

Anatomical Note
Scanning electron microscopic observations
on the opossum yolk sac chorion immediately prior to
uterine attachment

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INTRODUCTION

For 10 days after conception, opossum embryos float in the uterine secretions, enveloped by a shell membrane (Krause & Cutts, 1983). By the eleventh day, this membrane has degenerated and the yolk sac chorion and uterine epithelium soon make contact. In the opossum, the chorion shows distinct vascular and non-vascular regions, formed because mesoderm spreads about one third of the way around the developing blastocyst (McCrary, 1938). The vascular region consists of ectoderm, mesoderm and endoderm: the larger non-vascular area is never invaded by mesoderm and contains only ectoderm and endoderm as in the bilaminar blastocyst.

The present description of the surface contour of the yolk sac chorion in opossum embryos just prior to their attachment to the uterine epithelium appears to be the first such account of the yolk sac chorion in *Didelphis* or any other marsupial.

MATERIAL AND METHODS

Ten days after a sperm-positive date, female opossums were killed by ether asphyxia and the embryos recovered. Embryos were fixed for 4 hours at 4 °C, in 3·0% glutaraldehyde buffered to pH 7·4 with 0·1 M phosphate, followed by 1·0% osmium tetroxide for 2 hours and were then washed in buffer. The specimens were subjected to critical point drying using liquid CO₂ substitution, coated with gold to a depth of 20 nm, and examined in a JOEL M35 scanning electron microscope operated at 20 kV.

RESULTS AND DISCUSSION

Unfixed embryos appeared as translucent spheres, about 11 mm in diameter, floating in the uterine secretions. The vascular and non-vascular areas of the yolk sac were separated by a distinct sinus terminalis. The embryo was more closely related to the vascular area of the yolk sac chorion. The developing heart, eyes, pharyngeal arches and forelimb buds were present.

The external (ectodermal) and internal (endodermal) surfaces of the yolk sac chorion were markedly different, but there was little difference between vascular and non-vascular regions. The endoderm consisted of cuboidal cells whose relatively smooth luminal surfaces generally lacked specialisations (Figs. 1, 2) but an occasional cell showed more microplicae and microvilli than its neighbouring cells (Fig. 3).

