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HISTOLOGICAL STUDY OF THE VOMER NASAL ORGAN IN ADULT LOCAL BREED DOGS (*CANIS FAMILIARIS*)

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Abstract

The present study was carried out to investigate the vomer nasal organ of the indigenous dogs. Eight adult dogs were used. The tissue samples were fixed with 10% formalin solution, prepared for paraffin embedding technique and stained with Hematoxyline and Eosin stain, Masson trichrom stain and combine PAS - Alcian blue (2.5pH) stain. The results revealed that, the vomer nasal organ included two portions: Conducting and olfactory portions. The conducting portion has involved nasaopalatine duct which measured 623.8±12.1µm in diameter, had irregular shaped lumen and lined by thick stratified squamous epithelium, the epithelium was turned into ciliated pseudo stratified columnar epithelium at middle part of nasoplatine duct. The sub epithelial connective tissue of nasopalatine duct was occupied with wide veins and compound tubuloalveolar glands. The conducting portions involved vomeronasal duct which had significant mean diameter (985.79±13.1µm). The vomeronasal duct was incompletely surrounded by C- shape hyaline cartilage and the duct displayed medial and lateral walls. The medial wall was lined by neuro olfactory epithelium. The lateral wall was lined with respiratory epithelium. The lamina propria- submucosa was loose connective tissue contained several wide veins, nerves bundles and heavily occupied with compound tubuloalveolar mucous glands. The result concluded that the vomernasal organ of indigenous dog is similar that in farm animals except that the glandular tissue of Jacobson glands was distributed within subepithelial tissue of both wall and open throughout the lateral wall and dorsal and ventral commissars of duct.

***Key words**:* Vomernasal organ, Jacobson gland, Nasopalatine duct, neuroepithelium.

Introduction

The vomer nasal organ is regarded as part of the secondary or in most mammals, the vomer nasal system is responsible for detection the specific biological chemicals mainly pheromones (Brennan, 2001; Houpt, 2018). The accessory olfactory system composed groups of related sensory organs; vomer nasal organ. nerves, accessory olfactory bulb, olfactory tract and the amygdala, these organs are related to do their function (Brennan, 2001; Dulac and Torello, 2003). The vomernasal organ is a bilateral narrow tubular structure located on either side of the base of nasal septum, The caudal end of the organ is blind and opened rostrally with outer environment (Abood, 2017; Salazar *et al*., 2003). The vomer nasal organ is composed of vomer nasal cartilage and the vomer nasal duct, the vomer nasal duct possess a well vascular loose connective tissue which covered with sensory and respiratory epithelia those forms a hollow structure (Lee, 2003; Abood and Hussien, 2017). Vomer nasal organ of different animals has been investigated by (Al-Hussany *et al*., 2012) in Awassi sheep, (Altaii, 2010 and Abood, 2017) in ox and buffalo, (Abood and Hussien, 2017) in indigenous Gazelle. The current study was aimed to investigate the histological structure of vomer nasal organ in indigenous doges.

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Materials and Methods

Ethical approval

The design of the present study was approved by the Animal Care and Use Committee at the College of Veterinary Medicine, University of Baghdad. Baghdad, Iraq.

The animals

Eight heads of adult, healthy indigenous doges were

used for this study. Animals were obtained from the department of surgery and obstetrics in the Faculty of Veterinary Medicine, University of Baghdad. The study was carried out at department of Anatomy and Histology, College of Veterinary Medicine, during a period extended from April-December 2019. The animals were sacrificed,the heads were removed from the body and the nasal region included the hard palate was immersed in 10% formalin for 72 h. The nasal region was divided to five transversal sections (Each was about 1.30-1.50cm). The sectioning has made up through the hard palate from the level of the upper incisors to the level of the 5th transverse palatine ridge that included the vomer nasal organ. The 1st section represented the hard palate with vestibule region of nasal cavity (nasopalatine duct), the 2nd section was at the level of 2nd and 3rd transverse palatine ridge included the respiratory region of nasal cavity and anterior of vomernasal duct, while the 3rd section was represented the posterior part of vomeronasal duct (At level of 4th into 5th transverse palatine ridges). Each section has trimmed to achieve the region which included both tubes of the vomernasal organ and then all sections were transformed into decalcification (formic acid solution). It was composed of equal parts of formic acid solution (250 ml formic acid (98%) + 250 ml distal water) and Sodium citrate solution (50 gr. Sodium citrate +250 ml Distal water) (Luna,1968). The decalcified process take about 20 days, the solution was changed every 4 days. Paraffin embedding tissue preparation technique was fallowed to get (5-7)µm. serial section. The (Hematoxylin and Eosin stain, Masson's trichrom stain and PAS - Alcian blue pH 2.5 stain) were used (Bancroft, 2008). The Histometrical measurements included the diameters of vomernasal duct and the height of the epithelial lining in all region of vomernasal organ were done by using coulometer.

