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Research Article

LIGHT AND ELECTRON MICROSCOPY OF LENS OF BUFFALO FETUS (*Bubalus bubalis*)

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ABSTRACT

The present investigation was performed on eye lens of buffalo fetuses. These fetuses were divided in to three groups viz. <20.0 cm CVRL (Group I), >20.0-40.0 cm CVRL (Group II) and >40.0 cm CVRL (Group III). The lens of buffalo was composed of capsule, subcapsular epithelium and the lens fibers. The subcapsular epithelium was clearly seen at 1.6 cm CVRL stage onwards in all the groups. It was composed of multiple layers of cuboidal to columnar cells. These cells invaginated in the lens stroma at the postero-middle part and then elongated to form the lens fibers which were arranged at the equator of lens. At this point of invagination the cells became columnar with an elongated oval nucleus in the center of the cells. In group II, initially at 20.5 cm CVRL stage, the subcapsular epithelium was comprised of one or two cell layers but at 22.8 cm CVRL onwards, it became single cell layer. No blood vessels and nerve were observed in the developing lens in buffalo fetuses. Lens and its capsule depicted a moderate reaction for basic proteins whereas the subcapsular epithelium showed a strong reaction. The lens showed weak activity for LDH and NADPHD, moderate for SDH, G6PD and carbonic anhydrase, and no reaction for NADHD and nonspecific esterases. The scanning electron microscopic study revealed that the lens fibers were arranged parallel to each other and were intermingled together. Zonular fibers of variable size were attached to the lens tissue.

Key words: Buffalo fetus, Lens, Microscopy

The lens is biconvex, transparent refractive structure situated between the iris and the vitreous body. It is suspended by the zonular fibers arising from the ciliary body and attaching to the lens capsule at the lens equator. The lens is completely enclosed within a thick elastic capsule which plays major role in the vision by their accommodation capacity. Transparency of the lens is resulted from its relatively dehydrated state, lamellar arrangement of fibers, smooth and uniform thickness of the lens capsule and the epithelium in the pupillary region. The available literature on lens in buffalo is meager, thus the present investigation was planned to record histology, histochemistry, histoenzymology and electron microscopic details of lens of buffalo fetus.

MATERIALS AND METHODS

The present study was carried out on 6 early gestation (<20.0 cm CVRL), 6 mid gestation (between 20.0 – 40.0 cm CVRL) (collected from the Postmortem Hall of the university) and 6 full term fetuses (died during the correction of dystocia at TVCC) of buffalo. The age of all the fetuses was calculated by using by formula described by Soliman (1975): Y=28.66+4.496 X (CVRL< 20 cm); Y=73.544+2.256 X (CVRL≥20 cm) and Y=age in days; X=CVRL in centimeters

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The tissue samples of fetal lenses were immediately collected and fixed in Davidson's fixative for histology and histochemistry and at -20^{0}C for lipid moiety. Sections were obtained at 6 µ and were subjected to hematoxylin and eosin staining for detailed histomorphology and mercury bromphenol blue stain for basic proteins (Pearse, 1972). Fresh unfixed tissues from lens of prenatal buffalo eyes were collected and placed in a deep freezer under -20^{0}C. Frozen sections of 10 µ were obtained at -20°C on glass slides with a cryostat and incubated with different substrates for the demonstration of oxidoreductases (Pearse, 1972), phosphatases and esterases (Barka and Anderson, 1963) and carbonic anhydrase (Roy, 2002). The positive and negative controls were carried out wherever possible.

For scanning electron microscopy, the tissue samples were washed in chilled 0.1M cacodylate buffer at (pH 7.2) and were subjected for fixation in 2.5% gluteraldehyde (in 0.1M cacodylate buffer) for 4-6 hours. Fixed samples were washed in 0.1M cacodylate buffer with 3 changes of 15 min each at 4ºC. Thereafter, the samples were dehydrated in ascending grades of ethyl alcohol i.e. 30%, 50%, 70%, 80%, 90%, 95% and 100% ethyl alcohol at 4ºC. The specimens were then placed under vacuumed desiccation for 24 h. The processed tissues were observed under a scanning electron microscope (Hitachi).

