AGE RELATED CHANGES IN THE HISTOMORPHOLOGY OF MANDIBULAR GLAND IN PRENATAL BUFFALO (*BUBALUS BUBALIS*)

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ABSTRACT

The present work was undertaken on 42 buffalo embryos and fetuses ranging from 40 to 253 days to study the histomorphological features of mandibular salivary glands in the buffalo *(Bubalus bubalis)* during the prenatal period. The specimens were fixed and processed for serial paraffin sectioning and the sections were subjected to different staining methods. The primordium of mandibular gland appeared first as a solid epithelial bud from the oral epithelium at the base of the tongue at 40 days. Ductal Lumen formation was observed first in the terminal buds and primary cords of mandibular gland at 84 days. The differentiation of terminal buds into terminal tubules was completed at 91 days. The typical compound tubulo - alveolar architecture of the gland was attained first at 125 days. The gland was predominant in mucous type of acini from 140 day onwards. The formation of capsule around the gland was evident at 125 days and it was well developed at 197 days. Differentiation of intercalated, intralobular and interlobular ducts was possible at 125 days.

Keywords: histomorphology, mandibular gland, prenatal buffalo

INTRODUCTION

Major salivary glands of various domestic animals are paired structures, which includes parotid, mandibular and sublingual glands. Salivary glands fulfill important role in the oral biology by producing saliva to provide water for lubrication, as well as supplying electrolytes, mucus, antibacterial compounds and various enzymes to the oral cavity. Loss of salivary glands function can result in the wide spread deterioration of oral health (Hsu *et al.*, 2010). Study of normal development of salivary glands will be helpful for both developmental anatomists and clinicians as they are having important role in several dreadful diseases like rabies, foot and mouth disease and Herpes viral diseases.

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MATERIALS AND METHODS

Total 42 buffalo embryos and fetuses ranging from 40 to 253 day of gestation (2.5 to 79.5 cm) "Curve Crown-Rump Length" (CVRL) were collected from pregnant uteri immediately 1hr after the slaughter from slaughter house of Hyderabad irrespective of the the age and sex. The age of fetuses was determined on the basis of their CVRL by using Soliman's formula (1975). The whole embryos of earliest possible age (from 40 days i.e. 2.5cm CVRL) and fresh tissue pieces from the mandibular salivary gland of fetuses from 84 day (12.4 cm CVRL) to 253 day (79.5cm CVRL) were collected and fixed in 10% Neutral Buffered Formalin(pH 7). Bouin's fluids (Singh and Sulochana, 1997) also used for the fixation of the gland because bouins fluid allows crisper and better nuclear staining than 10% neutral-buffered formalin. The tissues were processed in routine for paraffin sectioning of 5 to 8µm thickness and subjected to Mayer's Haemotoxylin and Eosin method for routine histological developmental study, Van Gieson's technique for collagen fibres, Masson's Trichrome for connective tissue fibres (Singh and Sulochana, 1997) staining techniques to study the histomorphological changes in the mandibular salivary gland will be observed by using the Olympus microscope.

RESULTS AND DISCUSSION

The primordium of mandibular salivary gland developed as a solid epithelial bud from the oral epithelium at the base of the tongue in linguo-gingival space at 40 days (Figure 1). Contrary to this the primordium of the gland was reported to be observed at 45 days (Santhi, 2006) and 69 days (Venkatakrishnan, 1994) in buffalo and during 6th week (Arey, 1965) in human. The migration of epithelial bud into the surrounding mesenchyme as club shaped structure was noticed at 41 days (Figure 2) with surrounding mesenchyme in condensed form at 43 days. The appearance of the anlagen as a solid club shaped structure from the oral cavity in relation with the developing tongue was also reported by Venkatakrishnan (1994), Mc Geady *et al*., (2006) and Santhi (2006) in buffalo. The glandular mass was composed of undifferentiating basophilic epithelial cells. The epithelial bud was attached to oral epithelium by a single epithelial stalk. The glandular mass began to branch into several terminal epithelial buds at 45 days in dichomotous pattern surrounded with large amount of dense mesenchyme. The terminal buds were formed by multilayered polyhedral cells with basophilic cytoplasm and spherical nuclei. The gland reached the space between the tympanic bulla and the angle of the mandible at 54 days.

