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SCANNING ELECTRON MICROSCOPIC STUDY OF AMNION IN THE MICROCHIROPTERAN BAT, *HIPPOSIDEROS SPEORIS* AND HUMAN AT FULL TERM PREGNANCY

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ABSTRACT
On Scanning Electron Microscopy, at low magnification the single layered amniotic surface appeared to be thrown into folds in (Microcheropteran bat, Hipposideros Speoris Schneider) in near term and polygonal cell gave a mosaic or "cobblestone"appearance was due tot he presence of deep recesses or troughs between adjacent cells. In the intracellular gap openings or "ostia" were common. In human being on SEM four patterns were observed two were the pattern were surrounded by intracellular channels and in other large flat cells observed. The Microvilli in all four pattern, variable morphology in different samples observed. Possible functional implications of the findings are discussed.

INTRODUCTION

The amniotic cavity is lined by epithelium and supported by the amniotic chorion. The epithelium plays an important role since all fluid, other than that produced by foetus that enter the amniotic cavity pass through it (Sinha, 1971; Okazaki, 1981). Underlying the epithelium is the chorion whose function is complementary (Bourne, 1962; Thomas, 1965).

The studies on this aspect are basd on light microscopy with same recent major contribution by transmission or scanning electron microscopy (Anderson, 1969; Wynn and French, 1968; Okazaki, 1981).

There has been no comprehensive morphological study of the human amnion as well as bat. A detailed study of the fetal membrane from normal full term pregnancies using scanning electron microscopy has been done and campared with that of bat.

MATERIALS AND METHODS

Hipposideros speoris is a common bat. *Hipposideros speoris* is a monotoccous and monoestrous bat, breeding once in a year. The ovulation and copulation in females occur during mid December. The gestation period lasts for 135 $\pm$ 5 days. Parturition takes place from last week of April to 1st week of May and lactation extends upto June (Gopal Krishna *et al.*, 1991).

Collection of animals

The specimens of *Hipposideros* were collected during full term pregnancy (April/May) from abundant mines in Khapa, Nagpur with the help of mist nut.

Collection of amniotic fluid in bat

After anaesthising the animals with ether, abdomen walls was cut opened by amid incision. The gravid uteri of full term pregnant bats were slit opened without damaging the amnion. Amniotic fluid was collected and kept at -20ºC. Amnion from the species were cut into small pieces, were fixed for 1-2 hr. in cold 3% Gluteraldehyde in 0.1M phosphate or cacodylate HCl buffer (pH 7.2-7.4) and then rinsed over night in the same buffer, then was dehydrated in acetone. After drying tissues were mounted on stubs, lightly coated with gold and was examined on Phillips 501 Scanning electron microscope.

Collection of amniotic fluid in human beings

At the city hospital, immediately after the normal delivery at term, the placenta and membranes were washed in normal saline at room temperature to remove the surface blood and mucus. The pieces of the amnion were gently stripped the chorion and mounted with their epithelial surface and selected piece placed for two hours in fixative (3% Gluteraldehyde in 0.12 M Phosphate buffer). After fixation, they may be thoroughly in phosphate buffer and dehydrated in 50%, 70% and 90% absolute acetone.

RESULTS

The majority of the cells were flat or polygonal but irregularly

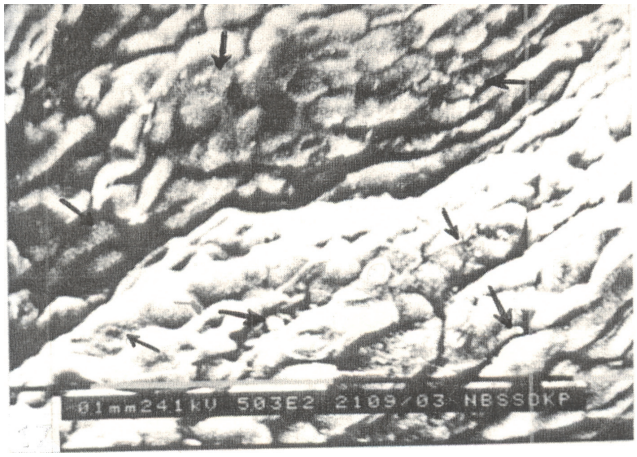

Figure 1: Bleb formation on the surface of epithelium along microvilli in *H. Speoris* consist of polygonal cells with definable boundaries

scattered through the cell sheets were other cells with different morphological features. These cells were slightly larger than neighboring cells, were usually oval and surrounded by an intercellular space crossed by strands of cytoplasm, dividing the space into series of channels. Cells with these morphological characteristics were designed as spider cells. These were mostly single. The intercellular junction appeared to be widely separated. The cells are faintly scattered. The interesting feature is deposition of extra cellular extrusion on the intercellular spaces probably on ostia.