Statistical analysis

Statistical analysis has been done by using The IBM/ SPSS statist version (24) to analyze the estimated data, the results were representing by Means and Standard Error (M±SE). One way analysis of Variance (ANOVA) has been used. The value ($P<0.05$) was considered to be significant.

Results and Discussion

In indigenous dogs the vomeronasal organ was a bilateral tubular organ located at the base of nasal septum and extended from level of the canine's teeth into level of 2nd upper molar teeth. Both tubes of the organ were appeared as hallow tube with crescent-oval shaped lumen. According to the differences in examined figures, the results showed that the vomer nasal organ was including two portions: conducting and olfactory portions. Such description has mentioned by (Al-Hussani *et al.,* 2012) (Besoluk *et al*., 2006) (Karimi *et al*., 2007), (Lee *et al*., 2003) and (Nunezchichet *et al*., 2007).

The conducting portion

This portion was represent the anterior of vomernasal organ and extended as bilateral ducts called nasopalatine ducts from floor of vestibule of the nasal cavity into level of 1st premolar teeth. The nasopalatine duct was measured 623.8±12.1µm in diameter, had irregular shaped wide lumen and lined with thick stratified squamous epithelium (Fig. 1). This result is similar to results of (Vaccarezza, 1981; Salazar *et al*., 1995; Sano and Okano 1995; Abood, 2010; Abood and Hussein, 2017; Abood, 2017; Karimi *et al.,* 2014) who mentioned that the anterior of the vomernasal organ is lines with stratified squamous epithelium, this suggested that all mammals which their vomer nasal organ has open into outer environment throughout the nasal cavity have the longest conducting part which extended into rostral portion and lined with protected epithelium (stratified squamous epithelium). The lamina propria- submucosa has composed of dense irregular collagenous connective tissue contained numerous of blood vessels (Fig. 2). At the middle region of the nasoplatine duct, the epithelium was turned into respiratory epithelium and the sub epithelial connective was showed many of wide veins and compound tubuloalveolar glands (Jacobson gland) (Fig. 3, 4). This type of epithelium is specifying with protection, thus this part not deal with olfaction. On other hand the present result part showed that the rest part of duct is lined by transitional zone to the respiratory epithelium. The present results are agreed with results of (Altaii, 2010; Abood, 2017).

Olfactory portion

This portion represents the middle and posterior end of vomernasal organ and called "Vomeronasal duct". The luminal diameter of vomer nasal duct in the middle part of vomernasal duct has a significant ($P<0.05$) diameter value (985.79±13.1µm) in compared with nasopalatine duct. The vomeronasal duct was incompletely surrounded by C- shape hyaline cartilage and the duct displayed two walls (medial & lateral walls), the walls were lined with two types of epithelia (Fig. 5). The incomplete status of hyaline cartilage helps to give the vomernasal duct a suitable space to distend during pumping mechanism, this suggestion has supported by results of (Kratzing, 1971; Bland and Cottrell, 1989) in sheep, on other hand the variable shape of the lumen of vomernasal duct is related with the action of pumping status of vomer nasal organ