Table1

Thickness of subcapsular epithelium of eye lens in buffalo fetuses of various age groups

Parameter	Thickness of eye lens in buffalo fetuses											
	1.6cm	4.0cm	5.1cm	7.1cm	8.0cm	20.5cm	22.8cm	24.5cm	89.0cm	90.0 cm	92.0cm	94.0cm
Thickness of subcapsular epithelium	34.23 ±1.10	27.39 ±2.12	23.28 ±1.53	17.36 ±1.23	16.241 ±1.65	6.23 ±1.47	13.36 ± 2.17	12.42 ±1.06	8.67 ±0.95	8.62 ±1.01	8.60 ±0.99	8.57 ±1.03

RESULTS AND DISCUSSION

Histology: In group I, the lens was composed of lens capsule, subcapsular epithelium and the lens fibers. The lens capsule was corresponding to the elastic basal lamina and zonular fibers inserted in to it. This layer was generated by cells of subcapsular epithelium. The subcapsular epithelium was clearly seen at 1.6 cm CVRL stage onwards (Figs. 1- 3). It was composed of multiple layers of cuboidal to columnar cells with round to oval, centrally placed nuclei. These cells invaginated the lens stroma at the postero-middle part and then elongated and formed the lens fibers which were arranged at equator of lens. At this point of invagination, the cells became columnar with an elongated oval nucleus in the center of the cells. In group II, initially at 20.5 cm CVRL stage the subcapsular epithelium was comprised of one or two cell layers but at 22.8 cm CVRL onwards, the subcapsular epithelium became single cell layered structure. The capsule increased in the thickness and became more refractile. The single layered subcapsular epithelium was also observed in group III. The invagination of this epithelium was observed in all the groups. No blood vessels and nerve were observed in the developing lens in buffalo fetuses (Figs. 8 – 10). The present observations were found to be similar with the findings of Carlson (1985). The mean thickness of subcapsular epithelium in fetuses under present investigation is summarized in table.

Histochemistry: The capsule depicted a moderate reaction for basic proteins whereas the subcapsular epithelium showed a stronger reaction but the nuclei of these cells could not be demonstrated by Mercury Bromphenol blue method and appeared as a transparent area within the epithelium. The lens fibers showed strong reaction indicating presence of basic proteins moieties in developing lens tissue at 5.1 cm CVRL stage of buffalo fetus.

Enzyme Histochemistry: The lens showed weak activity of LDH, moderate activity for SDH and G6PD (Fig. 13) and no reaction for NADHD. It showed weak reaction for NADPHD while had no activity for non-specific esterases. A moderate activity of the carbonic anhydrase was also noticed in lens.

Electron Microscopy: The SEM of the buffalo fetal lens revealed that the lens fibers were arranged parallel to each other and were intermingled together (Fig. 7). The intermingling of lens fibers has the support of Stirling and Griffiths (1991) and Canals *et al.* (1996) in human lens. The curved elongated fibers were the slender in shape which were arranged at equator of the lens and

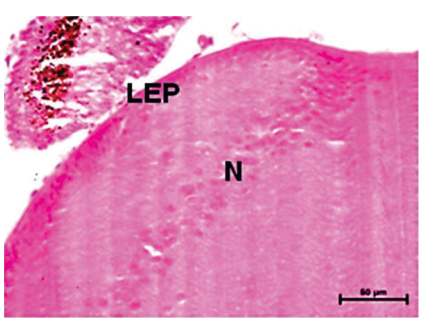

Fig.1: At 1.6 cm CVRL showing stratified sub capsular epithelium (LEP) and nuclei (N) arranged at equator of lens. (H. & E. X 400)

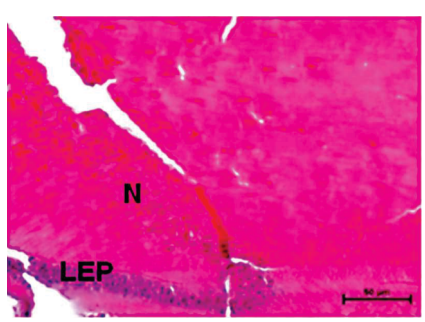

Fig.2: At 4.0 cm CVRL showing stratified sub capsular epithelium (LEP) and nuclei (N) arranged at equator of lens. (H. & E. X 400)

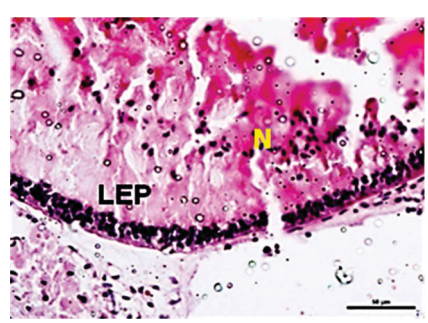

Fig.3: At 4.5 cm CVRL showing stratified sub capsular epithelium (LEP) and nuclei (N) arranged at equator of lens. (H. & E. X 400)

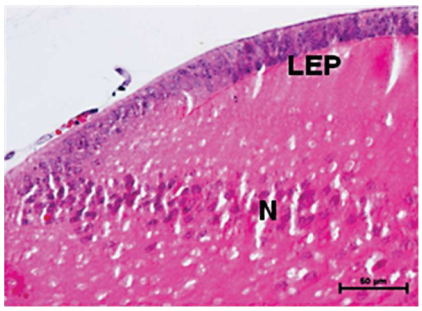

Fig.4: At 5.1 cm CVRL showing stratified sub capsular epithelium (LEP) and nuclei (N) arranged at equator of lens. (H. & E. X 400)

