Original Research Article

Prenatal development of submandibular salivary gland of New-Zealand rabbits

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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. Simileanously, 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.

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1. Introduction

The maintenance of normal oral biology is achieved by the saliva lubricating and antimicrobial actions (Hsu and Yama, 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 the 13th 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 illuminated (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 by high 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).
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 photomicrograph 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, illuminised developing acini (A) surrounded by primitive stroma (S). H&E, X 400.

**Fig. 9.** A photomicrograph of 18 days-old rabbit embryo submandibular salivary gland showing the 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), and well-developed duct system (arrows). H&E, X 200.

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. PAS stain, 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 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 et al., 1990). The present study results are in accordance with the previous reports indicating the formation of compact bulges of stratified epithelium that develop to the primitive acini of the 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 stage 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 acini 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).

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