The samples of the amnion shows wide range of appearances at low magnification however four basic patterns were observed. The four basic patterns were normally distributed over the amnion and were related to sample site.

Pattern 1 (Fig.1) consist of polygonal cells with definable boundaries usually slightly depressed below the rest of the

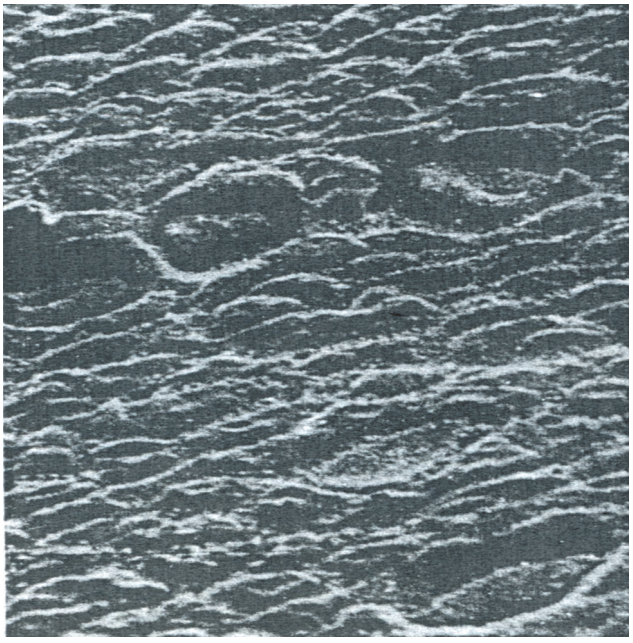

Figure 3: spider cells lying singly and in groups. Numerous intercellular canals are visible (Human being)

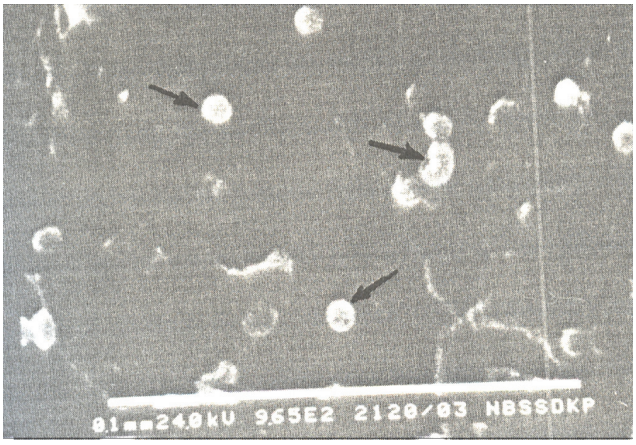

Figure 2: Scanning electron micrograph of amnion at term in *H. Speoris*. The cells are faintly demarcated. The interesting feature is deposition of extra cellular extrusion on the intercellular spaces probably ostia (arrow)

cell surface. Intercellular channels were present but not numerous and were seen in angles between cells and ran perpendicular to the surface penetrating right through the retiocular layer. The detailed pattern of these canals could be seen more clearly in pattern 2.

Pattern 2 (Fig. 3) showing the spider cells lying singly which are slightly larger than neighboring cells were usually oval and surrounded by an intercellular space crossed by thin strands of cytoplasm dividing the space into a series of channels.Cell with these morphological characteristics were designated 'spider cells'.

Pattern 3 (Fig. 4) was the least common pattern showing sheets rather than elongated cells tending to lie in parallel rows. Interspersed irregularly in the cell sheet were larger, flatter cells, lying sometimes singly, sometimes in small groups.

