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Original Research Article

Prenatal development of submandibular salivary gland of New-Zealand rabbits

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ABSTRACT

The present study aimed to ellucidate the prenatal developmental stages of the submandibular salivary gland of the New-Zealand rabbits. To conduct that, twenty New-Zealand rabbit fetuses ranged from 11 to 30 days-old were used. The head region of fetuses and gland specimens were fixed, processed and stained with histological stains to be examined by light microscope. The submandibular primordia was firstly seen at the 12th day of the prenatal life as bilateral invaginated epithelial buds from the linguo-gingival groove. At 15 days-old , such buds continued deep down growth forming cord-like structure ended by compact bulges that forming the future primitive acini. At 17 days, such cords were branched off forming the future primary ducts. Canalization of the ducts appeared at 18 days. At 22 days, the primitive capsule initiated around the gland and the lobulation was recognized. At 25 days, the capsule became well developed, the duct system was completed and the parenchyma occupied by serous adenomeres surrounded by myoepithelial cells. At the full term, the submandibular gland became fully developed and became typically compound tubulo-acinar nature, the parenchyma showed seromucoidadenomeres. Strong positive PAS reaction was noticed in the striated ducts, while the cytoplasm of the acinar cells reacted weakly.

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1. Introduction

The paired submandibular glands are one of the major salivary gland. They are considered as a key role in food digestion by producing substantial amount of saliva (Adnyane et al.,2010).The lubricating and the antimicrobial action of saliva maintain the oral biology (Hsu and Yamda, 2010).All major salivary glands are ectodermal in origin as a result of interacting with neural crest-derived mesenchyme (Jaskoll et al.,2002 ; Rothova et al.,2012).Other studies reported the submandibular and sublingual are endodermal in origin (Emami et al., 1991).

.The submandibular gland is the first major salivary gland that to be developed followed by the sublingual and parotid (Tucker, 2007). Various prenatal studies have been done on this gland in rat (Culter and Moordian, 1987); in mice (Jasckoll and Melnick, 1999); in pig (Pospienzy et al., 2010); in cat (Knospe and Bohme, 1995); in buffalo (Amanand Opinder, 2017) and in human (Velasco et al., 1993).the present study was intended to highlight the prenatal development stages of the submandibular salivary gland of New-Zealand rabbits.

2. Materials and methods

A total number of 20 New-Zealand normal and apparently healthy rabbit fetuses ranged from 11-20 days-old and the dissected glands of fetuses (20 – 0 days-old) were subjected to this study. The head regions of fetuses were fixed in 10% neutral formalin, Suza and Helley's fluid, processed and embedded in paraffin blocks. Cross and/or sagitalstepserial sections of 4-6 um thick were obtained and stained with Harris's Haematoxylin and Eosin , Masson's trichrome stain, Periodic acid Schiff technique (PAS) and Alcian blue method (PH 2.5) as outlined by (Bancroft and Gamble, 2008).

3. Results

The primordia of the submandibular salivary gland was firstly recognized at 12 days-old New-Zealand rabbit embryos as bilateral solid epithelial buds invaginated from the linguo-gingival groove at the base of the developing tongue (Fig.1). Such buds were formed of clusters of irregularly arranged undifferentiated cells which were rounded or ovoid in shape with rounded nuclei surrounded by faintly basophilic cytoplasm .Some of primordial cells showed mitotic activity (Fig.2).At the13th day rabbit embryo, the submandibular buds grew deeply throughout underlying mesenchymal tissue forming solid epithelial cords with closely packed cellular masses of ill-distincit cell boundaries (Fig.3&4). On reaching the 15th day, the developing cords continued their deep down growth and showed compact terminal bulges forming the primitive acini which surrounded by large amount of primitive stroma with many fibroblasts and mesenchymal cells (Fig.5). The primitive acini at this stage were lined by multilayered polyhedral cells of basophilic cytoplasm and darkly stained nuclei with loose cellular central mass (Fig.6). At 17 days-old rabbit embryo, progressive branching of the developing cords connected with primitive acini which surrounded by loose mesenchymal tissue (Fig.7).The primitive acini were lined by closely packed cells with numerous mitotic division. The developing cords at this stage showed more differentiation and designated to form the future primitive ducts. Their cellular clusters formed of outer regularly arranged closely packed layer of columnar cells with oval nuclei while the inner cells were loosely arranged (Fig.8). The primitive ducts began to be canalized at the 18th day of the prenatal life. They were lined by one to two layers of cuboidal to columnar cells housing rounded or oval nuclei surrounded by pale basophilic cytoplasm. The acini were still illuminized (Fig.9). On reaching 22 days, the embryonic mesenchymal tissue became differentiated into primitive capsule and trabeculae that divided the gland into different lobes and lobules. The

