Journal homepage:
http://www.bsu.edu.eg/bsujournals/JVMR.aspx

Online ISSN: 2357-0520 Print ISSN: 2357-0512

Original Research Article

Prenatal development of submandibular salivary gland of New-Zealand rabbits

Shehata M.M. Soliman, Khalid M. Mazher, Shimaa M. Essam*, Taghreed M. Nabil

Department of Histology, Faculty of Veterinary Medicine, Beni-Suef University, BeniSuef 62511, Egypt.

ABSTRACT

The paired submandibular gland plays a critical role in maintaining normal oral biology through the lubricating and antimicrobial actions of produced saliva. In this study, the developmental stages of the submandibular salivary gland of the New Zealand rabbits were investigated. Twenty New-Zealand rabbit fetuses of 11-30 days old were used. Samples of the head region and submandibular salivary glands were histologically stained and examined under a light microscope. Results revealed that the submandibular primordia appeared as bilateral invaginated epithelial buds from the linguo-gingival groove on the 12th day of prenatal life. By the 15th day, the buds deeply grow to form a cord-like structure that ends with compact bulges forming the future primitive acini. The formed cords are then branching to develop the primary glandular ducts by the 17th day, which are canalized on the 18th day. The primitive gland capsule was observed by the 22nd day. The lobulation was recognized and became well developed by the 25th day. Similetanouly, the glandular duct system is completely developed, and serous adenomeres fully occupied the parenchyma and are surrounded by myoepithelial cells. The full-term fetuses have fully developed submandibular glands with a typically compound tubulo-acinar nature and parenchymal seromucoid adenomeres. The PAS staining revealed a strong positive reaction in the striated ducts; however, weak PAS reactions were noticed in the cytoplasm of the acinar cells

ARTICLE INFO

Received 11/2018
Accepted 12/2018
Online 12/ 2018

Keywords:

salivary gland primordia of rabbit,

mandibular gland of rabbit,

primordia of mandibular salivary gland

***Corresponding author: e-mail:** sh.essam2009@yahoo.com

1. Introduction

The maintenance of normal oral biology is achieved by the saliva lubricating and antimicrobial actions **(Hsu and Yamda, 2010).** Additionally, food digestion by substantial saliva produced is an essential function of the major salivary glands. The paired submandibular gland is one of these major salivary glands **(Adnyane et al., 2010).** Studies reported that the submandibular and sublingual are endodermal in origin **(Emami et al., 1991).** However, the major salivary gland, including

the submandibular gland, is primarily ectodermal due to its interaction with neural crest-derived mesenchyme **(Jaskoll et al.,2002; Rothova et al.,2012).**

Prenatally, the development of major salivary glands starts with the submandibular gland, followed by sublingual and parotid glands **(Tucker, 2007).** The prenatal developmental stages of the submandibular gland were investigated in different animals, including the rats **(Culter and Moordian, 1987),** mice **(Jasckoll and Melnick, 1999),** pigs **(Pospienzy et al., 2010),** cats **(Knospe and Bohme, 1995),** buffalos **(Amanand Opinder, 2017)** and human **(Velasco et al., 1993).** However, little literature reported their development in rabbits. Therefore, the present study was undertaken with an aim to highlight the prenatal developmental stages of New-Zealand rabbits.

2. Materials and methods

Total number of 20 normal and healthy New-Zealand rabbit fetuses ranging from 11-30 days of gestation were used in this study. The head regions of fetuses (11-20 days of gestation), and the dissected glands of the fetuses (21–30 days old fetuses) were obtained, fixed in 10% neutral formalin, Suza, and Helley's fluid, processed and embedded in paraffin blocks. Cross and/or sagittal step serial 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 submandibular salivary gland's primordia were first recognized at 12 days-old New Zealand rabbit embryos as bilateral solid epithelial buds invaginated from the linguogingival 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 primordial cells showed mitotic activity (Fig.2). On the13th day of the rabbit embryo, the submandibular buds grew deeply throughout underlying mesenchymal tissue forming solid epithelial cords with closely packed cellular masses of ill-distinct 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 was surrounded by a 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 divisions. 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 on the 18th day of 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 capsules 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 a distinct increase in lobulation and

vascularity. The capsule became well-developed formed 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).

