A HISTOLOGICAL STUDY OF HUMAN OLFACTORY MUCOSA: REGIONAL DISTRIBUTION AND AGE RELATED CHANGES

by

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Dedicated to Late Prof. Mahmood Ahmad (HI, SI)

For his moral support, strong motivation and sympathetic attitude

and

To my Father, Dr. Mehram Khan Jafari, my wife Naheed Akhtar and to our children; Ayesha, Babar, Zain and Zeeshan

For their support, understanding and patience.

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ABSTRACT

The present study on the morphology of human olfactory mucosa was carried out with emphasis on its regional distribution, and changes related with age and gender. Eighty tissue samples (forty for either sex) were collected from cadavers ranging from 30 to 82 years of age, available in the mortuary of King Edward Medical College, Lahore. Individual age groups of males and females included 10 specimens from each sex. The histological study of the mucosa included morphology, regional distribution, quantitative analysis of all four major types of epithelial cells, height of epithelium and thickness of lamina propria in the roof, medial and lateral walls of both nasal cavities. A detailed study of the epithelium revealed the presence of classically known three cells: olfactory cells, sustentacular cells and basal cells and a fourth type, microvillar cells. In the age group 30-39 years (male and female) the mucosa was seen in the roof lying next to cribriform plate of the ethmoid bone and extending on both sides of the nasal septum and on the lateral walls of both nasal cavities. At places the respiratory epithelium was seen in the area of the olfactory epithelium which was much thicker. In the age group of 40-49 years, early age related changes were observed in the shape of occasional short epithelial invaginations, and disturbance of the zonal distribution of olfactory and supporting cells. In the age group 50-59 years, major morphological changes were observed like substantial reduction in the number of nuclei resulting in decreased height of the epithelium, disturbance of zonal distribution and presence of epithelial invaginations. The age group of 60 years onwards showed gradual thinning of the epithelium, epithelial invaginations, and in few cases atrophied olfactory epithelium devoid of olfactory cells. ANOVA showed significant age related decrease in the number of olfactory and sustentacular cells and in the height of the olfactory epithelium among the male and female groups. There was no significant age related decrease in the number of basal cells and thickness of the lamina propria. The number of microvillar cells was markedly less when compared to other cells of the epithelium. These results suggest that loss of olfactory and sustentacular cells becomes pronounced in individuals of both sexes of 50+ years of age. The results of the present study suggest that the reduction in the number of olfactory receptors and in the height of neuroepithelium with advancing age is associated

with impairment of olfactory sensibility. There was no evidence of significant sex related differences in the olfactory mucosa. These results are in the accordance with the previous observations in humans and other mammals showing a decline in the olfactory capacity with aging, mostly attributable to a decline in the number of olfactory cells. Contrary to earlier observations, the present study did not reveal any conclusive evidence that females had an increased sense of smell based on histological observations alone.

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INTRODUCTION

General:

We all have a unique sense of smell (Wysocki and Preti, 2004). The sense of smell is the act of perceiving and distinguishing odours (Anderson, 2003; Krieger and Breer, 1999). It is one of the primitive senses (Dominy *et al*., 2004; Buck, 2000; Laska and Teubner, 1998). Smell allows organisms with olfactory receptors to identify mates (Keller *et al*., 2006; Wedekind and Furi, 1997; Stengl, 1993), food, predators, and provides both sensual pleasure (e.g., odour of flowers) as well as warns of sources of danger (e.g., enemies) (Mandal *et al*., 2005; Preti *et al*., 2003; Belanger *et al*., 2003). It is one of the important means by which our environment communicates with us (Laska *et al*., 2005; Shepherd, 2004; Leffingwell, 2002; Lledo *et al*., 2005).

We perceive the external world around us via five separate modalities: touch, vision, taste, hearing and smell. Smells are all around us, and it is probably true to say that life is made rich by them (Lewis, 2006; Chen and Dalton, 2005; Miwa *et al*., 2001). Memory is also often associated with smell as smell evokes memories (Richardson and Zucco, 1989). Smell is involved in relaying emotions such as fear and anxiety (Chen *et al*., 2006; Caruso *et al*., 2004). Smell can provoke mating behavior (Jacob *et al*., 2002; Beauchamp *et al*., 1985) and is useful in identification of kin in a variety of species (Preti *et al*., 1997). Human mothers of newborn babies can recognize their offspring by odour alone (Beauchamp *et al*., 1995; Kaitz *et al*., 1987). The infants are attracted to breast odours of their mothers and move in the direction of the odour (Varendi and Porter, 2001). Smell receptors have been identified in human sperm, which function in sperm chemotaxis and may be a critical component of the fertilization process (Spehr *et al*., 2003). In certain diseases the smell given off by the patient can lead to a diagnosis. The reason that a person's body odour changes in disease is because there is an alteration in his normal physiology. For instance, the fruity, sweet odour of the breath of diabetics is caused by an excess of ketone, an aminoacid breakdown product of proteins that are being burnt off as fuel by the body (Kasper *et al*., 2005).

The nasal cavity, which contains the olfactory mucosa, extends from the nares, through the external nose and between the bones of the face as far back as the posterior nasal apertures or choanae, where the nasal cavity communicates with the nasopharynx (Agur and Dalley, 2005; Cummings *et al*., 1990). The nasal cavity consists of floor, medial and lateral walls and roof (Sing, 2003). It is divided sagittally into right and left halves by the nasal septum (Snell, 2004). Each half of the nasal cavity is approximately 5 cm in height, and 5-7 cm in length. It is narrow transversely, measuring approximately 1.5 cm at the floor, and only 1-2 cm at the roof (Sinnatamby, 1999). It is divisible into three regions, the nasal vestibule anteriorly, the respiratory region and the olfactory area (Bradbury, 1973). The vestibule forms the beginning of the nasal cavity anteriorly; the respiratory region constitutes the majority of the nasal cavity, while the limited and variable olfactory area is confined mainly to its posterosuperior parts including the upper regions of lateral and medial walls (Telford and Bridgman, 1990; Morrison and Costanzo, 1990; Basmajian, 1989).

The respiratory membrane extends from the limen nasi throughout the nose and into the upper half of the nasopharynx. It also extends into the sinuses, through their ostia, and is thinner there. It is also continuous with the epithelia of the nasolacrimal duct and Eustachian tube. Above, it is continuous with the olfactory mucosa of the nose. Anteriorly, at the limen nasi, it becomes continuous with the skin of the nasal vestibule (Gray and Hawthorne, 1992).

The interior of the nose is lined by four types of epithelium. The stratified squamous epithelium of the skin continues through the nares into the vestibule, where a few large stiff hairs project into the airway. These are believed to help exclude large dust particles in the inspired air. A few millimeters into the vestibule, stratified squamous epithelium gives way to a narrow transitional band of nonciliated cuboidal or columnar epithelium. This is continuous with ciliated pseudostratified columnar epithelium that lines the remainder of the nasal cavity, save for a small area in the dorsal wall, where it is replaced by the sensory olfactory epithelium (Kelly *et al*., 1984; Jafek *et al*., 2002; Gross *et al*., 1982). The respiratory epithelium consists of ciliated columnar cells, goblet cells,

and small basophilic cells that are regarded as stem cells for replacement of the more differentiated cell types. Beneath the epithelium there is a thick lamina propria containing glands made up of both mucous and serous cells (Fawcett, 1994; Kratzing, 1984).

The olfactory mucosa occupies an area of approximately 10 cm^2 covering the posterior upper parts of the lateral nasal walls, including the superior concha, the sphenoethmoidal recess, the upper part of perpendicular plate of the ethmoid (Fig. 1), and the roof of the nose arching between the septum and the lateral wall, including the underside of the cribriform plate of the ethmoid bone (Doty, 1990) (Fig. 2). It consists of a pseudostratified olfactory epithelium (Naguro and Iwashita, 1992), which contains the sensory receptors, and an underlying lamina propria, which overlies the dense connective tissue forming the periosteum of the cribriform plate of the ethmoid bone (Lane *et al*., 2002). The olfactory epithelium is considerably thicker (up to 100 µm) than the adjacent respiratory epithelium (Standring *et al*., 2005). It is composed of four principal types of cells: olfactory receptor cells (sensory cell), sustentacular cells (supporting cell), basal cells (Nomura *et al*., 2004; Polyzonis *et al*., 1979) and microvillar cells (Moran *et al*., 1982a,b; Jafek *et al*., 2002; Morrison and Costanzo, 1992).

The olfactory receptor cell is a bipolar neuron with its cell body generally located deeper in the epithelium than the sustentacular cells and a nerve fiber (axon) extends from its basal end. The nuclei of the sensory cells are elliptical and darkly stained. The dendrites of these sensory cells ascend toward the surface in the crevices between sustentacular cells terminate in a knob bearing olfactory cilia (Bloom and Engström, 1952; Graziadei, 1965; Telford and Bridgman, 1990). Gap junctions are present between sensory neurons, facilitating in the continual turnover and development of olfactory receptor cells (Delay and Dione, 2003). The olfactory nerve fibers enter the lamina propria forming fila olfactoria, which pass through the cribriform plate of the ethmoid bone to join the olfactory bulb (Fig. 3).

The sustentacular cells are irregular columnar cells separating and partially ensheathing the olfactory receptor cells. Their large, vertically elongated, euchromatic

nuclei form a layer superficial to the receptor perikarya (Weiler and Farbman, 1998). At the exposed surface of the epithelium they send numerous long, somewhat irregular microvilli into the mucous layer (Polyzonis *et al*., 1979), among the long trailing ends of the olfactory cilia. Their cytoplasm contains many mitochondria, granular and especially much agranular endoplasmic reticulum. Near the epithelial surface fine microfilaments attached to desmosomes give mechanical support to the epithelium. Tight junctions are present between the sustentacular cells and the olfactory receptor cells (Ross *et al*., 2003; Morrison and Costanzo, 1990).

The basal cells are small, round or cone-shaped cells that form a single layer resting on the basal lamina. They are stem cells; the source of new olfactory receptors and sustentacular cells that differentiate to replace cells lost during normal turnover or injury (Schwob, 2005; Costanzo and Graziadei, 1983; Farbman *et al*., 1988; Goldstein and Schwob, 1996; Ducray *et al*., 2002). There are two types of basal cells; basal cells proper and globose basal cells (Williams *et al*., 1995).

The microvillar cells are flask shaped cells that exhibit large, blunt microvilli at their apical surface, a feature that gives them their name. The nuclei of these cells lie close to the apical surface. The basal surface of these cells is in synaptic contact with nerve fibres that penetrate the basal lamina (Kwon *et al*., 2005; Moran *et al*., 1982a; Kratzing, 1982).

The basement membrane (basal lamina) usually consists of a well defined, homogenous structure, lying against the under surface of epithelial cells (Petruson, 1984; Frisch, 1967).

The lamina propria contains numerous olfactory nerve fascicles (Lovel *et al*., 1982) and sub-epithelial olfactory glands (glands of Bowman) which secrete through ducts on to the epithelial surface (Polyzonis *et al*., 1979). It also includes some pigment cells, lymphoid cells, and a rich plexus of blood capillaries. In its deeper portion, there is a plexus of large veins and numerous lymphatics (Williams *et al*., 1995).

The Bowman's glands are branched tubuloalveolar structures that lie beneath the olfactory epithelium and secrete onto the epithelial surface through narrow, vertical ducts (Nomura *et al.*, 2004; Frisch, 1967). Their secretions, which include defensive substances, lysozymes, lactoferrin, Immunoglobulin A (IgA) and sulphated proteoglycans (Okamura *et al*., 1999), bathe the dendritic endings and cilia of the olfactory receptors allowing their diffusion to the sensory receptors. The Bowman's glands are confined to the olfactory epithelium (Nakashima *et al*., 1984).

The dendrite of the sensory cells is the receptor portion. It terminates in a knob bearing several long cilia extending to the surface of the mucosa. Each receptor cell has 10-20 cilia (Ganong, 2005). These cilia contain the binding sites for attachment of odourants. A substance must be sufficiently volatile (easily vaporized) so that some of its molecules can enter the nose in the inspired air and should be sufficiently water soluble to dissolve in the mucous layer coating the olfactory mucosa (Sherwood, 2004; Johnson *et al*., 1998). The olfactory mucosa contains as many as 1,000 different odourant binding proteins (OBP) that concentrate the odourants and transfer them to the receptors (Fox, 2006). During smell detection, an odour is broken into various components. Each receptor responds to only one discrete component of an odour rather than to the whole odourant molecule. Accordingly, each of the various parts of an odour is detected by one of the thousand different receptors, and a given receptor can respond to a particular odour component shared in common by different scents (Sherwood, 2004). All the odourant receptors are coupled to Guanine nucleotide binding proteins (G-proteins) (Silverthorn, 1998). Binding of an appropriate scent signal to an olfactory receptor activates a G-protein, triggering a cascade of cyclic adenosine monophosphate (cAMP) dependant intracellular reactions that lead to opening of Na^+ channels (Bhandawat *et al*., 2005; Waxman and deGroot, 1995). The resultant ion movement brings about a depolarizing receptor potential that generates action potentials which are propagated through the axons of olfactory receptor cells into the two olfactory bulbs (Johnson *et al.*, 1998). The input layer of each bulb contains about 2000 spherical structures called glomeruli. Within each glomerulus, the endings of about 25,000 primary olfactory axons converge and terminate on the dendrites of about 100 second-order neurons. Each glomerulus receives input only

from receptor cells expressing a particular receptor protein gene (Widmaier *et al*., 2006). The output axons of the olfactory bulbs course through the olfactory tracts. Each olfactory tract projects directly into the primitive regions of the cerebral cortex; from here information passes to the thalamus and finally on to the neocortex (Fox, 2006).

There is growing evidence that as we get old, our sense of smell declines (Larsson *et al*., 2000; Ship *et al*., 1996; Farbman, 1994; Graziadei and Monti-Graziadei, 1978; Moulton, 1974). This also affects our sense of taste. Thus food loses its flavour (Duffy *et al*., 1999). By the age of 80 years, 80% of people have some major smell dysfunction and 50% are "anosmic" by the standards of young people (Doty *et al*., 1984; Stevens *et al*., 1982). Not only do we lose our sense of smell, but we also lose our ability to discriminate between smells. There is also evidence that women, while also losing smell sensitivity with age, perform better than men at all ages (Jacob, 2006), i.e. gender associated changes are also on record.

Age – related changes:

Steady loss and replacement of receptor cells throughout life is a normal process, and the stem cells undergo periodic mitotic division throughout life giving rise to new olfactory receptors (Mumm *et al*., 1996; Caggiano *et al*., 1994; Farbman, 1994; Graziadei, 1973). However as stated earlier, the olfactory function is markedly altered in old age and in a number of age related diseases (Rombaux *et al*., 2005; Feng *et al*., 1997; Doty, 1989) and aging can seriously blunt olfactory sensations mediated by the olfactory receptor system (Stevens *et al*., 1982). Head and facial injuries (Zusho, 1982; Jafek *et al*., 2000), medication (Douek *et al*., 1975) and environmental risk such as working in places where exposure to caustic fumes (e.g., formaldehyde) is common, constitute long term risk factors for odour identification (Elsner, 2001). Histological studies show that there is an age – related tendency to loose olfactory receptor cells, the sensory epithelial surface being replaced by ciliated respiratory epithelium (Legrier *et al*., 2001; Paik *et al*., 1992). Distortions to olfactory sensation can cause great disturbance to our lives (Doty and Mishra, 2001). There is a loss of quality of life and it can bring anxiety and loss of appetite. For example, the inability to detect smoke can be dangerous and may lead to

subsequent harm while food poisoning is more prevalent in patients who cannot detect rotten food. Age-related changes in the ability to perceive odours include deficits in olfactory sensitivity, odour discrimination, odour identification, odour recognition, odour memory, the perception of odour pleasantness and susceptibility to and recovery from olfactory adaptation (Doty, 1990; Stevens *et al*., 1989; Murphy, 1998; Weiffenback, 1984). According to Doty *et al*. (1984), the average ability to identify odours declines markedly after the seventh decade of life. This indicates that certain morphologic and or physiologic changes may occur in the olfactory system as part of the aging process, relating to lower probability of obtaining olfactory tissue from older subjects. There is an age-related decrease in the ability to identify or recognize odourants. Olfactory decrement is especially prominent in the elderly; as over three-fourths of individuals over 80 years of age are nearly anosmic and over one half of those between the ages of 65 and 80 years are similarly deficient (Doty *et al*., 1984). Such a decline may be universal, as it appears to occur in persons from all walks of life in all cultures (Gilbert and Wysocki, 1987; Doty *et al*., 1985; Doty, 1986).