At 84 days the gland was formed by groups of luminized terminal buds (terminal tubules) and primary cords (Intercalated ducts) (Figure 3) with dense mesenchyme and rich vasculature. However Venkatakrishnan (1994) reported the similar finding at 105 days in buffalo. The emergence of duct system was reported to be observed at 21 day in utero in the submandibular gland of rat (Ogawa *et al.*, 2000). Most of the terminal tubules were lined with 2 to 3 layers of cells with central lumen from 91 days. The terminal tubules attained the structure of acini at 115 days in which the lining epithelium was changed to single layer (Figure 4), which was reported to be established at 5 weeks postnatally in rat (Ogawa, 2000).

The cytoplasm of the acinar cells was lightly basophilic with spherical basal nucleus. Typical compound tubulo - alveolar nature of the

gland was attained first at 125 days of foetal life (Figure 5). From 140 day onwards the gland was predominantly mucous type along with serous demilunes. The cells of the mucous acini were pyramidal in shape with distinct cell borders and basement membrane. The cytoplasm was lightly basophilic with flattened basal nuclei. The cells lining the serous acini were pyramidal with spherical and darkly stained nuclei. Myoepithelial cells were observed first around the acini and intercalated ducts at 140 days between the basement membrane and acinar cells. The developing mandibular gland was compound tubulo-alveolar type with predominantly mucous alveoli during the late foetal stage, which was reported to occur in all foetal age groups of animals by Venkatakrishnan (1994) in buffalo.

The differentiation of embryonic mesenchymal tissue into stroma was observed first in foetal mandibular gland at 84 days (Figure 6). The gland showed primitive lobules separated by stroma with fine collagen fibers at 91 days. The lobulation of the gland was distinct at 101 days (Figure 7). The lobules of the gland were reported to be formed during 9^{th} to 10^{th} week (Merida-Velasco *et al*., 1993) in human beings and 14 to 26 days of prenatal development (Knopse and Bohme, 1995) in cat. The gland was highly vascular between 84 and 101 days. Dense lobulation of the gland with steep increase in the number of lobules and capsule formation was evident at 125 days (Figure 5). The connective tissue septa were formed at 131 days and were traversed by several blood vessels, nerves and ducts. Capsule showed large amount of collagen fibres and few elastic fibres at 197 days. The parenchyma of the gland was predominant in mucous acini from 125 days and 253 days (Figure 9). A large quantity of connective tissue was observed around the groups of interlobular ducts between 140 and 188 days of foetal life.

The developmental changes in the parenchyma and stroma of the mandibular salivary gland of fetuses were gradual in buffalo, which is in agreement with the findings of Venkatakrishnan (1994) in buffalo, Arey (1965) and El- Mohandes *et al.* (1987) in man. The stromal content was reduced and replaced by the parenchyma as the age of the foetus advanced. The parenchyma and the duct system of the foetal mandibular gland nearing the full term of pregnancy had achieved its identity to that of glands in adult buffalo.Differentiation of intercalated and intralobular ducts was observed at 84 days of foetal age. The intercalated ducts and striated ducts were lined by a double layered epithelium from 91 to 130 days with inner low columnar or cuboidal cells and outer flattened cells. All types of ducts i.e., intercalated (acinar ducts), intralobular (striated ducts) and interlobular ducts were observed at 125 days (Figure 6) in the developing mandibular gland. However the differentiation of striated ducts, intercalated ducts and terminal buds was reported to occur at the time of the birth (Cutler and Chaudhry, 1973) and at 16 weeks (El-Mohandes *et al*., 1987) in human being. The intercalated ducts were lined with single layered cuboidal epithelium and were closer nearest to the secretory end pieces from 140 days. The number of intercalated ducts increased as the foetal age increased.

