PRENATAL DEVELOPMENT OF BUFFALO MAJOR SALIVARY GLANDS: GROSS MORPHOLOGICAL AND BIOMETRICAL STUDIES

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ABSTRACT

The present study was aimed at elucidating the gross morphology, morphometry and biometry of major salivary glands of buffalo during different prenatal as well as neonatal stages. The study was conducted on major salivary glands of twenty-four buffalo foetuses as well as six neonatal buffalo calves. Three pairs of major salivary glands viz., parotid, mandibular and sublingual were distinguished during prenatal and neonatal life in the buffalo. The mandibular gland was the largest among the three major salivary glands in prenatal buffalo, which may be attributed to its early development among all the salivary glands. The sublingual gland was the smallest among the three major salivary glands in prenatal buffalo and measured about half the size of the mandibular gland. At 5.8 cm CVRL (54th day), the mandibular gland began to form an epithelial outgrowth into the mesenchyme, forming the floor of the mouth in the linguo-gingival groove. At 6 cm CVRL (55th day), the sublingual gland arose as a number of small epithelial thickenings in the linguogingival groove and each thickening formed its own canal. Grossly, the superior (polystomatic) and inferior (monostomatic) parts of the sublingual gland were distinguishable at 16.5 cm CVRL (102nd day). At 6.9 cm CVRL (60th day), the parotid gland was seen in the form of a small rod, extending dorsally from the lateral part of the oral cavity, just below and infront of the external ear and behind the facial nerve. Significant differences in the biometrical parameters of all major salivary glands between foetuses of Group I, II, III as well as neonatal buffalo were observed at $P \leq 0.05$ and $P \leq 0.01$ level.

Keywords: ontogeny, major salivary glands, buffalo, gross anatomy, biometry

INTRODUCTION

The study of prenatal development is prerequisite to understand the normal developmental biology of an organ. The documentation of normal foetal growth can serve as a guide for understanding the consequence of harmful influences at various stages of gestation. The study of the salivary glands forms an important link between the anatomy and surgery, as the salivary glands and associated ducts may be affected by inflammation, calculus formation, rupture or neoplasia. The secretion, saliva, contains water, various enzymes, mucopolysaccharides and lubricating glycoproteins. The gland has an important role to provide lubrication for eating and vocalization, aid digestion and supply saliva for pH buffering (Moghaddam *et al*., 2009). In general, the major salivary glands of the herbivores are better

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developed than those of the carnivores. Saliva is secreted into the oral cavity via a series of ducts in the ductal system. Dysfunction of salivary secretion (hyposalivation) causes xerostomia (dry mouth) and sequentially leads to severe dental caries as well as oral mucosal disorders (Featherstone, 2000).

The saliva, which plays important role in digestion, is secreted from major and minor salivary glands. The major salivary glands in domestic animals include the parotid, mandibular and sublingual salivary glands while the minor glands are the buccal, labial, lingual, palatine, molar and zygomatic salivary glands (Dellmann and Eurell, 1998). The minor salivary glands were embedded in the subepithelial tissues of the mouth and oro-pharynx. This wide distribution of the minor salivary glands is advantageous for the protection of the oral cavity against pathogens (Singh *et al*., 2011).

The prenatal development of major salivary glands had been studied in cat (Knospe and Bohme, 1995), human (Chi, 1996), rat (Wolff *et al*., 2002) and pig (Pospieszny *et al*., 2010), however, there is no detailed information about the gross morphology as well as biometry of major salivary glands of buffalo during prenatal life, therefore, the goal of this study was to describe the macroscopic ontogenetic events of major salivary glands of buffalo.

MATERIALS AND METHODS

The present study was conducted on major salivary glands of twenty-four buffalo foetuses, during different stages of prenatal development, as well as six neonatal buffalo calves. Immediately after collection, the foetus was measured for its curved crown rump length (CVRL) in centimetres with a calibrated inelastic thread. The approximate age of foetuses was calculated by using the following formula given by Soliman (1975) in buffalo:

$$Y = 28.66+4.496\ X \quad (CVRL < 20\ cm)$$
$$Y = 73.544+2.256\ X \quad (CVRL \geq 20\ cm)$$

Where Y is age in day(s) and X is curved crown rump length (CVRL) in cm(s). Depending upon CVRL, foetuses were divided into three groups with a minimum of eight samples in each group:

Group I : CVRL between 0 to 20 cm
Group II : CVRL >20 to 40 cm
Group III : CVRL >40 cm

Immediately after measuring CVRL, the foetuses as well as head of neonates were fixed in 10% neutral buffered formalin (NBF). After dissection, the skin over each gland was incised and the fascia was transected. The topography of the major salivary glands in relation to other structures was studied. Each gland was carefully freed from the adhering tissue and the length, breadth and weight were measured for each gland on two sides (left and right) and then compared. The data was subjected to statistical analysis for determining correlation between development of major salivary glands and the foetal age by using SPSS statistics software version 22.