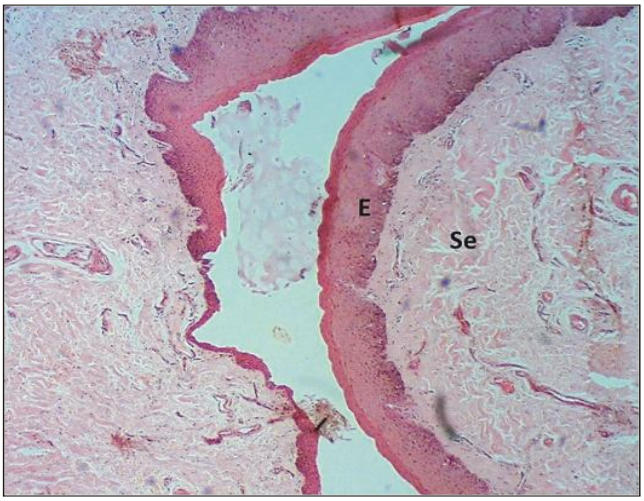

Fig. 1: Section of the opening of nasoplatine duct at the floor of the vestibule of nasal cavity show: epithelium (E) & sub epithelial connective tissue (Se). H&E stain. 100x.

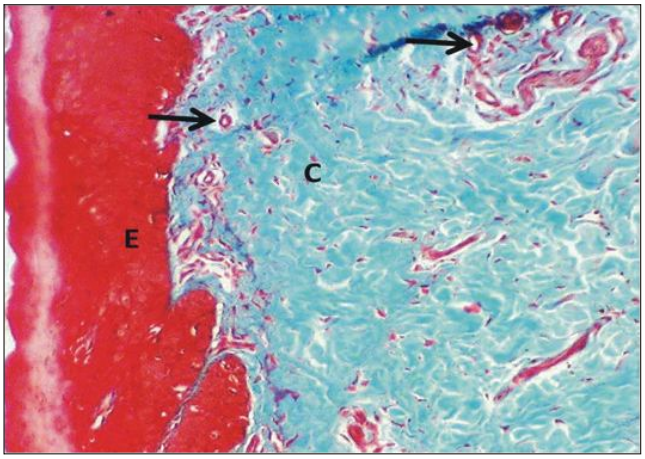

Fig. 2: Section of the opening of nasoplatine duct show: stratified squamous epithelium (E) & sub epithelial connective tissue with blood vessels (Black arrows) & collagen bundles (C). Masson's trichrom stain. 100x.

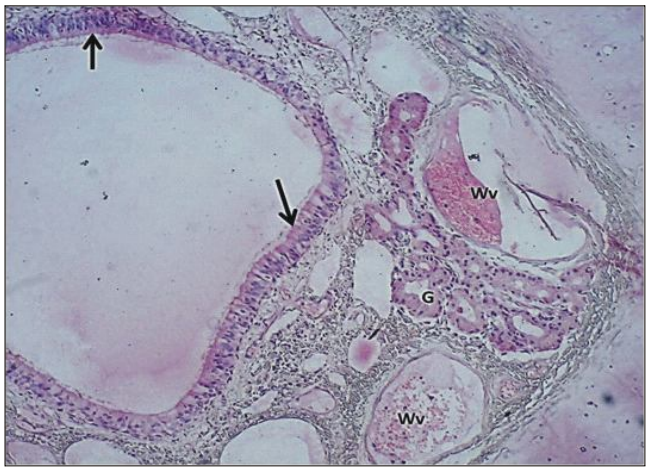

Fig. 3: Section at the middle part of the nasoplatine duct show: respiratory epithelium (Arrows) & sub epithelial connective tissue with wide vein (Wv) & serous glands (G). H&E stain. 100x.

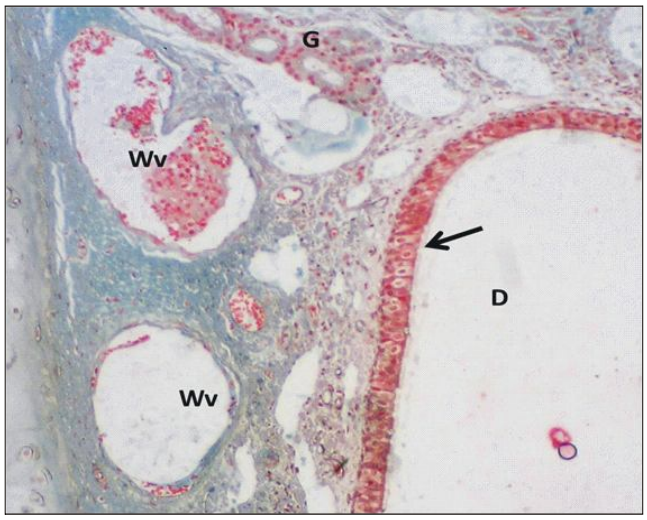

Fig. 4: Section at the middle part of nasoplatine duct show: lumina of duct (D), respiratory epithelium (Arrow), wide vein (Wv) & Jacobson glands (G). Masson trichrom stain. 100x.