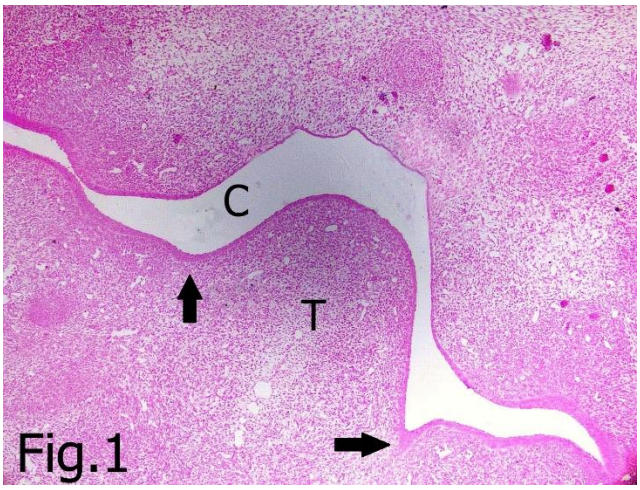

Fig.1

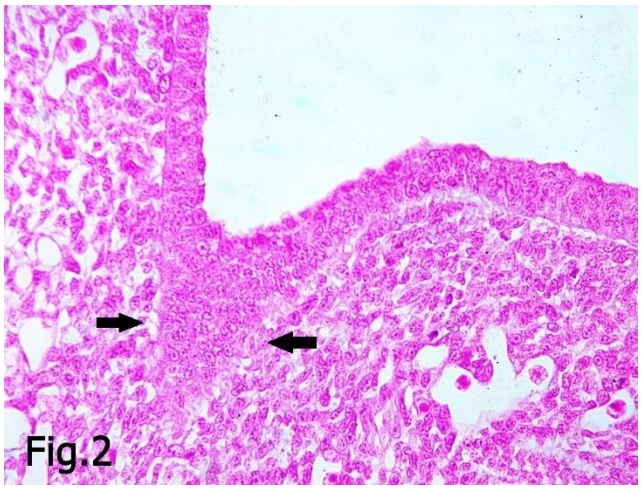
Fig.2

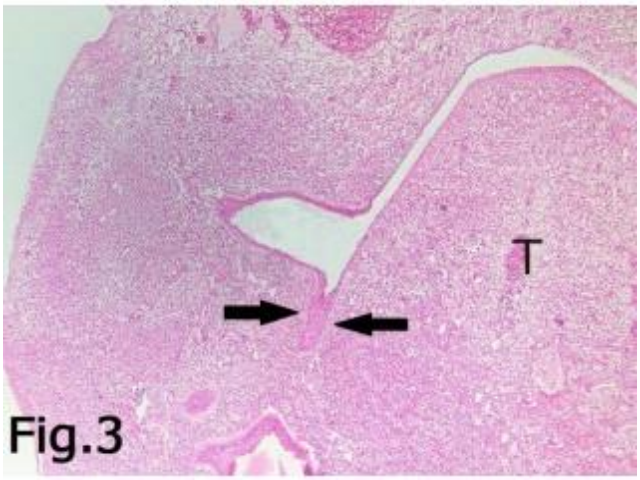

Fig.3

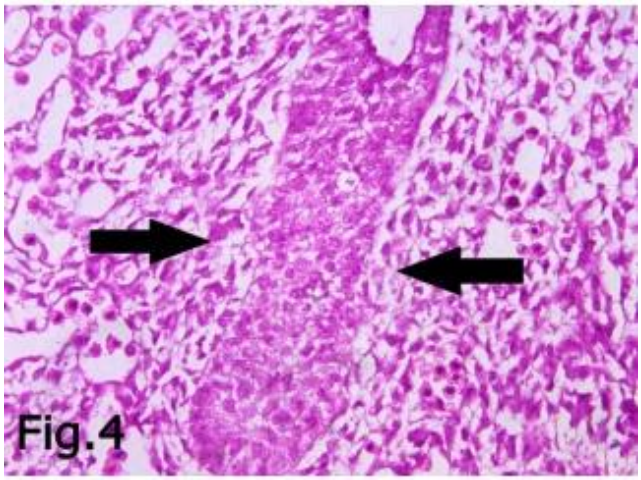
Fig.4

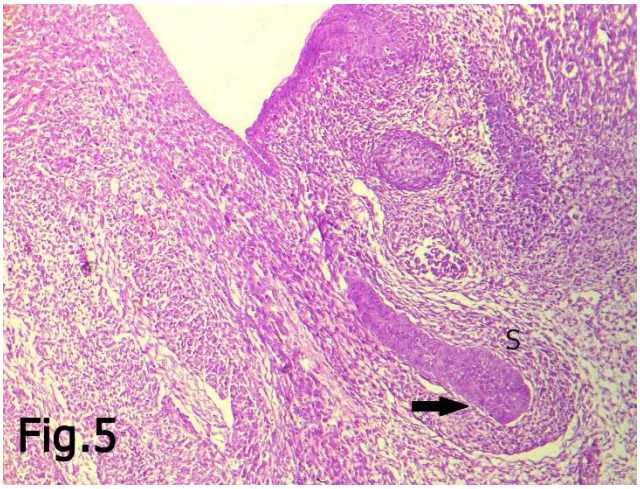

Fig.5

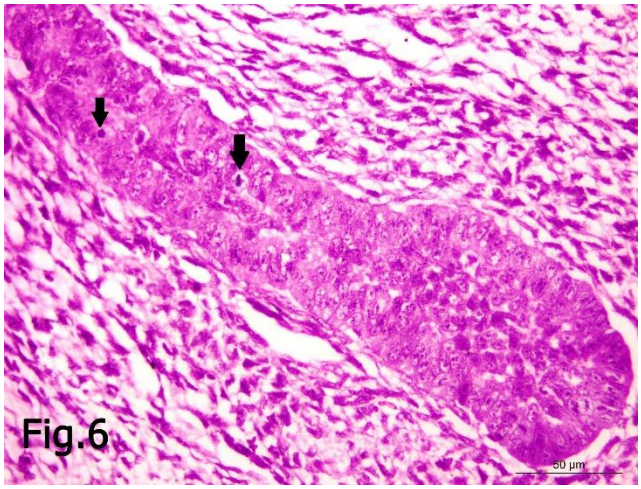
Fig.6