RESULTS AND DISCUSSION

Three pairs of major salivary glands viz., parotid, mandibular and sublingual were

distinguished during prenatal and neonatal life in the buffalo as reported in human beings (Chi, 1996). The gross morphological and biometrical observations were made on major salivary glands of buffalo during prenatal and neonatal life.

Parotid salivary gland

The gland was light yellow in colour and was seen in the form of a small rod, extending dorsally from the lateral part of the oral cavity, just below and infront of the external ear and behind the facial nerve at 6.9 cm CVRL (60th day). At this stage, the gland was devoid of superficial lobulations. At 9.2 cm CVRL (70th day), the gland was pyramidal in shape with loosely arranged lobules and situated along the caudal border of the masseter muscle extending from the region of the external auditory canal to the level above the angle of the mandible (Figure 1). The parotid gland was reported to be in the form of a epithelial rod from 56 to 95 days of prenatal life in human beings (Nayeem *et al*., 2000). The gland gradually attained triangular shape with a broad base and narrow apex at 11.5 cm CVRL (80th day). The base of the gland was superior with a notch placed around the external auditory canal, while the apex was situated little above the angle of the mandible. Well developed parotid duct (Stenson's duct) was observed from 12.5 cm CVRL (84th day) onwards, leaving the gland at apex and ascending in the groove along the rostral border of masseter muscle along with the facial vessels and ventral buccal nerve. In human beings, Stenson's duct was observed from 80th day onwards during the prenatal life (Attie and Sciubba, 1981). The left parotid gland of Group I foetuses on average measured 0.58±0.1 cm in length and 0.43±0.1 cm in breadth whereas right one was 0.60±0.1 cm in length and 0.45±0.1 cm in breadth. The mean weight of left and right parotid glands was 1.39±0.2 gm and 1.40±0.2 gm, respectively (Table 1).

The gland was enclosed within a fibro-adipose capsule and reached the space between the base of the ear and vertical ramus of the mandible at 25.9 cm CVRL (132nd day). The facial nerve was seen very superficially and passed through the parenchyma of the gland. The auriculo-temporal nerve and few branches of facial nerve were seen at this stage of gestation (Figure 3). Similar findings were reported in domestic animals by Getty (1975); Barnwal and Sinha (1982); Nayeem *et al*. (2000). The prenatal parotid gland attained the shape of narrow triangle in mid age groups (Figure 3a). The lobules of the gland were loosely arranged and separated by interlobular septa at 29.5 cm CVRL (140th day). The parotid duct was opened into the mouth cavity at the level of upper 2nd erupting cheek tooth till 32 cm CVRL (145th day). The same was evident at the level of upper erupting 3rd cheek tooth from 36.2 cm CVRL (155th day) to 80 cm CVRL (254th day) of foetal life. In Group II, morphometric studies revealed that on average left parotid gland was 1.68±0.4 cm in length, 1.45±0.1 cm in breadth and weighed 3.85±0.5 gm whereas right one was 1.70±0.4 cm in length, 1.50±0.1 cm in breadth and weighed 3.90±0.5 gm (Table 1).

The adult characteristic features of the parotid gland were attained from 45.2 cm CVRL (175th day) onwards. At 100 cm CVRL (299th day), the colour of the gland varied from light yellow to light brown. The lobules of the gland were distinctly visible (Figure 4a). The upper anterior part of the gland was loosely attached to the parotid lymph node, while the lower anterior part was in close contact with the masseter muscle (Figure 4). The middle part of the gland was penetrated by the maxillary vein from lateral to medial surface throughout the prenatal life. The lateral surface was

covered by parotid fascia, developing parotido-auricularis muscle and facial muscles. The medial surface was uneven and related to great cornu of hyoid bone, digastricus, occipito-hyoideus and sterno-mastoideus muscles, external carotid artery, external jugular vein and its tributaries, facial nerve and its branches. The dorsal border was related to the base of the external ear. The anterior border was in contact with the parotid lymph node above and masseter muscle below. The posterior border was related to the posterior auricular vein. Nayeem *et al*. (2000) reported that the human parotid gland, in later stages of prenatal development, was closely related to 9th, 10th and 11th cranial nerves. The facial nerve was also very superficial. The left parotid gland of Group III foetuses on average measured 2.95±0.6 cm in length and 2.72±0.2 cm in breadth whereas right one was 3.00±0.6 cm in length and 2.80±0.2 cm in breadth. The mean weight of left and right parotid glands was 8.65±1.1 gm and 9.00±1.1 gm, respectively (Table 1). Gradual increase in length, breadth and weight of the gland was due to increased proliferation of ducts, increased lobulation and connective tissue formation during the foetal stage.