The intralobular ducts appeared first at 84 days in the form of solid epithelial cell cords. Double layered intralobular ducts composed of inner and outer cuboidal cells were evident at 140 days of foetal life (Figure 8). The mature striated ducts were lined by a layer of tall inner columnar cells with flattened myoepithelial cells in the basal area. The inner cells lining the striated ducts

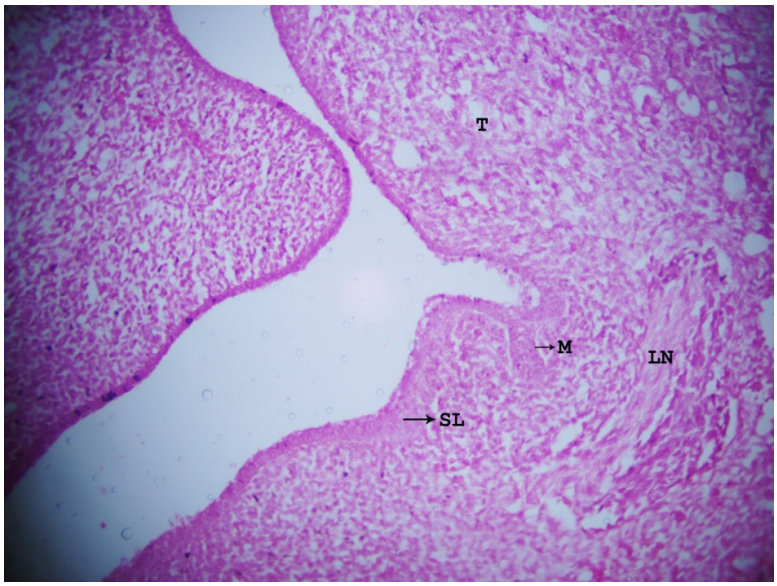

Figure 1. Photomicrograph of cross section of foetal head showing the primordium of mandibular (M) salivary gland at 40 days.
SL- Sublingual salivary gland , LN-Lingual nerve, T-Tongue H and E X 20.

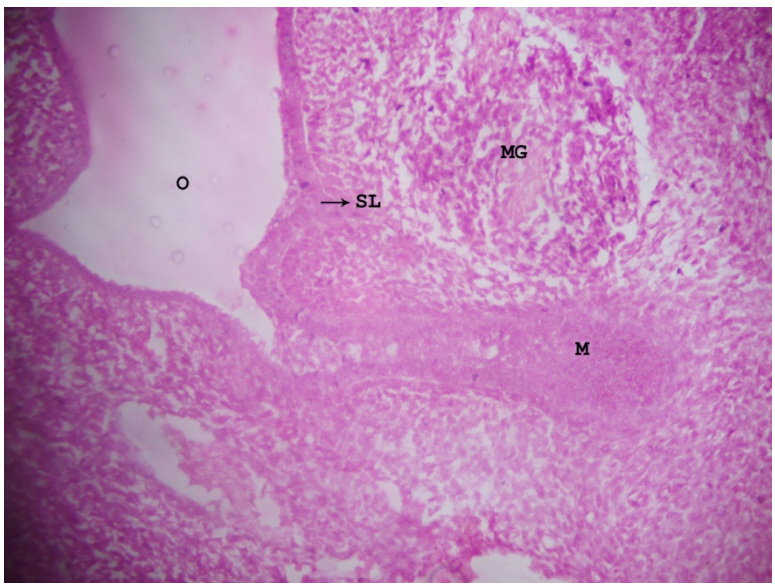

Figure 2. Photomicrograph of cross section of 41 day buffalo foetal head showing the migration of epithelial bud of mandibular (M) gland into the mesenchyme.
SL-Primordium of sublingual gland, O-Oral cavity, MG- Mandibular ganglion H and E X 20.