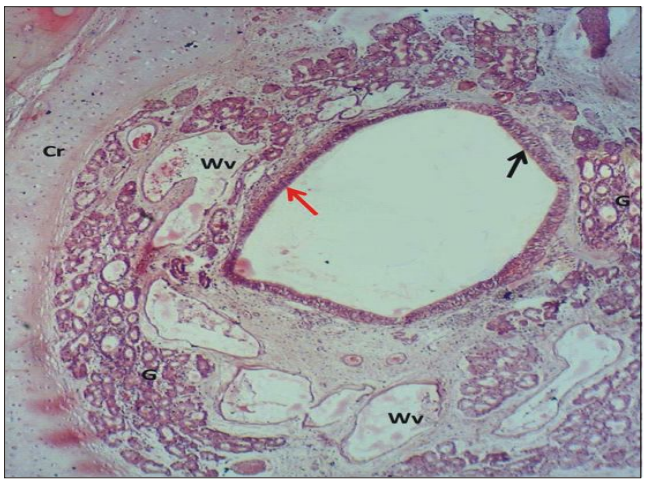

Fig. 5: Section of the vomernasal duct show: respiratory epithelium (Red arrow), Neuro olfactory epithelium (black arrow) wide vein (Wv) & Jacobson glands (G). H&E stain. 40x.

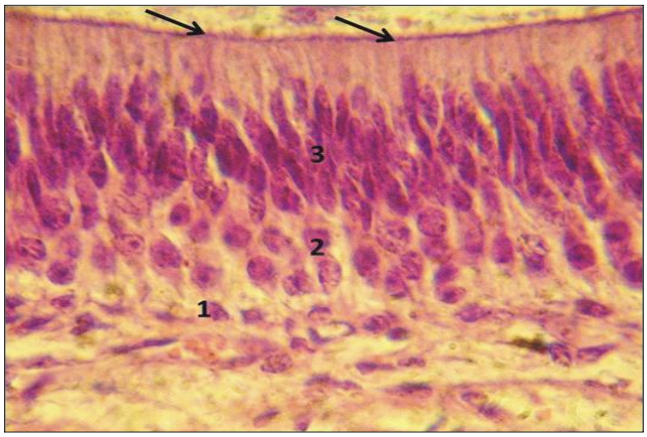

Fig. 6: Section of neuro olfactory epithelium show: Basal cells (1), neuro olfactory cells (2), sustentacular cells (3) & microvilli (arrows). H&E stain. 400x.

which is responsible for this variability (when the lumen of duct emptying from fluids the luminal shape is crescent and becomes oval shape when the lumen of duct is distended with fluids), the incomplete vomernasal cartilage represented by cleft which lead to entrance of blood supply into the soft tissue of the vomer nasal organ from nasal mucosa, this agrees with results (Salazar *et al*., 1995) in cattle, cat, dog, pig, cow and horse, (Abbasi and Khosravinia, 2002) in sheep,(Salazar *et al*., 2003) in pig and (Altaii, 2010) in Ox.