In neonatal buffalo, the parotid gland was long, narrow, pinkish brown in colour and triangular in shape with a wide thick dorsal end that reached the region of the temporo-mandibular joint. However, the gland was reported to be rectangular in sheep and goat (Getty, 1975) and pyramidal or triangular in buffalo calves (Barnwal and Sinha, 1982). The gland was located along the caudal border of the masseter muscle and extended from the zygomatic arch to the ramus of the mandible. The ventral aspect followed the caudal border of the mandible and was deeply related to the mandibular gland. The deep surface was related to the angle of the stylohyoid bone as well as the occipito-hyoideus and digastric muscles. The parotid duct left the gland ventrally with the facial artery as well as facial vein and ascended on the lateral surface of the masseter muscle to open near the posterior upper molar teeth in the oral cavity. The morphometric studies revealed that on average left parotid gland of neonatal buffalo was 5.50±0.8 cm in length, 3.00±0.2 cm in breadth and weighed 19.7±1.5 gm whereas right one was 5.80±0.8 cm in length, 3.10±0.2 cm in breadth and weighed 20.9±1.5 gm (Table 1). There was significant difference in the biometrical parameters of parotid gland between foetuses of Group I, II, III as well as neonatal buffalo at $P \leq 0.05$ and $P \leq 0.01$ level. The biometrical studies showed that there was no significant difference in the left and right parotid salivary gland within same group at $P \leq 0.05$ and $P \leq 0.01$ level (Graph 1).

Mandibular salivary gland

The mandibular gland was the largest among the three major salivary glands in prenatal buffalo, which may be attributed to its early development among all the salivary glands, while the parotid gland was reported to be the largest major salivary gland in human beings during prenatal life (Attie and Sciubba, 1981). The gland was light yellow in colour, long, narrow and curved in shape. It began to form an epithelial outgrowth into the mesenchyme, forming the floor of the mouth in the linguo-gingival groove at 5.8cm CVRL (54th day). At this stage, the gland was devoid of any superficial lobulations. The mandibular gland showed loosely arranged lobules at 9.2 cm CVRL (70th day) (Figure 1). The mandibular duct was well developed at 12 cm CVRL (82nd day). Budras and Habel (2003) reported similar findings in domestic animals. At 14 cm CVRL (91st day), mandibular gland was long, narrow and curved and enclosed

within a fibroadipose capsule. It was situated along the medial side of the angle of the mandible. In Group I, morphometric studies revealed that the mean length and breadth of left mandibular gland was 1.09±0.1 cm and 0.39±0.1 cm, respectively and average weight was 1.53±0.3 gm whereas right one was 1.10±0.1 cm in length, 0.40±0.1 cm in breadth and weighed 1.55±0.3 gm (Table 2).

The gland showed lobulations similar to that of parotid gland at 25.9 cm CVRL (132nd day). It extended from the region of tympanic bulla to the angle of the mandible behind the parotid salivary gland (Figure 3). At this stage, it was rough quadrilateral in shape (Figure 3b). The location was caudomedial to the parotid salivary gland in the mid and late foetal age groups. The left mandibular gland of Group II foetuses on average measured 2.38±0.6 cm in length and 1.19±0.1 cm in breadth whereas right one was 2.40±0.6 cm in length and 1.20±0.1 cm in breadth. The mean weight of left and right mandibular glands was 4.76±1.0 gm and 4.80±1.0 gm respectively (Table 2). Sivakumar *et al*. (2003) reported that in Group II (18 to 25 weeks), the length and breadth of human foetal submandibular gland increased 1.6 times and weight increased 2.4 times as compared to that of Group I.

At 42.7 cm CVRL (170th day), the mandibular gland attained the characteristics of adult gland. At 100 cm CVRL (299th day), the gland was elongated, curved and longer than the parotid gland and covered by fascia and the confluence of linguo-facial, occipital and maxillary veins with the external jugular vein laterally and developing thymus caudally. Medially, it was related to the larynx, division of the common carotid artery, external carotid artery, 9th, 10th, 11th cranial nerves, stylo-hyoideus muscle and great cornu of hyoid. The dorsal border was related to the duct of this gland and the ventral border was related to the external maxillary vein (Figure 4). Mandibular lymph node was placed above the anterior extremity of the gland. Dense compact lobulation of the gland was also observed at this stage (Figure 4b). In Group III, morphometric studies revealed that the mean length and breadth of left mandibular gland was 4.95±0.7 cm and 1.98±0.2 cm, respectively and average weight was 11.42±1.5 gm whereas right one was 5.00±0.7 cm in length, 2.00±0.2 cm in breadth and weighed 11.50±1.5 gm (Table 2). Gradual increase in length, breadth and weight of the gland was due to increased proliferation of ducts, increased lobulation and connective tissue formation during the foetal stage. Sivakumar *et al*. (2003) reported that in Group III (28 weeks-full term), the length and breadth of human foetal submandibular gland increased two times and weight increased five times than that of Group I.