A number of factors are likely to be responsible for age-related changes in human odour perception, e.g., vascular and metabolic insufficiency and loss of specific neurotropic factors leading to age related atrophy of the olfactory receptors, viral damage (Jafek *et al*., 1990; Douek *et al*., 1975), nutritional deficiencies and air pollution (Hudson *et al*., 2006). Anatomical changes in the olfactory region (Zhao *et al*., 2004; Mishra and Doty, 2002; Yamagishi *et al*., 1988) and alterations in the highly vascularized nasal respiratory epithelium, particularly the epithelium located on the nasal turbinates, can alter airflow patterns within the nose and presumably the amount of odourized air reaching the receptors through the small (<1 mm wide) superior meatus (Doty *et al*., 1988; Ghorbanian *et al*., 1983). Thus, age-related alterations in factors which influence airway patency, such as decreased mucosal thickness, nasal polyps, turbinal engorgement and inflammation, could influence odour perception in elderly individuals (Ge *et al*., 2002; Wolfenberger and Hummel, 2002; Larsson and Tjälve, 2000; Apter *et al*., 1999).

Environmentally induced damage to the receptor epithelium is probably the most common basis for age-related alterations in the olfactory function (Hinds *et al*., 1984). It is conceivable that age-related physiological and structural changes either directly damage the neuroepithelium or predispose it to damage from environmental insults. The olfactory receptor cells can differentiate from the basal cells of the olfactory epithelium in normal adult vertebrates (Hinds *et al*., 1984; Graziadei *et al*., 1978; Graziadei and DeHan, 1973). Such plasticity has been suggested to be an adaptation to the fact that the apical processes of these neurons are exposed almost directly to the outside environment, making them very susceptible to insult from bacteria, viruses and airborne toxins (Bonfanti and Fasolo, 2002; Hummel *et al*., 1998). In humans, histological studies of the olfactory epithelium suggest that the plasticity in receptor cell turnover fails to protect all sectors of the olfactory epithelium from destruction.

Nakashima *et al*. (1984) examined, by light microscopy, the olfactory epithelia of five aborted human fetuses and 21 adults ranging in age from 20 to 91 years at autopsy. They observed regions of disorganization or degeneration in adult tissue and found islands of respiratory epithelium in olfactory areas. The regions of disorganization and mixing made it difficult to distinguish respiratory and olfactory epithelium. Invasion of respiratory epithelium was more prominent in the roof of the nasal cavity compared with the other olfactory regions. They noted zonal degeneration of receptor cells in specimens of all ages, although the effect was more marked in the older specimens. The distribution of the basal, sustentacular and sensory receptor cells was often disturbed. Metaplasia of the respiratory epithelium was evident within the olfactory epithelium, suggesting that regions of the damaged olfactory epithelium were replaced with respiratory epithelium. In the fetus, the olfactory neuroepithelium extended from the roof of the nasal cavity to the mid portion of the nasal septum and onto the superior turbinates in a continuous fashion. In fetal life and early childhood, the olfactory epithelium was highly cellular and thicker than the respiratory epithelium, but in the adults, the olfactory epithelium was generally thinner than the respiratory epithelium (Naessen, 1970).

Specimens obtained from the olfactory region in human autopsy cases, sometimes contained only respiratory epithelium. The border between the respiratory and olfactory regions was irregular, with intermixed strands of respiratory and olfactory epithelia (Engström and Bloom, 1953). Naessen (1971a) observed that the olfactory epithelium in adults was mixed with the respiratory epithelium. There were alterations of the olfactory neuroepithelium in humans who did not have intranasal or intracranial diseases suggesting that these changes occur with aging. A reduction in thickness of the olfactory epithelium was evident in aging humans, with a concomitant loss of normal zonal distribution of sustentacular and sensory cell nuclei. A less well-defined boundary between respiratory and olfactory epithelia and an increase in pigment granules within the sustentacular cells were also seen. The olfactory epithelium was often intermixed with islets of ciliated respiratory tissue. Paik *et al*. (1992) obtained the specimens by biopsies and performed en-block removal of the olfactory area. They reported that the olfactory mucosa was replaced by respiratory epithelium with aging. They observed multiple patches of respiratory epithelium over the upper portion of nasal septum and superior turbinates. Frequent metaplasia was suggested with increase in age. Out of 36 specimens, only 17 had olfactory epithelium.

Age-related changes have been described in rat olfactory bulb, with decline in size in later life (Hinds and McNelly, 1981). However, this was thought to be secondary to the changes within the olfactory epithelium as changes in number of olfactory receptor neurons directly influence the size of the olfactory bulb. Thus the primary deficit in declining olfactory function may reside in the olfactory epithelium. Proliferation density in the olfactory epithelium of unperturbed rats declines dramatically with age (irrespective of body weight) from the neonate to the age of 11 months (Weiler and Farbman, 1997).

Sex – related changes:

Scores on the University of Pennsylvania smell identification test (UPSIT) demonstrate that peak performance in odour identification ability occurs in the 3rd through the 5th decades of life and markedly declines after the 7th decade, with women

generally out performing men at all ages. Women have greater sense of smell than men of the same age group (Doty *et al.*, 1984). Impairment in the ability to detect low concentrations of odourants occurs in later life. On the average the decline in sensitivity (i.e., rise in thresholds), while present in both sexes, begins at an earlier age in men than in women (Deems and Doty, 1987). The lower discrimination ability for odour mixtures indicates that human olfaction is reduced with age. Olofsson and Nordin (2004) investigated chemosensory gender differences and showed that compared to men, women gave higher intensity and unpleasantness ratings. Gender does not affect olfactory detection thresholds and discrimination (Kaneda *et al*., 2000) and is not the definitive factor in the age-related decline in olfactory function (Corwin *et al*., 1995). Women outperformed men in the tasks involving verbal processing i.e. memory for familiar odours and odour identification (Öberg *et al*., 2002).

Objectives of the study:

It is clear from the above review that olfactory function decreases significantly in older persons, and sex-related changes are also on record. Such decline can be detected by a variety of psychological tests, including the ones which assess odour detection, identification, discrimination, memory and adaptation. Such perceptual alterations are accompanied by changes in the anatomy and physiology of the olfactory mucosa. However, most of the research on olfactory mucosa has been conducted in the United States of America and Europe. There are marked geographical differences between these countries and Pakistan. There is also difference in the life expectancy of the populations of these countries. According to WHO health report 2006 for the year 2004, the life expectancy in Pakistan was 62 years for males and 63 years for females, as compared to USA where it was 75 years for males and 80 years for females. There is lack of studies on human olfactory mucosa, its morphology, gender distribution and changes related with age in this part of the world. Information generated through studies on populations in other geographic regions of the world can hardly be applied universally without direct evidence for local populations. While women have been considered in the existing literature to possess a sharper sense of smell compared to men, there have been no studies that show differences between men and women of the same age in the histology of the

olfactory mucosa. Moreover, most studies have been carried out on laboratory animals and even studies on humans have been confined to a limited number of cases (Nomura *et al*., 2004; Weiler and Farbman, 1997; Paik *et al*., 1992; Morrison and Costanzo, 1990; Nakashima *et al*., 1984; Moran *et al*., 1982a,b).

With these considerations in mind, the present work was planned to study the human olfactory mucosa in randomly selected indigenous male and female cadavers of different age groups and, to compare our findings with those from other parts of the world. It was also hoped that the study would provide base line data on age and sex related differences in its morphology to correlate with the functional aspect. The plan of work on the human olfactory mucosa included study of the following.

a. Morphology of the olfactory receptor cells, sustentacular cells, basal cells and microvillar cells.
b. Population of all major type of cells in the roof, medial and lateral walls of right and left nasal cavities.
c. Height of epithelium in the roof, medial and lateral walls of right and left nasal cavities.
d. Histological study of the lamina propria and its various components.
e. Thickness of lamina propria in the roof, medial and lateral walls of right and left nasal cavities.

MATERIALS AND METHODS

Tissue samples for study of nasal mucosa were collected from cadavers (The Human Tissue Act, 1961) available in the mortuary of King Edward Medical College, Lahore. The cadavers with the following conditions of nose were not included in the study.

a) Nasal injury,
b) Inflammatory and neoplastic conditions of nose identified on gross appearance,
c) Nasal polyps, and
d) Evidence of major rhinal surgery.

The nasal mucosa of all those cadavers was presumed to be normal which did not have any of the above mentioned conditions of the nose. Particulars of the cadavers regarding age, sex and septal position were noted (Appendix). The cadavers were placed in a cold room at 4^0C until the time of autopsy. The interval between death and collection of specimens did not exceed six hours. Tissue samples were obtained from the nasal septum, lateral wall and roof of the nasal cavity from 80 human adults. Eight groups of 10 specimens of each regional sample were made according to age and sex (Table 1).

Method of taking specimens:

Removal of scalp:

The scalp was removed after dividing it into four pieces by the following incisions. The first incision was given in the sagittal plane, cutting through the scalp from the root of the nose to the external occipital protuberance. The second incision was made in the coronal plane from the middle of the first incision to the root of each auricle, dividing the scalp into four pieces. All the five layers of these four pieces of the scalp were peeled off to the ends of the cut.

Removal of calvaria:

A thread was tied around the skull 1 cm above the orbital margins and the external occipital protuberance. A pencil mark was made on the bone all along the thread and the thread was removed. A saw cut along this line was made, but no deeper than the marrow cavity which was indicated by blood stained saw dust. The inner table of the skull was broken with chisel and mallet by a series of short sharp strokes and the calvaria was removed. This exposed the brain along with dura mater and endocranium with the superior sagittal sinus in the midline.

En - bloc removal of brain along with its coverings:

The brain along its meninges was removed to expose the cranial fossae in the following manner: The endocranium in the three cranial fossae was mobilized and detached manually. Falx cerebri was severed from the crista galli and the foramen caecum. The frontal lobes were pulled out with gentle force and attempt was made during this maneuver to preserve the soft tissue in the region of cribriform plates and the olfactory filaments. The following structures were cut carefully in sequence from front to back.

a. Optic nerves and ophthalmic arteries,
b. Infundibulum,
c. Abducent, Trochlear and Trigeminal nerves,
d. Facial and Vestibulocochlear nerves, and
e. IX, X, XI and XII cranial nerves and Internal Jugular Vein.

Following this, the medulla oblongata was separated with a long knife along with vertebral arteries as close to the foramen magnum as possible, and the brain was taken out to expose the anterior cranial fossa.

En - bloc removal of olfactory area:

With a swab soaked in 10 % formalin, the anterior cranial fossa was cleaned to remove any debris, especially in the vicinity of crista galli, cribriform plates and foramen

caecum. A rectangle measuring 4 x 3 centimeters was marked with lead pencil around the cribriform plates with its longer axis directed anteroposteriorly (Fig. 4).

This area encompassed the following:

a. Crista galli,
b. Cribriform plates of the ethmoid,
c. Narrow edge of orbital plates of the frontal bones, and
d. Four mm of jugum sphenoidale.

With a sharp chisel and mallet, the bone was cut along the demarcated area (Nakashima *et al*., 1984). This allowed access to the perpendicular plate of the ethmoid in the middle and the ethmoidal air cells on either side. The incision was deepened using a sharp scalpel to cut through the perpendicular plate of the ethmoid anteriorly and posteriorly, and on either side the incision was continued through the ethmoidal air cells about 4 centimeters to include the following:

a. Roof of the nasal cavity,
b. Sphenoethmoidal recess,
c. Superior concha,
d. Superior meatus, and
e. Upper part of the nasal septum.

The block of the tissue was separated by cutting the lateral nasal walls by repeated medial excursions of the knife from both sides and then cutting the nasal septum in the same dimensions and then it was lifted out (Figs. 5, 6). The bone on both sides adjacent to the roof, which was not required, was cut off by a bone cutter.

Fixation:

The specimen was immediately immersed in 10% formol saline fixative. The fixation time was seven days after which the specimen was processed for decalcification.

Decalcification of the bone:

The bone, which was part of the specimen, was decalcified in a glass jar containing ethylene diamine tetracetic acid (EDTA) (5.5 g) in 10% formol saline solution (Mukherjee, 1988). The solution was changed daily and the bone was manually checked for decalcification. Manual check revealed that on the twenty fourth day the bone showed flexibility and had the quality of a piece of cartilage indicating completion of decalcification.

Dissection of decalcified specimen:

The decalcified specimen was washed in running water; the roof of the nose on either side of crista galli was trimmed by a pair of scissors. Two coronal incisions, parallel to each other were made, first 8 mm behind the anterior end and the other one cm posterior to the first. These incisions were extended on each side to the extent of lateral nasal walls, and were carried vertically. Specimens thus obtained included the roof and both lateral walls and the septum of the nose. Anteroposterior identification of specimen was achieved by tying a thread passed with the help of fine sewing needle through the lower part of the posterior edge of lateral wall of the left nasal cavity.

Dehydration:

The tissue samples were dehydrated in ascending ethanol series, cleared in xylene and embedded in paraffin wax (56-58^{0}C melting point) according to standard histological procedures.

Embedding and orientation of the tissue:

For embedding, Leuckhart's moulds were used. The orientation thread was removed from the samples and a blunt nosed warm forceps was used for transferring them to the mould. Melted paraffin was poured over the tissue and tissue was oriented in the desired plane ensuring that the sections will be cut from the anterior surface.

Table 1. Schedule for staining sections of specimens taken from the nasal cavity of male (M) and female (F) subjects.

Group [1]	Stains and Sections Examined [2]		
	H&E	PAS	Silver Stain
30-39	3 sections	3 sections	3 sections
40-49	3 sections	3 sections	3 sections
50-59	3 sections	3 sections	3 sections
60 & over	3 sections	3 sections	3 sections

[1] Individual age groups (males & females) include 10 specimens from each sex.

[2] Thirty sections from each sex per age group were stained as indicated in Table 1.

Sectioning and staining:

Six micron thick consecutive sections were made on a rotary microtome (Leica RM 2125). The sections were affixed to precleaned albumenized glass slides and stained with Haematoxylin and Eosin (H & E) for general histological study (A), periodic acid Schiff (PAS) for demonstration of basement membrane and mucous cells (B), and Bielschowsky's silver stain, (Glees-Marsland modification) for demonstration of neurons (C) (Bancroft and Gamble, 2002).

Microscopic study:

The sections (see scheme in Table I) were studied under a light microscope (Leica DM 1000). Observations were made separately on sections taken from roof, medial and lateral walls of the right and the left nasal cavities.

In addition to the morphology of the mucosa in the areas mentioned above, quantitative measurements were made to determine.

a. Number of various cell types in the olfactory epithelium,
b. Height of the olfactory epithelium, and
c. Thickness of the lamina propria.