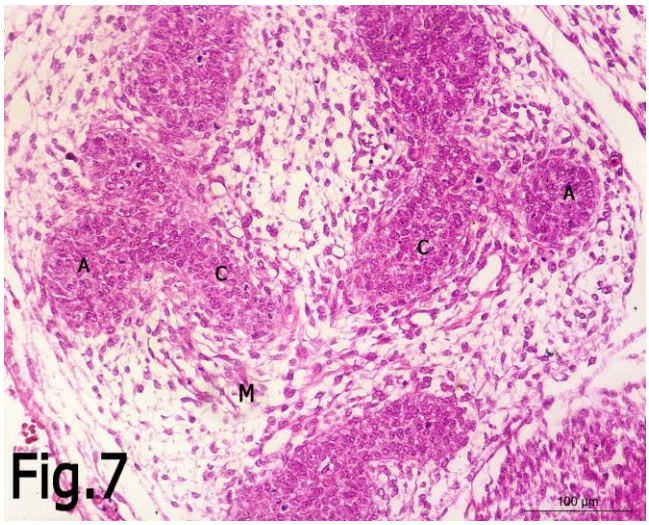

Fig.7

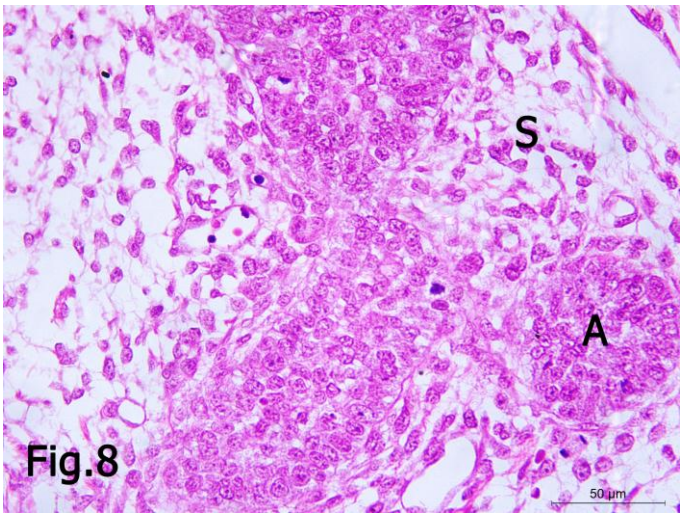

Fig.8

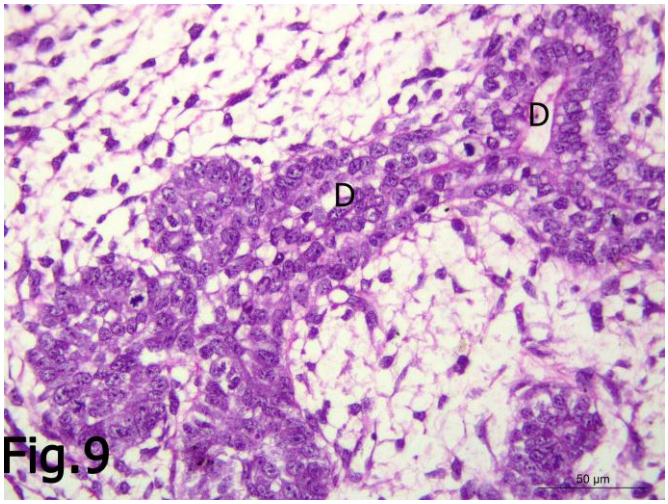

Fig.9

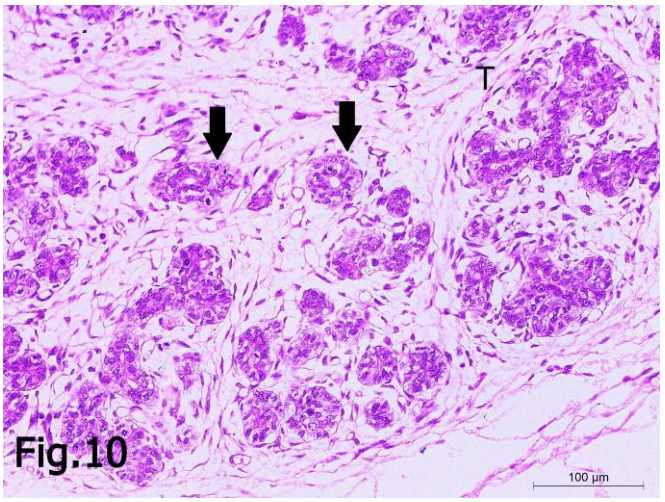

Fig.10

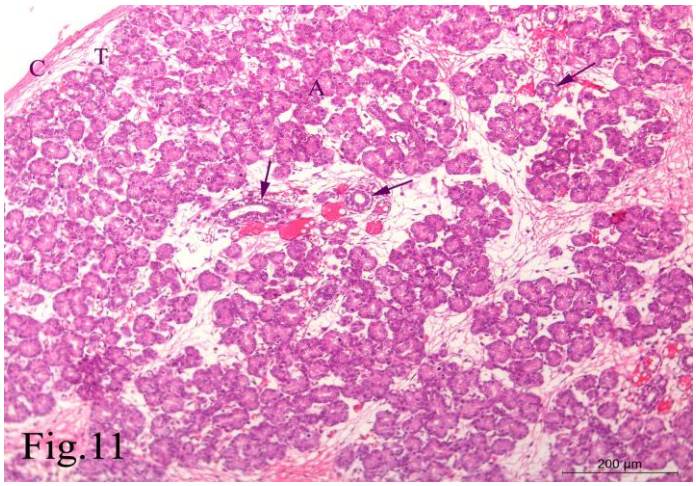

Fig.11

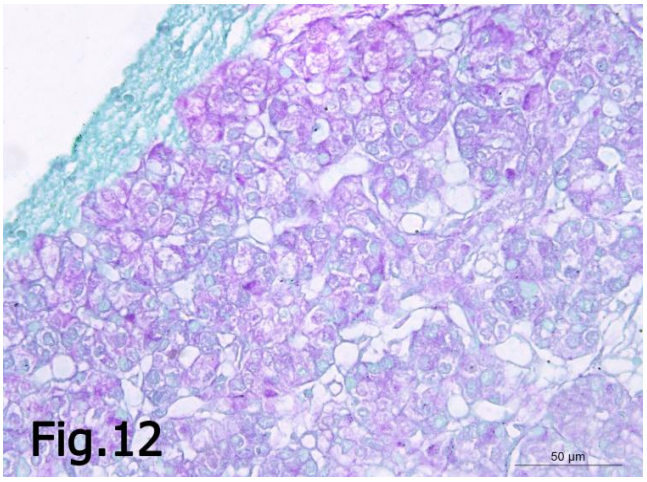

Fig.12