developing adenomeres continued to increase in number but loosely arranged within the lobules. The interlobular ducts were lined by double layers of epithelial cells with central lumina (Fig.10). At 25 days-old rabbit embryo, the submandibular gland became larger in size and highly organized into well- developed stroma and distinct increasing in lobulation and vascularity. The capsule became well-developed formed of dense collagenous connective tissue with many fibroblasts (Fig.11).The gland adenomeres were progressively increased and closely packed with each other. Most of them became luminized and lined by truncated pyramidal cells with basal basophilic cytoplasm and slightly acidophilic apically. An elongated curved myo-epithelial cells with flattened nuclei and scanty cytoplasm appeared to surround the adenomeres (Fig.12&13).Extensive branching of ducts were observed. All types of ducts including intercalated, striated and interlobular ducts were noticed at this stage. The lining epithelium of some striated ducts changed into single layer of columnar cells with oval nuclei (Fig.14).Both fibrous elements either in capsule or in the trabeculae and basal lamina of adenomeres and ducts showed positive PAS reaction, while the cytoplasm of acinar cells showed weak reaction (Fig.15). From 27 till the end of the prenatal life, submandibular gland became highly developed and became typical compound tubulo-acinar gland. The glandular lobules were increased on the expense of the interstitial tissue (Fig.16).The stroma became fully developed formed mainly of collagen fibers and fibroblasts. The trabeculae carried interlobular ducts (Fig.17). The glandular acini became differentiated into mucous and serous. The mucous adenomeres were more prominent and larger in size than the serous ones. They lined byhigh cuboidal cells with basally ovoid or flattened nuclei surrounded by vacuolated or foamy cytoplasm While the serous adenomeres appeared smaller in size and lined by truncated pyramidal cells with basal spherical nuclei and basophilic cytoplasm. Some serous demilunes showed to be capped the mucous adenomeres (Fig.18). Both types of gland adenomeres were surrounded by myoepithelial cells. During this stage, the fibrous stroma and the basement membrane of adenomeres and ducts showed positive PAS reaction. Also, the cellular cytoplasm of some striated ducts showed strong positive PAS reaction, while the cytoplasm of acinar cells still showed weak reaction (Fig.19).

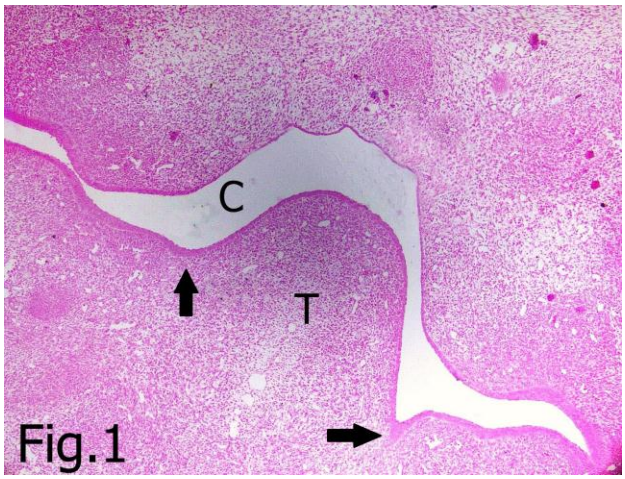


Fig.1

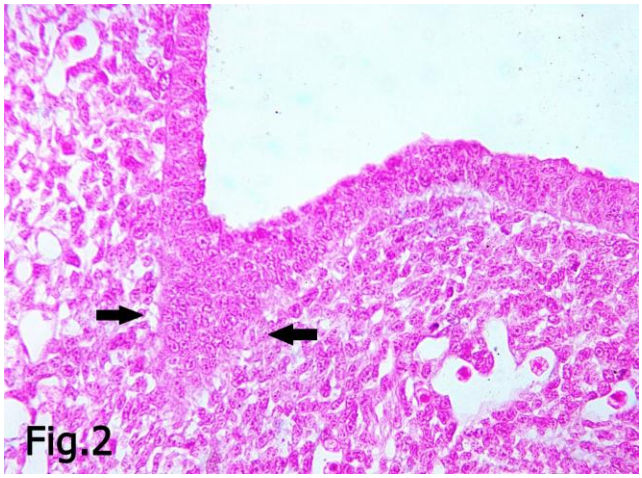
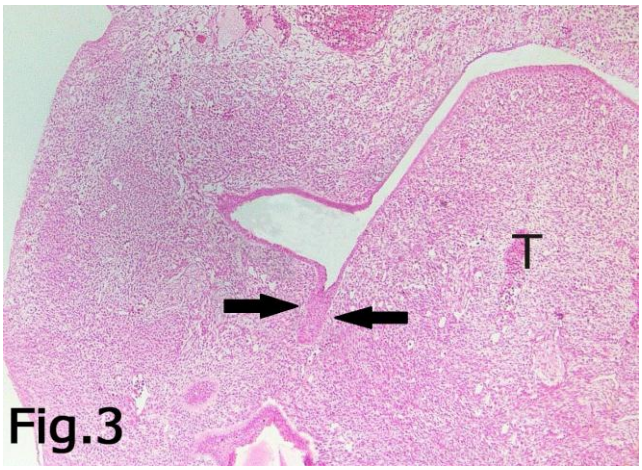