The vomernasal duct was displaying two walls (medial and lateral). The medial wall was lined by thick pseudo stratified columnar epithelium without goblet cells (Neuro olfactory epithelium). Olfactory epithelium has composed of basal, sustentacular and neuro olfactory cells. The basal cells were few cells, rested at the basement membrane and had oval-rounded shaped nucleus. Neuro-olfactory cells were occupied the middle region of the epithelium and had large spherical-oval shaped nuclei. Supporting cells (sustentacular cells) were columnar elliptical shaped cells with elongated darkly stained nuclei, they were occupied the upper region of the epithelium, the apical surface of neuro olfactory epithelium was well demarcated by apical dark line which represented the microvilli (Fig. 6). The neuro olfactory epithelium had significant epithelial height which measured (109.2±5.07µm). This result agrees with the results of all authors except the result of (Karimi *et al*., 2007) in Iranian goat, the present result revealed that, the vomer nasal duct start at the region lines with the neuro olfactory epithelium that considered the functional units of vomernasal organ, this result is consistent with the result of Vaccarezza, who remember that the vomernasal duct started at the middle portion of organ in rat (Vaccarezza, 1981). Also in horse, Okano, Salazar and Lee, remembered that the in horse the starting of vomer nasal duct is at the body portion of vomer nasal organ because this portion bore both respiratory and neuro-epithelium (Salazar *et al*., 1995), (Okano *et al*., 1998), (Lee *et al*., 2003). Also in canine, Sano mentioned that, the vomernasal duct has started at the body portion (Sano and Okano, 1995). In cat, the vomernasal duct has started at the middle portion (Salazar *et al*., 1996, 1997), (Al-Hussany *et al*., 2012) in Awassi sheep and (Altaii, 2010) in ox and (Abood and Hussein, 2017) in Gazelle. The current result is incompatible with results of (Zuri *et al*., 1998) who refereed that, the olfactory epithelium in rat has characterized by abundant of cilia and microvilli (Uraih and Maronpot, 1998); (Zuri *et al*., 2000). The lateral wall of vomernasal duct was lined by pseudo stratified columnar epithelium (respiratory epithelium). Respiratory epithelium has composed of ciliated, non-ciliated and basal

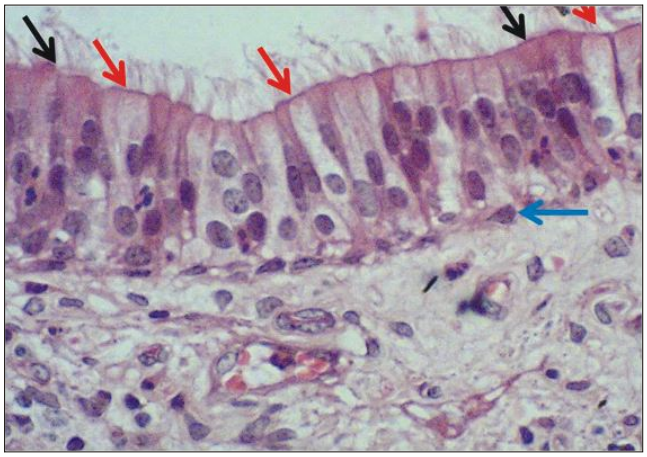

Fig. 7: Section of epithelium of lateral wall show: mucous secreting cells (Red arrows), ciliated cells with tall cilia (Black arrow) and basal cells (Blue arrow). H&E stain. 400x.

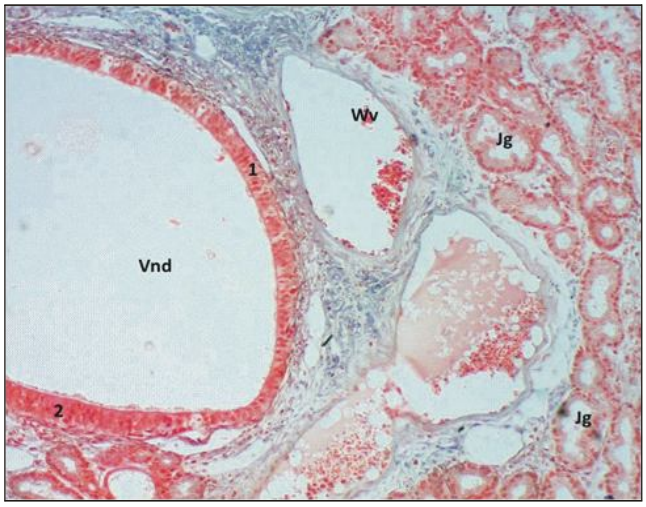

Fig. 8: Section of vomer nasal duct (Vnd) shows: respiratory epithelium (1), olfactory epithelium (2), wide vein (Wv) and serous Jacobson glands (Jg). Masson's trichrom stain. 100x.

