**Research Article**

# HISTOLOGICAL EVALUATION OF THE RAT CRANIAL REGION

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## Abstract

**Aim**: This study was to ascertain the histological evaluation of some organs in the cranial region of newborn *Wistar albino* rats, such as the tongue, palate, and Steno's gland in the nasal cavity, compared to adult rats.

**Method**: 5 healthy female newborn *Wistar albino* rats were used in the study. Tissues obtained from rats were fixed in 10% formalin for 2 days. The histological features of the tongue, palate and nasal cavity were revealed after Hematoxylin&eosin and Mallory's Azan stainings.

**Findings**: While there was fatty tissue in the submucosa layer in the ventral section of the soft palate, mucus glands were observed in the dorsal section. The main papillae were filiform and fungiform, and scattered foliate and circumvallate papillae were also present. The lateral nasal glands (Steno's glands) were well developed. In general, there were four ethmoturbinates in coronal and sagittal sections in the posterosuperior part of the unilateral sinus in adults, whereas there were two in neonatal rats. Unusually, a septal window was visible immediately rostral to the nasopharynx in the mouse, however, no septal window was observed in newborn rats.

**Results**: This study reports basic research features on the anatomy of the oral and nasal cavity of the newborn *Wistar albino* rat. Our data may shed light on other studies aimed at fully investigating the structure of these organs, which may be useful in subsequent experimental and morphological studies on newborn *Wistar albino* rats.

**Keywords:** Cranial region, Steno's gland, Rat

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# *Sıçan Kranial Bölgesinin Histolojik Olarak Değerlendirilmesi*

**Öz**

**Amaç**: Bu çalışma, neonatal *Wistar albino* sıçanların kranial bölgesindeki dil, damak ve burun boşluğundaki Steno bezi gibi bazı organların yetişkin sıçanlarla karşılaştırıldığında histolojik olarak değerlendirilmesini belirlemek amacıyla yapıldı.

**Yöntem**: Çalışmada 5 adet sağlıklı dişi yenidoğan *Wistar albino* sıçan kullanıldı. Sıçanlardan elde edilen numuneler %10'luk formalinde iki gün süreyle fikse edildi. Hematoksilen&eozin ve Mallary Azan boyama sonrasında dil, damak ve burun boşluğunun histolojik özellikleri ortaya çıkarıldı.

**Bulgular:** Yumuşak damağın ön kısmındaki submukoza tabakasında yağ dokusu bulunurken, arka kısmında mukus bezleri gözlendi. Ana papillalar filiform ve fungiform olup, dağınık foliat ve sirkumvallat papillalar da mevcuttu. Yan burun bezleri (Steno bezleri) iyi gelişmişti. Genel olarak yetişkinlerde tek taraflı sinüsün posterosuperior kısmında koronal ve sagittal kesitlerde dört adet etmoturbinat bulunurken, neonatal sıçanlarda iki tane etmoturbinat vardı. İlginç bir şekilde, farede nazofarenksin hemen rostralinde bir septal pencere mevcuttu, ancak yeni doğan sıçanlarda septal pencere görülmedi.

**Sonuç**: Bu çalışma, yeni doğmuş *Wistar albino* sıçanlarının ağız ve burun boşluğunun anatomisine ilişkin temel araştırma özelliklerini rapor etmektedir. Verilerimiz, bu organların yapısını tam olarak incelemeyi amaçlayan diğer çalışmalara ışık tutabilir ve yeni doğmuş *Wistar albino* sıçanlar üzerinde yapılacak daha sonraki deneysel ve morfolojik çalışmalarda faydalı olabilir.

**Anahtar Kelimeler:** Kranial bölge, Steno bezi, Sıçan

## 1. INTRODUCTION

Rats and mice are generally preferred in biomedical research animal models. The main reasons for its preference are that it resembles to humans anatomically, genetically and physiologically. Their short reproductive cycles and ability to survive even in the most adverse conditions provide unmatched convenience in experimental studies. Rats are also preferred in the experimental of some disease models, toxic or treatment effects of drugs, cancer studies, congenital anomalies, etc. (Wang et al., 2020). Histopathological studies are important in this type of research. Successful evaluation of histopathological and congenital anomaly studies depends on a good knowledge of the histological structure of the studied tissue (Charest et al., 2018). Considering that studies are carried out on experimental animals to reflect the structure of the human body, it becomes necessary to compare and estimate data regarding the formal qualities of animal and human organs. The most important element of conducting experimental research depends on the knowledge of the morphology of the unchanging organ.

The oral cavity (cavitas oris) is located at the beginning of the digestive tract. It extends from the outer lips and cheeks to the entrance of the oropharynx. The oral cavity includes the mouth, tongue, teeth, structures that support the teeth, salivary glands, uvula, palate, and tonsils. The oral cavity is divided into two; the vestibule (vestibulum oris) between the outside of the teeth and the lips and cheeks, and the true oral cavity (cavitas oris propria) behind the teeth. There are throat arches, between which the palatine tonsil is located in the passage between the mouth and pharynx (Abumandour, 2018; Madkour et al., 2021).

The palate is divided into two parts as hard (immobile) and soft (movable) (Madkour et al., 2021). The soft palate of the *Wistar albino* rat contains three types of keratinized stratified squamous epithelium. There is a rough epithelium with mushroom-shaped papillae and taste buds in a central region; it has a flat epithelium type on both sides and this epithelium is surrounded by an intermediate type. Deep within this epithelium, in the lamina propria containing loose connective tissue, is an elastic membrane that is thickest laterally and thinnest in the midline. A thick layer of mucous glands organizes the fundamental structure of the palate and contains the classical mucous acini, which flow through the ducts into the oral cavity. Deeply there is a dense collagen layer, the palatal aponeurosis, which is separated from the pseudostratified ciliary columnar epithelium of the nasal surface by another elastic lamina. Striated muscle is absent, except for the nasopharyngeal space, where a circular sphincter is visible (Obead et al., 2022).

The tongue is divided into three compartments; the apex, corpus, and radix (Akbari et al., 2018). The tongue is histologically composed of a central striated muscle and an epithelial covering that borders it from the outside. It is lined by keratinized squamous epithelium. It contains lingual papillae unlike the rostral area which is non-keratinized and does not contain papillae. The lamina propria is an intensive fibrous connective tissue containing in large quantities elastic fibers fused into similar tissue that spans between the lingual muscle fascicles. It includes numerous vessels and nerves that supply the papillae, as well as large lymphatic plexuses and lingual glands. The lingual mucosa of the lower surface is thin and smooth, as in most of the oral cavity. The mucosa of the tongue close to the pharynx has numerous lymphoid follicles. Several lingual glands, located between muscle bundles running in different directions, were remarkable.

This study was to survey this region histologically to guide researchers who will conduct experimental studies on the head region of newborn rats. While there are studies on the rat Steno's gland and other cranial region structures, the rarity of studies examining the changes in the region from the neonatal period to the adulthood has led to a more detailed examination of the subject. For these reasons, we determined structures such as the palate, tongue, nasal cavity and Steno's gland in the cranial region of the newborn rat and provided information about their histological appearances. The selected images may help to observe major structures at the basic developmental stages and to compare the normal morphology.

## 2. METHODS

The animals were obtained from the Sivas Cumhuriyet University Medical Faculty Experimental Animal Research Laboratory. Five healthy female newborn *Wistar albino* were included in the study. In rats, gender discrimination is based on the size of the genital papilla and the anogenital gap. Gender discrimination in newborn rats was made by experts under stereomicroscope looking at the anogenital gap. The rats (20.5 ± 2.8 g, mean weight ± SEM) were fed with ad libitum on a 12-h periodic cycle and at room temperature of 24°C. 7-day-old neonatal rats were then euthanized with an i.p. injection of 200 mg/kg sodium pentobarbital. Samples were fixed in 10% formalin for 2 days (Develioglu et al., 2006). All of the tissues were paraffin embedded and were sectioned 3-5 µm taken by a rotary microtome (Leica RM 2125RT, Germany) for the Hematoxylin (Bio-Optica, catalog no: 05-M06007)&Eosin (Bio-Optica, catalog no:05-M10002) (H&E) and Mallory-Azan (M-A) stainings. Photographs from the convenient fields of view were taken using an Olympus BX51 (Tokyo, Japan).