Fig.2



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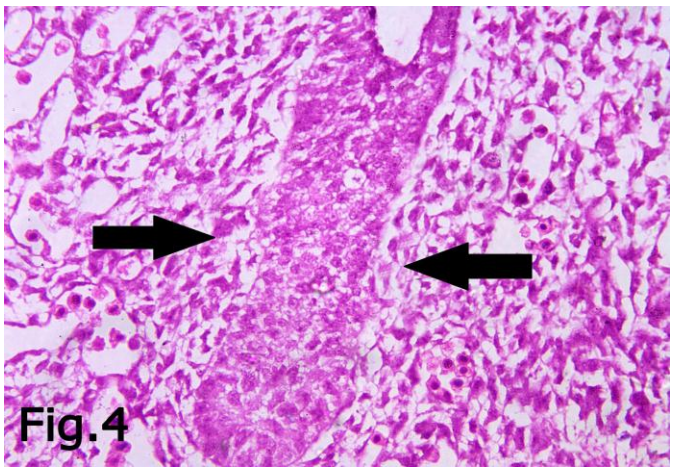
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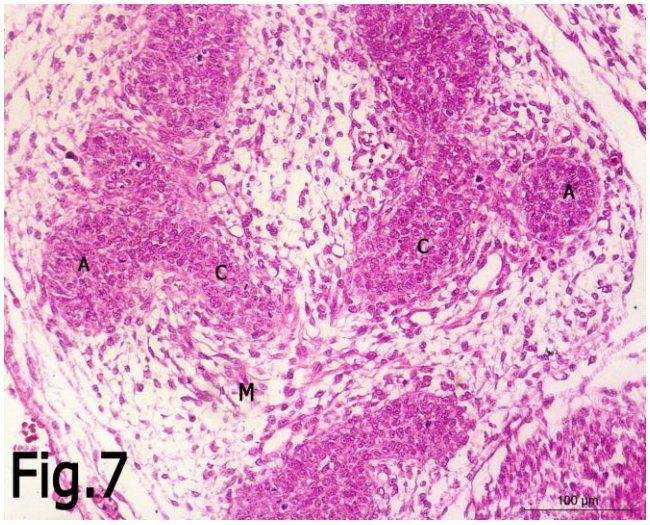