Quantitative measurements were made under appropriate magnification using a precalibrated ocular micrometer (10X objective: 1 oc. div = 9.89 µm; 40X objective: 1 oc. div = 2.4 µm). The number of various cell types in the olfactory epithelium was counted in H & E stained sections at the diameter of the field i.e., 0.4 mm (400 µm) under 40X objective (Drury and Wallington, 1967). This area was selected randomly from the epithelium. The number was counted from three different fields and mean was calculated. The height was measured with the help of a 40X objective from three different fields and mean was calculated. Thickness of the lamina propria was measured with 10X objective from three different fields and mean was calculated.

Statistical analysis:

Statistical analyses were conducted using the statistical package for social sciences (SPSS version 10.0). Age-related differences between the age groups of each sex (Group I: 30-39 years; Group II: 40-49 years; Group III: 50-59 years and Group IV: 60 years onwards) were tested for significance separately in the males and females using ANOVA (Analysis of variance). Tukey honestly significant difference was used as the post-hoc test before calculating the differences, Kolmogorov-Smirnov test of normality and Levene test of homogeneity of variance was calculated from the data to assess the normality of the data (Kuzma and Bohnenblust, 2000).

Similarly gender related differences were tested for significance by using t-test, probability less than 0.05 was considered as significant.

RESULTS

Observation of the Mucosa in the age group 30-39 years (male and female groups):

The olfactory mucosa was observed in the right and the left nasal cavities. At low magnification, the mucosa was seen in the roof area of the nasal cavities lying next to the cribriform plate of the ethmoid bone, extending on both sides of the nasal septum (medial wall) and on the lateral walls of the right and the left nasal cavities. General histological study of the olfactory mucosa revealed it to consist of the usually well known layers, namely, pseudostratified epithelium, basement membrane beneath the epithelial cells and lamina propria (Fig. 7).

The transition from the respiratory to the olfactory epithelium was observed in the superior region of the nasal cavity (Fig. 8). At places, the respiratory epithelium was seen in the area of the olfactory epithelium (Fig. 9). Patches of respiratory epithelium characterized by the deeply stained goblet cells were seen replacing the olfactory epithelium. Serous acini were also seen in the lamina propria (Fig. 10). The olfactory epithelium was located in the roof, medial and lateral walls of the right and the left nasal cavities resting upon a uniform basement membrane. It was observed that the olfactory epithelium was much thicker compared to the respiratory epithelium. A detailed study of the epithelium revealed the classically known four major types of cells (Fig. 11).

Olfactory receptor cells:

The cell bodies of the receptor cells were generally located in the middle and lower half of the epithelium. The cell bodies appeared round or oval. The nuclei were mostly elliptical and deeply stained; some of them were spherical and lightly stained as observed in haematoxylin and eosin stained sections. From the apical portion of the receptor cells a single extension was seen projecting towards the epithelial surface; likewise, the basal cytoplasm of these cells was seen forming a basal process (Fig. 12). In sections stained with Bielschowsky's silver method, the dark brown oval cell bodies were seen placed in the middle and basal two thirds of the epithelium. The apical and the basal processes could be distinguished as dendrites and axons respectively giving the cells the

typical appearance of bipolar neurons. Olfactory vesicles (dendritic knobs) were evident arising from the apical aspect of the dendrites of the receptor cells, projecting into the surface layer lining the nasal cavity (Fig. 13).

Statistical analysis of age-related changes in the number of olfactory receptor cells in males:

ANOVA showed that there was significant age-related decrease in the number of olfactory cells per 0.4mm among the groups of males ($P < 0.001$). Post-Hoc test, using the Tukey honestly significant difference (HSD), also showed that this difference was statistically significant ($P < 0.001$) (Table 2) among all the male groups revealing gradual decrease in mean number of olfactory cells with advancing age (Group I Mean = 59.04, SE = 0.1376; Group II Mean = 48.93, SE = 0.1886; Group III Mean = 19.62, SE = 0.2215; Group IV Mean = 14.84, SE = 0.2733). However, the greatest decrease in the number of olfactory cells was between the male groups of ages 40-49 years and 50-59 years (Mean Difference = 29.31) as compared to age groups 30-39 years and 40-49 years (Mean Difference = 10.11) and age groups 50-59 years and 60 years onwards (Mean Difference = 4.78). These results suggest that there was a significant decrease in the number of olfactory receptor cells in the male population throughout all ages (Fig. 14).

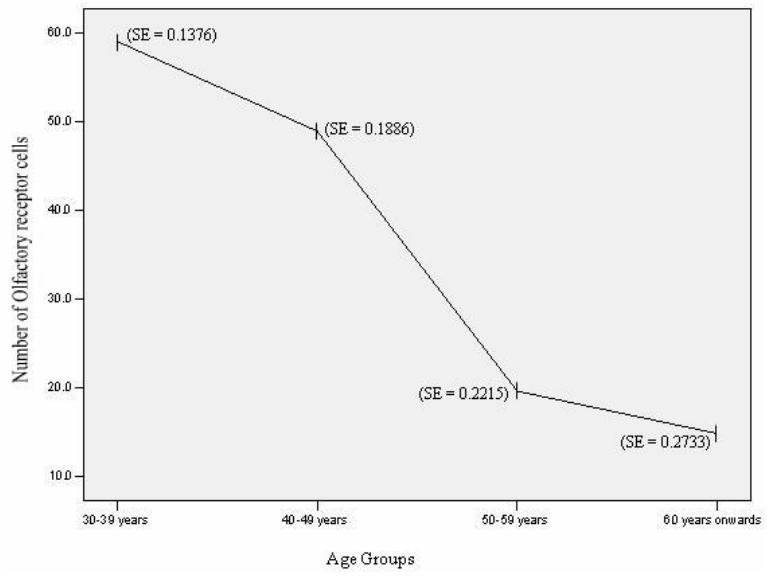

Fig. 14. Mean Number of Olfactory Receptor Cells in the Four Age Groups of Males.

Table 2. Multiple Comparison of Number of Olfactory Receptor Cells among the Age Groups of Males.

Comparison among age groups		Mean Difference (I-J)	Std. Error (SE)	Level of Significance (P-value)
Age group compared (I)	Age group compared with (J)			
30-39 years	40-49 years	10.11	0.2986	0.001
	50-59 years	39.42	0.2986	0.001
	60 years onwards	44.20	0.2986	0.001
40-49 years	30-39 years	-10.11	0.2986	0.001
	50-59 years	29.31	0.2986	0.001
	60 years onwards	34.09	0.2986	0.001
50-59 years	30-39 years	-39.42	0.2986	0.001
	40-49 years	-29.31	0.2986	0.001
	60 years onwards	4.78	0.2986	0.001
60 years onwards	30-39 years	-44.20	0.2986	0.001
	40-49 years	-34.09	0.2986	0.001
	50-59 years	-4.78	0.2986	0.001

Statistical analysis of age-related changes in the number of olfactory receptor cells in females:

ANOVA showed that there was marked age-related decrease in the number of olfactory cells among the female groups ($P < 0.001$). Post-Hoc test, using the Tukey HSD, also showed that this difference was statistically significant ($P < 0.001$) (Table 3) among all groups (Group V Mean = 59.37, SE = 0.1719; Group VI Mean = 49.35, SE = 0.1809; Group VII Mean = 19.80, SE = 0.1874; Group VIII Mean = 14.39, SE = 0.1935). However, the greatest decrease in the number of olfactory cells was between the female groups of age 40-49 years and 50-59 years (Mean Difference = 29.55) as compared to age groups 30-39 years and 40-49 years (Mean Difference = 10.02) and 50-59 years and 60 years onwards (Mean Difference = 5.41). These results show that there was a marked

decrease in the number of olfactory receptor cells in the female subjects throughout all ages (Fig. 15).

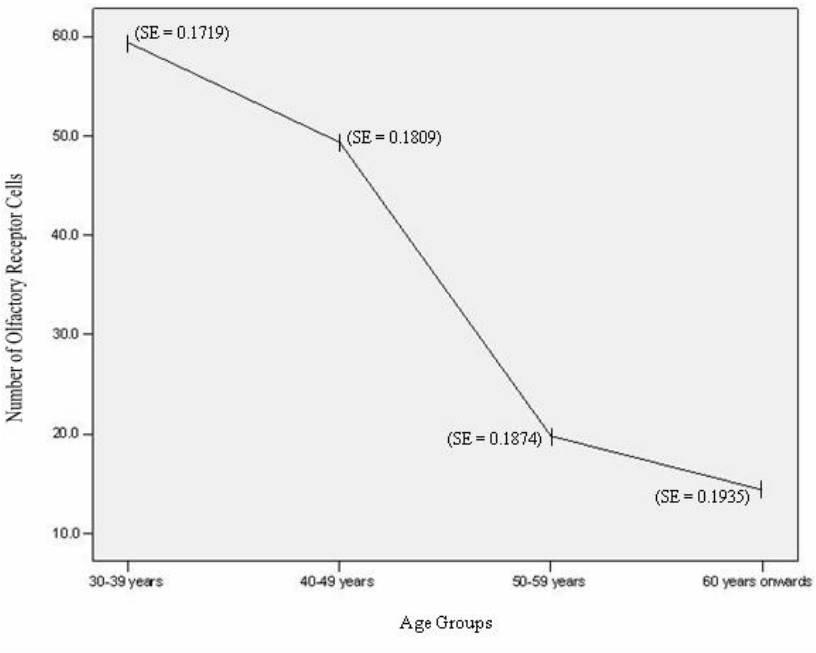

Fig. 15. Mean Number of Olfactory Receptor Cells in the Four Age Groups of Females.

Table 3. Multiple Comparison of Number of Olfactory Receptor Cells among the Age Groups of Females.

Comparison among age groups		Mean Difference (I-J)	Std. Error (SE)	Level of Significance (P-value)
Age group compared (I)	Age group compared with (J)			
30-39 years	40-49 years	10.02	0.2596	0.001
	50-59 years	39.57	0.2596	0.001
	60 years onwards	44.98	0.2596	0.001
40-49 years	30-39 years	-10.02	0.2596	0.001
	50-59 years	29.55	0.2596	0.001
	60 years onwards	34.96	0.2596	0.001
50-59 years	30-39 years	-39.57	0.2596	0.001
	40-49 years	-29.55	0.2596	0.001
	60 years onwards	5.41	0.2596	0.001
60 years onwards	30-39 years	-44.98	0.2596	0.001
	40-49 years	-34.96	0.2596	0.001
	50-59 years	-5.41	0.2596	0.001

Statistical analysis of gender related differences in the number of olfactory receptor cells:

The analysis of gender related differences was calculated using the independent sample t-test between the corresponding male and female groups. Comparison of age groups 30-39, 40-49, and 50-59 years showed that the numbers of olfactory cells in the two sexes were not significantly different (p-value > 0.05). However, in the age group 60 years and above the males had greater number of olfactory cells (Mean Difference = 0.45, p-value > 0.05) (Table 4).

Table 4. Comparison of Number of Olfactory Receptor Cells among the Age Groups of Males and Females.

Age group	Mean (Males)	Mean (Females)	Standard Error	t-score	p-value
30-39 years	59.04	59.37	0.22	-1.498	0.151
40-49 years	48.93	49.35	0.26	-1.607	0.125
50-59 years	19.62	19.80	0.29	-0.62	0.543
60 years onwards	14.84	14.39	0.33	1.344	0.196

Sustentacular cells:

These cells were tall and cylindrical; lying perpendicular to the surface, surrounding the olfactory receptor cells. Their large, spherical, lightly stained nuclei were observed superficial to the nuclei of the receptor cells as seen in the haematoxylin and eosin stained sections. Apical projections (microvilli) forming a brush border on the surface of these cells were also observed (Fig. 16).

Statistical analysis of age-related changes in the number of sustentacular cells in males:

ANOVA showed that there was no significant age-related decrease in the number of sustentacular cells among the groups of males of 30-39 years, 40-49 years and 50-59 years ($P > 0.05$) respectively. Post-Hoc test, using the Tukey HSD, also showed that this difference was not statistically significant ($P > 0.05$) (Table 5) among these age groups (Group I Mean = 71.36, SE = 0.4375; Group II Mean = 70.25, SE = 0.4204; Group III Mean = 69.50, SE = 0.3902; Group IV Mean = 66.38, SE = 0.3447). However, there was a significant decrease in the number of sustentacular cells among the groups of males of 50-59 years and 60 years onwards ($P < 0.001$).

Table 5. Multiple Comparison of Number of Sustentacular Cells among the Age Groups of Males.

Comparison among age groups		Mean Difference (I-J)	Std. Error (SE)	Level of Significance (P-value)
Age group compared (I)	Age group compared with (J)			
30-39 years	40-49 years	1.110	0.5653	0.221
	50-59 years	1.86	0.5653	0.012
	60 years onwards	4.98	0.5653	0.001
40-49 years	30-39 years	-1.11	0.5653	0.221
	50-59 years	0.75	0.5653	0.552
	60 years onwards	3.87	0.5653	0.001
50-59 years	30-39 years	-1.86	0.5653	0.012
	40-49 years	-0.75	0.5653	0.552
	60 years onwards	3.12	0.5653	0.001
60 years onwards	30-39 years	-4.98	0.5653	0.001
	40-49 years	-3.87	0.5653	0.001
	50-59 years	-3.12	0.5653	0.001

Statistical analysis of age-related changes in the number of sustentacular cells in females:

ANOVA showed that there was no significant age-related decrease in the number of sustentacular cells among the groups of females of 30-39 years, 40-49 years and 50-59 years ($P > 0.05$) respectively. Post-Hoc test, using the Tukey HSD, also showed that this difference was not statistically significant ($P > 0.05$) (Table 6) among these age groups (Group V Mean = 72.44, SE = 0.3933; Group VI Mean = 71.37, SE = 0.3867; Group VII Mean = 69.92, SE = 0.3589; Group VIII Mean = 67.34, SE = 0.4438). However, there was a significant decrease in the number of sustentacular cells among the groups of females of 50-59 years and 60 years onwards ($P < 0.001$).

Table 6. Multiple Comparison of Number of Sustentacular Cells among the Age Groups of Females.

Comparison among age groups		Mean Difference (I-J)	Std. Error (SE)	Level of Significance (P-value)
Age group compared (I)	Age group compared with (J)			
30-39 years	40-49 years	1.07	0.5613	0.243
	50-59 years	2.52	0.5613	0.001
	60 years onwards	5.10	0.5613	0.001
40-49 years	30-39 years	-1.07	0.5613	0.243
	50-59 years	1.45	0.5613	0.064
	60 years onwards	4.03	0.5613	0.001
50-59 years	30-39 years	-2.52	0.5613	0.001
	40-49 years	-1.45	0.5613	0.064
	60 years onwards	2.58	0.5613	0.001
60 years onwards	30-39 years	-5.10	0.5613	0.001
	40-49 years	-4.03	0.5613	0.001
	50-59 years	-2.58	0.5613	0.001

Statistical analysis of gender related differences in the number of sustentacular cells:

The analysis of gender related differences was calculated using the independent sample t-test between the corresponding male and female groups. Comparison of age groups 30-39 years showed that although the females had greater number of sustentacular cells as compared to the males (Mean Difference = 1.08) the difference was not significant (p-value > 0.05). The same results were obtained for comparison of age groups 40-49 years, 50-59 years and 60 year onwards (Mean Difference = 1.12, p-value > 0.05; Mean Difference = 0.42, p-value > 0.05; Mean Difference = 0.96, p-value > 0.05) respectively (Table 7).

Table 7. Comparison of Number of Sustentacular Cells among the Age Groups of Males and Females.

Age group	Mean (Males)	Mean (Females)	Standard Error	t-score	p-value
30-39 years	71.36	72.44	0.58	-1.836	0.083
40-49 years	70.25	71.37	0.57	-1.961	0.066
50-59 years	69.50	69.92	0.53	-0.792	0.439
60 years onwards	66.38	67.34	0.56	-1.708	0.105

Basal cells:

These short and broad cells were seen disposed along the deeper part of the epithelium, resting on the basement membrane and did not reach the apical surface. In haematoxylin and eosin stained sections, the nuclei of these cells were oval to round, some of them were stained lightly while others appeared deeply stained. The shape of some of the cells was polygonal with darkly stained cytoplasm; however some of them were rounded in appearance with pale cytoplasm (Fig. 16).