The experimental stages were ratified by the Sivas-Cumhuriyet University Animal Ethics Committee (approval No: 65202830-050.04.04-75). This study complied with Directive 2010/63/EU on the care, use and protection of animals used in scientific studies and the ethical standards of Low animal welfare No. 41/2009.

## 3. RESULTS

### 3.1. Palate

The soft palate consisted of mucosa covering the connective tissue, mucous glands, and thin skeletal muscle. It was lined with variably keratinized squamous epithelium. In the ventral part of the soft palate, adipose tissue was observed in the submucosa layer (Figure 1), while there were mucous glands in the posterior part (Figure 2a,b; Figure3a-d). Bone tissue formed the upper side of the hard palate (Figure 4a-c; Figure 5). The epithelium of the oral cavity was keratinized stratified squamous epithelium (Figures 1-5) and the nasal cavity was lined with respiratory epithelium, which also contained goblet cells. Numerous taste buds were observed within the epithelium (Figure 3a-d). The submucosa layer was dense connective tissue and contained a thin bone area. The oral submucosa region contained a thick collagen layer. There was a thinner and more compact layer of collagenous tissue under the nasal epithelium (Figures 4, 5). The nasopharyngeal duct structure just above the palatal region of newborn rats was also notable (Figures 2,4,5). It was determined that the epithelium lining of the nasopharyngeal duct had a simple columnar epithelium with cilia.

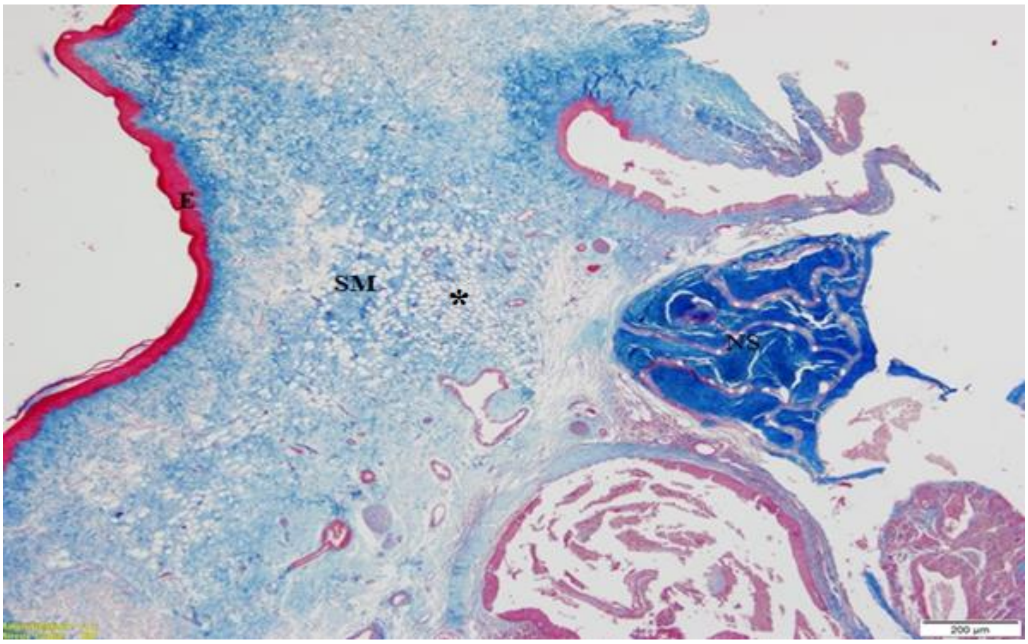


**Figure 1. Anterior soft palate region. E: Keratinized stratified squamous epithelium, SM: submucosa, (*): Adipose tissue, NS: Nasal septum. Mallory's Azan staining.**

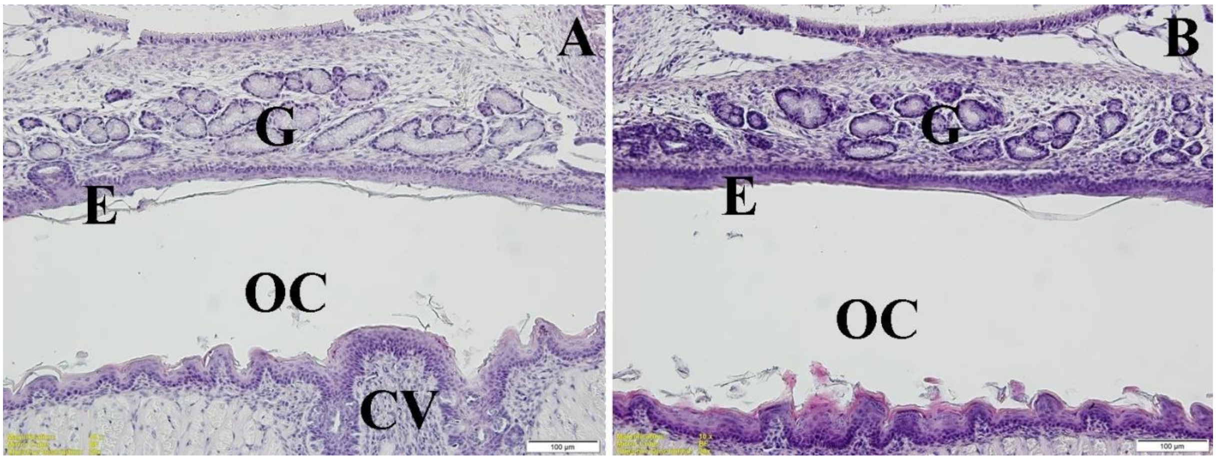


**Figure 2. Posterior soft palate region. E: Stratified squamous keratinized epithelium, G: Mucous glands, OC: Oral cavity, CV: Circumvallate papillae H-E.**

