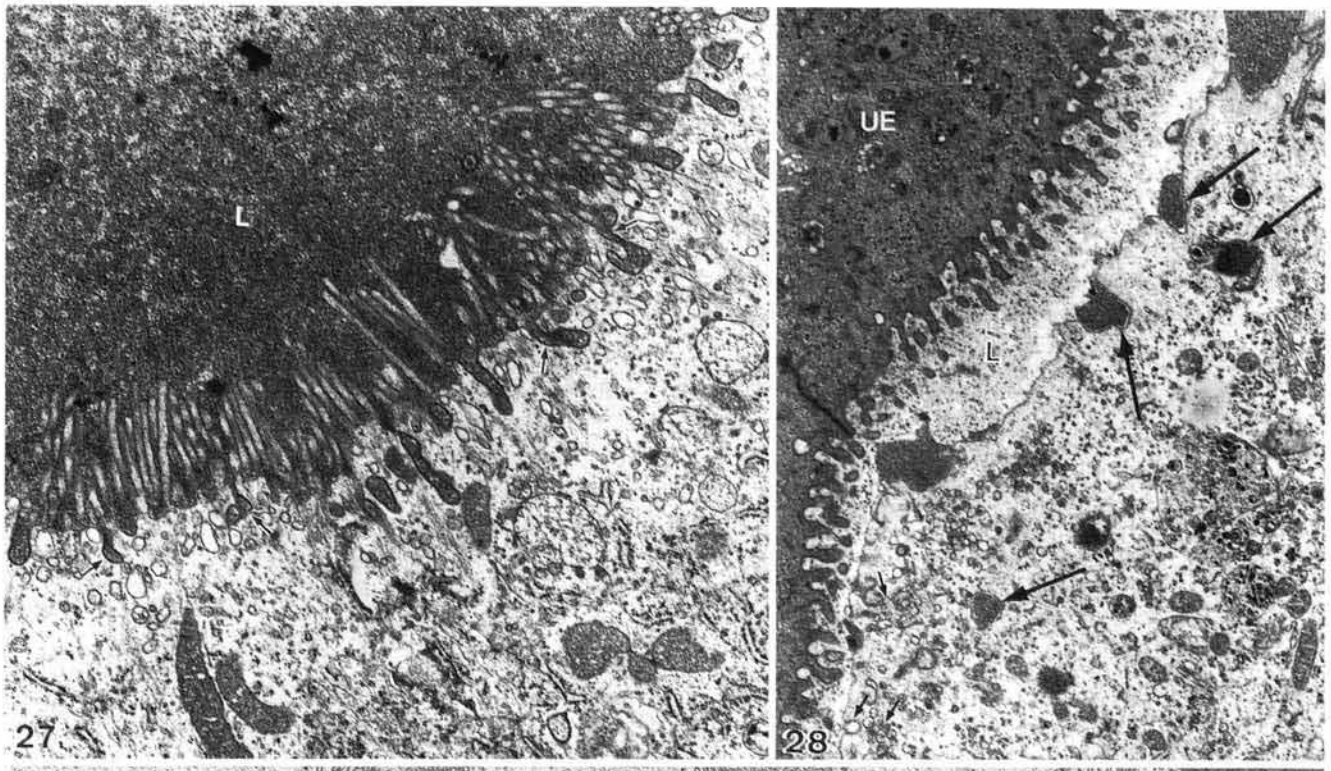
Fig. 22. The luminal surfaces of epithelial cells lining the uterus during the 12th day of gestation are devoid of cilia. ×120.