The mandibular duct was seen leaving the gland at lower third of the inferior border between 12.5 cm CVRL (84th day) to 22 cm CVRL (123rd day) and middle of the concave border between 29.5 cm CVRL (140th day) to 78.7 cm CVRL (250th day). The duct running on the floor of the mouth cavity in close association with the sublingual duct and opened closely along the side of monostomatic sublingual duct at the caruncula sublingualis. Similar findings were described in domestic animals by Budras and Habel (2003); Akers and Denbow (2008). Pospieszny *et al*. (2010) observed that in pig, the location of the mandibular gland changed in respect to the mandibular angle during the prenatal period. With age its location changed from lateral to more medial, and from caudal to more rostral.

In neonatal buffalo, the mandibular gland was larger than the parotid gland. It was pale yellow to pinkish brown in colour, distinctly

lobulated and extended in a curve medial to the angle of the mandible from the atlantal fossa to the basihyoid muscle. Caudally, the gland was partly covered by the parotid gland. The mandibular duct left the gland from the middle of its rostral border and extended laterally along the digastric muscle, then passed forward on the deep surface of the mylohyoid and opened lateral to the sublingual caruncle. These observations were in partial agreement with the findings of Rauf *et al*. (2004) in one day old kid. The left mandibular gland of neonatal buffalo on average measured 9.8±1.0 cm in length and 2.8±0.2 cm in breadth whereas right one was 10.1±1.0 cm in length and 3.0±0.2 cm in breadth. The mean weight of left and right mandibular glands was 28.8±1.5 gm and 30.1±1.5 gm, respectively (Table 2).

Significant differences in the biometrical parameters of mandibular gland between foetuses of Group I, II, III as well as neonatal buffalo were observed at $P \leq 0.05$ and $P \leq 0.01$ level. The biometrical studies showed that there was no significant difference in the left and right mandibular salivary gland within same group at $P \leq 0.05$ and $P \leq 0.01$ level (Graph 2).

Sublingual salivary gland

The sublingual gland was the smallest among the three major salivary glands in prenatal buffalo and measured about half the size of the mandibular gland. The gland arose as a number of small epithelial thickenings in the linguogingival groove and on the outer side of the groove at 6 cm CVRL (55th day). Each thickening formed its own canal. At 9.2 cm CVRL (70th day), the gland was devoid of any superficial lobulations (Figure 2). The left sublingual gland of Group I foetuses on average measured 1.49±0.3 cm in length and 0.20±0.2 cm in breadth whereas right one was 1.50±0.3 cm in length and 0.20±0.2 cm in breadth. The mean weight of left and right sublingual glands was 1.34±0.1 gm and 1.35±0.1 gm, respectively (Table 3).

The sublingual gland was composed of two parts, the superior (polystomatic) and inferior (monostomatic) parts. Grossly, the superior and inferior parts of the gland were distinguishable at 16.5 cm CVRL (102nd day). At 25.9 cm CVRL (132nd day), the colour of the superior part varied from light yellow to light brown (Figure 3c). The superior part of the gland was arranged in a chain of lobules and extended from the palato-glossal arch to the incisive part of the mandible. The colour of the inferior part varied from light yellow to light brown. It was elongated and situated beneath the mucosa of the floor of the mouth, above the mylohyoideus muscle, between the mandible laterally, and the muscles of tongue medially. Pospieszny *et al*. (2010) reported that in the initial developmental periods of pig, the elongation of sublingual salivary ducts was higher than in mandibular gland. In Group II, morphometric studies revealed that the mean length and breadth of left sublingual gland was 3.78±0.5 cm and 0.50±0.3 cm, respectively with mean weight was 1.93±0.1 gm whereas right one was 3.80±0.5 cm in length, 0.50±0.3 cm in breadth and weighed 1.95±0.1 gm (Table 3).

At 45.2 cm CVRL (175th day), the sublingual gland attained the characteristic shape, colour and position similar to adult gland. At 100 cm CVRL (299th day), the gland showed distinct lobulations (Figure 5a). The superior part was light brown in colour and extended from the mandibular symphysis to the palatoglossal arch. The ducts of the superior part were not visible during the prenatal period. The inferior part was also light brown in colour and extended from the mandibular symphysis to the level of the last premolar tooth.