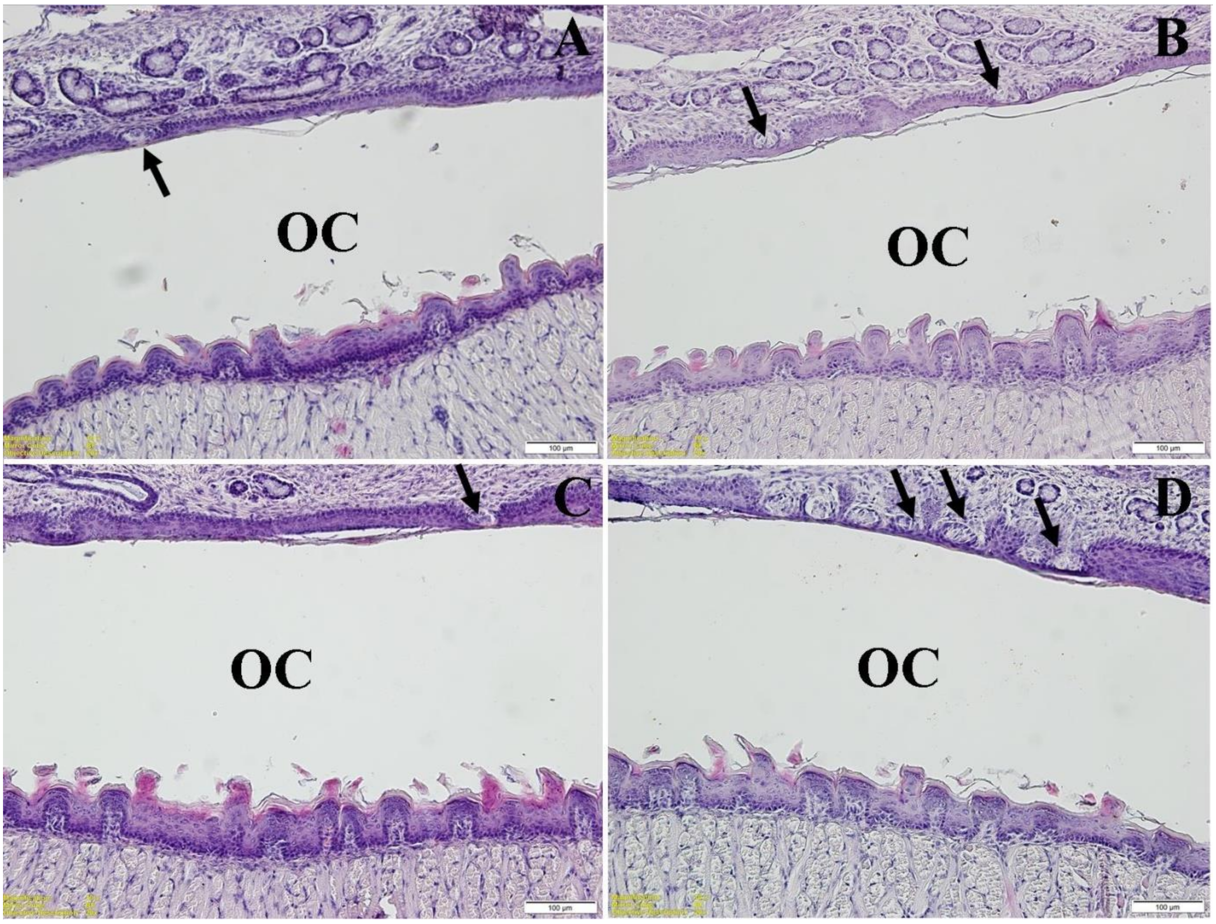


**Figure 3. Taste buds (arrows) on the soft palate. OC: Oral cavity. H-E.**

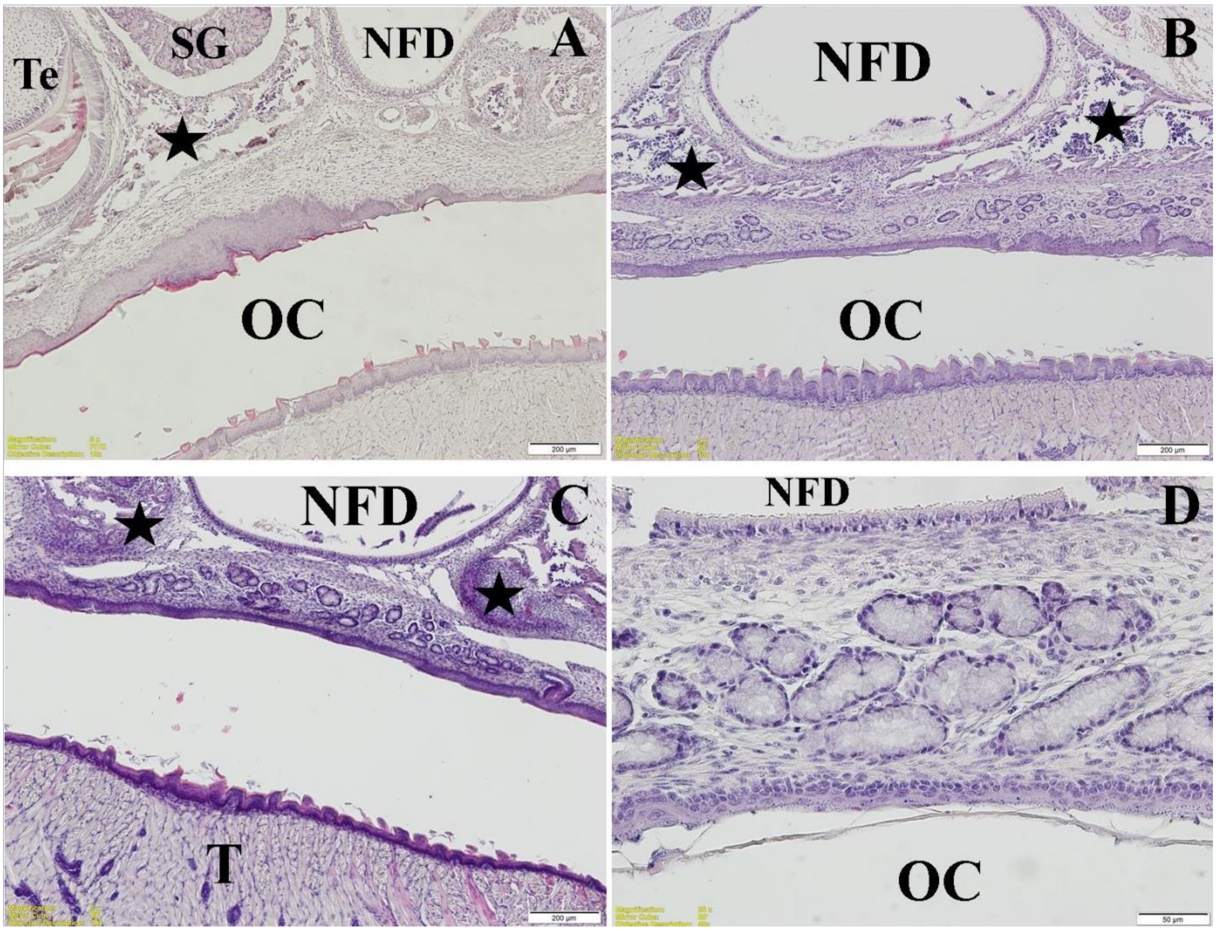


**Figure 4. The hard palate and dorsal tongue (T). OC: Oral cavity, NFD: Nasopharyngeal duct, SG: Steno's gland, Te: Teeth, (Asterisk): Bone, H-E.**