Fig.7

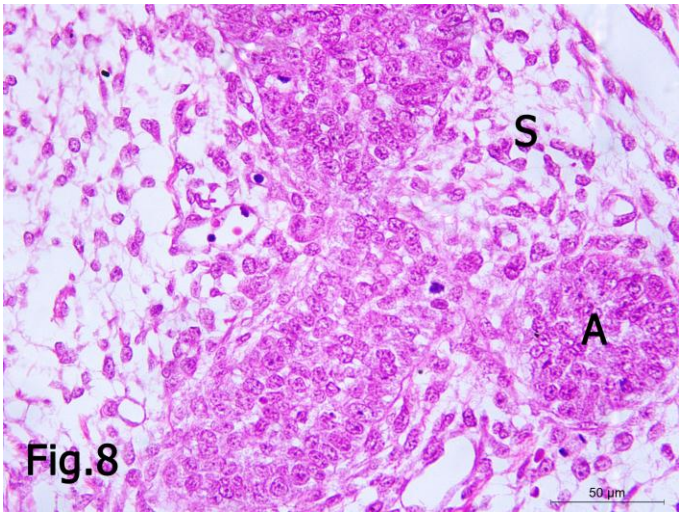


Fig.8

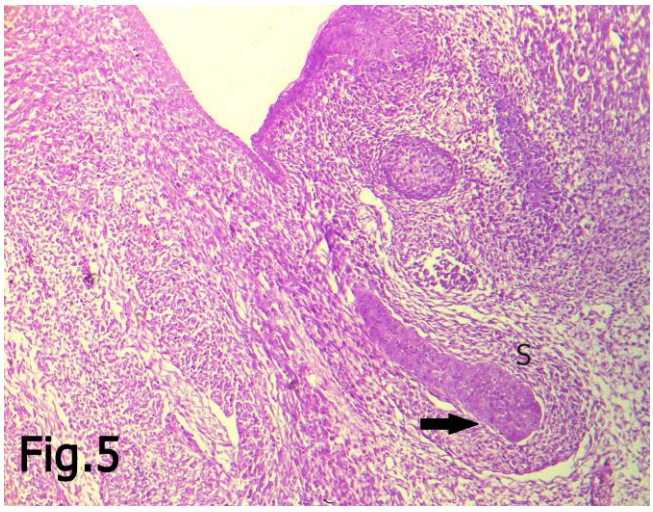


Fig.5

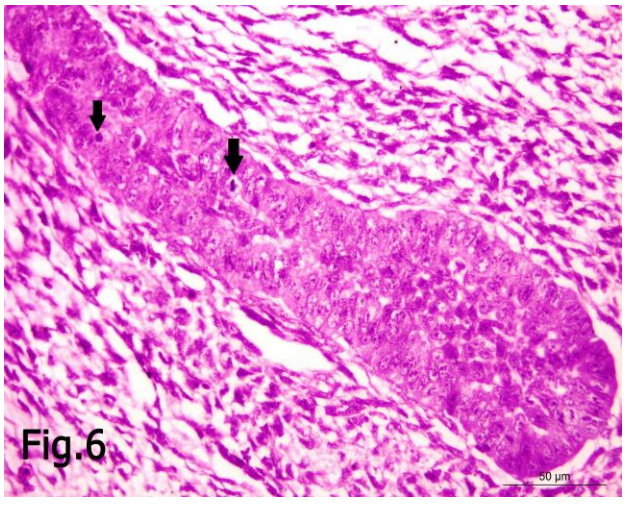


Fig.6

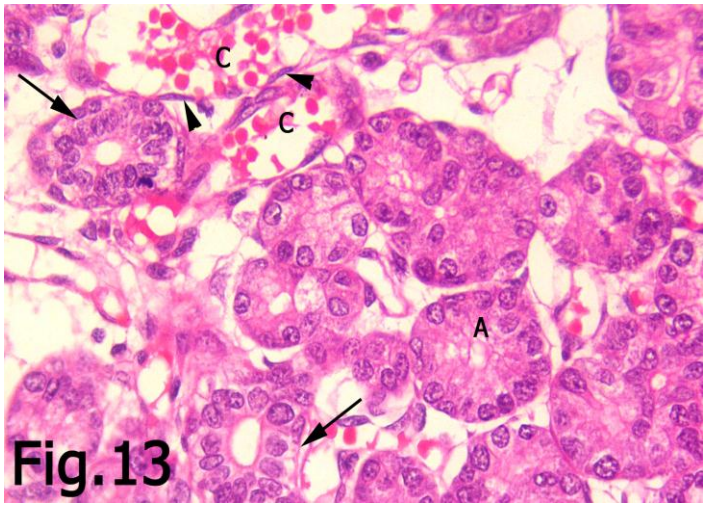


Fig.13

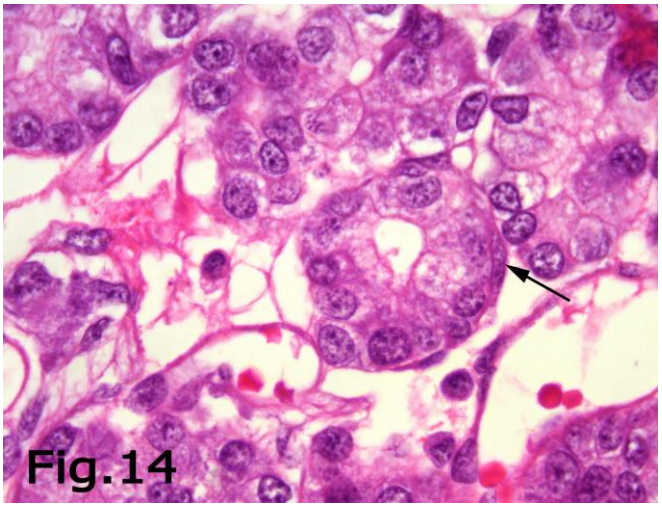


Fig.14

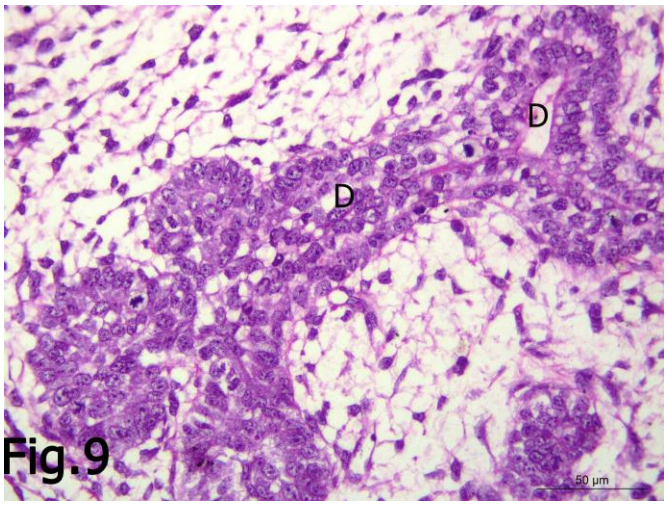


Fig.9

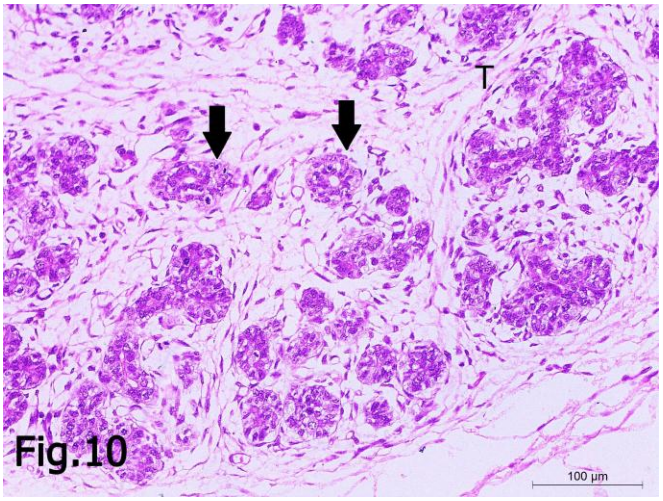


Fig.10

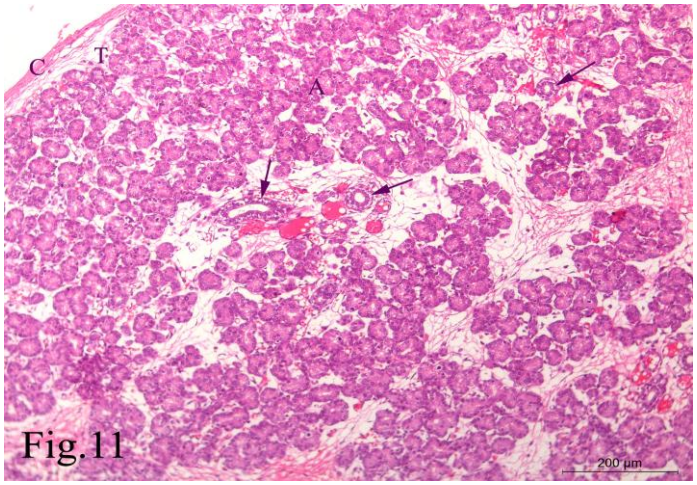


Fig.11

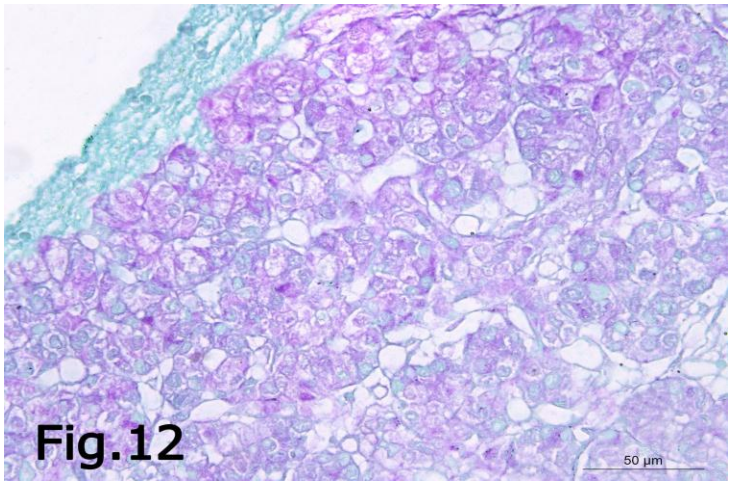


Fig.12

Figure legends

Fig. 1.A sagital section through the head region of 12 days-old New-Zealand rabbit embryo showing bilateral solid