It was thicker and shorter than the superior part and was situated below it. The inferior part of the sublingual gland was drained by only one duct, which opened along the side of mandibular duct at caruncula sublingualis (Figure 5). These features were in agreement with the reports of Arey (1965) in human beings and Latshaw (1987) in domestic animals. The location of foetuses in the horns of uterus and their sex did not have any significant impact on the morphology and development of salivary glands (Pospieszny *et al*., 2010). The left sublingual gland of Group III foetuses on average measured 5.98±0.6 cm in length and 1.19±0.3 cm in breadth whereas right one was 6.00±0.6 cm in length and 1.20±0.3 cm in breadth. The mean weight of left and right sublingual glands was 2.86±0.1 gm and 2.90±0.1 gm, respectively (Table 3). Gradual increase in length, breadth and weight of the gland was due to increased proliferation of ducts, increased lobulation and connective tissue formation during the foetal stage.

In neonatal buffalo, the superior part of sublingual gland was composed of a chain of lobules. It was pale yellow in colour and situated under the floor of the mouth. It extended from the incisive area of the mandible to the palato-glossal arch and was drained by many small ducts which opened along rows of long papillae found in the lateral sublingual recess of the mouth. The inferior part was extended from the incisive area of the mandible to the midline of the superior part of gland. A single excretory duct passed forward medial to the gland and accompanied the mandibular duct to the sublingual caruncle located on the floor of the mouth behind the incisor teeth. Collectively, the sublingual glands were related on their lateral aspects to the mylohyoid muscle and the sublingual nerve, medially to the hyoglossus, styloglossus and genioglossus muscles and ventrally to the geniohyoid muscle. These observations were in total agreement with the findings of Attie and Sciubba (1981) in human beings and Budras and Habel (2003) in domestic animals. The morphometric studies revealed that the mean length and breadth of left sublingual gland of neonatal buffalo was 10.4±1.0 cm and 1.35±0.5 cm, respectively and average weight was 6.7±1.0 gm whereas right one was 10.6±1.0 cm in length, 1.35±0.5 cm in breadth and weighed 6.9±1.0 gm (Table 3).There was significant difference in the biometrical parameters of sublingual gland between foetuses of Group I, II, III as well as neonatal buffalo at $P \leq 0.05$ and $P \leq 0.01$ level. The biometrical studies showed that there was no significant difference in the left and right sublingual salivary gland within same group at $P \leq 0.05$ and $P \leq 0.01$ level (Graph 3).

CONCLUSION

Three pairs of major salivary glands viz., parotid, mandibular and sublingual were distinguished during prenatal and neonatal life in the buffalo. The mandibular gland was the largest among the three major salivary glands in prenatal buffalo, which may be attributed to its early development among all the salivary glands. The sublingual gland was the smallest among the three major salivary glands in prenatal buffalo and measured about half the size of the mandibular gland. At 5.8 cm CVRL (54^{th} day), the mandibular gland began to form an epithelial outgrowth into the mesenchyme, forming the floor of the mouth in the linguo-gingival groove. The sublingual gland arose as a number of small epithelial thickenings in the linguogingival groove and each thickening formed its own canal at 6 cm CVRL (55^{th} day). The parotid gland was first seen in the form of a small rod,

Table 1. Gross biometrical observations on parotid salivary gland of buffalo foetuses and neonates.

Parotid Salivary Gland	Prenatal age groups									Neonatal group		
	Group I**			Group II**			Group III**			Group IV**		
	Length (cm)	Breadth (cm)	Weight (gm)	Length (cm)	Breadth (cm)	Weight (gm)	Length (cm)	Breadth (cm)	Weight (gm)	Length (cm)	Breadth (cm	Weight (gm)
Left*	0.58±0.1	0.43±0.1	1.39±0.2	1.68±0.4	1.45±0.1	3.85±0.5	2.95±0.6	2.72±0.2	8.65±1.1	5.50±0.8	3.00±0.2	19.7±1.5
Right*	0.60±0.1	0.45±0.1	1.40±0.2	1.70±0.4	1.50±0.1	3.90±0.5	3.00±0.6	2.80±0.2	9.00±1.1	5.80±0.8	3.10±0.2	20.9±1.5

Significant (P≤0.05), Highly Significant (P≤0.01).

*Non-significant difference within groups at 0.05 and 0.01 level.

**Significant difference between groups at 0.05 and 0.01 level.

Table 2. Gross biometrical observations on mandibular salivary gland of buffalo foetuses and neonates.

Mandibular Salivary Gland	Prenatal age groups									Neonatal group		
	Group I**			Group II**			Group III**			Group IV**		
	Length (cm)	Breadth (cm)	Weight (gm)	Length (cm)	Breadth (cm)	Weight (gm)	Length (cm)	Breadth (cm)	Weight (gm)	Length (cm)	Breadth (cm)	Weight (gm)
Left*	1.09±0.1	0.39±0.1	1.53±0.3	2.38±0.6	1.19±0.1	4.76±1.0	4.95±0.7	1.98±0.2	11.42±1.5	9.8±1.0	2.8±0.2	28.8±1.5
Right*	1.10±0.1	0.40±0.1	1.55±0.3	2.40±0.6	1.20±0.1	4.80±1.0	5.00±0.7	2.00±0.2	11.50±1.5	10.1±1.0	3.0±0.2	30.1±1.5

Significant ($p \leq 0.05$), Highly Significant ($p \leq 0.01$).