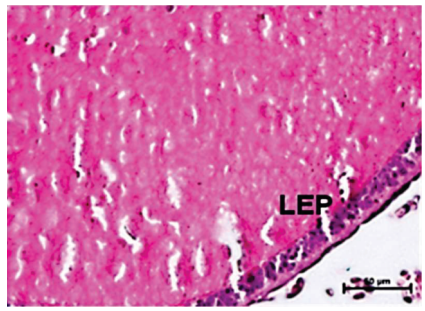

Fig.5: At 7.1 cm CVRL showing stratified sub capsular epithelium (LEP) and nuclei arranged at equator of lens. (H. & E. X 400)

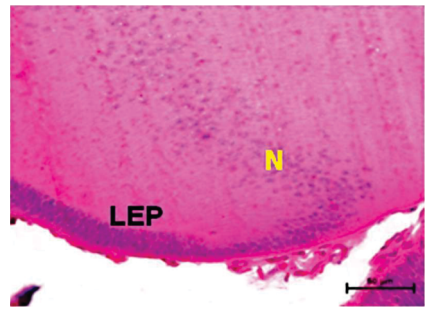

Fig.6: At 8.0 cm CVRL showing stratified sub capsular epithelium (LEP) and nuclei (N) arranged at equator of lens. (H. & E. X 400)

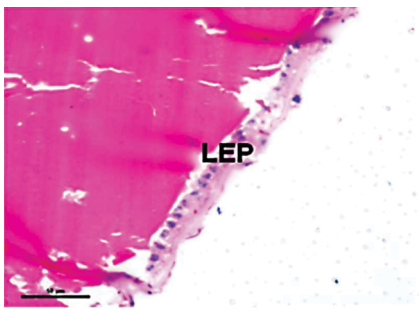

Fig.7: At 20.5 cm CVRL showing sub capsular epithelium (LEP) which was simple cuboidal. (H. & E. X 400)

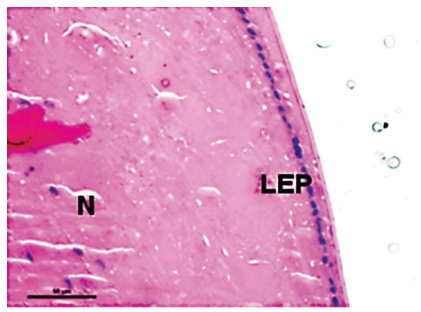

Fig.8: At 22.8 cm CVRL showing simple cuboidal sub capsular epithelium (LEP) and nuclei (N) (H. & E. X 400)

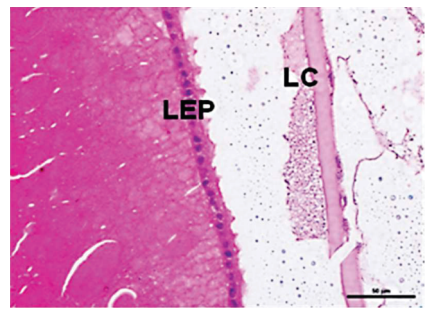

Fig.9: At 24.5 cm CVRL showing simple cuboidal sub capsular epithelium (LEP) and acellular lens capsule (LC) (H. & E. X 400)

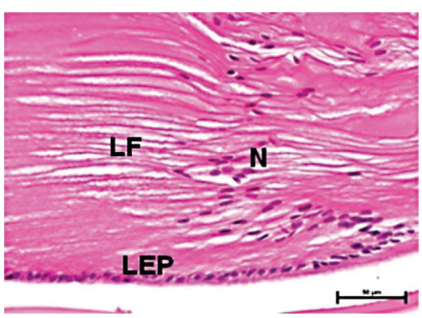

Fig.10: At 94.0 cm CVRL showing sub capsular epithelium (LEP) [in general simple cuboidal but towards equator it became simple columnar], lens fibers (LF) and nuclei (N) arranged at equator of lens (H. & E. X 400).

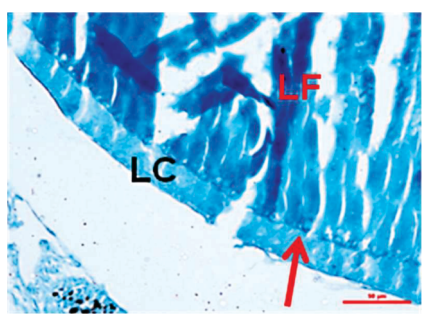

Fig.11: 5.1 cm CVRL fetus showing moderate activity in lens capsule (LC) strong reaction in basement membrane (arrow) of lens epithelium and lens fibers (LF) (Mercury Bromphenol Blue method X 400).