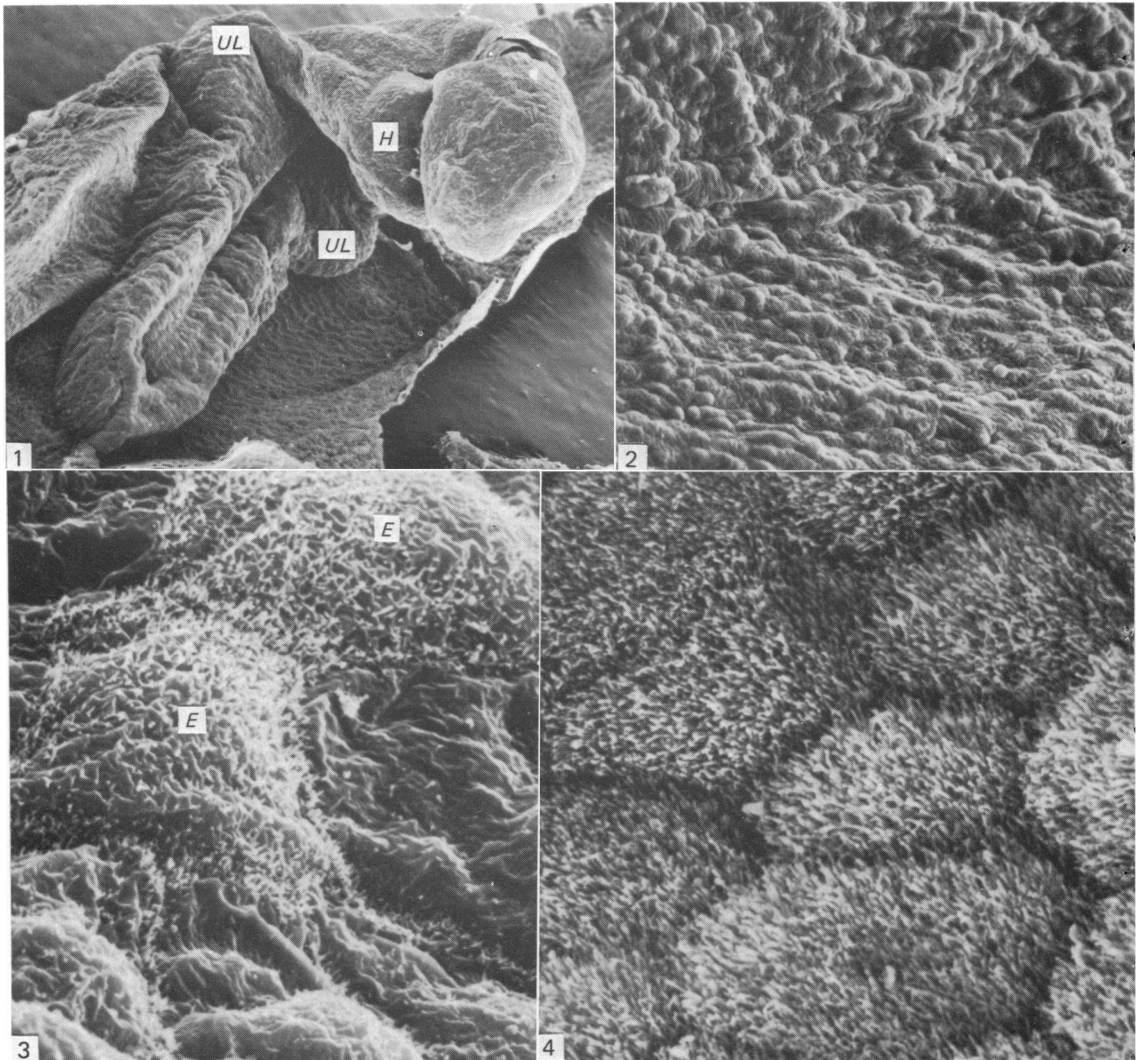


Fig. 1. A low power scanning electron micrograph shows the interior of the yolk sac chorion and the embryo within a surrounding amniotic membrane. The region of the upper limb bud (*UL*) and heart (*H*) are visible. $\times 20$.

Fig. 2. At increased magnification, the interior of the vascular yolk sac chorion shows ridges created by underlying vessels. The surface of endodermal cells comprising the interior of the yolk sac chorion appears smooth without numerous specialisations. $\times 200$.

Fig. 3. Occasional endodermal cells (*E*) exhibit an increased number of microplicae and microvilli. Surrounding cells appear relatively free of such surface specialisations. $\times 3000$.

Fig. 4. In contrast to the interior, ectodermal cells lining the external surface of the yolk sac chorion show numerous, elongated microvilli. $\times 3000$.

Ectodermal cells possessed an extensive apical concentration of long microvilli (Fig. 4).

Until contact between the fetal and uterine tissues occurs, developing embryos are thought to obtain their nutrition from uterine secretions which pass through the shell membrane. The uterine fluids of *Didelphis* are rich in protein, chiefly albumin and several types of pre-albumins not found in blood serum (Renfree, 1975). The

array of microvilli on the outer surface of the yolk sac chorion supports the view that absorption from uterine fluids occurs prior to contact between fetal and maternal tissues.

By the eleventh prenatal day, folds of the vascular chorion sink into crypts in the uterine mucosa, thus holding the embryo in place. Fusion of fetal and maternal tissues is said not to occur but they are firmly adherent (McCrary, 1938; New, 1973). The non-vascular chorion abuts the uterine lumen and never establishes a relationship with the uterine mucosa.

The present study reveals that vascular and non-vascular regions of the yolk sac chorion are well established prior to contact with the uterine mucosa and that absorption from the uterine secretions is enhanced by numerous, long microvilli on the ectodermal surface of the chorion.

SUMMARY

The 10 day opossum embryo is enclosed by a yolk sac chorion in which distinct vascular and non-vascular regions are established before attachment to the uterine epithelium. The embryo is closely associated with the vascular region which becomes adherent to the uterine lining. Cells of the external layer bear abundant, closely packed, long microvilli, which may serve to absorb nutrients from the uterine secretions. Cells of the inner surface show only scattered microvilli and microplacae on their apical surfaces.

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REFERENCES

- KRAUSE, W. J. & CUTTS, J. H. (1983). Ultrastructural observations on the shell membrane of the North American opossum (*Didelphis virginiana*). *Anatomical Record*. (In press.)
- MCCRADY, E. (1938). The embryology of the opossum. *American Anatomical Memoirs* 1-233.
- NEW, D. A. T. (1973). Studies on mammalian fetuses *in vitro* during the period of organogenesis. In *The Mammalian Fetus in Vitro* (ed. C. R. Austin), pp. 15-65. London: Chapman & Hall.
- RENFREE, M. B. (1975). Uterine proteins in the marsupial, *Didelphis marsupialis virginiana*, during gestation. *Journal of Reproduction and Fertility* **42**, 163-166.