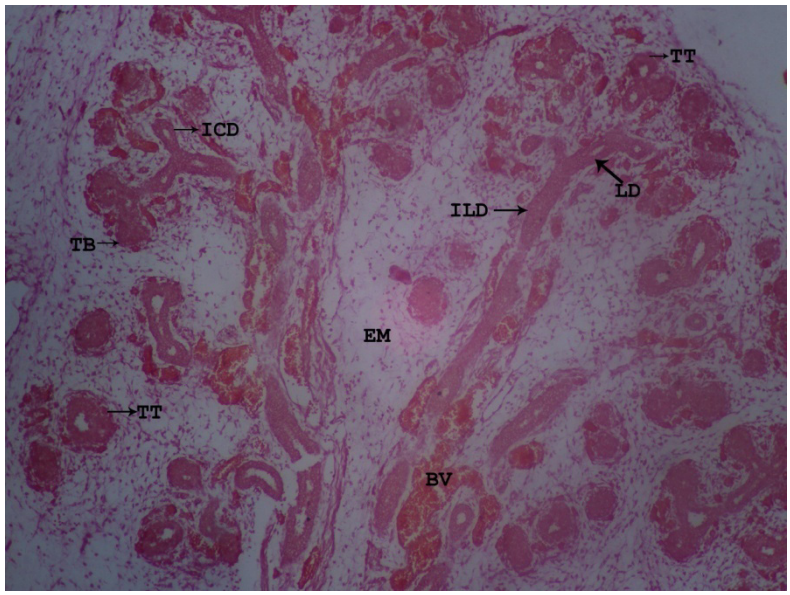

Figure 3. Photomicrograph of cross section of 84 day foetal mandibular gland showing groups of terminal buds (TB), terminal tubules (TT) with dichomotus intercalated (ICD) and intralobular (LD) ducts. ILD-Interlobular ducts, EM-Embryonal mesenchyme, BV- Blood vessels

Van Gieson's method X 10.

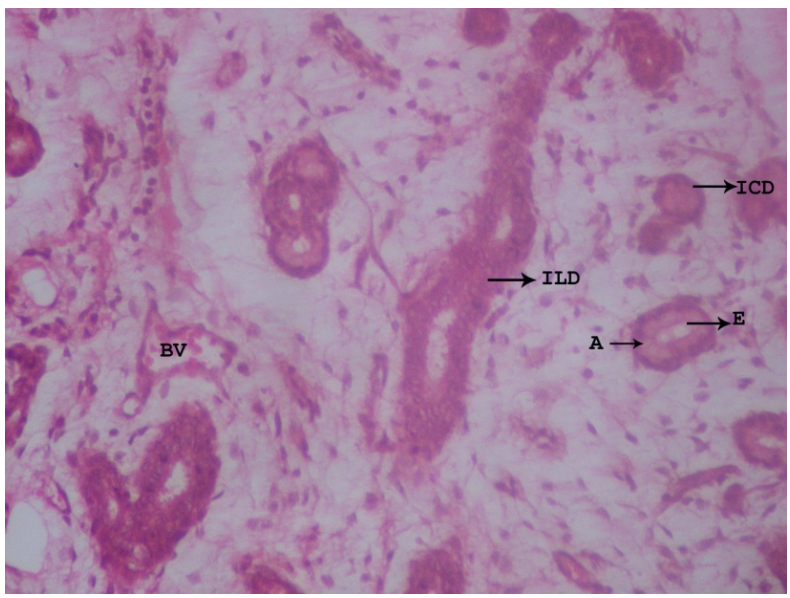

Figure 4. Photomicrograph of cross section of 115 day foetal mandibular gland showing the earliest appearance of the mucous acini (A) lined with single layer epithelium (E).

ICD-Intercalated duct, ILD-Interlobular duct, BV-Blood vessel H and E X 20.

