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# A Light Microscopic Study Of The Amniotic Membrane And Fluid In The Microchiropteran Bat Hipposideros Speoris

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**Abstract:**
The study based on light microscopic examination of amniotic membrane and fluid of hipposiderosspeoris bat may be useful in assessing the fetal maturity. The cells were morphological examine were 3 types, Type – 1 nucleated round or oval shape with densely stained cytoplasm sometimes vacuolated with well defined edge of variable size, 6 – 8 µ in diameter. Type – 2: cells showing flattening of the outline of nucleus faintly granular less densely stained 10 – 15 µ. Type – 3: Cells of similar size of type – 2 with poorly stained cytoplasm was no longer granular and enucleated. Transverse section amnion has single layer of epithelium with single nucleus with underline basement membrane and complex network of reticular fibers. The fine structure of amniotic epithelium with a single nucleus. The blank spaces in the cells are vesicular spaces and intracellular channels. Most of the cells are anucleated. The cells are densely adherent to the underlying basement membrane, which is followed by compact layer, totally devoid of cells, and consist of a complex network of reticular fibers. The fibroblast layer follows the compact layer; this is the thickest layer of the amnion. This is composed of loose fibroblast network embedded in a mass of reticulum. (Gordon L Bourne 1960).

**Keywords**: Microscopic study, bat, amnion, amniotic fluid

**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 fetus 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). They also considered that the orange staining cells in liquor amnii originate in the fetal. They also considered that the orange staining cells in liquor amnii originate in the fetal sebaceous glands and the percentage of the cells take the orange colour is directly related to the age of the pregnancy. (Borosens and Gordon, 1966, Meroni, 1969)

These cells can be used as a source of information of fetal maturity, antenatal diagnosis of sex of foetus, hemophilia, hereditary sex linked muscular atrophy and testicular feminization. (Hoyes, 1968). The primary purpose of this study is to review the architectural features of the amnion through the application of certain cytological techniques since these have recently become an important technique in assessing the status of the fetus. Such an investigation may help to elucidate the nature of the functional activity of the epithelium and the composition of the fluid in correlation of fetal health, maturation of fetus and of courses its resemblance with other mammalian species. There has been no study till today on the amnion and amniotic fluid of any chiropteran.

**Material and method:**

Hipposiderosspeoris 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 ± 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)[1]

**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 and amnion:**

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 drawn with 2ml sterile syringe and was collected into Eppendorff'S tube for biochemical estimations. The tubes were collected in deep freezer at -20O centigrade until estimations to be performed. Amnion from the species were cut into small pieces, were fixed for 1-2 hrs. in cold 3% Gluteraldehyde in 0.1 m phosphate or cacodylate HCL buffer ( PH 7.2-7.4).

**Observation:**

Following parameters were studied in detail for comparing the amnion and amniotic fluid in the Indian leaf – nosed bat, *Hipposiderosspeoris*.

**Disposition of amnion:**

With a view to study the fate of amnion at full term pregnancy schematic diagrams were used (Gopalakrishna and Karim, 1979) beside observing the uteri in situ. It was observed that the amniotic sac was moderate in size and does not touches to the chorion and was avascular.

**Light microscopic studies:**

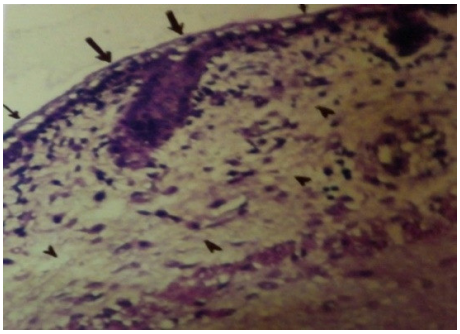
In the amnion which was normally 0.02 – 0.05 nm in thickness, consisted of four layers. These were, from within outward : A) epithelium; B) basement membrane; C) compact layer; D) fibroblast layer.

A) **The epithelium –** This is the innermost layer and is in contact with the amniotic fluid. It consisted of a single layer of cells which were usually cuboidal or columnar or squamous. On the embryonic side in the vicinity of placenta these cells were usually cuboidal or columnar but towards the abembryonic half mostly squamous. The lines of division between the cells were, as a rule, only faintly marked. The height of the cell in *speoris*varied in width from 2-5 µ and in length from 2-3 µ. They normally contained a single nucleus which occupied the greater portion of the cell and were densely adherent to the underlying basement membrane. No mitotic figures were seen. Double nuclei, however, some of which were only partially separated from one another, were evident with considerable frequency. The nucleus in*H. speoris* occupied greater part of the cell. Its diameter being approximately one-third of the total cell length. The nucleus contained coarsely granular chromatin material with one or two nuclei. Under high power on their free, normally convex surface they were surmounted by microvilli to form a brush border. Fat droplets predominated in the perinuclear zone. Intercellular canals were visible between most of the cells which had a very complex structure. The canalicular apparatus were prominent in the basal region.