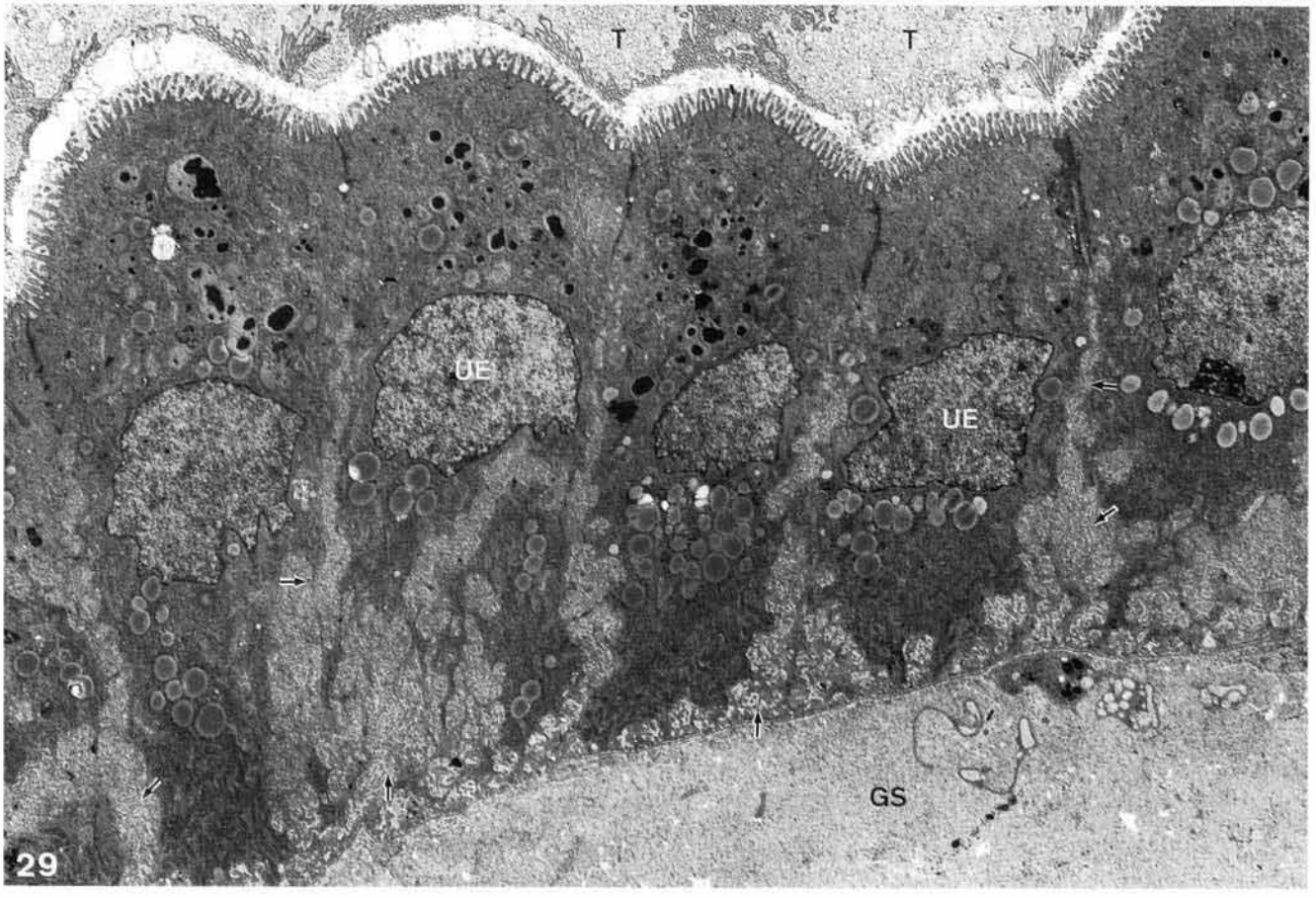
Fig. 23. A region of the endometrium intimately associated with the vascular portion of the yolk sac placenta. Note the abundant ground substance (GS) underlying the uterine epithelium. Nucleated erythrocytes (arrow) lie in thin-walled vessels between cells of the trophoblast (T) and yolk-sac endoderm (E). Epon 812–toluidine blue. ×250.

Fig. 24. A section through the vascular portion of the yolk-sac placenta shows: yolk-sac endoderm (E), a blood vessel (BV), mesenchymal cells (M), and epithelial cells of the trophoblast (T). Note the close relationship of the yolk-sac placenta with the adjacent uterine epithelium (UE). ×2,000.









phis is thought to be the more generalized marsupial and *Simpson* [1945] considered *Didelphis* to be ancestral to other marsupials. Although large, the allantois does not contribute to the formation of the placenta in the opossum and it remains an independent structure [*Krause and Cutts,* 1985a]. The placenta formed in *Didelphis* is epitheliochoroidal in nature and lies within the uterine lumen with no direct attachment to, or erosion into, the uterine mucosa by the trophoblast. Although *Didelphis* has one of the shortest gestation times (12.5 days) among marsupials, only during the last 3 days of this period, when the surrounding shell membrane disappears, does close apposition of the vacular portion of the yolk-sac placenta and uterine epithelium occur [*Krause and Cutts,* 1983, 1984]. Prior to this, the developing embryonic spheres float freely in the uterine secretions and presumably obtain their nutrition from these secretions which, at this time of gestation, contain much protein, particularly albumins and pre-albumins [*Renfree,* 1975].

Fig. 25. The relationships between the trophobast (T) and the uterine epithelium (UE) are shown at higher magnification. The luminal surface of cells comprising the trophoblast show areas with numerous microvilli as well as regions where the cell membrane is relatively smooth (arrows). A well-developed endocytic complex is present in the apices of cells of the trophoblast. Junctional complexes unite cell apices. ×8,000.