epithelial buds (arrows) on either sides of primitive tongue (T). Notice the oral cavity (c). H&E , X 40**.**

Fig. 2. A higher magnification of figure.1 showing primordial submandibular buds formed of clusters of undifferentiated epithelial cells with rounded nuclei and faintly basophilic cytoplasm(arrows).H&E , X 400.

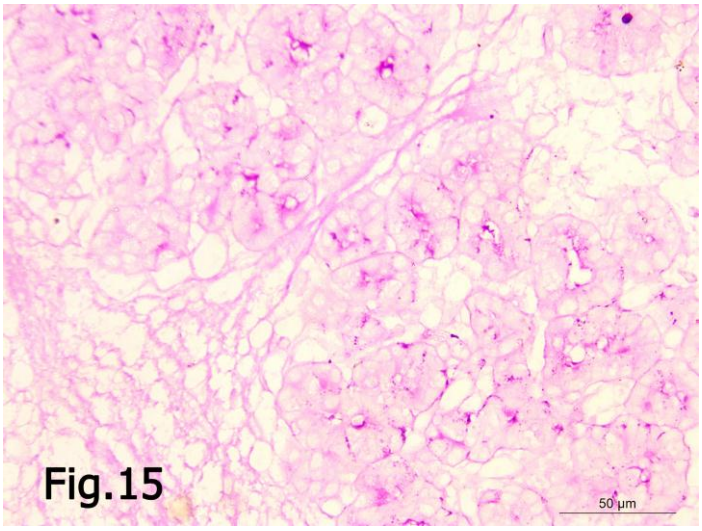


Fig. 3.A sagital section through 13 days-old rabbit embryo showing a deep invagination of submandibular primordia forming cord -like structure (arrows) from the surface epithelium on either sides of the primitive tongue (T).H&E , X 40.