Statistical analysis of age-related changes in the number of basal cells in males:

ANOVA showed that there was no significant age-related decrease in the number of basal cells among the groups of males ($P > 0.05$). Post-Hoc test, using the Tukey HSD, also showed that this difference was not statistically significant ($P > 0.05$) (Table 8) among the age groups (Group I Mean = 140.39, SE = 0.4076; Group II Mean = 139.96, SE = 0.4888; Group III Mean = 140.56, SE = 0.4354; Group IV Mean = 140.33, SE = 0.4731).

Table 8. Multiple Comparison of Number of Basal Cells among the Age Groups of Males.

Comparison among age groups		Mean Difference (I-J)	Std. Error (SE)	Level of Significance (P-value)
Age group compared (I)	Age group compared with (J)			
30-39 years	40-49 years	0.43	0.6397	0.907
	50-59 years	-0.17	0.6397	0.993
	60 years onwards	0.06	0.6397	1.000
40-49 years	30-39 years	-0.43	0.6397	0.907
	50-59 years	-0.60	0.6397	0.785
	60 years onwards	-0.37	0.6397	0.938
50-59 years	30-39 years	0.17	0.6397	0.993
	40-49 years	0.60	0.6397	0.785
	60 years onwards	0.23	0.6397	0.984
60 years onwards	30-39 years	-0.06	0.6397	1.000
	40-49 years	0.37	0.6397	0.938
	50-59 years	-0.23	0.6397	0.984

Statistical analysis of age-related changes in the number of basal cells in females:

ANOVA showed that there was no significant age-related decrease in the number of basal cells among the groups of females ($P > 0.05$). Post-Hoc test, using the Tukey HSD, also showed that this difference was not statistically significant ($P > 0.05$)

(Table 9) among the age groups (Group I Mean $\bar{x}$ = 140.12, SE = 0.2715; Group II Mean = 140.89, SE = 0.4632; Group III Mean = 139.58, SE = 0.4816; Group IV Mean = 140.04, SE = 0.3896).

Table 9. Multiple Comparison of Number of Basal Cells among the Age Groups of Females.

Comparison among age groups		Mean Difference (I-J)	Std. Error (SE)	Level of Significance (P-value)
Age group compared (I)	Age group compared with (J)			
30-39 years	40-49 years	-0.77	0.5797	0.551
	50-59 years	0.54	0.5797	0.778
	60 years onwards	0.08	0.5797	0.999
40-49 years	30-39 years	0.77	0.5797	0.551
	50-59 years	1.31	0.5797	0.127
	60 years onwards	0.85	0.5797	0.468
50-59 years	30-39 years	-0.54	0.5797	0.778
	40-49 years	-1.31	0.5797	0.127
	60 years onwards	-0.46	0.5797	0.857
60 years onwards	30-39 years	-0.08	0.5797	0.999
	40-49 years	-0.85	0.5797	0.468
	50-59 years	-0.46	0.5797	0.857

Statistical analysis of gender related differences in the number of basal cells:

The analysis of gender related differences was calculated using the independent sample t-test between the corresponding male and female groups. Comparison of age groups 30-39 years, 50-59 years and 60 years onwards showed that although the males had greater number of basal cells as compared to the females (Mean Difference = 0.27; Mean Difference = 0.98; Mean Difference = 0.29) respectively the difference was not significant (p-value > 0.05). However comparison of age groups 40-49 years showed that

females had more number of basal cells as compared to males but the difference was not significant (Mean Difference = 0.93, p-value > 0.05) (Table 10).

Table 10. Comparison of Number of Basal Cells among the Age Groups of Males and Females.

Age group	Mean (Males)	Mean (Females)	Standard Error	t-score	p-value
30-39 years	140.39	140.12	0.48	0.551	0.588
40-49 years	139.96	140.89	0.67	-1.381	0.184
50-59 years	140.56	139.58	0.64	1.509	0.149
60 years onwards	140.33	140.04	0.61	0.473	0.642

Microvillar cells:

These cells were quite distinct. In haematoxylin and eosin stained sections, large nuclei were observed close to the epithelial surface. The cells appeared pear-shaped having a clear cytoplasm. Microvilli were present on the apical surface of these cells. The number of microvillar cells was markedly less when compared to the other cells of the epithelium (Fig. 16).

Basement membrane:

In routine H & E sections, the basement membrane appeared as a well-defined homogenous structure (Figs. 7, 9), lying against the under surface of the epithelial cells becoming more prominent in PAS stained sections (Figs. 10, 17).

Lamina Propria:

The lamina propria was quite thick. It consisted of a framework of collagenous connective tissue containing fibroblasts, lymphocytes and neutrophils (Fig. 18), nerve bundles, blood vessels and serous acini (Fig. 19).

Bowman's glands as well as serous acini were present in all the regions of the lamina propria. The serous acini were almost circular in cross section (Fig. 17). The secretory cells were pyramidal in shape, the apical portion filled with discrete eosinophilic zymogen granules, and encircling a narrow lumen. The darkly stained round nucleus was present in the basal portion of the cell, having a comparatively basophilic cytoplasm. The duct of Bowman's gland was also observed in the lamina propria (Figs. 11, 20). At places, the openings of these ducts were seen at the free surface of the olfactory mucosa, traversing the entire length of the epithelium (Fig. 21). In the regions of the olfactory mucosa where respiratory epithelium was present, the lamina propria also showed characteristic features of the respiratory epithelium. Mucous acini with serous demilunes were also observed in such places. They were lined by columnar cells containing faint eosinophilic cytoplasm, which was vacuolated at places. Their nuclei were flat and pushed peripherally (Fig. 22).

Nerve fibres (axon of the receptor cells) appeared forming nerve bundles of various thicknesses in the lamina propria. The nerve fibres were also seen traversing the bone in the area of the roof of the nasal cavity (Fig. 23).

Statistical analysis of age-related changes in the thickness of lamina propria in males:

ANOVA showed that there was no significant age-related change in the thickness of lamina propria among the groups of males ($P > 0.05$). Post-Hoc test, using the Tukey HSD, also showed that this difference was statistically not significant ($P > 0.05$) (Table 11) among all the male groups. (Group I Mean = 142.8, SE = 0.611; Group II Mean = 141.9, SE = 0.547; Group III Mean = 141.2, SE = 0.629; Group IV Mean = 141.5, SE = 0.637).

Table 11. Multiple Comparison of the Thickness of Lamina Propria among the Age Groups of Males.

Comparison among age groups		Mean Difference (I-J)	Std. Error (SE)	Level of Significance (P-value)
Age group compared (I)	Age group compared with (J)			
30-39 years	40-49 years	0.90	0.858	0.722
	50-59 years	1.60	0.858	0.261
	60 years onwards	1.30	0.858	0.440
40-49 years	30-39 years	-0.90	0.858	0.722
	50-59 years	0.70	0.858	0.847
	60 years onwards	0.40	0.858	0.966
50-59 years	30-39 years	-1.60	0.858	0.261
	40-49 years	-0.70	0.858	0.847
	60 years onwards	-0.30	0.858	0.985
60 years onwards	30-39 years	-1.30	0.858	0.440
	40-49 years	-0.40	0.858	0.966
	50-59 years	0.30	0.858	0.985

Statistical analysis of age-related changes in the thickness of lamina propria in females:

ANOVA showed that there was no significant age-related change in the thickness of lamina propria among the groups of females ($P > 0.05$). Post-Hoc test, using the Tukey HSD, also showed that this difference was statistically not significant ($P > 0.05$) (Table 12) among all the female groups (Group I Mean = 142.1, SE = 0.605; Group II Mean = 141.7, SE = 0.423; Group III Mean = 140.4, SE = 0.476; Group IV Mean = 140.4, SE = 0.600).

Table 12. Multiple Comparison of the Thickness of Lamina Propria among the Age Groups of Females.

Comparison among age groups		Mean Difference (I-J)	Std. Error (SE)	Level of Significance (P-value)
Age group compared (I)	Age group compared with (J)			
30-39 years	40-49 years	0.40	0.752	0.951
	50-59 years	1.70	0.752	0.127
	60 years onwards	1.70	0.752	0.127
40-49 years	30-39 years	-0.4	0.752	0.951
	50-59 years	1.30	0.752	0.324
	60 years onwards	1.30	0.752	0.324
50-59 years	30-39 years	-1.70	0.752	0.127
	40-49 years	-1.30	0.752	0.324
	60 years onwards	0.00	0.752	1.000
60 years onwards	30-39 years	-1.70	0.752	0.127
	40-49 years	-1.30	0.752	0.324
	50-59 years	0.00	0.752	1.000

Statistical analysis of gender related differences in the thickness of lamina propria:

The analysis of gender related differences was calculated using the independent sample ŧtest between the corresponding male and female groups. Comparison of age groups 30-39 years, 40-49 years, 50-59 years and 60 years onwards showed that although the males had increased thickness of lamina propria as compared to the females (Mean Difference = 0.7; Mean Difference = 0.2; Mean Difference = 0.8; Mean Difference = 1.1) respectively the difference was not significant (p-value > 0.05) (Table 13).

Table 13. Comparison of the Thickness of Lamina Propria among the Age Groups of Males and Females.

Age group	Mean (Males)	Mean (Females)	Standard Error	t-score	p-value
30-39 years	142.80	142.10	0.86	0.814	0.426
40-49 years	141.90	141.70	0.69	0.289	0.776
50-59 years	141.20	140.40	0.78	1.014	0.324
60 years onwards	141.50	140.40	0.87	1.257	0.225

Age-related changes in the Mucosa in the age group 40-49 years (male and female groups):

In this age group, morphological changes were evident in some of the specimens. Early age-related changes were observed in both males and females in the shape of occasional short epithelial invaginations and incidence of mucoserous glands instead of pure serous glands. However, the olfactory epithelium appeared quite normal at this stage (Fig. 24). Disturbance of the zonal distribution of the olfactory receptor cells and the sustentacular cells was observed occasionally (Fig. 25).

Age-related changes in the Mucosa in the age group 50-59 years (male and female groups):

Major morphological changes were observed in this group. In some places, a substantial reduction in the number of nuclei (and hence cells) was noted, which resulted in decreased height of the epithelium (Fig. 26). There were changes in the form of reduction in the height of olfactory epithelium, and disturbance of zonal distribution (Fig. 27). The olfactory mucosa was seen in the roof of nasal cavity as a continuous sheet. When viewed over the medial and lateral nasal walls, several patches of respiratory epithelium were found distributed in the olfactory area. Another change observed in this age group was decrease in the height of the olfactory epithelium compared to respiratory

epithelium (Fig. 28). Surface epithelial invaginations of the epithelium into the lamina propria were also found quite frequently in this age group (Fig. 29).

Age-related changes in the Mucosa in age group 60 years onwards (male and female groups):

In this age group, gradual thinning of the olfactory epithelium was seen, with a concomitant loss and derangement of the normal zonal distribution. Mucous acini along with serous acini were present in the lamina propria (Fig. 30). The epithelial invaginations were frequent in the advanced age groups (Fig. 31). In few cases, atrophied olfactory epithelium devoid of olfactory cells was seen (Fig. 32). Olfactory receptors were scarce in this group. Ultimately, in advanced stages the atrophy and decrease in receptor cells led to gradual thinning of the olfactory epithelium (Fig. 33).

Statistical analysis of age-related changes in the height of epithelium in males:

ANOVA showed that there was significant age-related decrease in the height of epithelium among the groups of males ($P < 0.001$). Post-Hoc test, using the Tukey HSD, also showed that this difference was statistically significant ($P < 0.001$) (Table 14) among all the male groups revealing gradual decrease in mean height of epithelium with advancing age (Group I Mean = 53.2, SE = 0.742; Group II Mean = 43.5, SE = 0.671; Group III Mean = 27.2, SE = 0.416; Group IV Mean = 20.4, SE = 0.499). However, the greatest decrease in the height of epithelium was between the male groups of ages 40-49 years and 50-59 years (Mean Difference = 16.3) as compared to age groups 30-39 years and 40-49 years (Mean Difference = 9.7) and age groups 50-59 years and 60 years onwards (Mean Difference = 6.8). These results suggest that there was a significant decrease in the height of epithelium in the male population throughout all ages (Fig. 34).

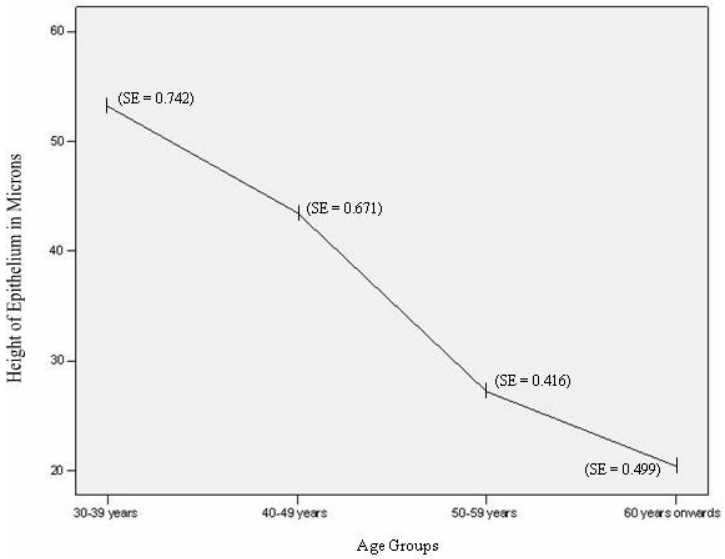

Fig. 34. Mean Height of Epithelium in the Four Age Groups of Males.

Table 14. Multiple Comparison of the Height of Epithelium among the Age Groups of Males.

Comparison among age groups		Mean Difference (I-J)	Std. Error (SE)	Level of Significance (P-value)
Age group compared (I)	Age group compared with (J)			
30-39 years	40-49 years	9.70	0.844	0.001
	50-59 years	26.0	0.844	0.001
	60 years onwards	32.8	0.844	0.001
40-49 years	30-39 years	-9.7	0.844	0.001
	50-59 years	16.3	0.844	0.001
	60 years onwards	23.1	0.844	0.001
50-59 years	30-39 years	-26.0	0.844	0.001
	40-49 years	-16.3	0.844	0.001
	60 years onwards	6.80	0.844	0.001
60 years onwards	30-39 years	-32.8	0.844	0.001
	40-49 years	-23.1	0.844	0.001
	50-59 years	-6.8	0.844	0.001

Statistical analysis of age-related changes in the height of epithelium in females:

ANOVA showed that there was significant age-related decrease in the height of epithelium among the groups of females ($P < 0.001$). Post-Hoc test, using the Tukey HSD, also showed that this difference was statistically significant ($P < 0.001$) (Table 15) among all the female groups revealing gradual decrease in mean height of epithelium with advancing age (Group I Mean = 52.5, SE = 0.619; Group II Mean = 43.9, SE = 0.504; Group III Mean = 26.6, SE = 0.400; Group IV Mean = 20.0, SE = 0.422). However, the greatest decrease in the height of epithelium was between the female groups of ages 40-49 years and 50-59 years (Mean Difference = 16.3) as compared to age groups 30-39 years and 40-49 years (Mean Difference = 9.7) and age groups 50-59 years and 60 years onwards (Mean Difference = 6.8). These results show that there was a significant decrease in the height of epithelium in the female population throughout all ages (Fig. 35).