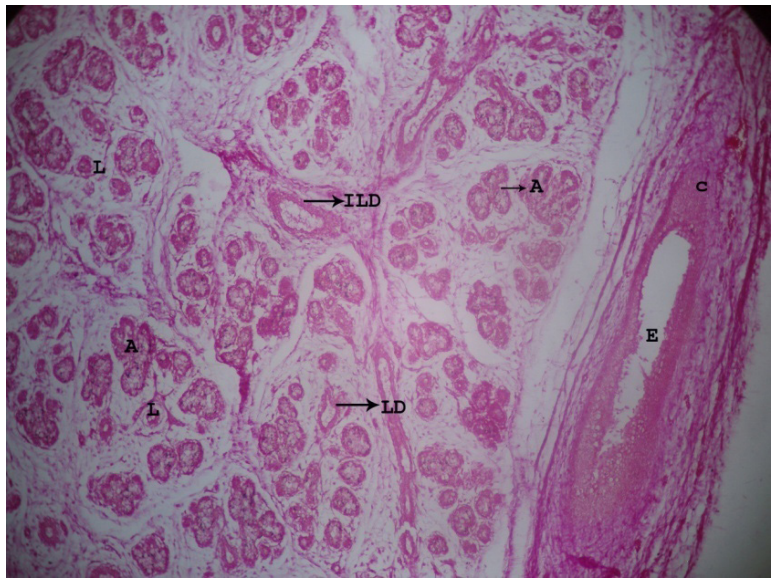

Figure 5. Photomicrograph of cross section of 125 day foetal mandibular gland showing compound tubulo-alveolar architecture predominant in mucous acini (A) and developing capsule (C) around the gland.
LD-Intralobular duct, ILD-Interlobular duct, E- Excretory duct, L-Lobule
Van-Gieson's method X 10.

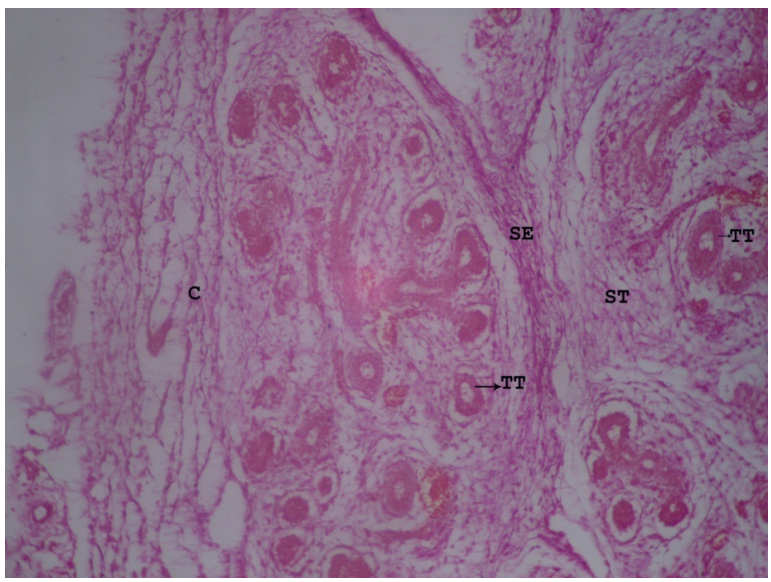

Figure 6. Photomicrograph of cross section of 84 day foetal mandibular gland showing the differentiation of stroma (ST), capsule (C) and septa (SE) from embryonic mesenchyme.
TT-Terminal tubule Van-Gieson's method X 10