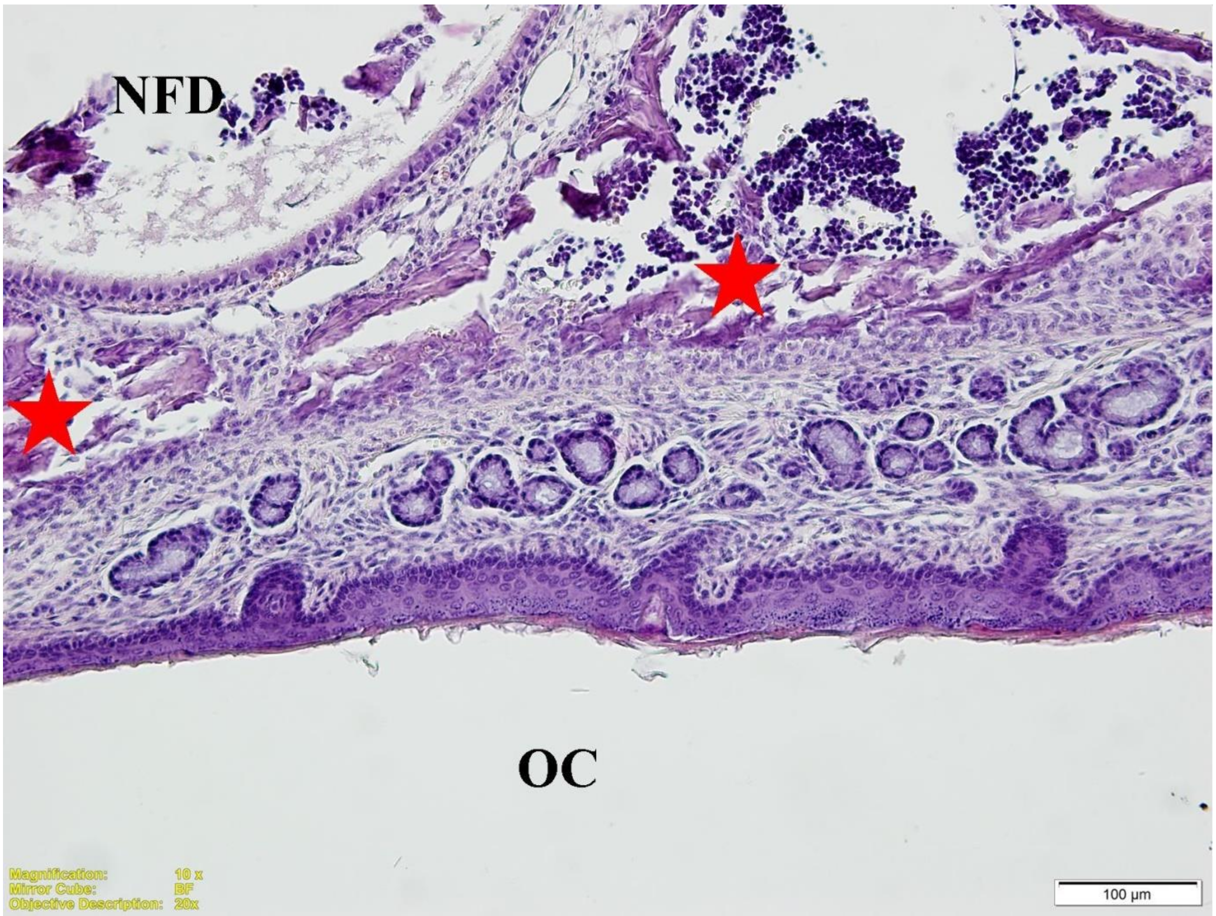


**Figure 5. Posterior hard palate region. NFD: Nasopharyngeal duct, (Red asterisk): Bone OC: Oral cavity, H-E.**

### 3.2. Tongue

The tongue contained striated muscle bundles running in different directions and the continuation of the lamina propria extending between these bundles contained loose connective tissue (Figure 6-10). There were serous and mucous glands between the muscle bundles (Figure 7a-d; Figure 10a,b). The excretory ducts of the glands were opened to the surface epithelium (Figure 7a,b). The epithelium of the dorsal surface of the tongue was keratinized stratified squamous. There were papillae on the dorsal surface. The epithelium and lamina propria folded together towards the surface to form the papillae. There were 4 types: filiform, fungiform, foliate, and circumvallate papillae (Figure 8-10). It was determined that filiform papillae at the apex of the tongue were directed towards the posterior (Figure 6b-d; Figure 8a,b). Filiform papillae were lined with keratinized stratified squamous epithelium. Fungiform papillae were scattered among the filiform papillae on this surface. Fungiform papillae were observed intensively on the dorsal surface of the tongue (Figure 8a,b). Only one circumvallate papilla (CVP) was found on the midline of the body root border (Figure 10a-d). Fungiform papillae were located in filiform papillae. The fungiform papilla was lined with keratinized stratified squamous epithelium and it did not contain keratohyaline granules. However, it was observed that the labial mucosa contained keratinized stratified squamous epithelium and showed keratohyaline granules (Figure 6d). A single taste bud was observed intraepithelial in the part of the fungiform papillae close to the epithelium (Figure 8b). A thin keratinized stratified squamous epithelium was detected on the foliate papillae. It was observed that microscopic papillae had not yet developed in the foliate papillae (Figure 9). The CVP were covered with keratinized stratified squamous epithelium. Numerous intraepithelial taste buds were seen on both surfaces of the papillar groove (Figure 10d).

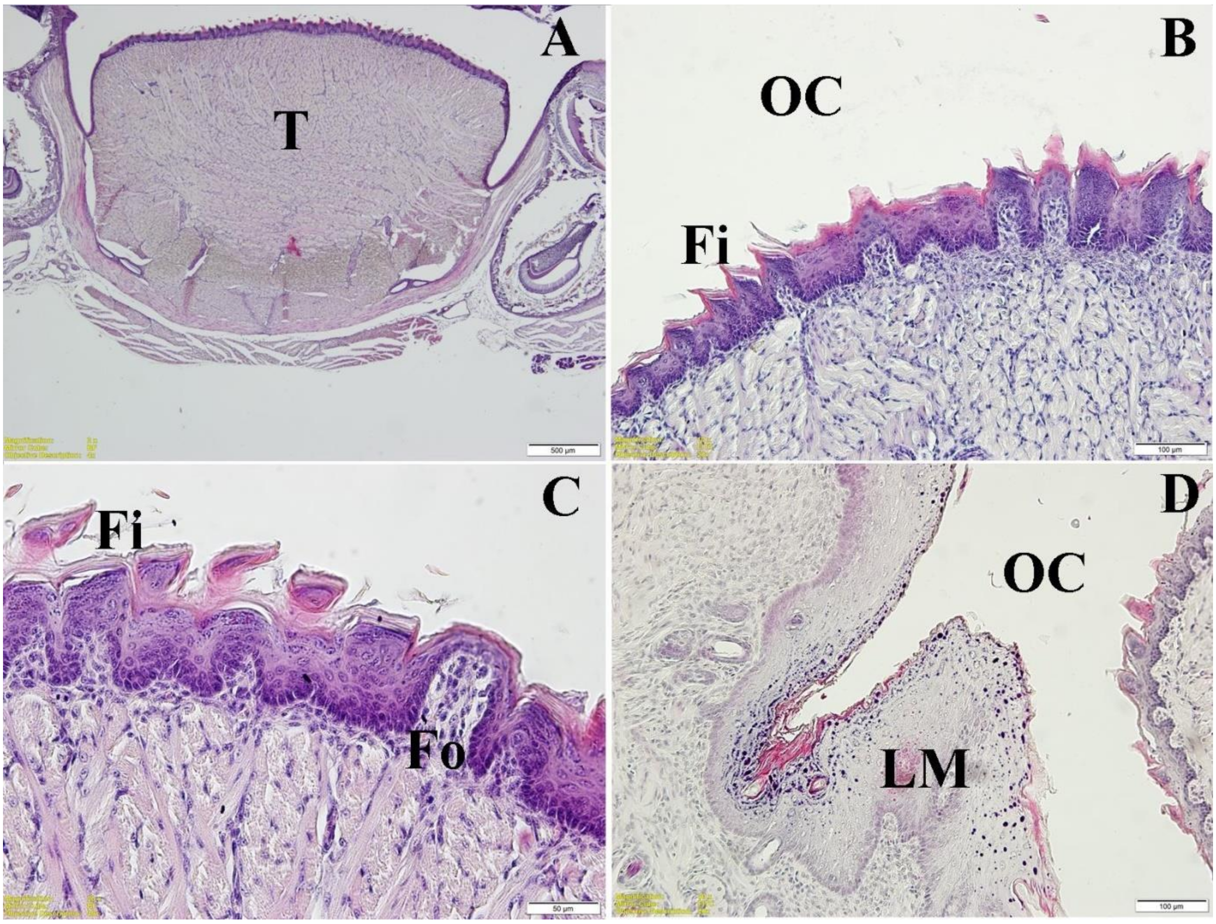


**Figure 6. New born rat tongue (T). Fi: Filiform papillae, Fo: Foliat papillae, OC: Oral cavity, LM: Labial mucosa. H-E.**