Fig. 26. The lateral and basal plasmalemmae of the cells of the trophoblast usually show elaborate infoldings (arrows). In addition to profiles of granular endoplasmic reticulum and numerous mitochondria, cytoplasmic vacuoles (V) are present and may contain a light-staining amorphous material. Electron-dense crystalline structures are also seen frequently. ×8,000.

Fig. 27. The apical endocytic complex in cells of the trophoblast appears to be actively involved in sequestering electron-dense material from the uterine lumen (L). Numerous small vesicles and invaginations form from the cell membrane between microvilli (arrows). 12th day of gestation. ×10,000.

Fig. 28. Apical regions of trophoblastic cells devoid of microvilli also show invaginations and formation of numerous small vesicles (small arrows). Larger invaginations appear to form vacuoles (large arrows) that contain electron-dense material acquired from the uterine lumen (L). A portion of the uterine epithelium (UE) is shown at the upper left of the electron micrograph. 12th day of gestation. ×12,800.

Fig. 29. The uterine epithelium (UE) is simple columnar at the 12th day of gestation and for the most part lacks cilia and rests on a distinct basal lamina. The lamina propria contains abundant ground substance (GS). Note the abundant inclusions and in particular the elaborate infoldings of the lateral and basal cell membranes (arrows). Mitochondria appear to be concentrated in the basal half of the cell. Cells of the trophoblast (T) are shown near the top of the micrograph. ×3,000.

As the vascular portion of the yolk-sac placenta increases in size during the latter part of the 10th day, not only is the surface area associated with the uterine epithelium increased, it also sinks into deep folds in the uterine mucosa. Neither invasion of the trophoblast into the uterine epithelium nor attachments between cells were seen in *Didelphis*. Invasive properties of the trophoblast in the region of the sinus terminalis have been observed ultrastructurally in some Australian marsupials [*Padykula and Taylor,* 1977] and in at least one New World didelphid, the *Philander opossum* [*Enders and Enders,* 1969]. In *Perameles* (bandicoots) and *P. opossum,* areas of adhesions and desmosome-like structures form between the trophoblast and uterine epithelium [*Enders and Enders,* 1969; *Padykula and Taylor,* 1977]. This feature may represent an initial step in implantation in those mammals that develop chorioallantoic placentae [*Enders and Schlafke,* 1969], and perhaps reflects a slight evolutionary advance over the more ancestral marsupials.

In *Didelphis,* trophoblastic cells show ultrastructural features that can be associated with a high absorptive activity, particularly of macromolecules. *Enders and Enders* [1969] reported similar features for the trophoblastic cells of the *P. opossum*. Both species show elaborate infoldings of the lateral and basal cell membranes, a provision for rapid transport of materials through the trophoblast layers. Trophoblastic cells associated with the non-vascular regions of the yolk-sac placenta are somewhat more cuboidal in shape and the vacuoles and inclusions are larger than in cells of the vascular region. *Enders and Enders* [1969] have suggested that in *Philander opossum* the accumulation of larger inclusions in the cells of this region of the trophoblast may result from slower turnover because of the lack of an associated vasculature.

Cells of the yolk-sac endoderm of *Didelphis* and *Philander* show little morphological evidence that would support absorptive or transport activities. In contrast, the yolk-sac endoderm of eutherians such as the rat [*Lambson,* 1966; *Padykula* et al., 1969; *Seibel,* 1974], rabbit [*Deren* et al., 1966], and guinea pig [*King and Enders,* 1970] show large cells that are active in the transport of materials.

By the 9th day of gestation, the endometrium of *Didelphis* is very glandular and consists of a thick, pseudostratified columnar epithelium that covers large folds onto which the uterine glands empty. The glands consist of tall secretory cells with basally located nuclei and of ciliated cells [*Padykula and Taylor,* 1971]. The