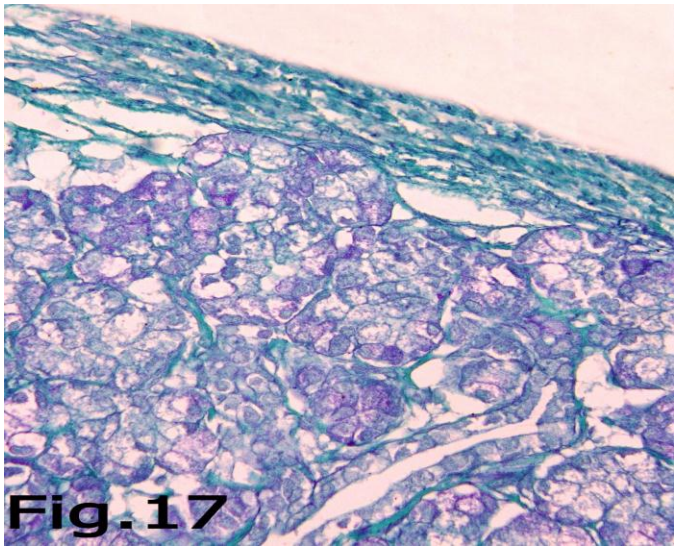
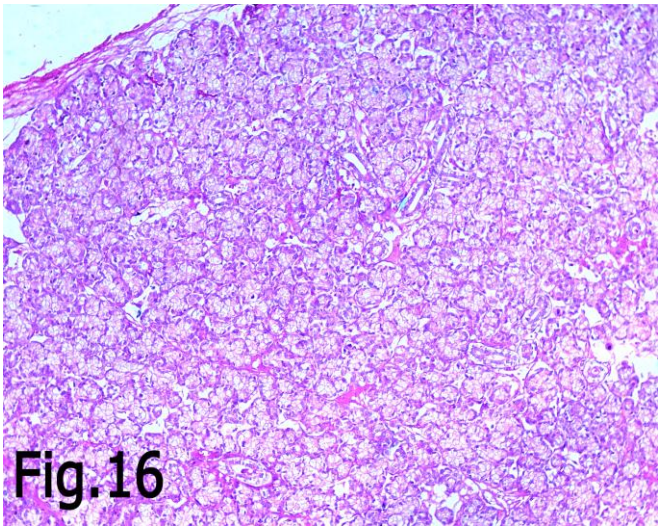
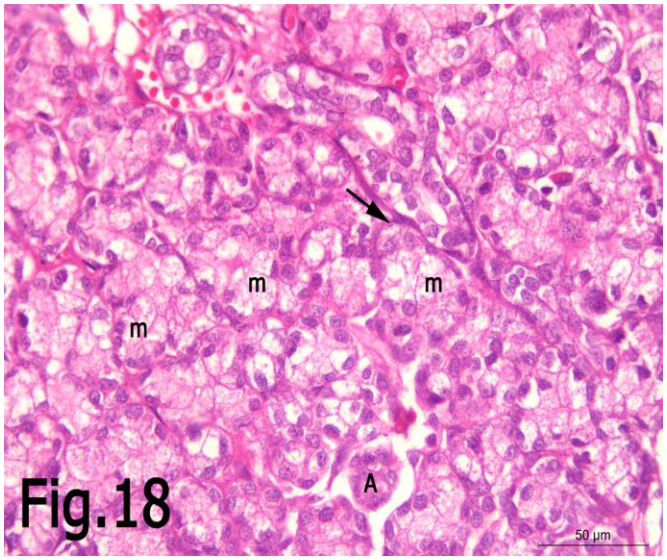


Fig. 4. A higher magnification of figure.3 showing the epithelial cords formed of clusters of closely packed cellular masses with ill-distinct cell boundaries(arrow).H&E , X 400.

Fig. 5.A photomicrogragh of the head region of the 15th day of rabbit embryo showing deep down growth of developing cords with terminal bulges (arrows) surrounded by primitive stroma





(s).H&E , X 100.

Fig. 6.A higher magnification of figure. 5 showing the primitive acinus lined by multilayered polyhedral cells of basophilic cytoplasm and darkly stained nuclei .Notice, the cells of both primitive duct and acini showing highly mitotic activity (arrows). H&E , X 400.

Fig. 7. Aphotomicrogragh of the developing submandibular salivary gland of 17 days-old rabbit embryo showing progressive branching of developing ducts (c) and primitive acini (A) surrounded by mesenchymal tissue (M). H&E , X 200.

Fig.8. A higher magnification of figure.7 showing the cellular elements of developing ducts became differentiated into outer regularly arranged closely packed polyhedral cells and inner loose cellular masses forming primitive ducts (D). Notice, illuminized developing acini (A) surrounded by primitive stroma (S). H&E , X 400.

Fig.9. A photomicrograph of the developing submandibular salivary gland of 18 days-old rabbit embryo showing the beginning of canalization in the duct system (D).H&E , X 400.

Fig.10. A photomicrograph of the developing submandibular salivary gland of 22 days-old rabbit embryo showing different lobules separated by developing trabeculae (T). Notice: the inter lobular ducts (arrows) appeared canalized and lined by double layer of epithelial cells. H&E , X 200.