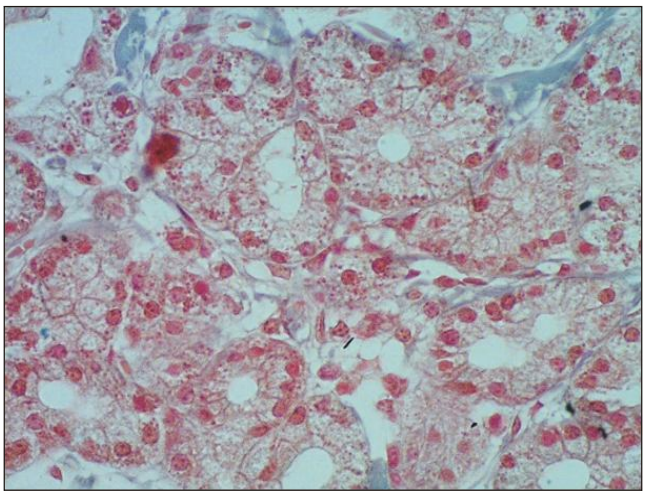

Fig. 9: Section of Jacobson glands show: secretory units of Jacobson glands. Masson's trichrom stain. 400x.

cells (Fig. 7) which measured about (43.4±3.6µm). This result suggests that this type of epithelium is has a supportive role in provide adequate secretory product and the cilia serve in mixing the mucus fluids along the lumen of duct, so that the cilia improved good contact between molecule of pheromones and the microvilli of olfactory neurons and this opinion is supported by (Adams and Wiekamp, 1984); (Abood, 2010); (Abood and Hussein, 2017).

The sub epithelial connective tissue (lamina propria submucosa) of both lateral and medial walls was highly vascularized and occupied by groups of Jacobson's glands in addition for many of axon of accessary olfactory nerve and wide veins (Fig. 8, 9). The vomernasal glands (Jacobson's glands) were compound tubuloalveolar type and their common ducts have opened through the respiratory epithelium and at the dorsal and ventral commissure of vomernasal duct. This result is supported by the results of (Kratzing, 1971) in sheep, (Taniguchi and Mikami, 1985) in horse, (Adams, 1986) in cattle, (Abbasi, 2007) in buffalo, (Salazar *et al*., 2003) in pig, (Karimi *et al*., 2007) in Iranian goats, (Yilmaz, 2008) in dog, (Adams and Wiekamp, 1984). in canine and (Altaii, 2010) in Ox, the present results suggest that the increased population of glands in this portion is important, the secretion of these glands acts as dissolving media for pheromones which sniffed and passed through the vomernasal duct, the pheromones easily dissolved in the sero-mucus which covered microvilli (Charles and McGinley, 2000). The secretory units of the Jacobson's glands have showed both reactions; neutral secreting cells and acidic secreting cells (Fig. 10). The result showed that at the ends of the lumen of vomernasal ducts had oval shape and measured 612.9±18.03µm in diameter, this result agree with results of (Adams, 1986) in cattle,

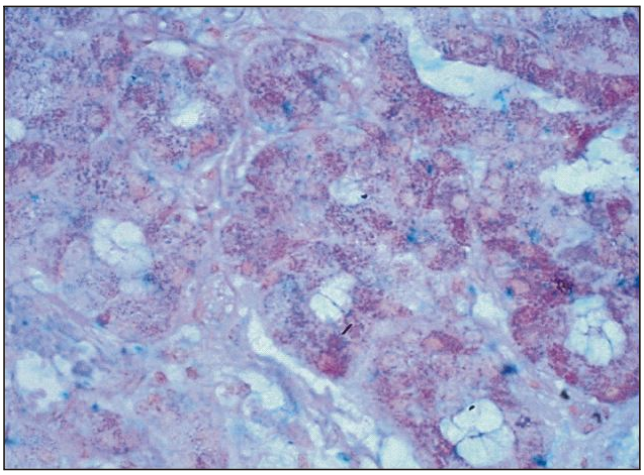

Fig. 10: Section of Jacobson's glands shows: acidic mucous secreting cells, neutral secreting cells .combine AB (pH2.5) & PAS stain. 400x.

(Okano *et al*., 1998) in horse, this is an important feature, this result is paralleled with the results of (Vaccarezza *et al*., 1981) in rat, (Salazar *et al*., 1995) in horse and cattle, (Sano and Okano 1995) in canine, (Salazar *et al*., 2003) in pig, (Salazar, 1996) in cat, in these animals the caudal portion of vomer nasal duct, the epithelium gradually changed into simple columnar epithelium and ciliated pseudo stratified columnar epithelium.

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