

## ULTRASTRUCTURAL STUDIES ON THE MAJOR SALIVARY GLANDS OF THE PRENATAL BUFFALO (*Bubalus bubalis*)

K. Raja\*, M. Santhi Lakshmi<sup>1</sup>, M. Lakshman<sup>2</sup>, G. Purushotham<sup>3</sup> and  
K.B.P. Raghavender<sup>4</sup>

\*Assistant professor, Department of Veterinary Anatomy NTR College of Veterinary  
Science, Gannavaram

<sup>1</sup>Professor, College of Veterinary Science, Department of Veterinary Anatomy, Tirupati

<sup>2</sup>Associate professor, Incharge of Ruska Lab, Rajendranagar, Hyderabad

<sup>3</sup>Professor, Department of Veterinary Anatomy, College of Veterinary Science,  
Rajendranagar, Hyderabad

<sup>4</sup>Professor & University Head, Department of Veterinary Surgery & Radiology, College of  
Veterinary Science, Rajendranagar Hyderabad

E-mail: raja.vet04@gmail.com (\*Corresponding Author)

**Abstract:** Ultrastructurally, the terminal buds of parotid gland showed inner luminal and outer myoepithelial precursor cells. The cytoplasmic organelle were poorly developed in the cells of terminal tubules of early age groups. Increase of rough endoplasmic reticulum and development of the Golgi complexes were noticed during the differentiation from terminal buds into acini during the development of the gland. The rough endoplasmic reticulum elongates into spiral form in the infranuclear cytoplasm of the acinar cells at 145 days. The terminal buds of mandibular and sublingual glands showed undifferentiated epithelial cells with a large heterochromatic nucleus in early stages. The secretory granules of the salivary glands were distinct in the acinar cells of mid and late foetal age groups.

**Keywords:** Prenatal, Buffalo, Ultra structure, Salivary glands.

### MATERIALS & METHODS

Total 49 buffalo embryos and foetuses ranging from 40 to 253 days (2.5 to 79.5 cm CVRL) were used for the present study. The age of foetuses was determined on the basis of their CVRL by using Soliman's formula and divided them in 3 age groups. Fresh tissue pieces of parotid, mandibular and sublingual salivary glands from 3 age groups were collected and fixed immediately in 2.5% glutaraldehyde in phosphate buffer for 3 hr at 4° c. The tissue pieces were thoroughly washed in Phosphate buffer (pH 7.2) and post fixed in 1% Osmium tetroxide at 4° c for one hr. The tissue samples were dehydrated and embedded in Epon-Araldite. Semithin sections were made and stained with a 2% Toluidine blue - borax mixture served for orientation and for the light microscopic investigation. Ultrathin sections were picked upon Grids and stained with 10% Uranyl acetate and 1% Lead citrate and examined

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with a Zeiss EM 109 Electron microscope (Zeiss, Oberkochen, Germany) to identify the early appearance of secretory granules.

## RESULTS & DISCUSSION

### Parotid Salivary gland

Ultra structurally, the terminal buds were in the form of cell clusters, which consisted of glycogen rich inner luminal and outer myoepithelial precursor cells. Contrary to this Yaku (1983) also reported the accumulation of glycogen particles in the cytoplasm of terminal tubules at 16<sup>th</sup> week of age in human beings. More glycogen and mitochondria were noticed in both striated and interlobular ducts during the development of the gland as reported in bovines (Eisenbruckner *et al.*, 2003). The cytoplasmic organelles were poorly developed in the cells of terminal tubules of early age groups, which agree with the findings in human beings by Yaku (1983). In the present study most of the acini were composed of two cell layers with inner prismatic cells and cubic myoepithelial precursor cells. A large heterochromatic nucleus surrounded by a narrow band of cytoplasm was evident, while the cytoplasm of the acinar cells showed some organelles such as mitochondria, rough endoplasmic reticulum and Golgi profiles in late age groups, which agree with the reports of Eisenbruckner *et al.* (2003) in bovines.

At 84 days the epithelial cells were undifferentiated. The cytoplasmic organelles were poorly developed in the cells of terminal tubules at 115 days foetal age. Secretory granules with medium electron density first appeared in the cell apex of the luminal acinar cells at 125 days (Fig.1). However in human beings they were reported to be formed in acinar cells of parotid gland during 35<sup>th</sup> week of pregnancy (Donath *et al.*, 1978) and one day after birth in rats (Redman and Sreebny, 1970). The rough endoplasmic reticulum elongated into spiral form in the infranuclear cytoplasm of the epithelial cells as reported in bovines (Eisenbruckner *et al.*, 2003). Lumen formation was first observed in the primary duct when compared to terminal buds. Most of the acini were two cell layered with inner prismatic cells and outer cuboidal myoepithelial precursor cells during the development of the gland.