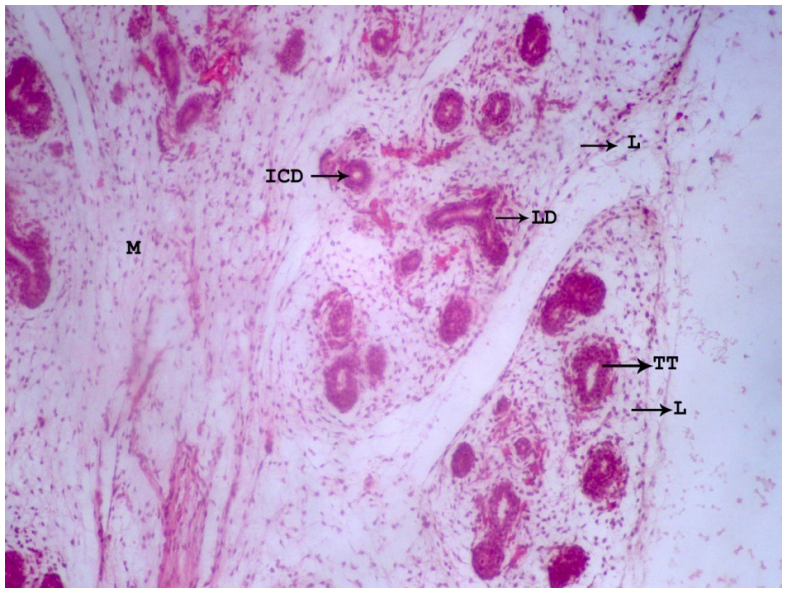

Figure 7. Photomicrograph of cross section foetal mandibular gland showing distinct lobulation (L) at 101 days.
TT- Terminal tubule, ICD-Intercalated duct, LD-Intralobular duct, M-Mesenchyme
H and E X 10.

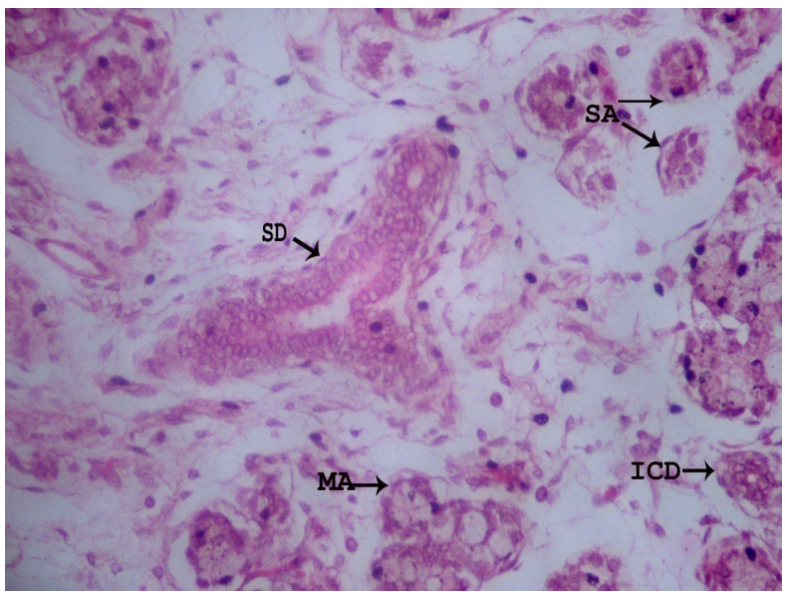

Figure 8. Photomicrograph of cross section of 140 day foetal mandibular (M) gland showing Two layered intralobular ductal structures composed of inner and outer cuboidal cells.
MA-Mucous acini, SA-Serous acini, SD-Serous demilune ---Goblet cells H and E X 20.

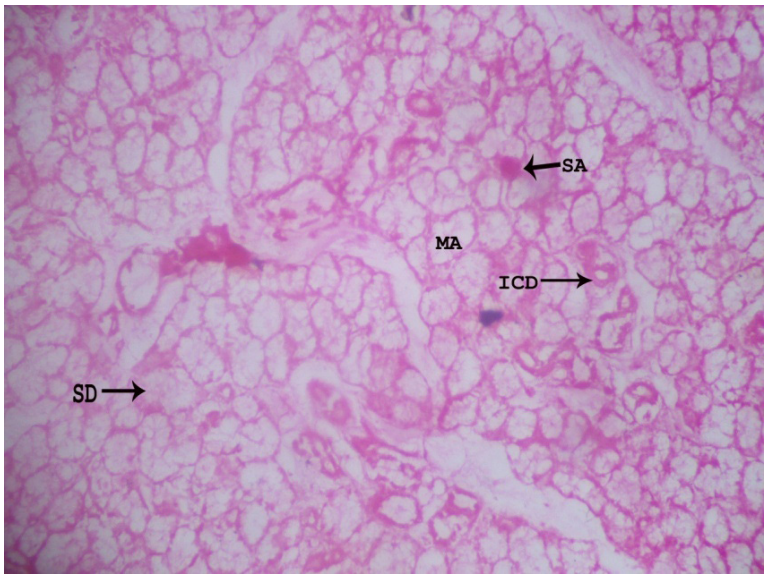

Figure 9. Photomicrograph of cross section of 253 day foetal mandibular gland showing parenchyma predominant in mucous acini (MA).
ICD-Intercalated duct, SD-Serous demilune, SA- Serous acini H and E X 10.