Fig.11. A photomicrograph of the developing submandibular salivary gland of 25 days-old rabbit embryo showing a well developed capsule (c), trabeculae (T), distinct parenchymal lobulation occupied by glandular adenomeres(A) and well developed duct system (arrows). H&E , X 200.

Fig.12. A photomicrograph of the developing submandibular salivary gland of 25 days-old rabbit embryo showing well developed capsule formed of collagen fibers. Masson's trichromestain , X 400.

Fig.13. A photomicrograph of the developing submandibular salivary gland of 25 days-old rabbit embryo showing most of glandular serous adenomeres were luminized, lined by truncated pyramidal cells with oval basally situated nuclei and well developed striated ducts lined by single layer of columnar cells (arrows).Notice,well developed blood capillaries lined by endothelial cells (arrow heads).H&E , X 400.

Fig.14.A photomicrograph of the developing submandibular salivary gland of 25 days-old rabbit embryo showing the glandular myoepithelial cells (arrow) surrounded adenomeres.(H&E ,X 1000).

Fig.15. A photomicrograph of the developing submandibular salivary gland of 25 days-old rabbit embryo showing PAS positive reaction in the fibrous stromainaddition to basal lamina of alveoli while the cells of both ducts and acini showed weak reaction. PAS stain , X 400.

Fig.16.A photomicrograph of the developing submandibular salivary gland of full term rabbit embryo showing progressive growth of the gland parenchyma . H&E , X 200.

Fig.17. A photomicrograph of the developing submandibular salivary gland of full term rabbit embryoshowing well developed fibrous capsule and septa . Masson's trichromestain , X 400.

Fig.18. A photomicrograph of the developing submandibular salivary gland of full term rabbit embryoshowing both mucous (M) and serous(A) adenomeres. Notice, some serous demilunes were present (arrow). H&E stain , X 400.

Fig.19.A photomicrograph of the developing submandibular salivary gland of full term rabbit

embryo showing strong PAS positive reaction in the duct system and weak reaction in the cytoplasm of acinarcells.PASstain , X 400.

4. Discussion

There is a general agreement that the submandibular salivary gland morphologically developed from the epithelial lining of the linguo -gingival groove (William et al., 1989; Klein, 2002; Sivakumar,et al., 2003; Patel and Hoffman, 2014; and Kwon &larsen, 2015).The authors supported our results as the submandibular salivary gland primordiaarised as an epithelial bud formed of clusters of epithelial cells. The present study revealed that the primordia of submandibular salivary gland began to develop at 12 days-old New-Zealand rabbit embryo. On the other hand Soliman (2006) recorded that the rabbit submandibular primordia firstly recognized at the 13th day of prenatal life. In another line, mice submandibular primordia could be detected at 11.5th (Tucker, 2007), while that of rat could be noticed at the 12thday(Kleinman, 2003). As mentioned in the present study, Melnick, et al., (2001) recorded that the epithelial bud developed progressively with underlying mesenchyme and became branched. As developement proceeded the present study revealed that the epithelial buds showed more deep down growth throughout the underlying mesenchymal tissue forming cord like structure which branched off forming the future primitive ducts and acini as recorded by (Bath-Balogh&Fehrenbach, 2011; Abuzaid et al.,1990).Our results are in accordance with that of Soliman(2006) and Patel(2014) as the primitive acini of developing of submandibular salivary gland are formed as compact bulges of stratified epithelium. In addition, Klein (2002) augment the fact that the developing gland either duct or acini are surrounded by continuous basal lamina. The present investigation revealed highly mitotic activity in the cellular elements of the developed gland. This result is in accordance with that of Noden and Lahunta (1985), soliman (2006) and Teshima et al.(2015). In the present study, canalization began at th 18th day of fetal life in the duct system as also mentioned by Kwon and larsen (2015).While Soliman (2006) noticed canalized ducts at the 17th day of fetal life. There is ageneral agreement that canalization of the developing gland parenchyma is caused by apoptosis of the centrally located cells (Teshima et al., 2015).In the present work , the gland stroma differentiated from the mesenchymal tissue surrounding the develoing parenchyma as also mentionedby Soliman (2006).Later on , the mesenchymal tissue became condensed and changed gradually to fibrous tissue with many fibroblasts (Melnick et al.,2001).At 22 days-old rabbit embryos, the developing gland was surrounded by prominent continuous fibrous capsule as mentioned by Soliman (2006).In agreement with our study, the developing gland increased progressively in size and number of alveoli due to progressive branching of the cord and formation of new alveoli (Noden and Lahunta,1985 ; Soliman, 2006).At the day 25, mostly of alveoli became canalized and lined by pyramidal cells as also recorded by Soliman (2006).The myoepithelial cells could be firstly recognized at the 25th day of pregnancy as flattened curved cells with elongated nuclei. The cells surrounded the secretory acini and increased in number toward the full-term as mentioned by Sivakumar et al.,(2003) and Patel and Hoffman (2014).On the other hand Soliman(2006) recorded this myoepithelial cells

at the 29th day. In the opinion of most of histologists , the myoepithelial cells have a contractile function leading to evacuation of alveolar lumen in to the ducts (Sivakumar et al., 2003; Soliman et al., 2006 and Teshima et al., 2015).