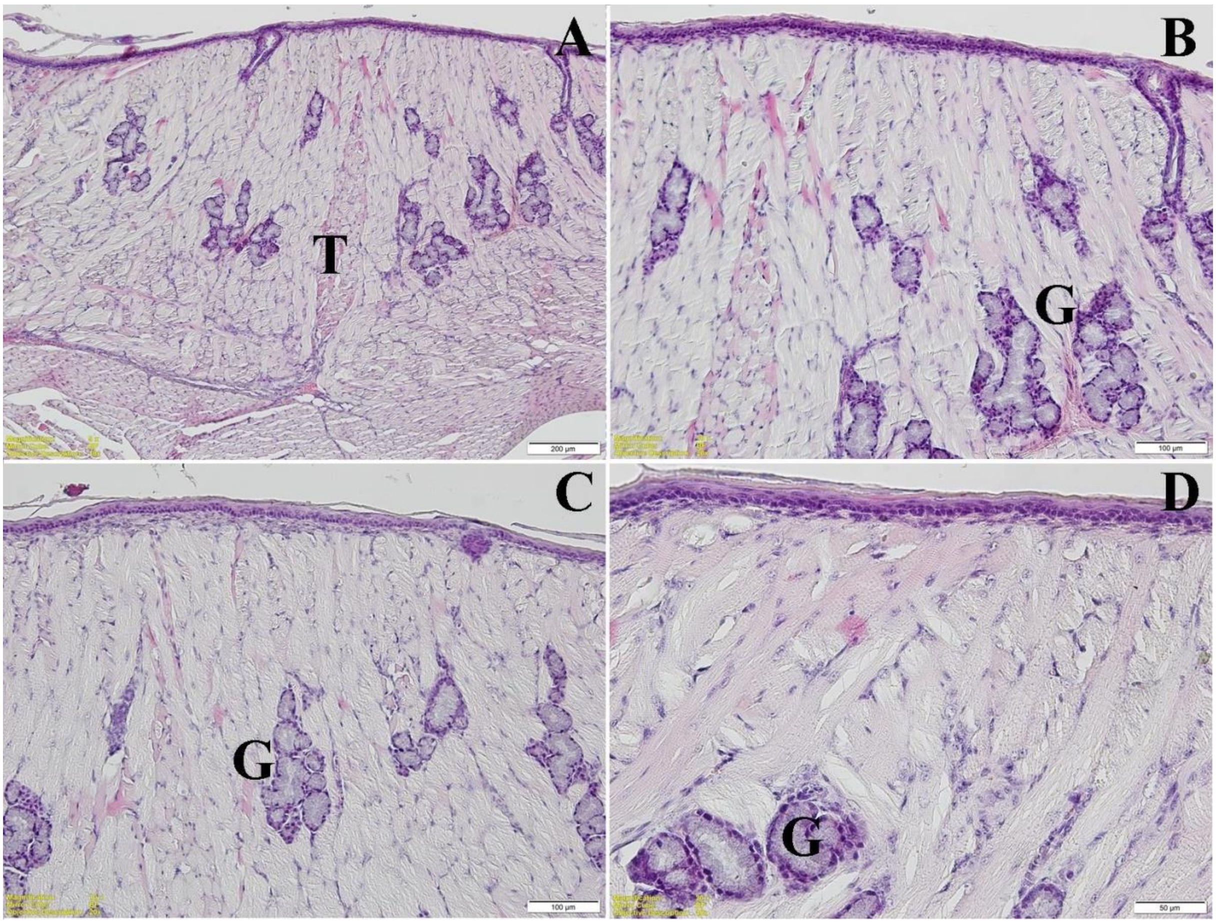


**Figure 7. Serous and mucous glands (G) surrounded by the muscle bundles in the tongue (T), H-E.**

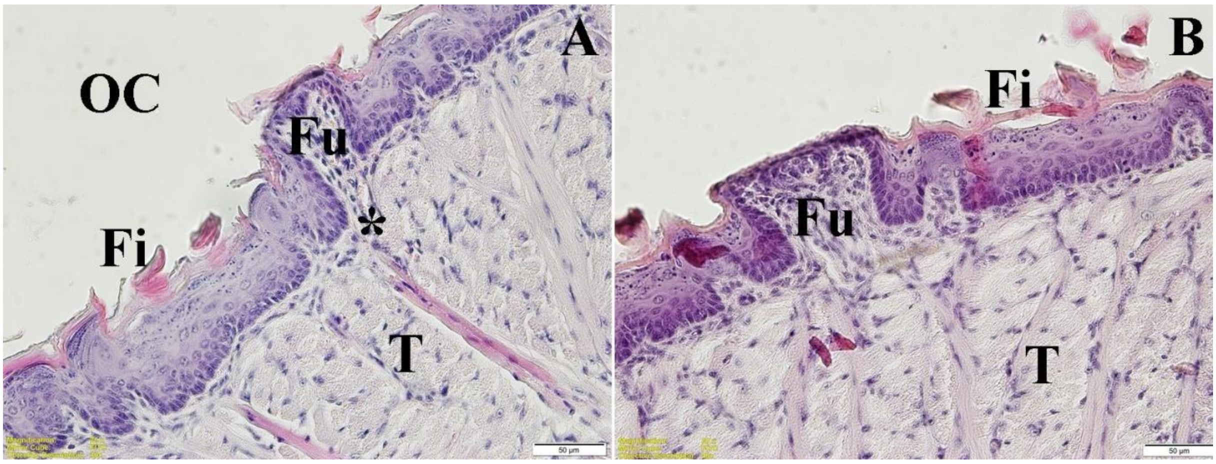


**Figure 8. Filiform (Fi) and fungiform (Fu) papillae. OC: Oral cavity, T: Tongue, (*): Blood vessel H-E.**

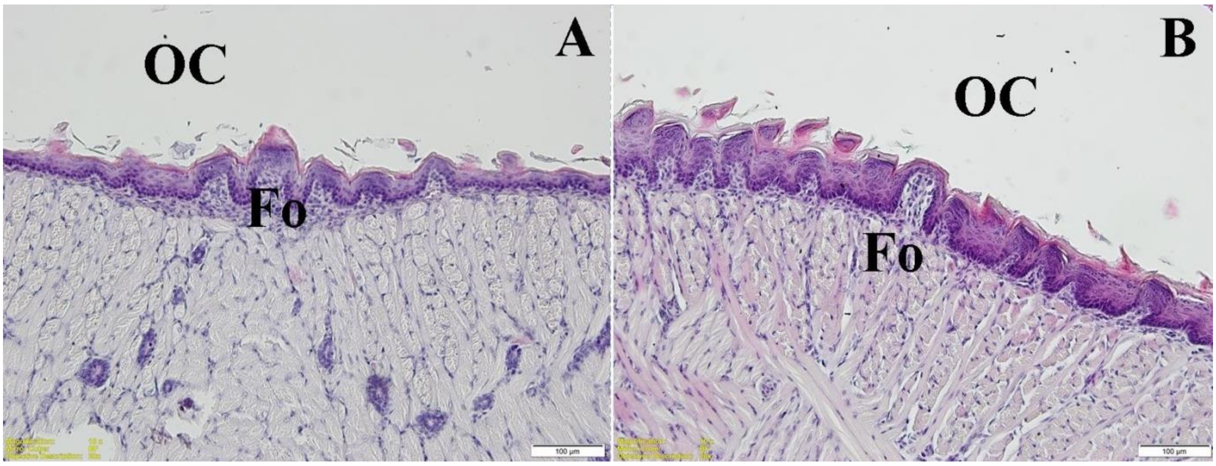


**Figure 9. Foliate papillae (Fo). OC: Oral cavity, H-E.**

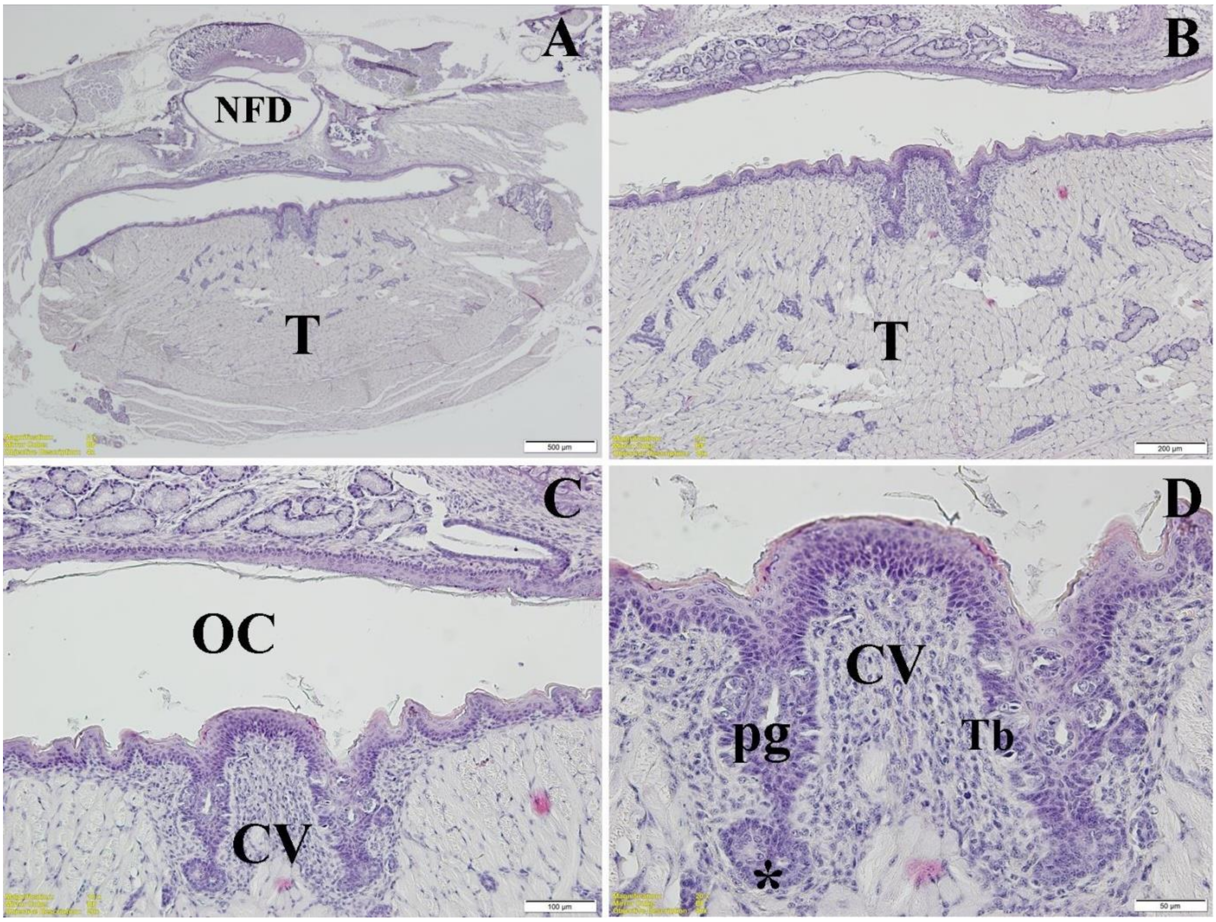


**Figure 10. Circumvallate papillae (CV). Pg: Papillar groove, T: Tongue, Tb: taste buds, (*): Ducts of the serous von Ebner gland, OC: Oral cavity, NFD: Nasopharyngeal duct, H-E.**

### 3.3. Steno's Gland (The Lateral Nasal Gland)

Light microscope images of the coronal section showed normal sinonasal structure. Consecutive sections in the coronal plane revealed the nasal septum (NS), vomeronasal organ (VNO), lateral nasal glands (Steno's glands (SG)), maxillary sinus (MS), ethmoturbinates (ETs), maxilloturbinates (MTs), lateral meatus (LM), dorsal medial meatus (DMM), both parts of the ethmoidal sinus (ES), nasopharyngeal ducts (NFDs) and soft palate (SP) (Figure 11,12). In general, four ETs were found in adults in the posterosuperior part of the unilateral sinus on coronal and sagittal sections, while only two were found in neonatal rats (Figure 11; Figure 12a,b). Complicated turbinate regions expanded the surface area of the olfactory epithelium in rodents. Unusually, a septal window was visible just proximal to the nasopharynx in mouse, however, the nasal septum completely separated the nasal cavity into two symmetrical chambers in humans, rats and monkeys. However, no septal window was observed in newborn rats (Figure 11-13).