*Non-significant difference within groups at 0.05 and 0.01 level.

**Significant difference between groups at 0.05 and 0.01 level.

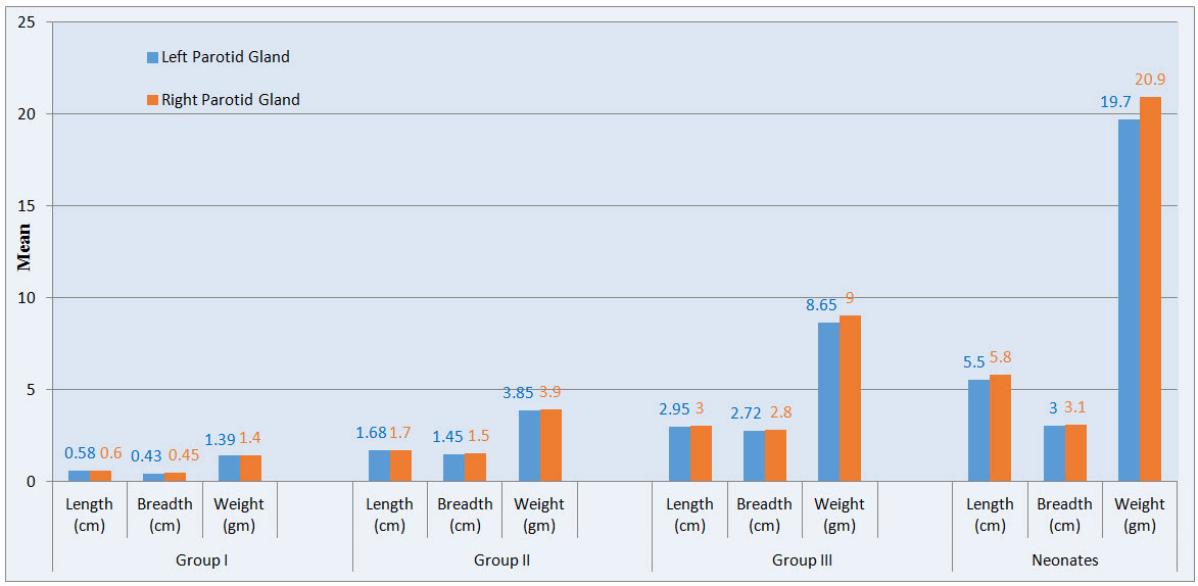
Table 3. Gross biometrical observations on sublingual salivary gland of buffalo foetuses and neonates.

Sublingual Salivary Gland	Prenatal age groups									Neonatal group		
	Group I**			Group II**			Group III**			Group IV**		
	Length (cm)	Breadth (cm)	Weight (gm)	Length (cm)	Breadth (cm)	Weight (gm)	Length (cm)	Breadth (cm)	Weight (gm)	Length (cm)	Breadth (cm)	Weight (gm)
Left*	1.49±0.3	0.20±0.2	1.34±0.1	3.78±0.5	0.50±0.3	1.93±0.1	5.98±0.6	1.19±0.3	2.86±0.1	10.4±1.0	1.35±0.5	6.7±1.0
Right*	1.50±0.3	0.20±0.2	1.35±0.1	3.80±0.5	0.50±0.3	1.95±0.1	6.00±0.6	1.20±0.3	2.90±0.1	10.6±1.0	1.35±0.5	6.9±1.0

Significant (P≤0.05), Highly Significant (P≤0.01).

*Non-significant difference within groups at 0.05 and 0.01 level.