References

AbuzaidS.M.,Osman A.K.,ELNahlaS.M.,&Nada M.S (1990).Prenatal development of the mandibular salivary gland of the one-humped camel.Egyptian journal of anatomy.

Adnyane I.K.M., Zuki A.B., Noordin M.M. and Agungpriyono, S (2010).Histological study of the parotid and mandibular glands of barking deer (Muntiacusmuntjak) with special reference to the distribution of carbohydrate content. Anat. Histol. Embryol., 39: 516–520.

Aman DS.andOpinder S (2017).Prenatal and neonatal development of mandibular salivary gland of Indian buffalo. Journal of applied animal research.45:373-383.

Bancroft JD, Gamble M (2008).Theory and practice of histological techniques.6th ed., North Hollywood, USA.

Bath-Balogh and Fehrenbach.(2011).Illustarted dental embryology,histology& anatomy.page135.

Culter LS. And Mooradiam BA.(1987). Lumen formation during development of the rat submandibular gland. Journal of dental research.66(10):1559-1562.

Emami B., Lyman J., Brown A., Coia L.,Goitein M., Munzenrider J.E., et al.(1991).Toleranceof normal tissue to therapeutic irradiation. IntJ RadiatOncolBiol Phys.21:109–22.

Hsu J.Ch., Yamada K.M.(2010).Salivary Gland BranchingMorphogenesis — Recent Progress and Future Opportunities , Int J Oral Sci , 2(3): 117–126.

Jaskoll T., Zhou Y-M., Chai Y., Makarenkova H.P., Collinson J.M., West J.D., Hajihosseini M.K., Lee J., Melnick M (2002). Embryonic submandibular gland morphogenesis: stage-specific protein localization of FGFs, BMPs, Pax 6 and Pax 9 and abnormal SMG phenotypes in Fgf/R2- IIIc, BMP7 and Pax6 mice. Cells, Tissues, Organs, 270:83-98.

Jaskoll T., Melnick M (1999).Submandibular gland morphogenesis: stage-specific expression of TG Falpha, EGF, IGF, TGF-beta, TNF and IL-6 signal transduction in normal mice and the phenotypic effects of TGF-beta2, TGF-beta3, and EGF-R null mutations. Anat. Rec. 256:252-268.

Klein R.M (2002). Development, structure, and function of the salivary glands. In : Avery JK, Steele PF, Avery N, eds. Oral development and histology (3rd ed.), New York, Thieme, pp.292-330.

Kleinman H.K., Philp D., Hoffman M.P (2003).Role of the extracellular matrix in morphogenesis.CurrOpinBiotechnol14 : 526-532.

Knospe C and Bohme G (1995).Prenatal development of the mandibular gland and parotid gland in cats.AnatHistolEmbryol. 24:1–6.

Kwon H.R. and Larsen M (2015).The contribution of specific cell subpopulations to submandibular salivary gland branching morphogenesis.CurrOpin Genet Dev. 32: 47–54.

Melnick M., Chen H., Zhou Y-M., Jaskoll T (2001).Embryonic mouse submandibular salivary gland morphogenesis and the TNF/TNF-R1 signal transduction pathway. Anat. Rec. 262:318-320.

Noden D.M. and Lahunta A (1985). The embryology of domestic animals ,developmental mechanisms and malformations.

Patel V.N. and Hoffman M.P (2014). Salivary gland development: a template for regeneration. Semin Cell Dev Biol.25-26:52–60.

Pospienzy N., KurysizkoJ. ,Juszczyk M. and Adamski M (2010).Morphological and

histological analysis of the mandibular gland and sublingual glands in prenatal period of the pig.

Rothova M., Thompson H., Lickert H. and Tucker A. S (2012). Lineage tracing of the endoderm during oral development. Dev. Dyn. 241, 1183-1191.

Sivakumar M.,Suden M. and Vathsala V (2003). Histogenesis and morphometric study of human fetal submandibular salivary gland.J.Anat.soc.India 52(1) 3-6.

SolimanSh.M (2006).Histogenesis of mandibular salivary gland in New-Zealand white rabbits, Minufiya Vet.J.vol.4 No.2.

Teshima T.H, Wells K.L, Lourenço S.V, Tucker A.S (2015). Apoptosis in Early Salivary Gland Duct Morphogenesis and Lumen Formation. J Dent Res 95 (3), 277-283.

Tucker A.S (2007).Salivary gland development.Semin Cell DevBiol18 : 237-244.

Velasco J.A., Montesinos I., Espin J., Garcia J.D., Gomez S., Roldan V (1993). Development of the human submandibular salivary gland. J Dent Res. 72:12–27.

Williams P. L., Warwick R., Dyson M., Bannister L. H (1989).37th ed. Churchill Livingstone; Edinburgh.Gray's Anatomy; pp. 1290–1298.