The rough endoplasmic reticulum elongated into spiral form in the infranuclear cytoplasm of the epithelial cells at 145 days (Fig.2). The luminal diameter of acini was larger and had more of secretory granules than the intercalated ducts. The secretory granules had membrane bound with homogenous content as reported in human foetal parotid gland (Yaku, 1983), but in this study they were more distinct in late age groups with medium electron density. However the number of secretory granules was increased during mid and late age groups of

foetal life. The membrane bound medium electron dense secretory granules were distinct with inner homogenous content in late age groups and their number increased during mid and late foetal age groups (Fig.3). The secretory cells contained moderate amount of the dilated cisternae of rough endoplasmic reticulum. Increase of rough endoplasmic reticulum and development of the Golgi complexes characterize the differentiation from terminal buds into acini during the development of the gland. Similar findings were reported in the bovine parotid gland at the end of fourth month of gestation (Eisenbruckner *et al.*, 2003).

### **Mandibular salivary gland**

Ultrastructurally, the epithelial cells forming the terminal buds were undifferentiated with a large heterochromatic nucleus at 115 days (Fig.4). The cytoplasmic organelles were poorly developed. Massive accumulation of glycogen particles were found in the cytoplasm. There were intercellular spaces in the junctions between the acinar cells.

Mucous acinar cells contained electron lucid granules, while serous secretory cells showed electron dense granules. The accumulations of secretory granules were distinct in the acinar cells of late foetal age group. Small amount of cytoplasm between the granules contained some rER cisternae and mitochondria. Secretory granules were also located in the apical portion of the epithelial cells of the intercalated ducts, which were reported to be source of epidermal and nerve growth factors and as a possible source contributing in the formation of amniotic fluid in human foetuses (El-Mohandes *et al.*, 1987). The Golgi complex, mitochondria and rough endoplasmic reticulum were markedly developed in the cells of terminal tubules. The increased volume of rough endoplasmic reticulum in the cytoplasm of secretory cells was reported to be observed in rat by Cutler and Chaudhry (1973). The lateral infoldings of plasma membrane and junctional complexes were clearly evident. The secretory granules had appeared first in the form of membrane bound secretory vesicle with homogenous electron dense content (Fig.5). However the secretory granules had increased in number during the late age group.

Flattened myoepithelial cells with long cytoplasmic processes were observed between the epithelial cells and basement membrane of terminal tubules and intercalated duct (Fig.4) as reported in buffalo (Venkatakrishnan, 1994) and human beings (Yaku, 1983). The feature of cells containing myofilaments in the cytoplasmic processes and more of mitochondria in the striated ducts agree with the reports of Yaku (1983) in human beings.

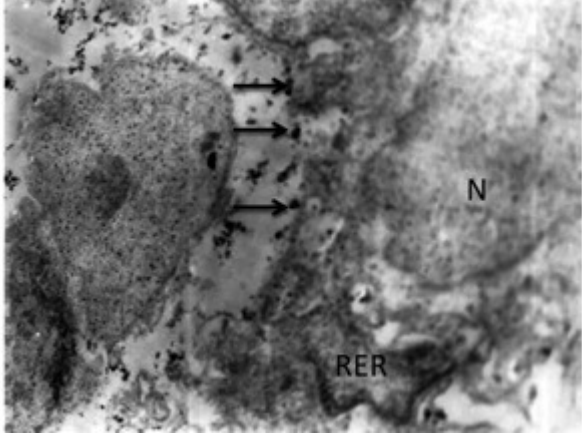
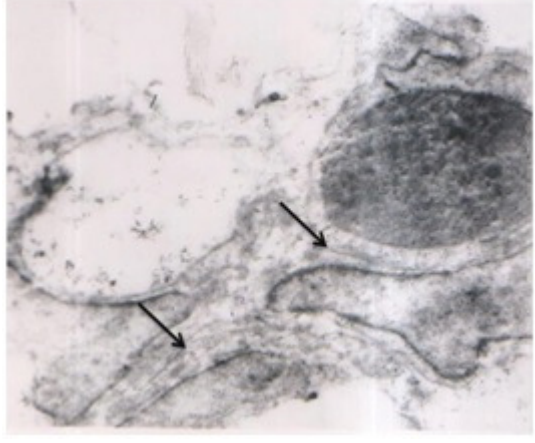
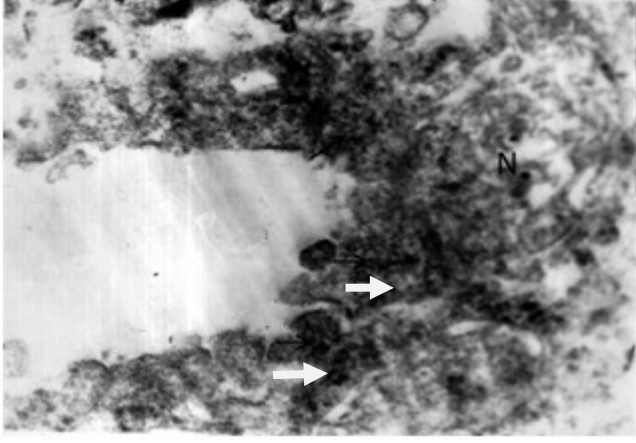
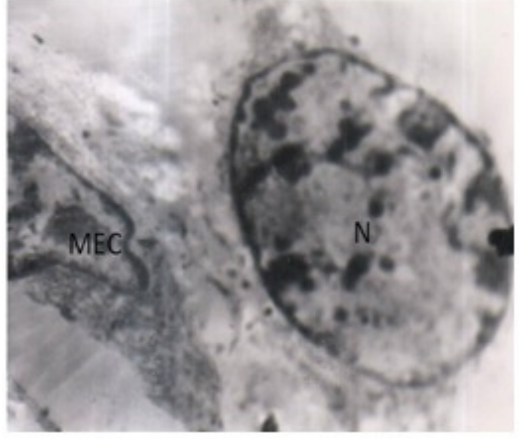
## SUBLINGUAL SALIVARY GLAND