Rats have a pair of paranasal sinuses (maxillary sinuses) visible on the lateral walls of the nasal cavity (Figure 11; Figure12a,b; Figure 13a-d). The maxillary sinuses were lined by ciliated columnar epithelium containing few goblet cells (Figure 12b; Figure 13a-d). Unlike humans, rats had multiple submucosal glands lined up around the MS. The lamina propria surrounding the sinuses contained prominent Steno's glands (Lateral nasal glands) that extended deep into the connective tissue rostral to the lateral wall of the maxillary sinus (Figure 11; Figure 12a,b,d; Figure 13a-d). These glands were submucosal glands. Humans and monkeys do not have shorthand glands. The ducts of the glands opened into the nasal vestibule. The surrounding epithelium was observed to be isoprismatic (cuboidal) epithelium no matter how large the diameter of the ducts (Figure 13a-d). This submucosal gland was also surrounded by ciliated cuboidal epithelium as well (Figure 13). Coronal H&E sections clearly revealed the entire nose, sinuses, and vital anatomical points.

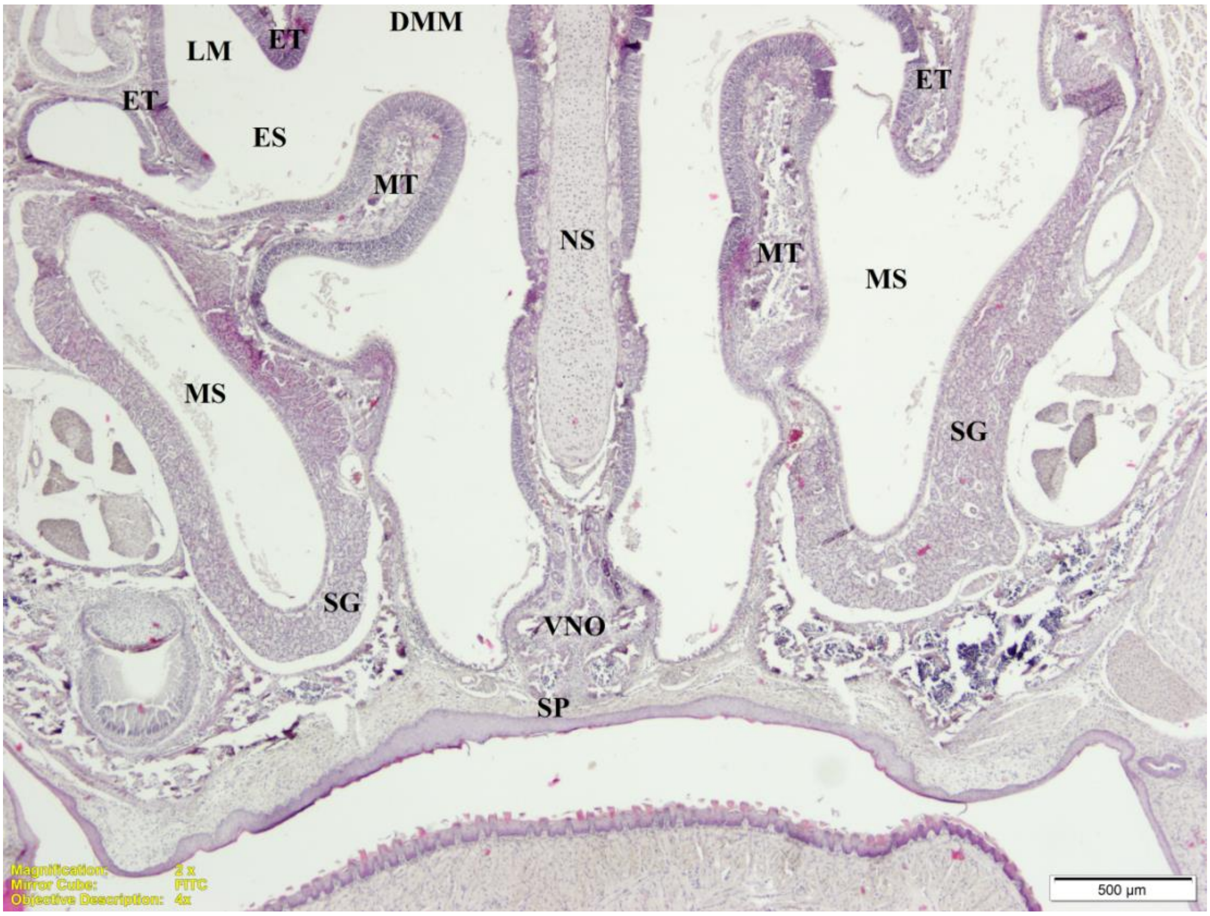


**Figure 11. General view of nasal parts in the coronal (frontal) section in newborn rats. DMM: Dorsal medial meatus, NS: Nasal septum, SG: Lateral nasal glands (Steno's glands), VNO: Vomeronasal organ, MS: Maxillary sinus, ETs: Ethmoturbinates, MTs: Maxilloturbinates, LM: Lateral meatus, ES: Both parts of the ethmoidal sinus, SP: Soft palate. H&E.**