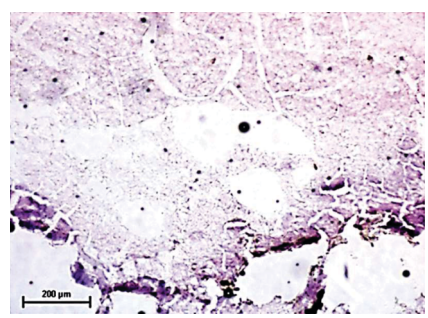

Fig.12: 94.0 cm CVRL fetus showing moderate activity in lens capsule and weak activity in stroma (Lead nitrate method X 100).

their nuclei appeared as swelling arranged in a single line (Fig. 8). Zonular fibers were of variable size and they were attached to the lens tissue (Fig. 9) as observed by Davanger and Ringvold (1978) and Canals *et al.* (1996). The thickness of lens fibers was more in the center of the lens (equator) and thinner towards periphery (Bow region). Masters *et al.* (1997) also reported the banding of lens fibers in the bow region. Under higher magnification, the lens fibers had globular structure at their surface in the area towards the center of the lens, which was not observed in fibers located towards the periphery of the lens. These fibers were connected to each other as reported by Kuwabara (1975) in human and monkey and Harding *et al.* (1976) in rabbit.

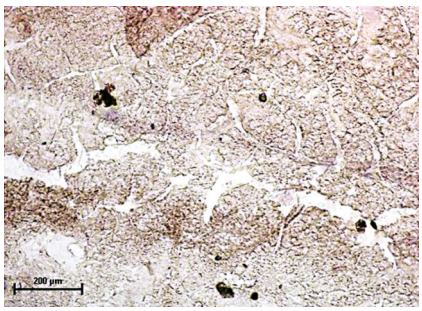

Fig.13: 94.0 cm CVRL showing moderate activity in lens (Nitro BT method X 100).

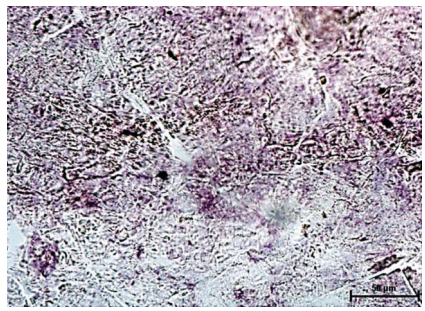

Fig.14: 89.0 cm CVRL fetus showing moderate activity in lens (Nitro BT method X 400).

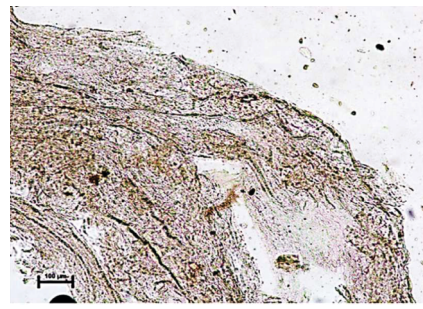

Fig.15: 89.0 cm CVRL fetus showing moderate to strong activity of CAse in lens (X 100).

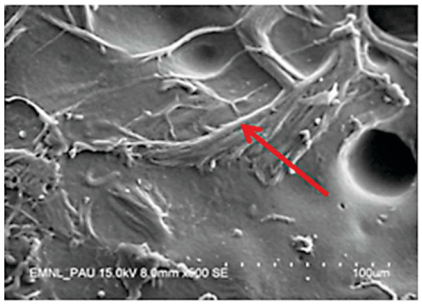

Fig.16: 92.0 cm CVRL fetus showing attachment of zonular fibers (arrow) at annular surface of lens (X 500).

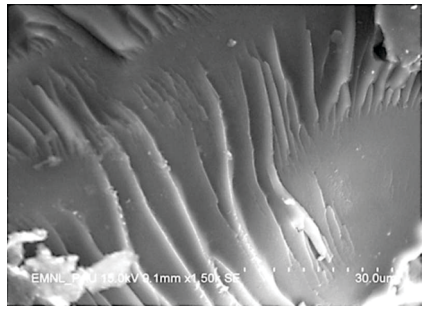

Fig.17: 94.0 cm CVRL fetus showing parallel arrangement of lens fibers (X 150).

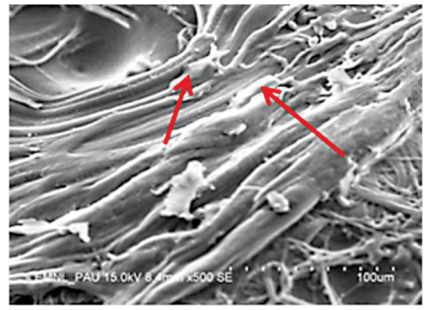

Fig.18: 92.0 cm CVRL fetus showing parallel arrangement of lens fibers with nuclei (as swellings) arranged at equator (arrows) (X 500).

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