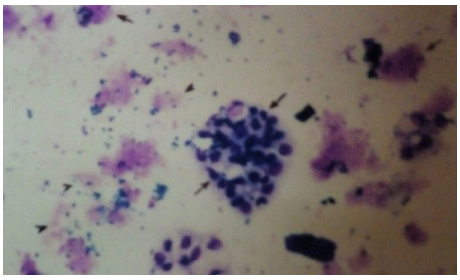
B) **The basement membrane** -This is a thin layer composed of a network of reticular fibers. The superficial or inner aspect of this layer showed a complex relationship with the epithelial cells. Short, blunt processes from the bases of the epithelial cells interdigitated with similar processes that arise from the basement membrane.

C) **The compact layer** – This relatively dense layer is almost completely devoid of cells and consisted of a complex network of reticular fibers, which is probably the strongest of the amniotic layers, appears to resist, to some extent, penetration of leukocytes.

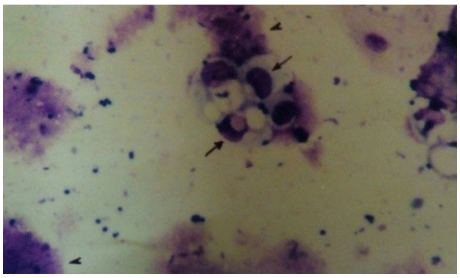
D) **The fibroblast layer** - This is the thickest layer of the amnion. It is composed of loose fibroblast network embedded in a mass of reticulum. The amnion has no blood supply of its own, nor does it contained blood vessels even over the placenta.



Transverse section of amnion from H.speoris at term. Arrow marks the single layer of epithelium with a single nucleus. The blank spaces in the cells are vesicular spaces and intracellular channels. Most of the cells are anucleated (small arrow). The cells are densely adherent to the underlying basement membrane, which is followed by compact layer, totally devoid of cells, and consist of a complex network of reticular fibers. The fibroblast layer follows the compact layer; this is the thickest layer of the amnion (arrow head). This is composed of loose fibroblast network embedded in a mass of reticulum X 400.



Low power picture of amniotic fluid from H.speoris at term. Please note three types of cells in the fluid (arrow). Beside type – 1 and type – 2 this photograph shows type – 3 cells (arrow head) which are similar to type – 2, but with poorly stained cytoplasm, which is no longer granular and anucleated, these are keratinized squamous cells X180.



Further magnified. Type – 1 cells (arrow) are nucleated either round, oval, oblong or egg-shaped, with densely stained cytoplasm. Sometimes vacuolated with a well-defined edges of variable size (6-8 µ in diameter). The nucleus was either pyknotic or vesicular. Type – 2 cells (arrow head) showing flattening of the outline,

nucleus still persisting, cytoplasm faintly granular and less densely stained than type – 1 (10 – 15 μ in diameter). Type – 3 cells are not visible in this picture X 400.

**Discussion**:

Our study of examination of exfoliated cells in the amniotic fluid are in accord with the previous workers mainly in human and other mammalian species. (Anderson and Griffths, 1968, Bergstrom, et al., 1982, Brosens and Gorden, 1966, Gorden and Brosens, 1967, Huisjes 1968, Tyden et al., 1981 who reported three distinct cell types at term pregnancy as described in Hipposidersosspeoris for the present work.

The examination suggests their origin either from fetal skin or amniotic membrane after their exfoliation into amniotic membrane. Some of the cells of the first type (large often polygonal, anucleate, in those nucleus was often pyknotic with less deposits of glycogen appeared flattened and surface membrane was thickened) are identical with the structures" Bladder cells" formed on the surface of the cells of the superficial layer of the epidermis.

There is evidence that these cells rapidly degenerate after their release in to the fluid. Cells of the stratum corneum and from the emergent hairs are also probably shed into the amniotic fluid, and at term cells derived from the epithelia lining the mouth and the urinogenital tract together with the sebaceous material. (Honen et al., 1955) The site of the origin of the second type of cells present in the amniotic fluid ( about half the first type, sometimes in groups with prominent central or eccentric nucleus, rather dense, sometimes vacuolted cytoplasm with microvilli with much more variable form, normal golgi bodies, containing numerous membrane bound bodies with intracellular debris) On the basis of their morphology, relative size and paucity in the specimen obtained at term pregnancy these cells can be equated with those as being amniotic in origin (Hoyes, 1968, Van Leeuwen et al., 1965) These are macrophages, although exhibit marked phagocytic activity. It is possible that they are derived from Hofbauer cells present in the amniotic mesenchyme. (Bourne, 1962, Hoyes, 1968).

The fine structure of amniotic epithelium with a single nucleus. The blank spaces in the cells are vesicular spaces and intracellular channels. Most of the cells are anucleated. The cells are densely adherent to the underlying basement membrane, which is followed by compact layer, totally devoid of cells, and consist of a complex network of reticular fibers. The fibroblast layer follows the compact layer; this is the thickest layer of the amnion. This is composed of loose fibroblast network embedded in a mass of reticulum. (Gordon L Bourne 1960).

**Conclusion:**

The anatomical structure of the human amnion and chorion is complex and complicated. It is considered physiologically capable of taking an active part in some of the amniotic fluid exchange mechanism.

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