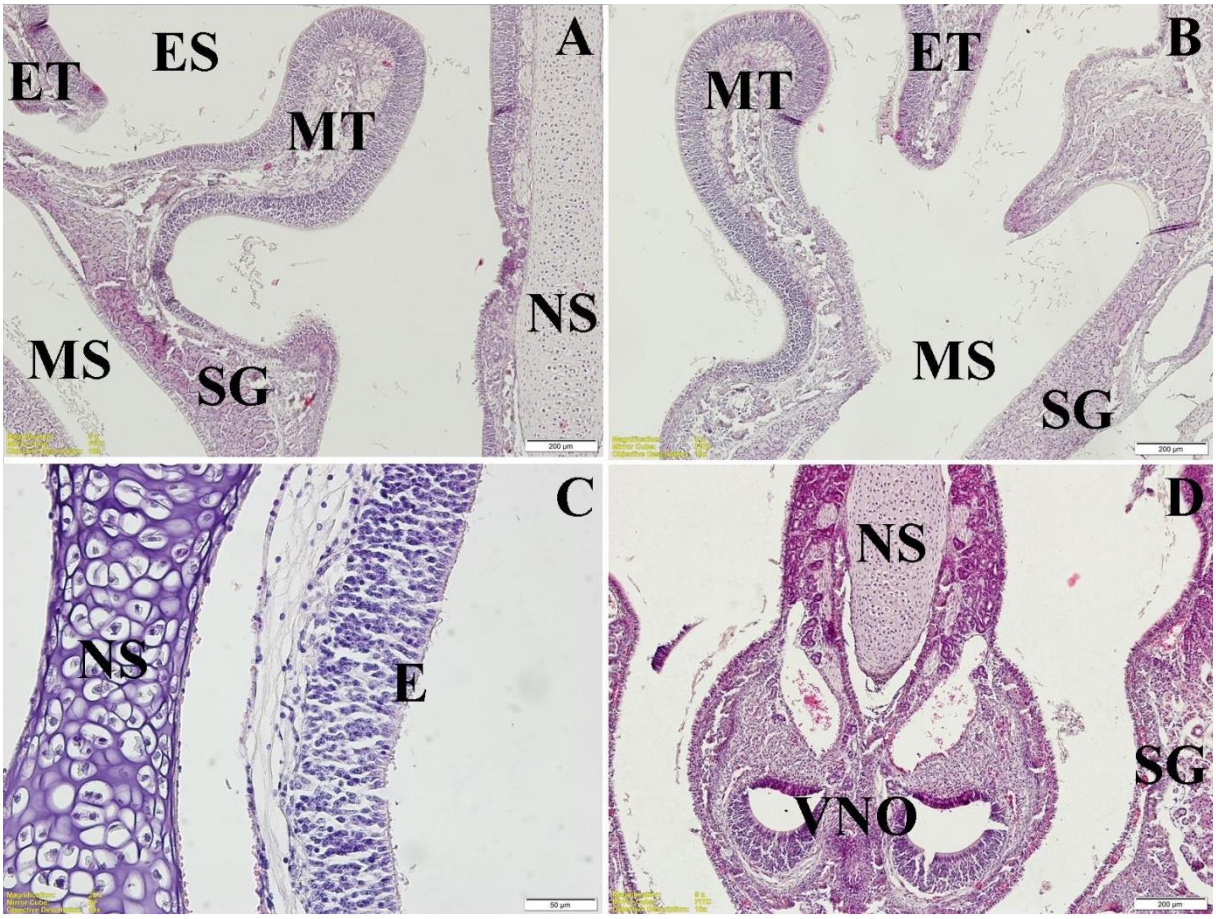


**Figure 12. Higher magnification of nasal parts in the coronal (frontal) section in newborn rats. NS: Nasal septum, E: Respiratory epithelium, VNO: Vomeronasal organ, SG: Lateral nasal glands (Steno's glands), MS: Maxillary sinus, ETs: Ethmoturbinates, MTs: Maxilloturbinates, ES: Ethmoidal sinus. H&E.**

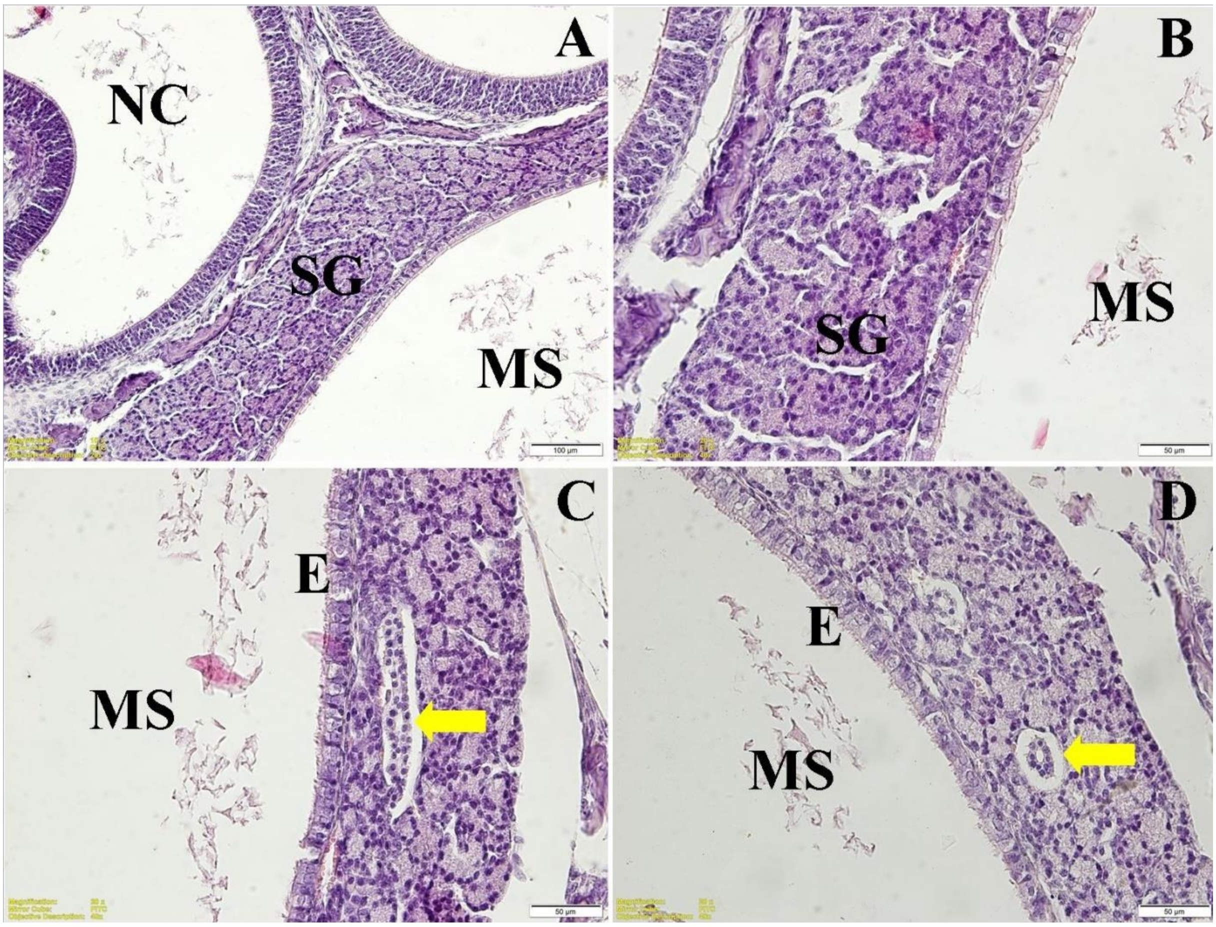


**Figure 13. Steno's gland (SG) surrounds the maxillary sinus (MS) in the coronal plane in newborn rats. Yellow arrows: The ducts of Steno's gland, E: Epithelium of Steno's gland, NC: Nasal cavity. H&E.**

## 4. DISCUSSION

Rats are preferred in various histological studies related to palate, tongue and many organs. The histological features of the Steno's gland, tongue along with soft and hard palates of newborn *Wistar albino* were revealed in this study.