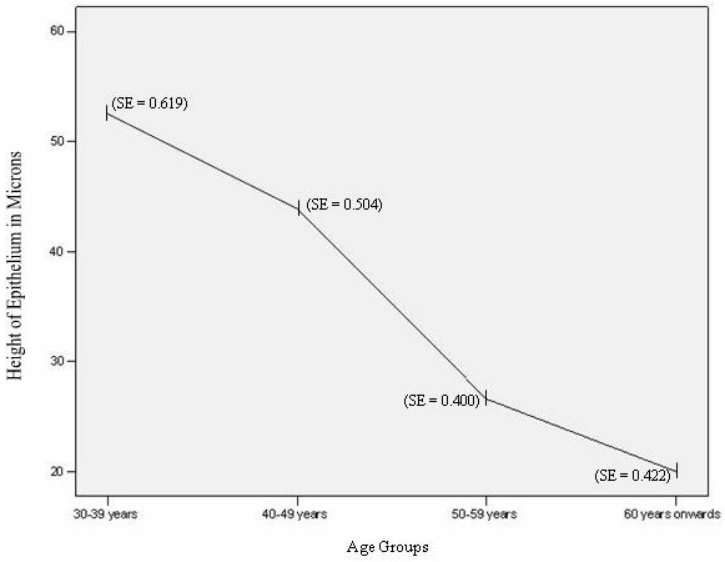

Fig. 35. Mean Height of Epithelium in the Four Age Groups of Females.

Table 15. Multiple Comparison of the Height of Epithelium among the Age Groups of Females.

Comparison among age groups		Mean Difference (I-J)	Std. Error (SE)	Level of Significance (P-value)
Age group compared (I)	Age group compared with (J)			
30-39 years	40-49 years	8.60	0.689	0.001
	50-59 years	25.9	0.689	0.001
	60 years onwards	32.5	0.689	0.001
40-49 years	30-39 years	-8.60	0.689	0.001
	50-59 years	17.3	0.689	0.001
	60 years onwards	23.9	0.689	0.001
50-59 years	30-39 years	-25.9	0.689	0.001
	40-49 years	-17.3	0.689	0.001
	60 years onwards	6.60	0.689	0.001
60 years onwards	30-39 years	-32.5	0.689	0.001
	40-49 years	-23.9	0.689	0.001
	50-59 years	-6.60	0.689	0.001

Statistical analysis of gender related differences in the height of epithelium:

The analysis of gender related differences was calculated using the independent sample t-test between the corresponding male and female groups. Comparison of age groups 30-39 years, 50-59 years and 60 years onwards showed that although the males had increased height of epithelium as compared to the females (Mean Difference = 0.7; Mean Difference = 0.6; Mean Difference = 0.4) respectively the difference was not significant (p-value > 0.05). However, comparison of age groups 40-49 years showed that the females had increased height of epithelium as compared to the males but the difference was not significant (Mean Difference = 0.4, p-value > 0.05) (Table 16).

Table 16. Comparison of the Height of Epithelium among the Age Groups of Males and Females.

Age group	Mean (Males)	Mean (Females)	Standard Error	t-score	p-value
30-39 years	53.20	52.50	0.96	0.724	0.478
40-49 years	43.50	43.90	0.83	-0.477	0.639
50-59 years	27.20	26.60	0.57	1.039	0.312
60 years onwards	20.40	20.00	0.65	0.612	0.548

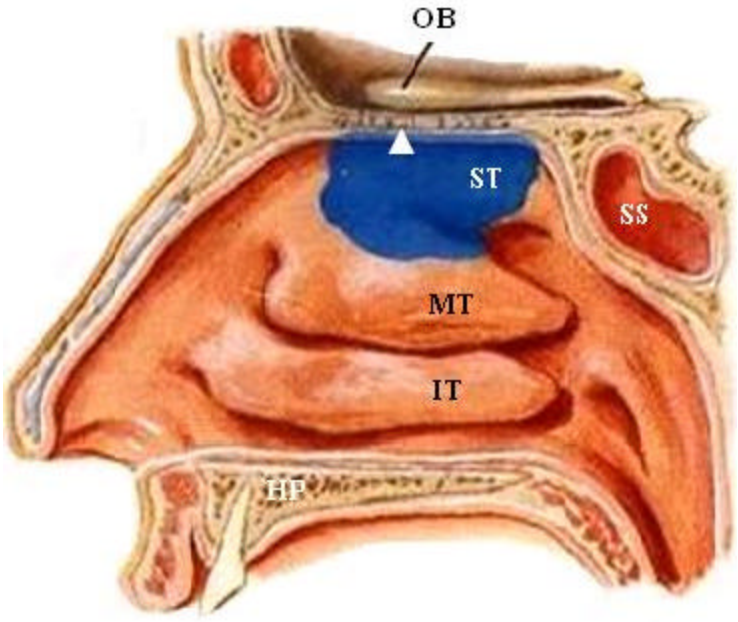

Fig. 1. Location of olfactory area (blue) at the lateral wall of the nose. Photograph showing: the superior turbinate (ST), middle turbinate (MT) and inferior turbinate (IT) at the lateral wall of the nose. Olfactory bulb (OB) lying above the cribriform plate (arrowhead) of the ethmoid bone, sphenoidal air sinus (SS) and hard palate (HP) are also visible. (Netter's Atlas of Human Anatomy, 2003).

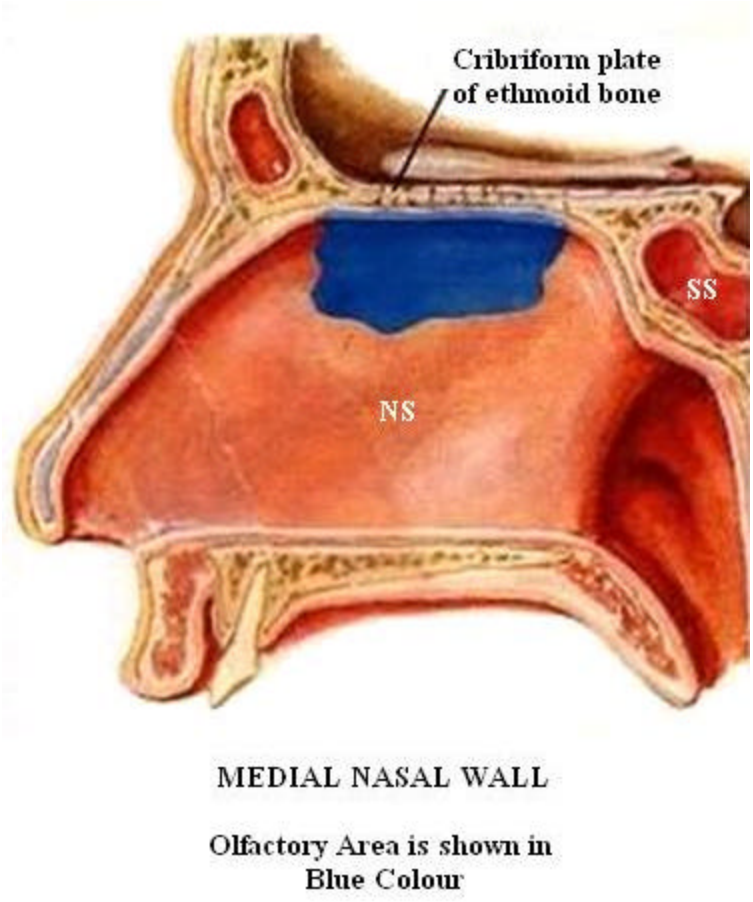

Fig. 2. Location of olfactory area (blue) at the medial wall (Septum) of the nose Photograph showing the nasal septum (NS) and sphenoidal sinus (SS). (Netter's Atlas of Human Anatomy, 2003).

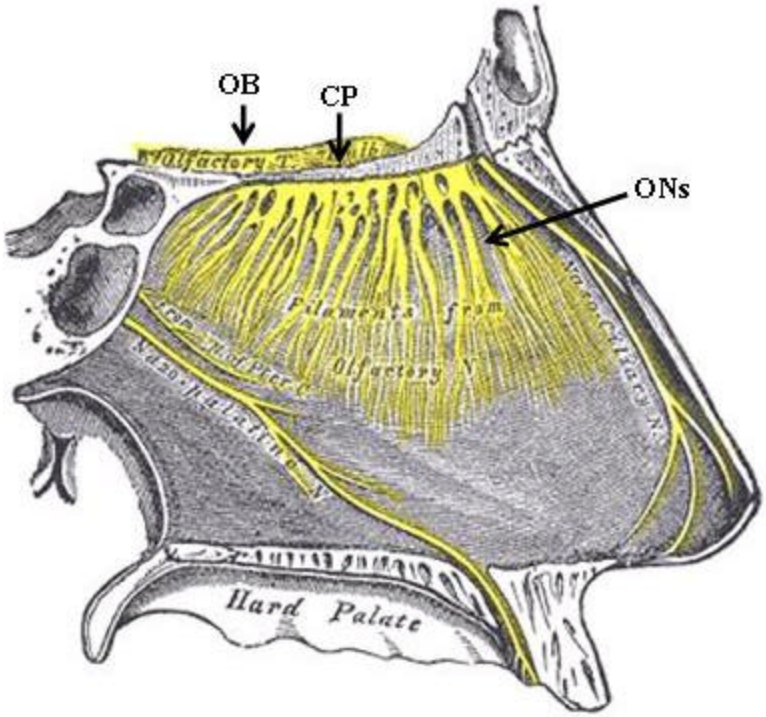

Fig. 3. Olfactory nerves (ONs) passing through the cribriform plate (CP) of the ethmoid bone to enter the olfactory bulb (OB) (Gray's anatomy: The Organ of Smell, 1918, Netter's Atlas of Human Anatomy, 2003).

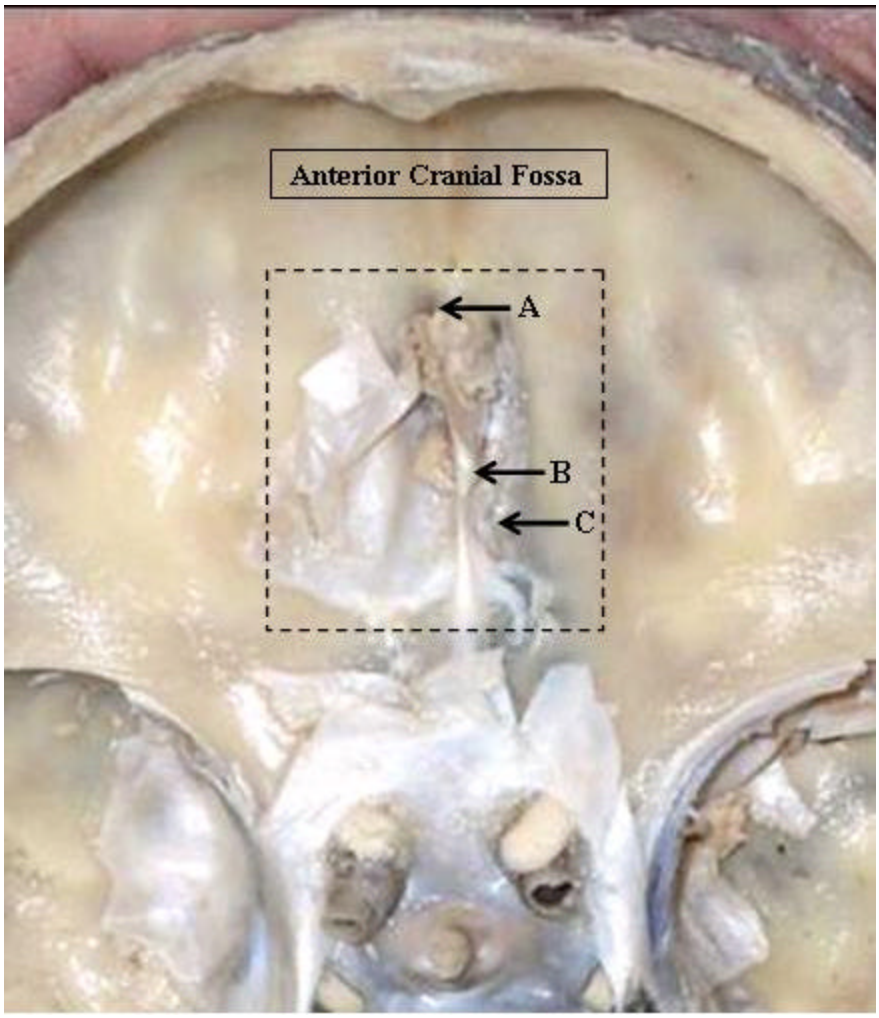

Fig. 4. Olfactory area shown in rectangle containing foramen caecum (A), crista galli (B) and cribriform plate (C) for en-bloc removal of the specimen through the anterior cranial fossa.

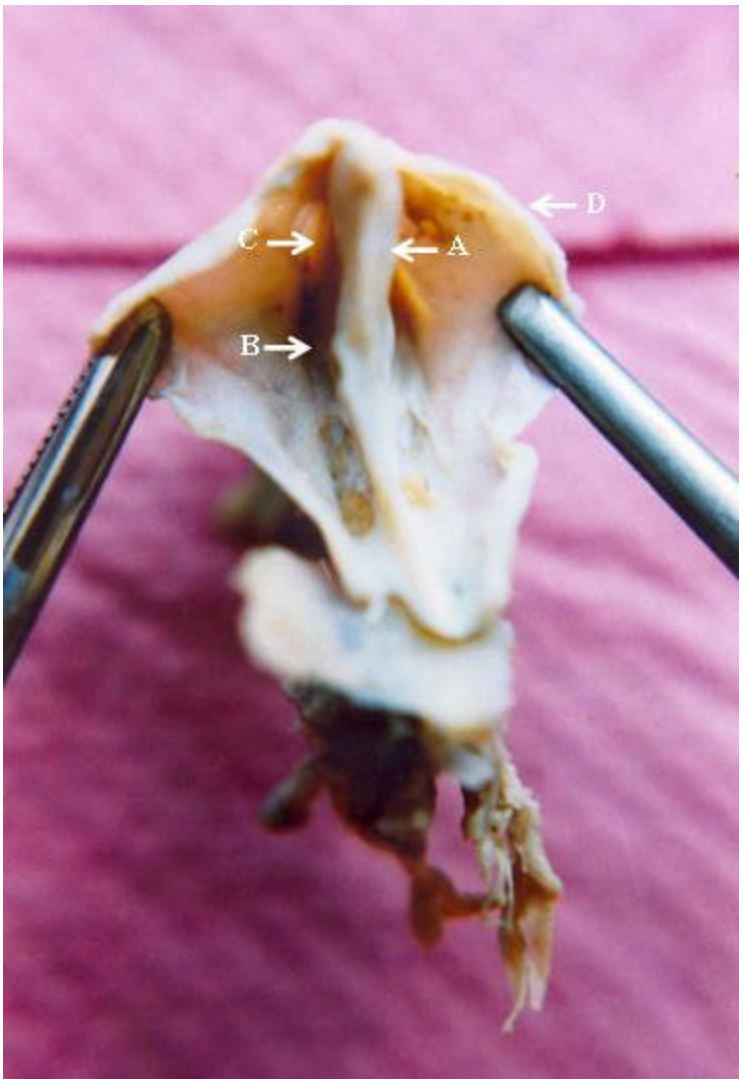

Fig. 5. A photograph of anterior cranial fossa around crista galli (A) showing cribriform plate (B), olfactory bulb (C) and cut edge of duramater (D).