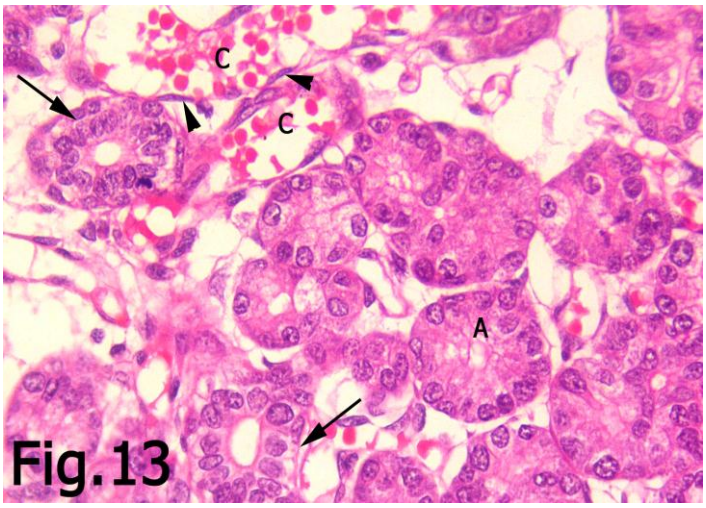

Fig.13

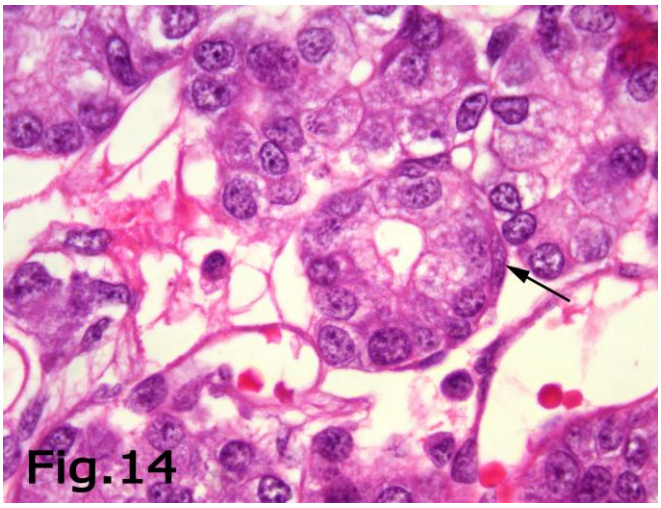
Fig.14

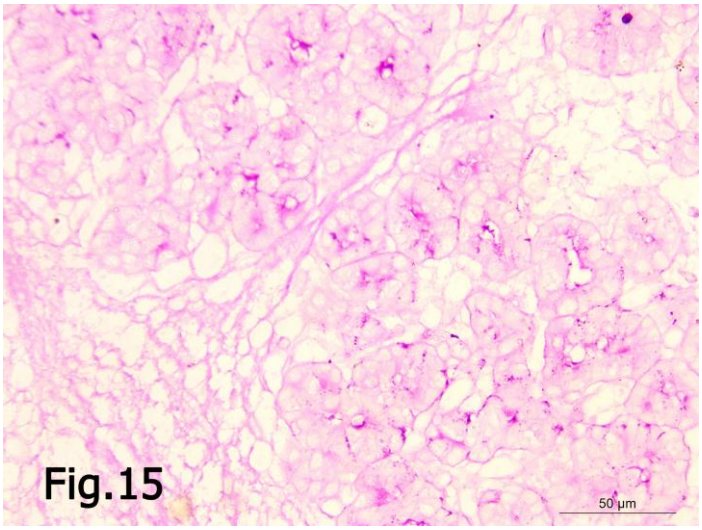

Fig.15

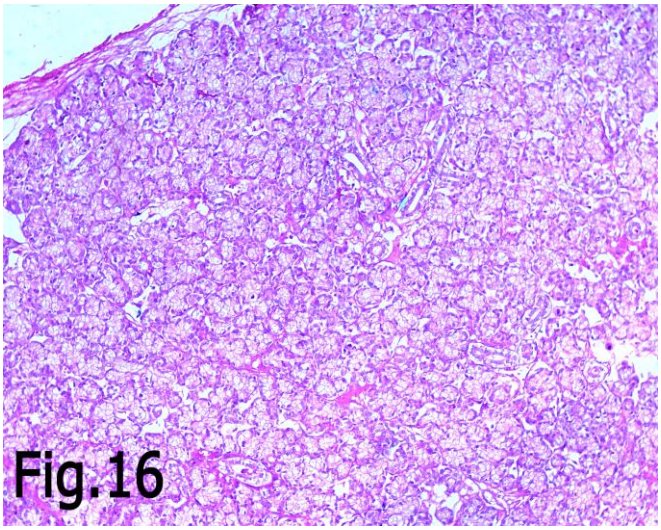
Fig.16

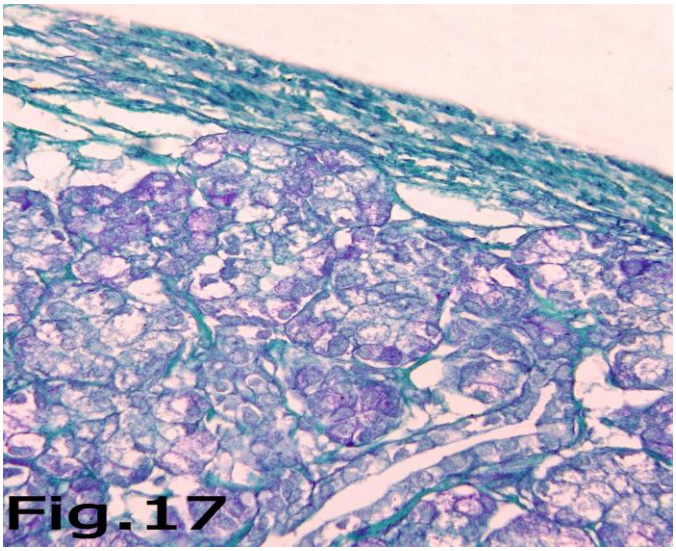
Fig.17

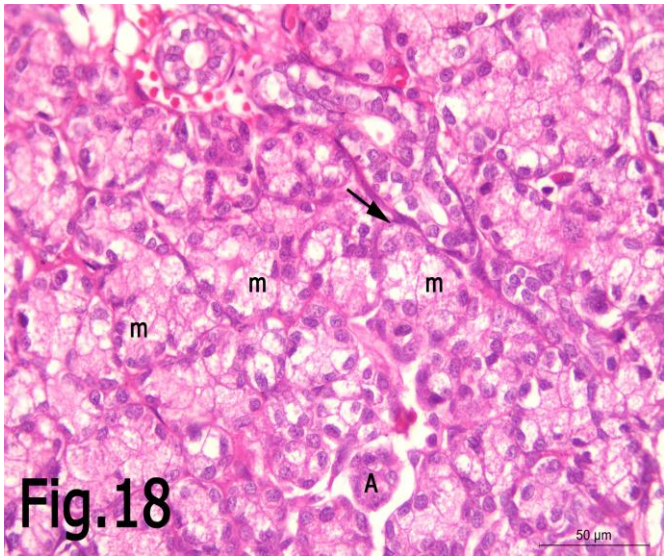

Fig.18