In our study, although the soft and hard palates show adult characteristics in terms of histological layers, they contain some differences. It was remarkable that the number of acini of the glands in both palatal regions was quite low. The glands located on the palate are combined glands consisting predominantly of mucous acini and several crescents of Giannuzzi. The nuclei of the acinar cells of the mucous glands in both palatal regions were round and their cytoplasms were eosinophilic. As is known in adults, the nuclei of mucous cells are flat (Hakami et al., 2015). Another remarkable feature was the presence of a thin keratin sheet in the hard palate epithelium. There were wide spaces between the bony trabeculae forming the upper side of the hard palate, and the trabeculae were quite thin.

Studies on the comparative morphology of tongue in vertebrates have disclosed how differences in organ morphology and function may be related to evolutionary events (Davydova et al., 2017; Abumandour, 2018; Igbokwe and Mbajiorgu, 2019; Farrag et al., 2022; Hutanu et al., 2022). All papillae on the tongue of the neonatal rats were detected on the dorsal surface, as in the adult rat (Nguyen et al., 2021; Demirci et al., 2023). However, the histological features of the papillae were different from those of adults. While there were microscopic papillae in foliate papillae in adult rats (Nguyen et al., 2021; Demirci et al., 2023), it was observed that these structures were not yet developed in our newborn rats. It is known that there is variation in the amount and dispersion of taste buds in postnatal mammals (Yamaguchi et al., 2001). In our study, taste buds in newborns were intensely observed in the soft palate, but not in foliate and fungiform papillae. However, taste buds were identified in the circumvallate papillae. Changing the amount of taste buds in the postnatal period may affect a change in a gustatory function with increasing age.

Humans have different features in nasal anatomy from other living things. Unlike humans and monkeys, rodents and dogs use their noses primarily for smelling. Humans have unbranched turbinates. There are detailed comparative data on rat sinuses in the literature for researchers to construct animal models (Wang et al., 2020). The nasal conchae, also known as the nasal turbinates, which in most individuals contain 3 pairs of turbinates: lower, middle and upper (some individuals may also have a 4th pair called the "uppermost"), protrude from the lateral walls of the nasal cavity. The nasal turbinates have crucial functions such as warming and humidifying air, regulating airflow, immune surveillance, and olfaction in the respiratory system (Fakoya et al., 2024). Dysfunction of the inferior turbinates, which most people are unaware of when they are functioning properly, can significantly impair their quality of life. These hypertrophied structures cause anatomical obstruction. If these turbinates are removed or their mucosa is severely injured, a feeling of nasal congestion may occur. A dysfunctional olfactory epithelium causes loss of smell and decreased taste, which can significantly affect the patient's well-being (Huang et al., 2019). The relevant terminology for rat sinuses is still yet confusing and vague. The morphology of the nasal cavity in *Wistar albino* varies greatly along its length. The nature of the nasal passages is best understood by the combined observation processes during dissection and histological examination. The nasal passages consist of two main chambers separated by the sensitive nasal septum, found in almost 70% of the dorsal part of the nasal cavity, by three well-developed turbinates, nasoturbinates, maxilloturbinates, and ethmoid turbinates that protrude into the lumen of each chamber. The septal window in the caudoventral part of the nasal cavity provides a straight communication between the nasal cavities and the nasopharyngeal duct. The septal window is the confluence of both air and mucus flows. The lateral meatus has a characteristic curved medial profile ventrally (Alvites et al., 2018; Herbert et al., 2018).

The vestibule is mainly surrounded by cartilage. The ducts of the nasolacrimal and Steno's glands open into the vestibule ventrally and dorsally (Crisler et al., 2020). The vestibule accumulates secretions produced by the serous glands. Unlike humans, rats have ethmoid turbinates (ET). While ETs originate from the ethmoid bone or the lamina cribrosa of the ethmoid bone, humans only have three nasal conchae. Complicated turbinate sections expanded the surface area of olfactory epithelium in rodents. The gross anatomy revealed some important anatomical points, but some points were not clearly shown. H&E staining filled this gap. The maxillary sinuses (MS) are lined by ciliated columnar epithelium containing few goblet cells. Unlike humans, rats have multiple submucosal glands lined up around the MS. Numerous submucosal glands called Steno's glands (lateral nasal glands) were monitored on the lateral wall of the MS in neonatal rats (Harkema et al., 2018). The Steno's gland has similar cytological characteristics to the major serous salivary glands and is homologous to the salt gland in marine birds. Humans and monkeys do not have Steno's glands. The development and maturation of Steno's gland in neonatal rats are equally intriguing. In this research article, we aim to examine the distinct features of the Steno gland in newborn rats to provide a comprehensive understanding of its structure at this critical stage of life. The gland is composed of both serous and mucous acinar cells, which work in tandem to produce a complex secretory mixture. The presence of gap junctions between these adjacent cells suggests a high degree of functional coordination, allowing for efficient regulation of the gland's secretory activity. Furthermore, the gland is innervated by both sympathetic and parasympathetic nerves, which play a crucial role in modulating its secretory function (Emmelin, 1987). Bryche et al. (2020) performed the entire tissue in adult mice with a light-sheet imaging, allowing 3D visualization extending from the outer half of the head along the lacrimal gland and Steno's gland (Bryche et al., 2020). Through this innovative 3D visualization, they revealed the size of the Steno's gland and highlighted that it corresponds to the rostral nasal glands (May and Tucker, 2015). They stated that the Steno's gland extends from the incisor root to under the most dorsal turbinate in the rostro-caudal axis and opens into the central part of the nasal cavity. Moreover, in order to develop ideas about this gland structure, their histological characterization was revealed by special (PAS) staining of semi-cranial sections in the sagittal plane. As a result, they confirmed the 3D data showing that the Steno's gland was located next to the endoturbinates, suggesting that the gland could be divided into two parts.

Steno's gland both humidifies the air and controls mucus viscosity (Hidayat and Wulandari, 2021). Male rat lateral nasal gland has high testosterone levels, while females cannot detect testosterone (Zhou et al., 2009; Abaffy et al., 2023). These results indicate sexual dimorphism within the lateral nasal gland. The lateral nasal gland is the main site for the synthesis and secretion of odor-binding proteins that act as odor carriers in nasal mucus (Harkema et al., 2018; Crisler et al., 2020). Steno's gland is also referred to as a potent immunoglobulin A secretion site and thus contributes to the protection of the olfactory mucosa against foreign substances (Pevsner et al., 1988). A recent study highlights that the vertebrate odorant-binding proteins may act as antimicrobial components that help maintain immunity, which may be crucial to consider the Steno's glands in studies based on respiratory infections (Bianchi et al., 2019). Steno's gland can be damaged by exposure to toxic chemicals that are inhaled or ingested due to high metabolic action. Due to the numerous autonomic nerves associated with the acinar cells, it is also thought that it is likely for the secretory activity of the Steno's gland to be adjusted by the nervous system depending on changes (Moe and Bojsen-Moller, 1971).

## 5. CONCLUSION

Due to rapid scientific progress and the perfection of research techniques, the need to improve the data obtained continues. There is a need to classify the data obtained to study language at all morphological levels and to have a clear understanding of organ structure and further morphological and experimental research on white laboratory rats in light of the above statement. In this study, we obtained data for a partial picture of the structure of these organs, which will be useful in experimental and morphological studies on *Wistar albino* rats.

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## Conflict of Interest

"No conflict of interest was declared by the authors".

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