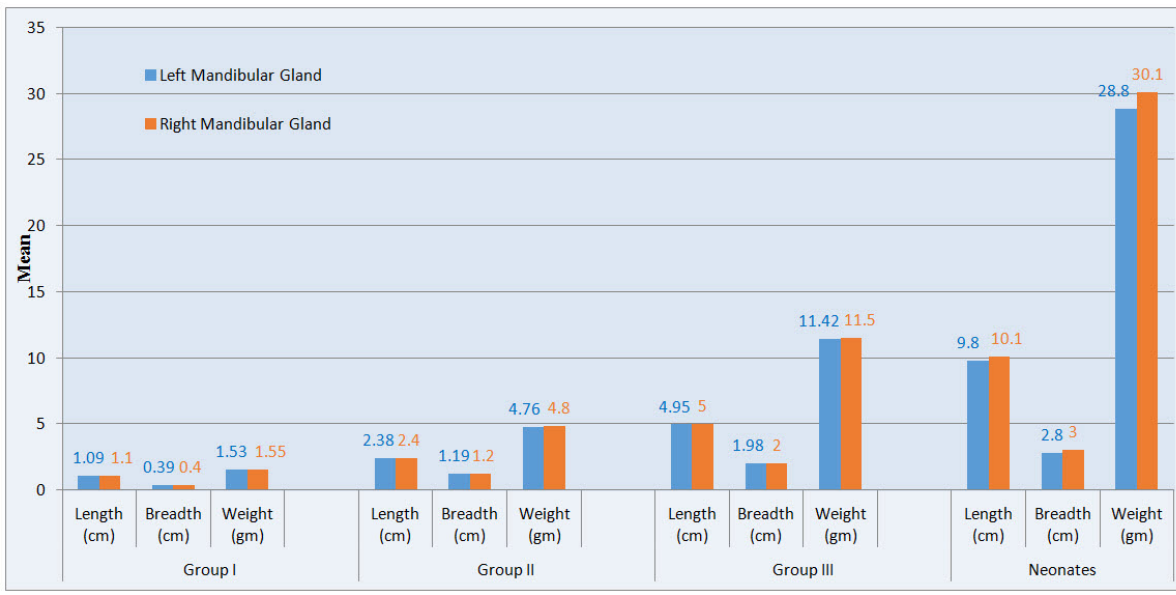
**Significant difference between groups at 0.05 and 0.01 level.



Graph 1. Showing variations in mean values of length, breadth and weight of parotid salivary gland of buffalo fetuses.

*Non-significant difference within groups at 0.05 and 0.01 level.

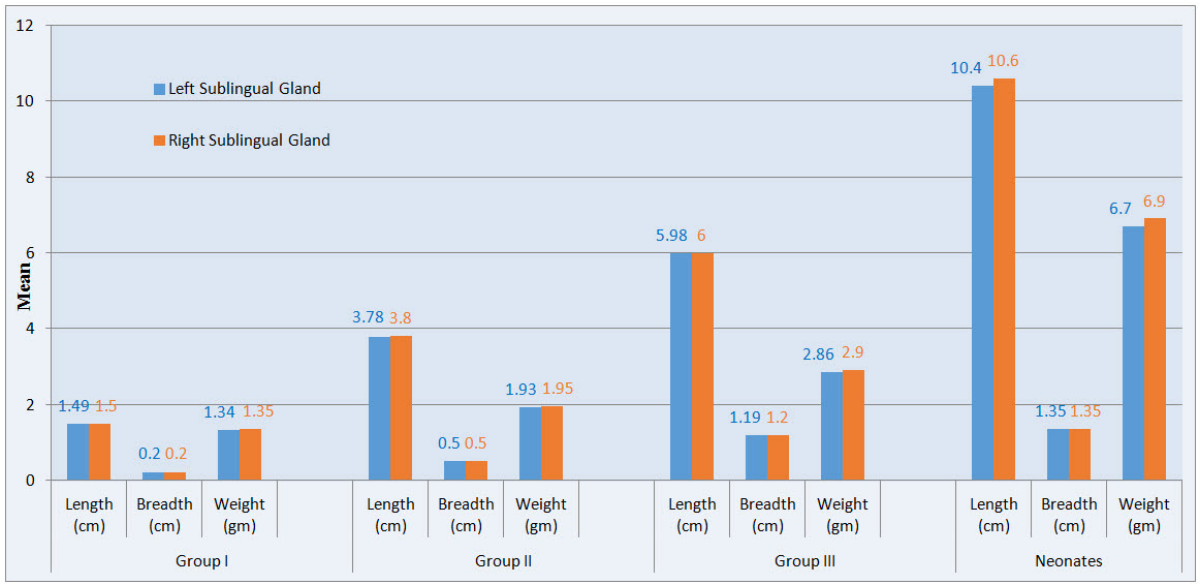
**Significant difference between groups at 0.05 and 0.01 level.



Graph 2. Showing variations in mean values of length, breadth and weight of mandibular salivary gland of buffalo fetuses.

*Non-significant difference within groups at 0.05 and 0.01 level.

**Significant difference between groups at 0.05 and 0.01 level.



Graph 3. Showing variations in mean values of length, breadth and weight of sublingual salivary gland of buffalo fetuses.

*Non-significant difference within groups at 0.05 and 0.01 level.