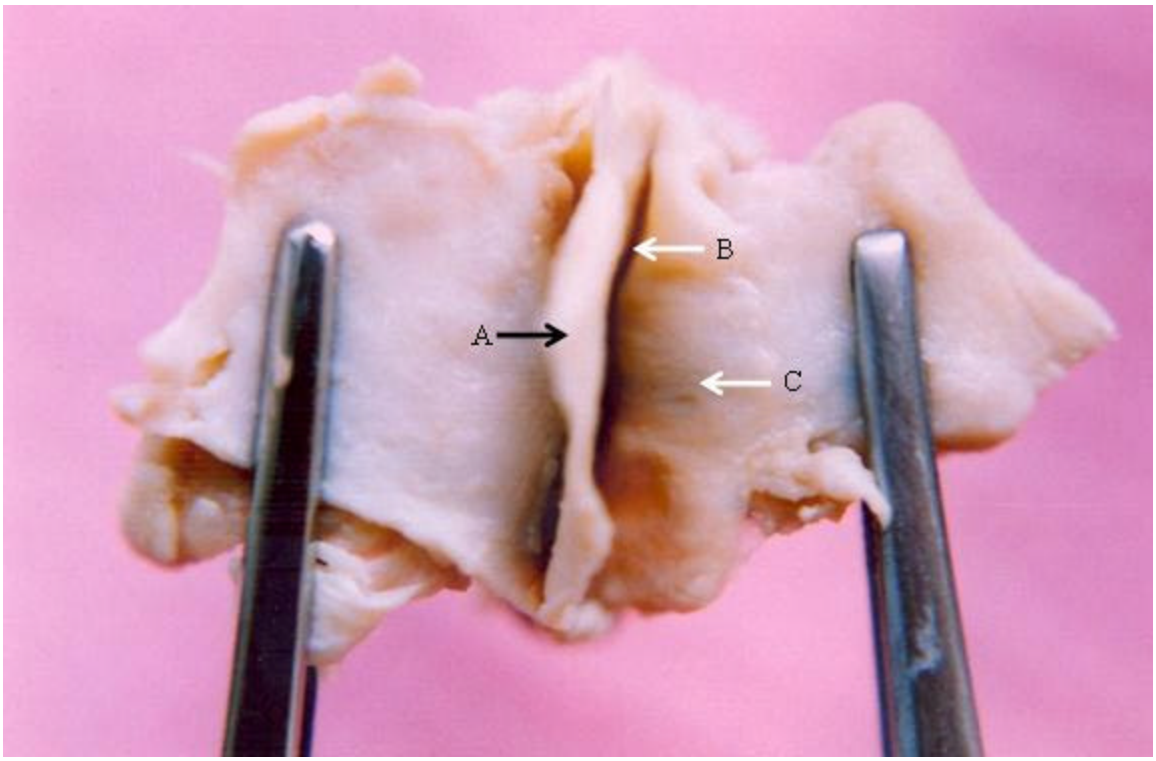

Fig. 6. Photograph of inferior aspect of olfactory part of the nasal cavity; the lateral walls (C) are stretched out showing roof as cribriform plate (B) and nasal septum (A).

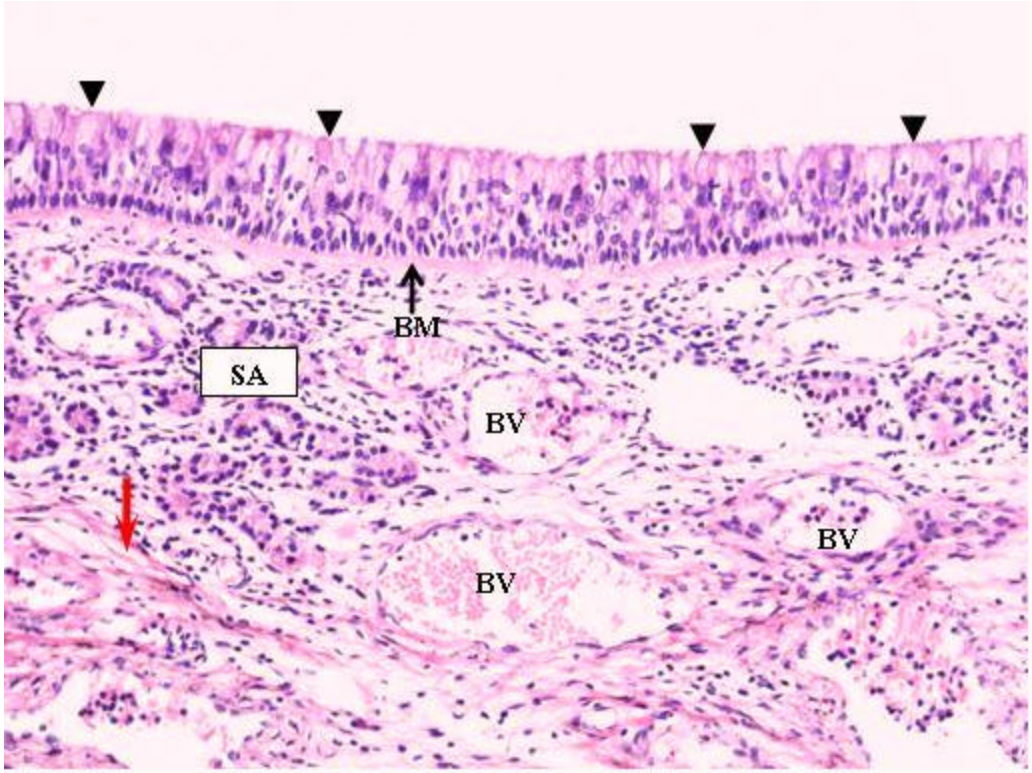

Fig. 7. Photomicrograph of human olfactory mucosa (Gp.I). Pseudostratified olfactory epithelium (Arrowheads) resting on basement membrane (BM). The lamina propria contains serous acini (SA), blood vessels (BV) and collagen fibres (Red arrow). H and E stain. X 200.

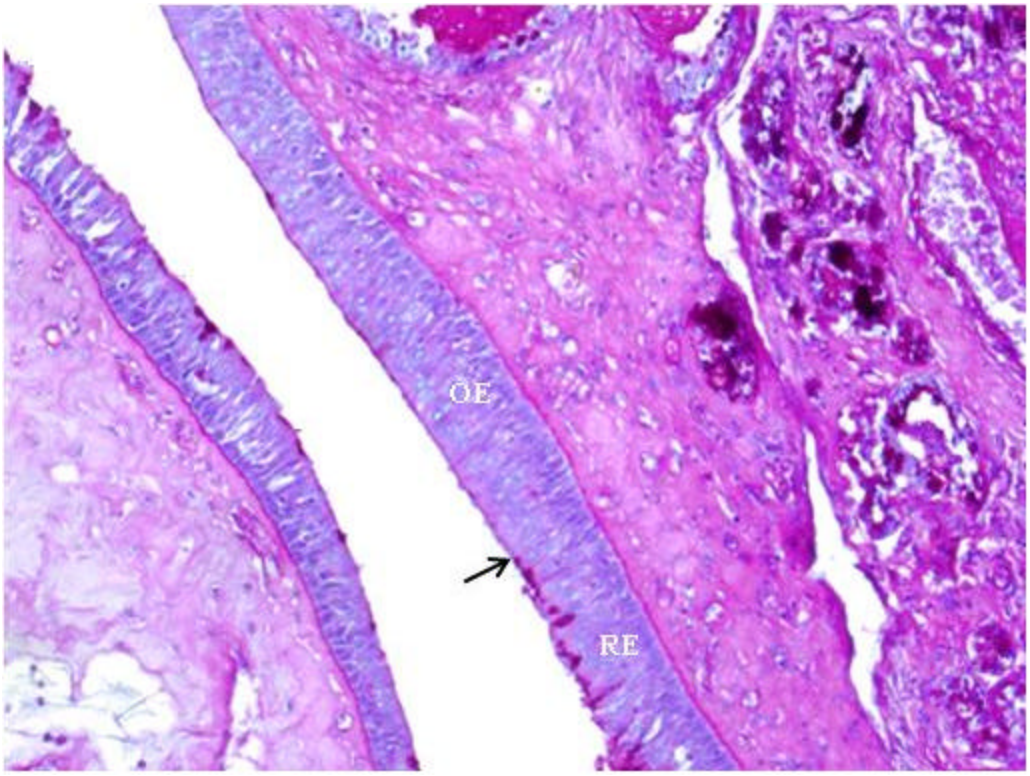

Fig. 8. Photomicrograph of human olfactory mucosa (Gp.I). The transition (Arrow) from the respiratory epithelium (RE) to the olfactory epithelium (OE). The presence of goblet cells (magenta red) is a characteristic feature of respiratory epithelium. PAS stain. X 200.

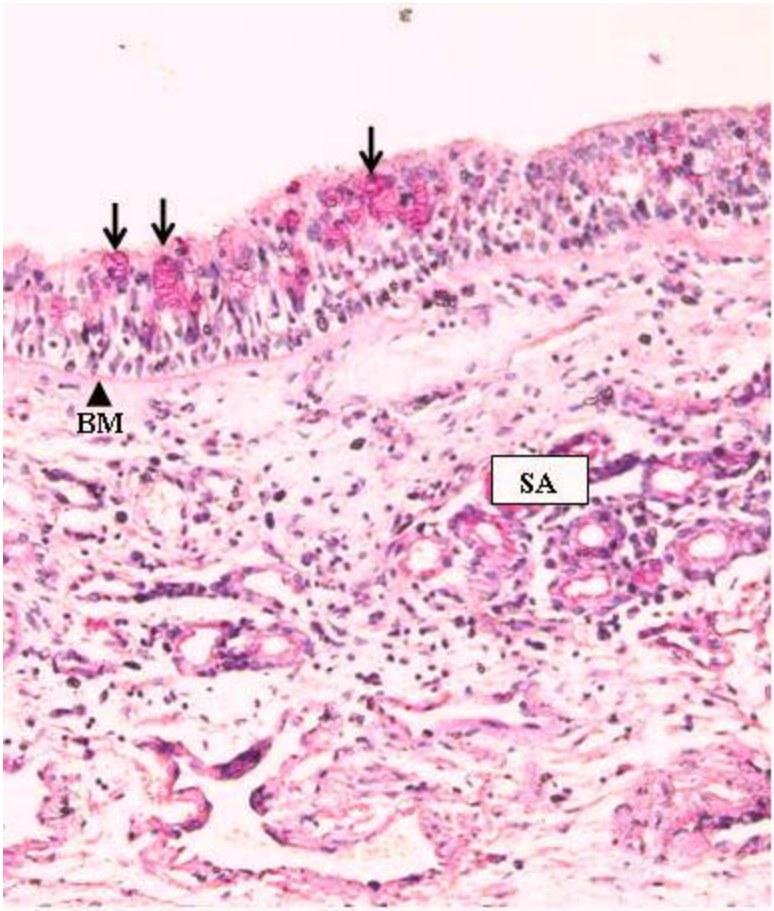

Fig. 9. Photomicrograph of human olfactory mucosa (Gp.I). Respiratory epithelium (left side) containing goblet cells (Arrows) are shown next to the olfactory epithelium (right side). Basement membrane (BM) is seen under the epithelium. In the lamina propria serous acini (SA) are present. PAS stain. X 200.

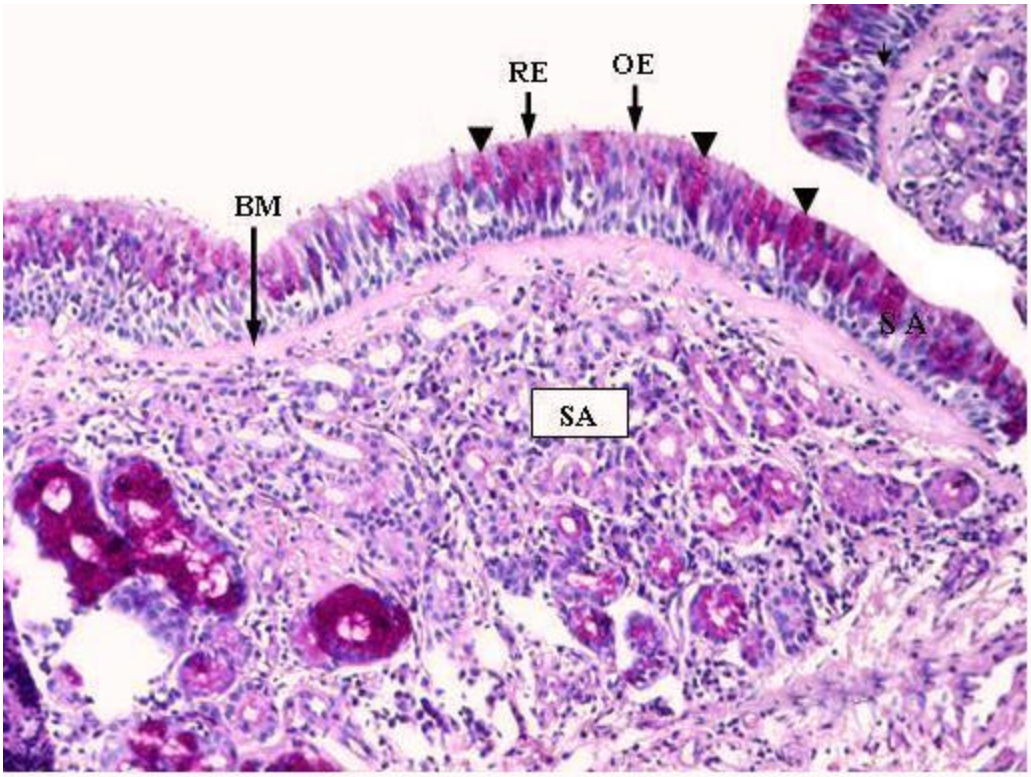

Fig. 10. Photomicrograph of human olfactory mucosa (Gp.V). Patches of respiratory epithelium (RE) characterized by the deeply stained goblet cells (Arrowheads) are seen replacing the olfactory epithelium (OE). Basement membrane (BM) is visible underneath the epithelium. Serous acini (SA) are also seen in the lamina propria. PAS stain. X 200.

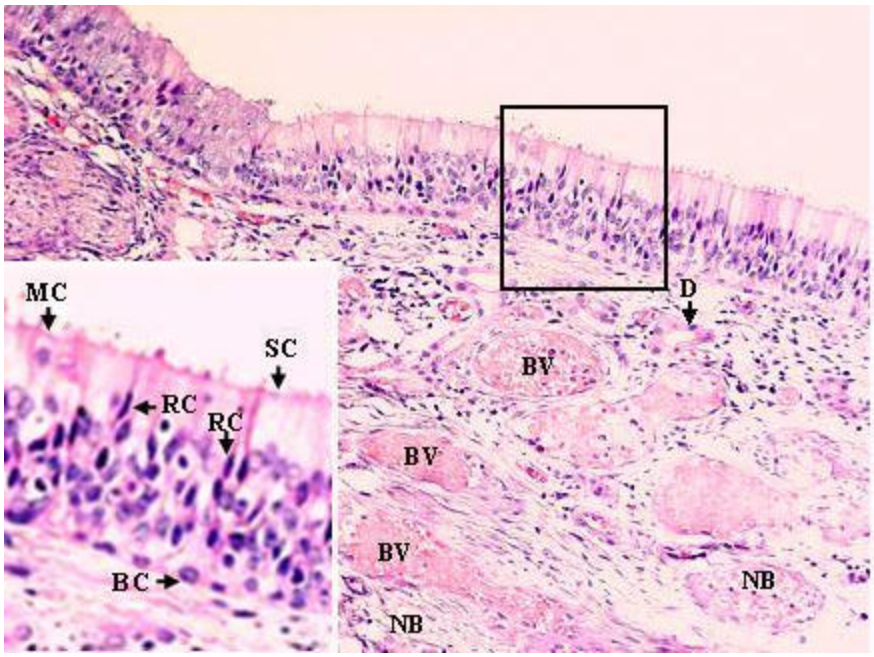

Fig. 11. Photograph of human olfactory mucosa (Gp.I). The olfactory epithelium showing classical cell types. The lamina propria contains nerve bundles (NB), blood vessels (BV) and duct of the Bowman's gland (D), lined by cuboidal epithelium. H and E stain. X 200. Inset showing receptor cell (RC), microvillar cell (MC), sustentacular cell (SC), and basal cell (BC). H and E stain. X 400.