In early stages, the cells lining the terminal tubules were undifferentiated with poorly developed cytoplasmic organelles but lacked secretory granules. However the apical ends of cells showed electron lucid and some electron dense granules as reported in rat (Wolff *et al.*, 2002). The accumulations of secretory granules were distinct in the acinar cells of mid and late age groups. The Golgi complex, mitochondria and rough endoplasmic reticulum were markedly developed in the cells of acini, which agree with the findings of Wolff *et al.* (2002) in rat. Flattened myoepithelial cells with long cytoplasmic processes containing homogenous cytoplasm were observed between the epithelial cells and basement membrane of terminal tubules and intercalated ducts at 115 days (Fig.6).

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<p>Fig.1 TEM of foetal Parotid gland showing the earliest appearance of secretory granules (Arrow) &amp; nucleus (N) in acinar cells at 125 days. N-Nucleus                      RER-Rough endoplasmic reticulum. <b>Mag 12,530 x</b></p>	<p>Fig.2 TEM of foetal Parotid gland showing spiral form of rough endoplasmic reticulum (Arrow) in the infra nuclear cytoplasm of epithelial cells at 145 days. <b>Mag 10740 X</b></p>
	
<p>Fig.3 TEM of foetal Parotid gland showing increased number of secretory granules (Arrow) in apical portion of acinar cells at 253 days.    N-Nucleus <b>Mag 6265 X</b></p>	<p>Fig.4 TEM of foetal mandibular gland showing myoepithelial cell (MEC) with long cytoplasmic process and large heterochromatic nucleus (N) in the acinar cells at 115 days. <b>Mag 8950 X</b></p>

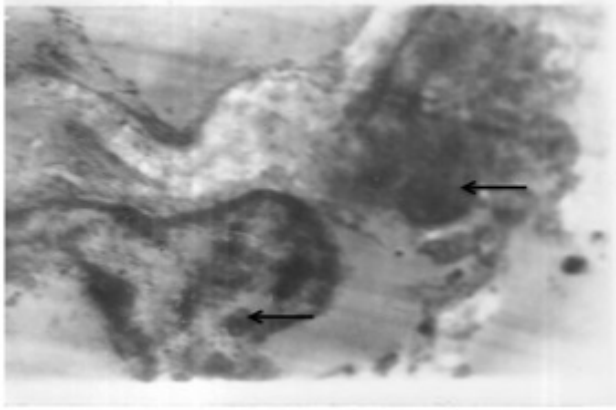


Fig.5 TEM of foetal mandibular gland showing membrane bound secretory vesicles in the acinar cells (Arrow) at 125 days.

**Mag 12530 X**

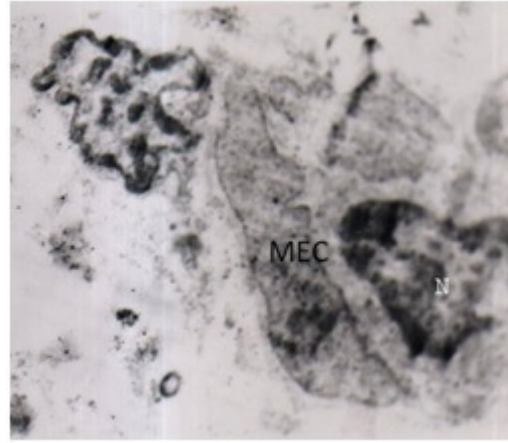


Fig.6 TEM of foetal Sublingual gland showing flattened myoepithelial cell (MEC) around the developing acinar cells at 115 days. **Mag 10740 X**