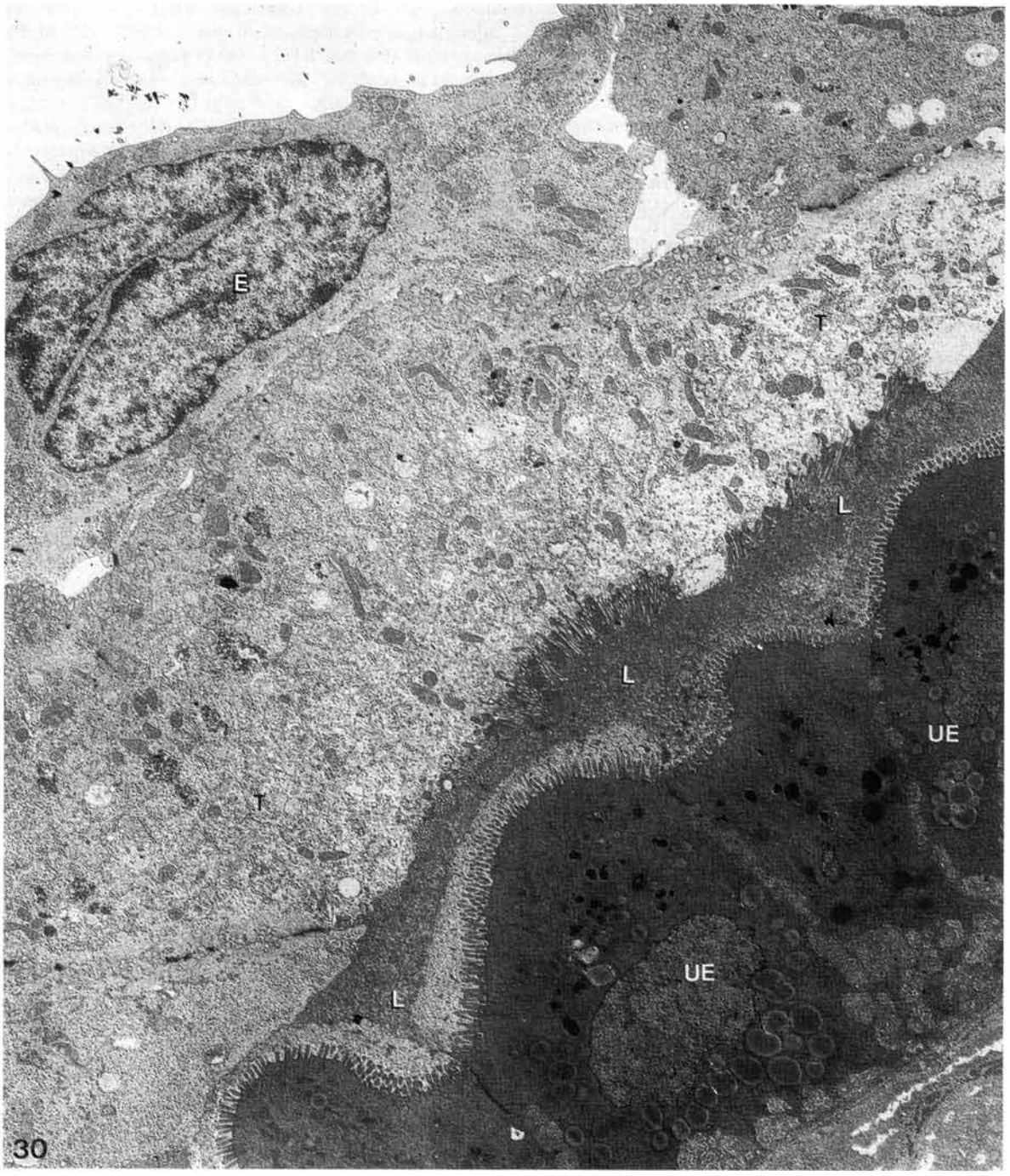


Fig. 30. A section through the non-vascular region of the yolk-sac placenta just beyond the sinus terminalis demonstrates that endodermal cells (E) of the yolk-sac lie immediately adjacent to cells of the trophoblast (T). The uterine epithelium (UE) is seen at the lower right. Electron-dense material is abundant in the uterine lumen (L). 12th day of gestation. ×3,000.

secretory cells contain numerous profiles of rough and smooth endoplasmic reticulum and large Golgi complexes. The surface epithelium also shows scattered ciliated cells.

By the 11th gestational day, most of the ciliated cells have disappeared from the uterine epithelium which now is simple columnar. The component cells show marked structural changes leading to the appearance of

a cell that is active in the transport of materials into the uterine lumen. The number of uterine glands declines after mid-gestation, whereas the total uterine volume continues to increase, suggesting that the nutritive role of the glandular epithelium decreases late in gestation [*Fleming and Harder*, 1981b]. Thus, the uterine glands may be the primary source of nutrients early in gestation when the embryonic spheres float within the uterine lumen, whereas by day 11, nutritive support appears to be taken over or is supplemented by the uterine epithelium. This view is supported by the ultrastructural appearances.

Prior to apposition of the fetal membranes, the embryo proper is engaged mainly in the formation of the three germ layers and in the initial formation of their derivatives [*Krause and Cutts*, 1984]. During the last 3 days of gestation when the vascular yolk-sac placenta is associated with the endometrium, development proceeds at an astonishing rate to produce a fetus-like individual that is capable of survival in the external environment. This would suggest that the nutritional needs during the last 3 days are greater than can be met by the uterine glands alone. The ultrastructural observations in this study reveal that both the uterine epithelial cells and the cells of the trophoblast are active in the transport of material, especially during the last 3 days of gestation. It would seem logical that the uterine epithelium would be active in moving materials into the uterine lumen from which they would be absorbed by the adjacent trophoblast.

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