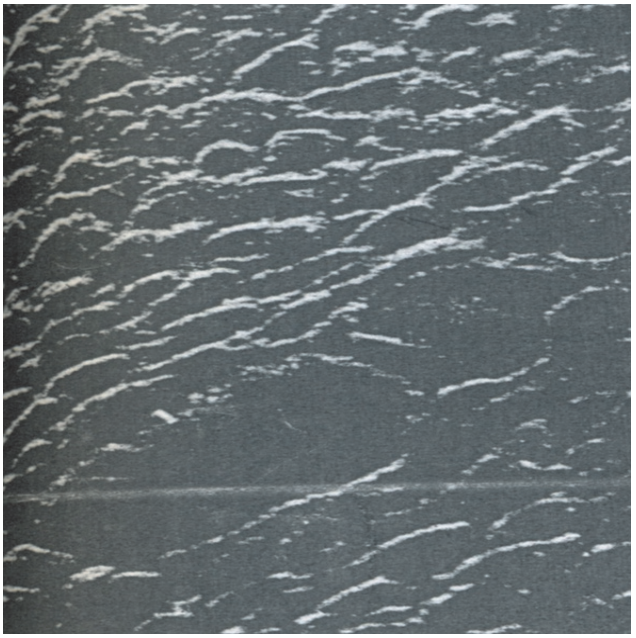

Figure 4: Group of large flat cells (Human being)

Intercellular canals not present around these flat cells, and only few could be found elsewhere in the cell sheet. This was least common pattern.

Pattern 4 (Fig. 2) showing irregular sheets of round cells with deep clefts between adjacent cells, nor crossed by cytoplasmic strands. In the depth of some of the clefts, strands of the reticular layers could be seen. In some areas there was more cohesion between groups of cells and the appearance suggested that the cell sheet was progressively breaking up.

DISCUSSION

Basic pattern of single layer of amniotic epithelium with apical microvillus surface either thrown into folds as noted or remaining smooth. The occurance of ostia in the intercellular gap in both the species reflects the continuity between amniotic fluid and intercellular canals. In *H. Speoris*, some bleb like extracellular extrusions was distributed on the intercellular ditches correlating to its secretory functions.

A well defined system of cell shedding is observed in the scanning studies as observed by surface only with basement membrane indicating its primordial role in preserving the integrity of the amniotic fluid cavity and possibly an analogue to the kidney, may very well have an important filtering role (Ludwig, 1974; Pollard, 1976 and van Herendael, 1978). The shedded cells become the plaques as described in white tailed deer and human (Sinha, 1970; Sinha, 1971) but such plaques have not been observed adhering to amnionic epithelium in both due to their fall in the amniotic fluid.

In human beings, regular association of the patterns with group of large flat cells and paucity of intercellular cannles compared to other areas suggest that pattern was entirely an artifact (ludwigs, 1971). The findings of the two basic patterns show gradual transmissions in morphology such a transmission was indeed visible in samples pollard S.M.1962 The distribution of the cells as single scattered cells in rows suggest that they may corr4espond to degenerate cells described by Bourne (1962). Also a layer of microvilli could occasionally be seen beneath the process of a spider cells suggesting the existence of another cell beneath (Thomas, 1965; Sinha, 1971).

The scattered cell of different morphology has not been described previously as distinct as electron microscopy; though a cells illustrated in the Ludwig's (1971) could be interpreted spider cells. These cells are unlikely to be artifacts spread during stripping of amnion, since they occur in an otherwise sheet of cells s showing no evidence of tearing. In a study of surface morphology only it is not possible to tell whether such cells might have specialized function or whether they are degenerated. Transmission electron microscopic studies have failed to agree on whether different functioning cell types can be distinguished in amnion (Thomas, 1965; Sinha, 1971; Bourne, 1992).

Microvilli exposing the fibrous basement membrane of interest were presence of spider cells in H.Speoris. These cells were slightly larger than neighboring cells, usually oval, surrounded by an intercellular spaces crossed by strands of cytoplasm, dividing the space into series of channels. Noteworthy features were seen in human beings as both are the mammals showing the same type of feathers in the amniotic fluid on scanning electron microcopy Pollard 1976.

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