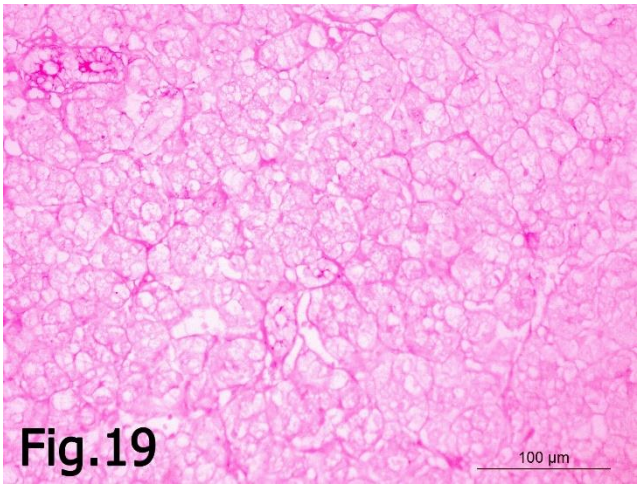

Figure legends

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

Fig. 2. 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 sagittal 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. Higher magnification of figure. 3 showing the epithelial cords formed of clusters of closely packed cellular masses with ill-distinct cell boundaries (arrows). H&E, X 400.

Fig. 5. A photomicrograph of the head region of the 15th day of rabbit embryo showing deep down growth of developing cords with terminal bulges (arrow) surrounded by primitive stroma (S). H&E, X 100.