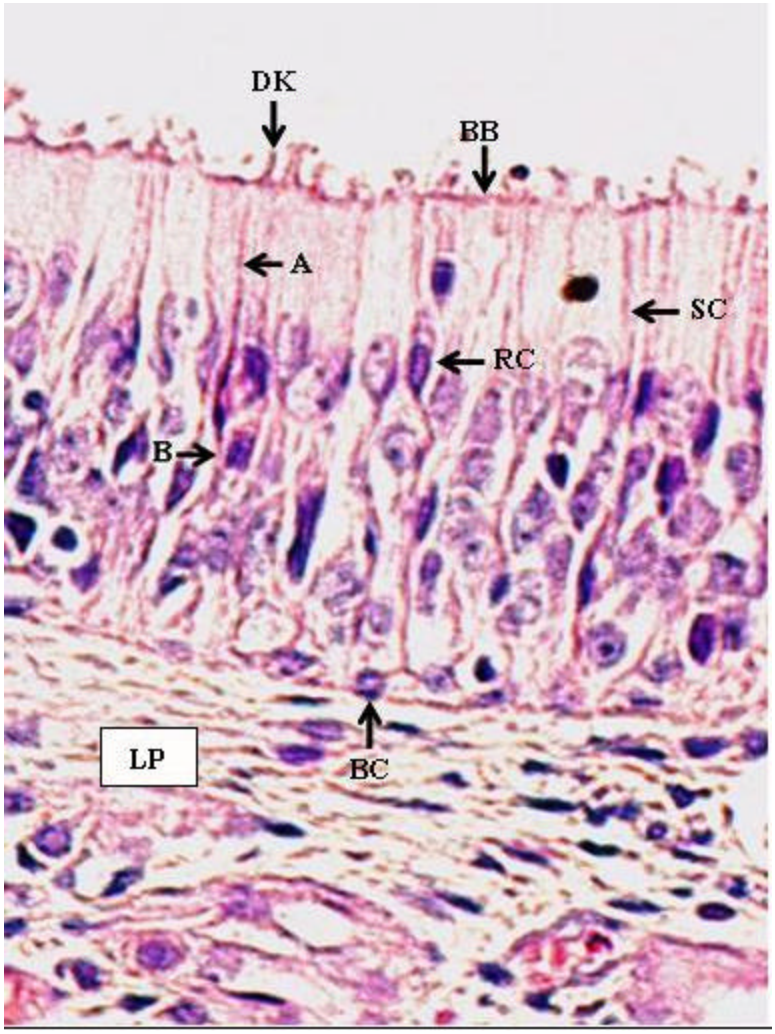

Fig. 12. Photomicrograph of human olfactory mucosa (Gp.I). Darkly stained olfactory receptor cell nuclei (RC) are present at different levels. A dendrite (A) is seen running towards the epithelial surface ending as a dendritic knob (DK). An axon (B) is present at the basal end of the cell body. Also present are: sustentacular cell (SC) having a brush border (BB) at the apical surface and basal cells (BC) lying at the deepest part of epithelium. Lamina propria (LP) lies beneath the epithelium. H and E stain. X 1000.

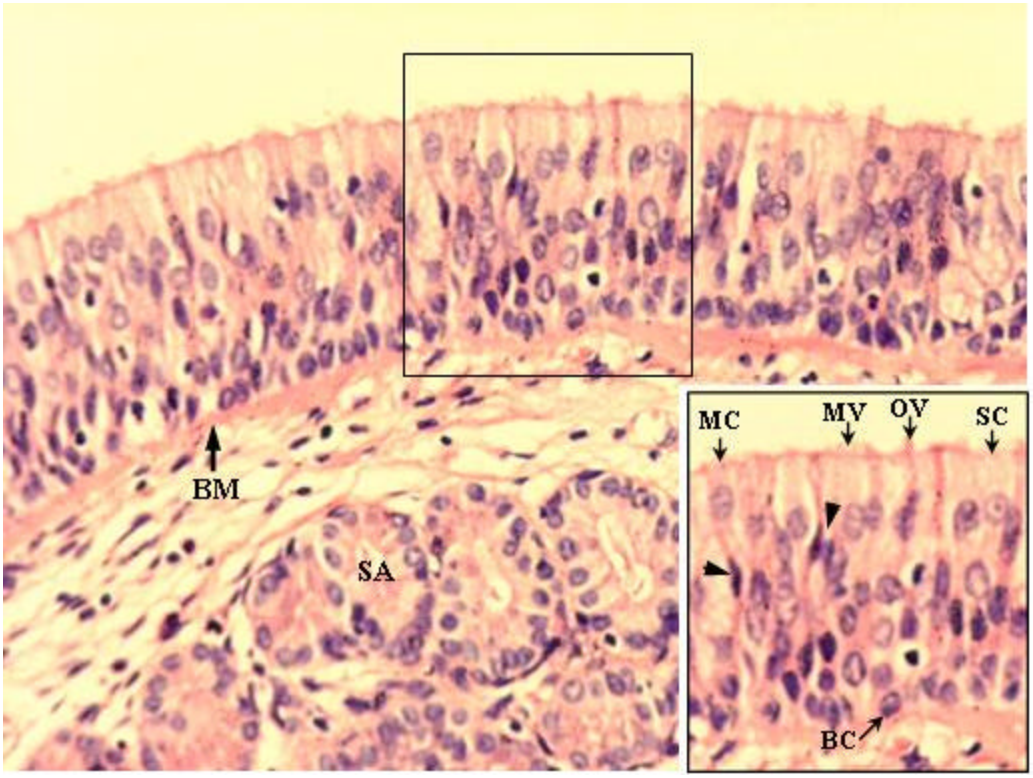

Fig. 13. Photomicrograph of human olfactory mucosa (GP.V). The pseudostratified olfactory epithelium showing distribution of olfactory receptor cells at different levels. Basement membrane (BM) is also visible. Lamina propria is studded with serous acini (SA). Bielschowsky's silver stain. X 400. Inset showing: receptor cell (Arrowheads), sustentacular cell (SC), microvillar cell (MC), and basal cell (BC). The microvilli (MV) and olfactory vesicles (OV) are seen at the apical surface. Bielschowsky's silver stain. X 600.

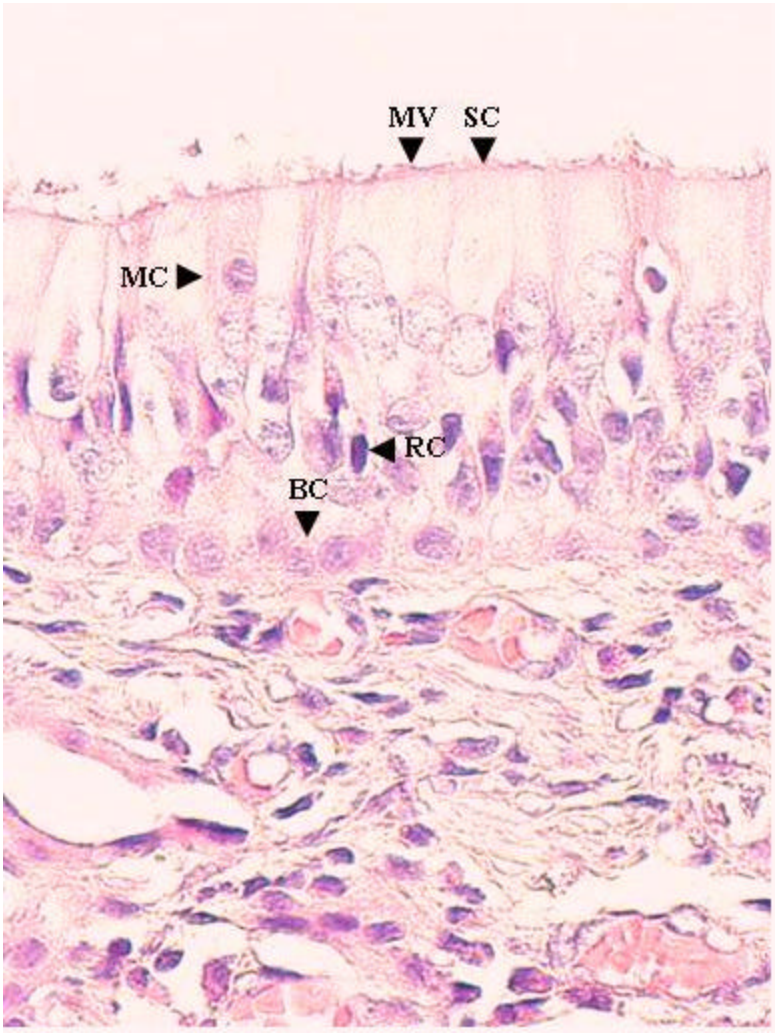

Fig. 16. Photomicrograph of human olfactory mucosa (Gp. I.), showing the four classical cell types, namely: sustentacular cell (SC), microvillar cell (MC), receptor cell (RC), and basal cell (BC). On the apical surface of the epithelium microvilli (MV) are also visible. H and E stain. X 1000.

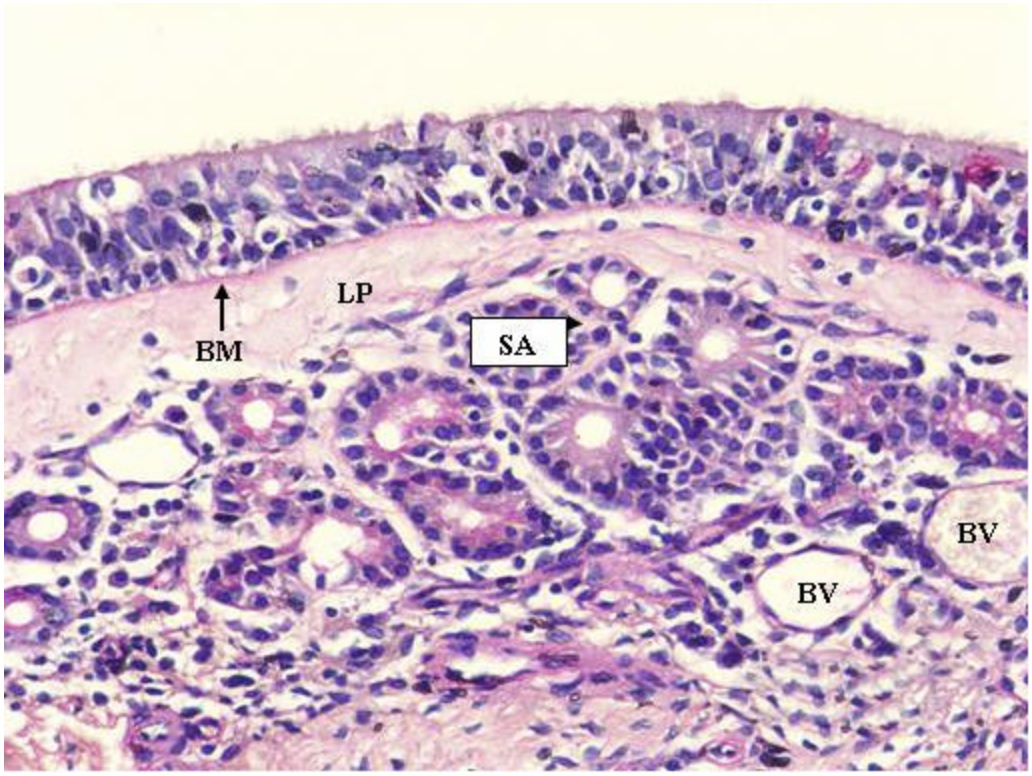

Fig. 17. Photomicrograph of human olfactory mucosa (Gp.I). Distinctly stained basement membrane (BM) is present underneath the olfactory epithelium. In the lamina propria (LP) numerous serous acini (SA) and blood vessels (BV) are seen. PAS stain. X 200.

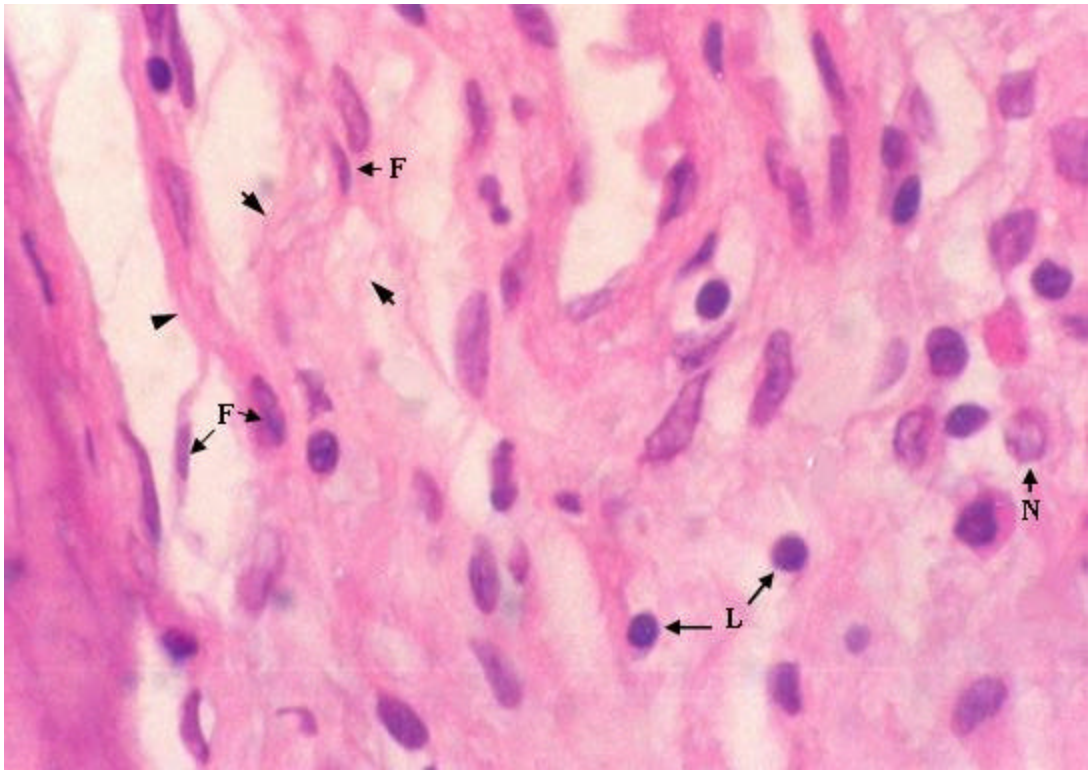

Fig. 18. Photomicrograph of lamina propria of human olfactory mucosa (Gp.I), showing collagenous fibres (Arrowheads), fibroblasts (F), lymphocytes (L) and neutrophils (N). H and E stain. X 1000.