**Significant difference between groups at 0.05 and 0.01 level.

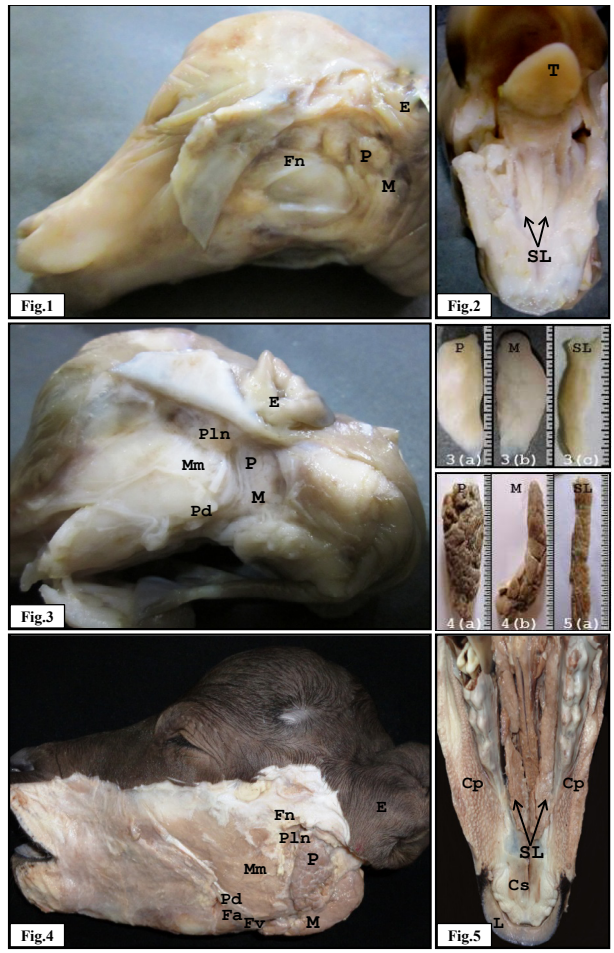


Figure 1. Photograph of 9.2 cm CVRL (70th day) buffalo foetal head showing loosely arranged lobules of parotid (P) and mandibular (M) salivary glands. (Fn- facial nerve; E- external ear).

Figure 2. Photograph of head of 9.2 cm CVRL (70th day) buffalo foetus showing sublingual glands (SL) were devoid of superficial lobulations. (T-tongue).

Figure 3. Photograph of 25.9 cm CVRL (132nd day) buffalo foetal head showing the location of parotid (P) and mandibular (M) glands and their relations. (Pln- parotid lymph node; Mm-masseter muscle; Pd- parotid duct; E- externar ear).

3(a) Photograph of prenatal parotid gland showing roughly triangular shape at CVRL of 25.9 cm (132nd day).

3(b) Photograph of prenatal mandibular gland showing roughly quadrilateral shape at 25.9 cm CVRL (132nd day).

3(c) Photograph of prenatal sublingual gland showing elongated chain of lobules at CVRL of 25.9 cm (132nd day).

Figure 4. Photograph of head of 100 cm CVRL (299th day) buffalo foetus showing distinct lobulations of well developed parotid (P) and mandibular (M) salivary glands. (Pln- parotid lymph node; Fn- facial nerve; Mm- masseter muscle; Pd- parotid duct; Fa- facial artery; Fv- facial vein; E- externar ear).

4(a) Photograph of prenatal parotid gland showing distinct lobulations at CVRL of 100 cm (299th day).

4(b) Photograph of prenatal mandibular gland showing dense compact lobulations at 100 cm CVRL (299th day).

Figure 5. Photograph of 100 cm CVRL (299th day) buffalo foetal head showing distinct lobulations of sublingual glands (SL) in the form of elongated chain of lobules. (Cp- conical papillae; Cs- caruncula sublingualis; L- lower lip).

5(a) Photograph of prenatal sublingual gland showing distinct lobulations at CVRL of 100 cm (299th day).

extending dorsally from the lateral part of the oral cavity, just below and infront of the external ear and behind the facial nerve at 6.9 cm CVRL (60th day). The mandibular duct was well developed at 12 cm CVRL (82nd day), however, parotid duct (Stenson's duct) was observed from 12.5 cm CVRL (84th day) onwards. Grossly, the superior (polystomatic) and inferior (monostomatic) parts of the sublingual gland were distinguishable at 16.5 cm CVRL (102nd day). At 42.7 cm CVRL (170th day), the mandibular gland attained the characteristics of adult gland, whereas in parotid and sublingual glands, the adult characteristic features were attained from 45.2 cm CVRL (175th day) onwards. Significant differences in the biometrical parameters of all major salivary glands between foetuses of Group I, II, III as well as neonatal buffalo were observed at $P \leq 0.05$ and $P \leq 0.01$ level. The biometrical studies showed that there was no significant difference in the left and right sets of all major salivary glands within same group at $P \leq 0.05$ and $P \leq 0.01$ level.

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