showed intensely eosinophilic cytoplasm and darkly stained nuclei. These ducts showed a well developed adventitia with abundant vasculature.

Two layered immature interlobular ducts were evident at 125 days. The tunica adventitia was prominent around the interlobular ducts with well developed collagen fibres, elastic fibres, blood vessels and nerves at 131 days. Groups of mature interlobular ducts were also prominent in the interlobular space from 140 days and lined with multilayered cuboidal or columnar epithelium with abundant goblet cells (Figure 8).

The excretory ducts were lined by a double layered columnar epithelium at 54 days. The caruncula sublingualis was distinct at 56 days as reported by Santhi (2006) in prenatal buffalo. The excretory ducts were larger than the other ducts in all age groups studied and were lined by stratified cuboidal or columnar epithelium in advanced age groups. Vacuolated cells were seen occasionally. The ducts had a well developed adventitial layer with collagen fibres, elastic fibres, blood vessels and nerves. Findings of this work gave a valuable informations and tremendous scope for the clinicians and Developmental anatomists.

REFERENCES

Arey, L.B. 1965. *Developmental Anatomy,* 7th ed. W.B. Saunders Company, Philadelphia. p. 411-419.

Cutler, L.S. and A.P. Chaudhry, 1973. Differentiation of myoepithelial cells in the rat submandibular gland in vivo and in vitro-An ultrastructural study. *J. Morphol.*, **140**: 343-354.

El-Mohandes, E.A., K.G. Botros and A.A. Bondok. 1987. Prenatal development of the human submandibular gland. *Acta Anatomica*, **130**(3): 213-218.

Hsu, J.C.F., M. Kenneth and Yamada. 2010.

Salivary gland branching morphogenesis, recent progress and future opportunities. *International Journal of Oral Science*, **2**(3): 117-126.

Knospe, C. and G. Bohme. 1995. Zur Prsanatalen Entwicklung Derglandula Manibularis und Glandula parotis der katze. *Anatomia, Histologia and Embryologia*, **24**: 1-6.

Mc Geady, T.A., P.J. Quinn, E.S. Fitzpatrick and M.T. Ryan. 2006. *Veterinary Embryology*. Wiley-Blackwell Publishers, UK. p. 184-204.

Merida-Velasco, J.A., I. Sanchez-Montesinos, J.F. Espin, J.D. Garcia-Garcia, S. Garcia-Gomez and V. Roldan-Schilling. 1993. Development of the human submandibular salivary gland. *Journal of Dental Research*, **72**(8): 1227-1232.

Ogawa, Y., S. Toyosawa, T. Ishida and N. Ijuhin. 2000. Keratin 14 Immunoreactive cells in Pleomorphic Adenomas and Adenoid Cystic carcinomas of Salivary glands. *Virchows Arch*., **437**(1): 58-68.

Santhi, L., 2006. *Prenatal development of the skull in the buffalo (Bubalus bubalis)*. Ph.D. Thesis, Sri Venkateswara Veterinary University.

Singh, U.B. and S. Sulochana. 1997. *A Practical Manual of Histopathological and Histochemical Techniques*. Kothari Publication, Bombay. p. 1-41.

Soliman, M.K. 1975. Studies on the physiological chemistry of the allantoic and amniotic fluids of buffaloes at the various periods of pregnancy. *Indian Vet. J.*, **52**: 106-112.

Venkatakrishnan, 1994. *Morphogenesis, histomorphology and histochemistry of the mandibular gland of the buffalo (Bubalus bubalis)*. Thesis, TANUVAS, Chennai, India.