Fig. 6. 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. A photomicragh 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. 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. Notice, illuminized developing acini (A) surrounded by primitive stroma (S). H&E, X 400.

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

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

Fig.11. A photomicrograph of 25 days-old rabbit embryo submandibular salivary gland showing a well-developed capsule (C), trabeculae (T), distinct parenchymal lobulation occupied by glandular adenomeres (A), andwell-developed duct system (arrows). H&E, X200.

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

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

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

Fig.15. A photomicrograph of 25 days-old rabbit embryo showing PAS-positive reaction in the fibrous stroma and alveolar basal lamina of the submandibular salivary gland while the cells of both ducts and acini revealed a weak reaction. PAS stain, X 400.

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

Fig.17. A photomicrograph of full-term rabbit embryo submandibular salivary gland showing well-developed fibrous capsule and septa. Masson's trichrome stain, X 400.

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

Fig. 19. A photomicrograph of full-term rabbit embryo submandibular salivary gland showing strong PAS-positive reaction in the duct system and weak reaction in the cytoplasm of acinar cells. PASstain, X 400. (arrows) from the surface epithelium on either side of the primitive tongue (T). PAS stain, X 400.

4. Discussion

In the current study, the submandibular salivary gland primordia arise as an epithelial bud formed of epithelial cell clusters. These findings further supporting the previously known morphological development of the submandibular salivary gland 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 submandibular salivary gland's primordia development began as early as 12 days in the New Zealand rabbit embryos. The development on the 12th day is similar to that observed in rats (Kleinman, 2003). However, Soliman (2006) first recognized the submandibular primordia development in rabbits by the 13th day of prenatal life. Compared to mice, the submandibular primordia development could be seen at an earlier age, "11.5th days" (Tucker, 2007).

The epithelial buds progressively developed with underlying mesenchyme and branch, as previously reported by Melnick et al. (2001). The epithelial buds initially grow deeply down through the underlying mesenchymal tissue to form cord-like structures that branched off as the glandular primitive ducts and acini (Bath-Balogh & Fehrenbach, 2011; Abuzaid etal., 1990). The present study results are inaccordance with the previous reports indicating the formation of compact bulges of stratified epithelium that develop to the primitive acini ofthe submandibular salivary gland (Soliman,2006; Patel, 2014).

additionally, Klein (2002) augments that a continuous basal lamina primarily surrounds the developing ducts or acini of the submandibular salivary gland. We observed a highly mitotic activity in the developed gland's cellular elements that indicate a proceeding development process (Noden and Lahunta, 1985; Soliman, 2006) and (Teshima et al., 2015).

In this study, the canalization of the glandular duct system began on the 18th day of prenatal life (Kwon and Larsen, 2015). In contrast, Soliman (2006) reported the canalization of the ducts at earlier stagy by the 17th day. The developing salivary gland parenchymal duct canalization occurred due to the centrally located cell apoptosis (Teshima et al., 2015).

The rabbits developing gland parenchyma surrounded by mesenchymal tissue from which the glandular stroma was differentiated (Soliman, 2006). Upon development proceeding, the mesenchymal tissue underwent condensing and gradually shifted to a fibrous tissue containing many fibroblasts (Melnick et al.,2001). The developing gland became then surrounded by a prominent, continuous fibrous capsule by the 22nd day of the rabbit embryo's prenatal life (Soliman, 2006). The sizes and the numbers of the secretory alveoli increase. Subsequently, the developing gland size increased gradually due to the cord's progressive branching and new alveoli formation (Noden and Lahunta, 1985; Soliman, 2006).

By day 25 of prenatal life, the developed alveoli are canalized and lined by pyramidal cells as previously recorded (Soliman, 2006). The myoepithelial cells were first observed as flattened curved cells with elongated nuclei on the 25th day of pregnancy. These cells surrounded the secretory acini, and their numbers increased gradually toward the full-term as previously reported by Sivakumar et al., (2003) and Patel and Hoffman (2014).

Conversely, late development of the myoepithelial cells was recorded on the 29th day of the embryonic rabbit life (Soliman, 2006). Most histological studies showed that the myoepithelial cells have a contractile function that evacuates the saliva in the alveolar lumen into the alveolar ducts (Sivakumar et al., 2003; Soliman et al., 2006 and Teshima et al., 2015).

References

Abuzaid, S.M., Osman A.K., ELNahla, S.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, TGFbeta, 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.

Soliman Sh.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.