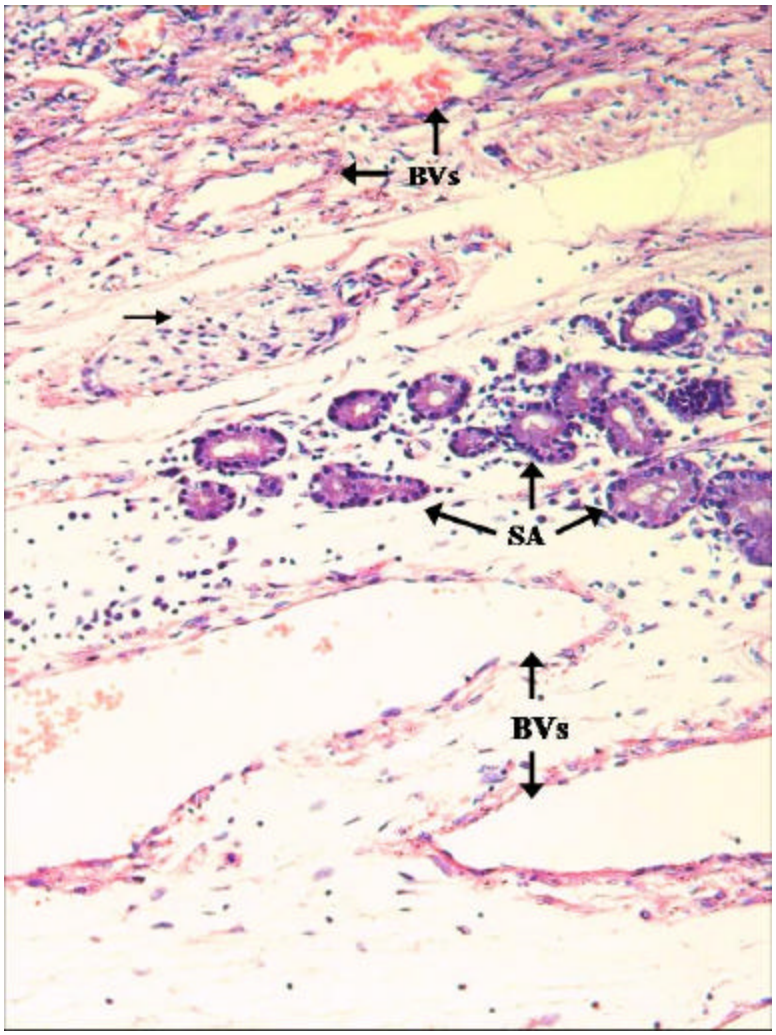

Fig. 19. Photomicrograph of lamina propria of human olfactory mucosa (Gp.I). Many blood vessels (BVs), a nerve bundle (Arrow), and numerous serous acini (SA) are visible. H and E stain. X 200.

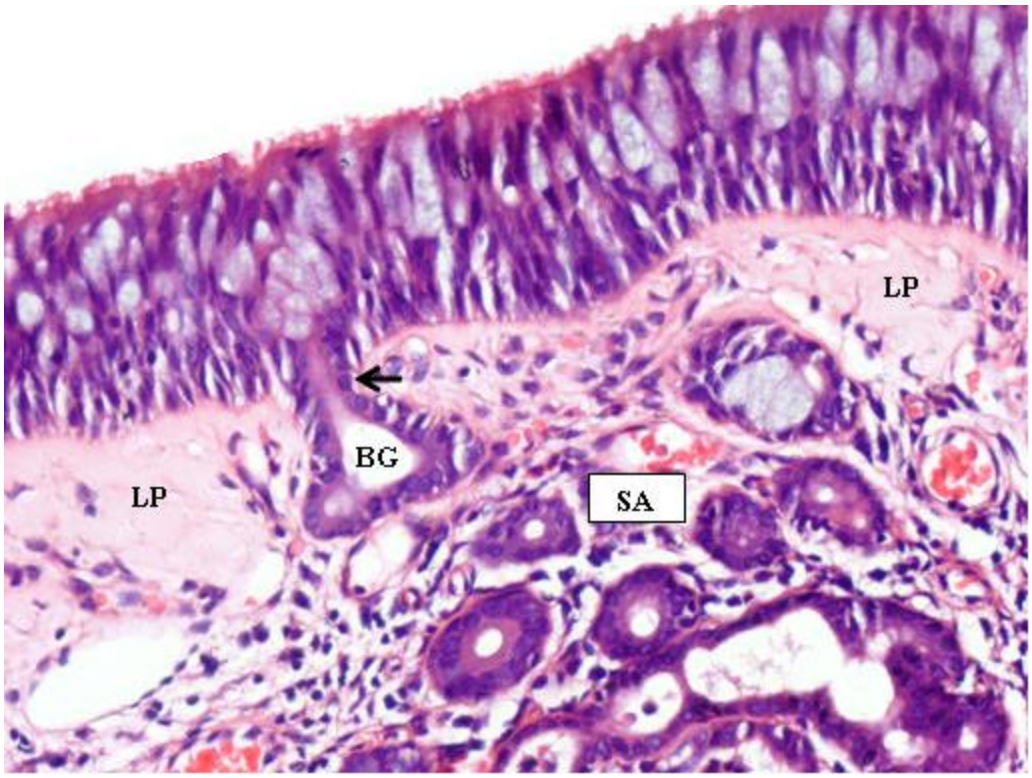

Fig. 20. Photomicrograph of human olfactory mucosa (Gp.V). In the lamina propria (LP) Bowman's gland (BG) and serous acini (SA) are present. A duct (Arrow) lined by cuboidal epithelium can be seen piercing the basement membrane. PAS stain. X 400.

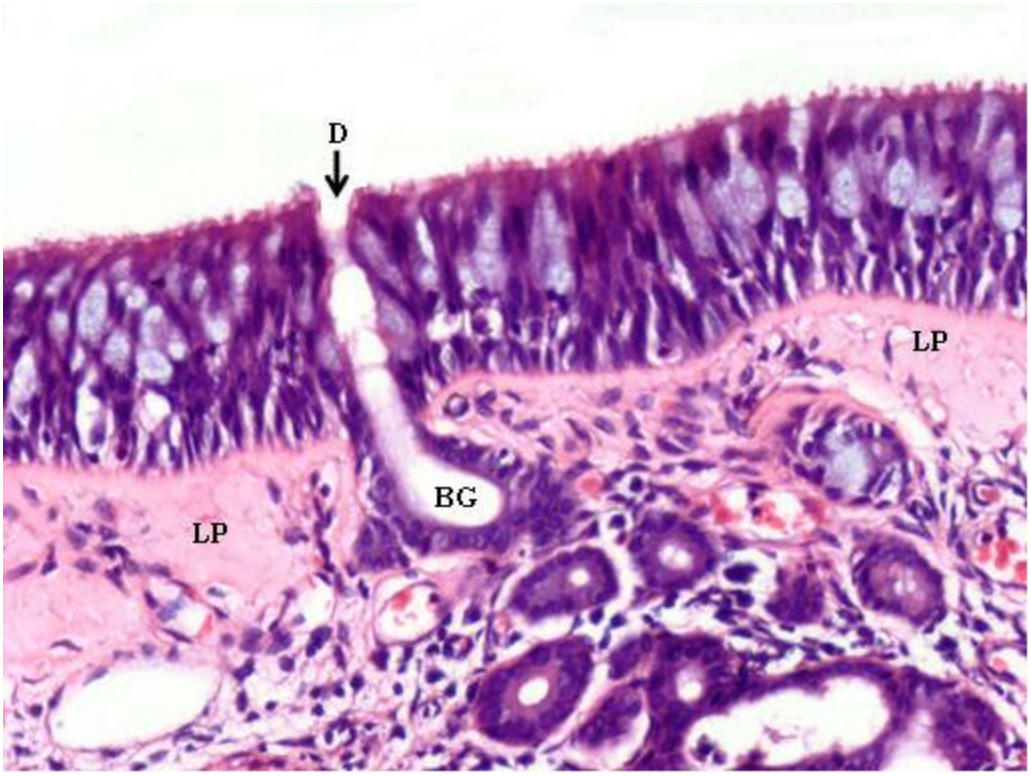

Fig. 21. Photomicrograph of human olfactory mucosa (Gp.V). Bowman's gland (BG) is visible in the lamina propria (LP). Duct (D) of the Bowman's gland can be seen traversing the whole thickness of the olfactory epithelium to open at the apical surface. PAS stain. X 400.

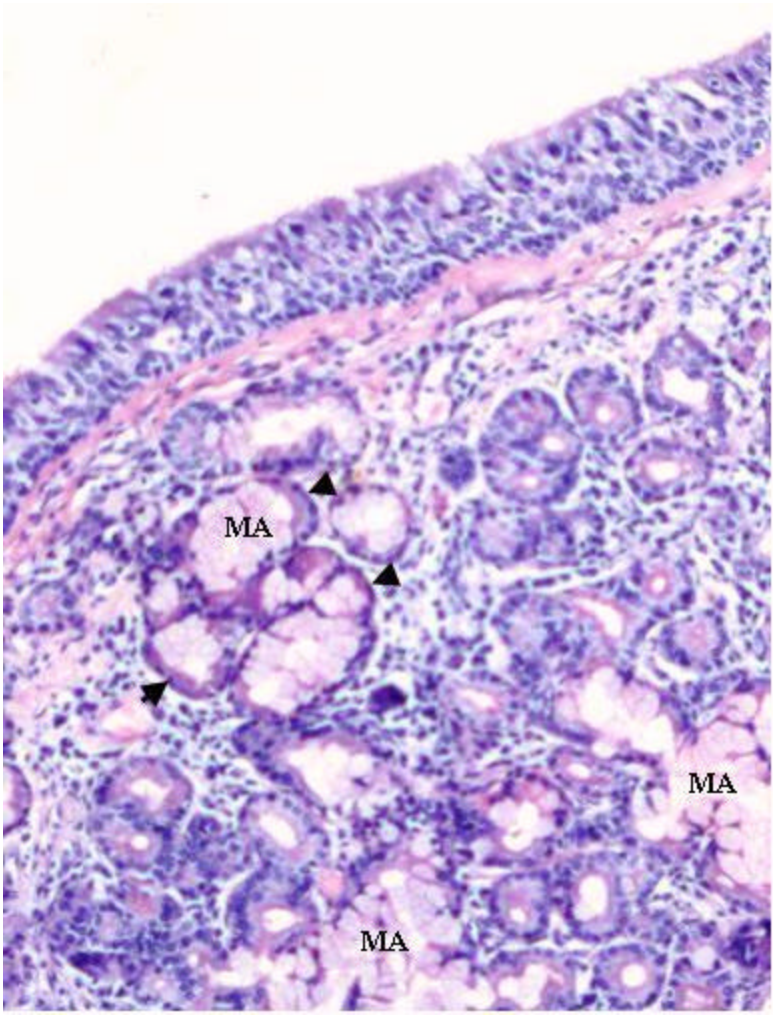

Fig. 22. Photomicrograph of human olfactory mucosa (Gp.V). Pseudostratified olfactory epithelium is seen at the top. Thick lamina propria is showing mucous acini (MA) with serous demilunes (Arrowheads). H and E stain. X 200.

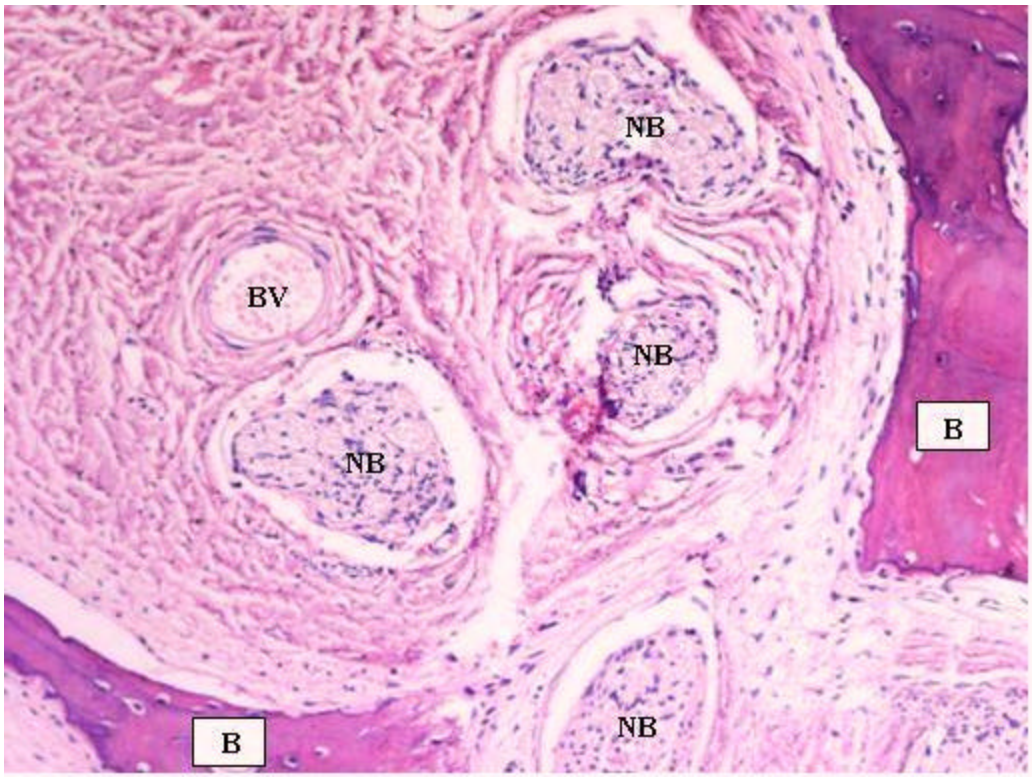

Fig. 23. Photomicrograph of human olfactory mucosa (Gp.I). Many bundles of nerve fibres (NB) can be seen traversing the bone (B) in the roof of the nasal cavity and a blood vessel (BV) is evident. PAS stain. X 400.

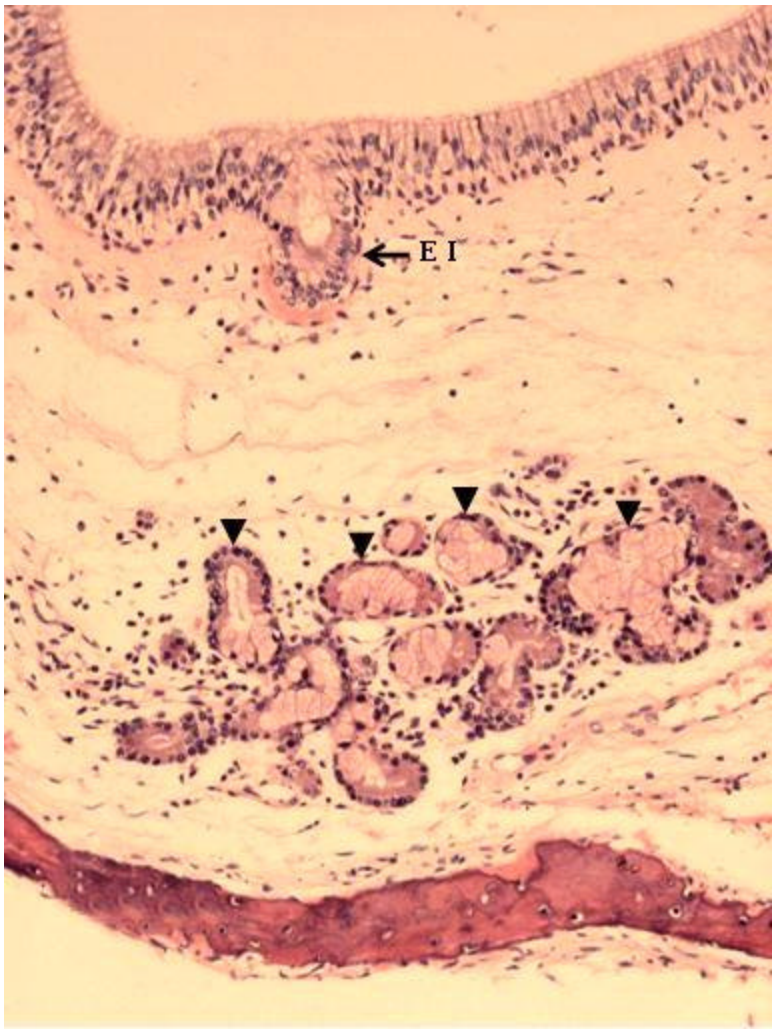

Fig. 24. Photomicrograph of human olfactory mucosa (Gp.VI). Early age related changes showing normal olfactory epithelium with occasional epithelial invagination (EI) and mucoserous glands (Arrowheads) in lamina propria. Bielschowsky's silver stain. X 100.

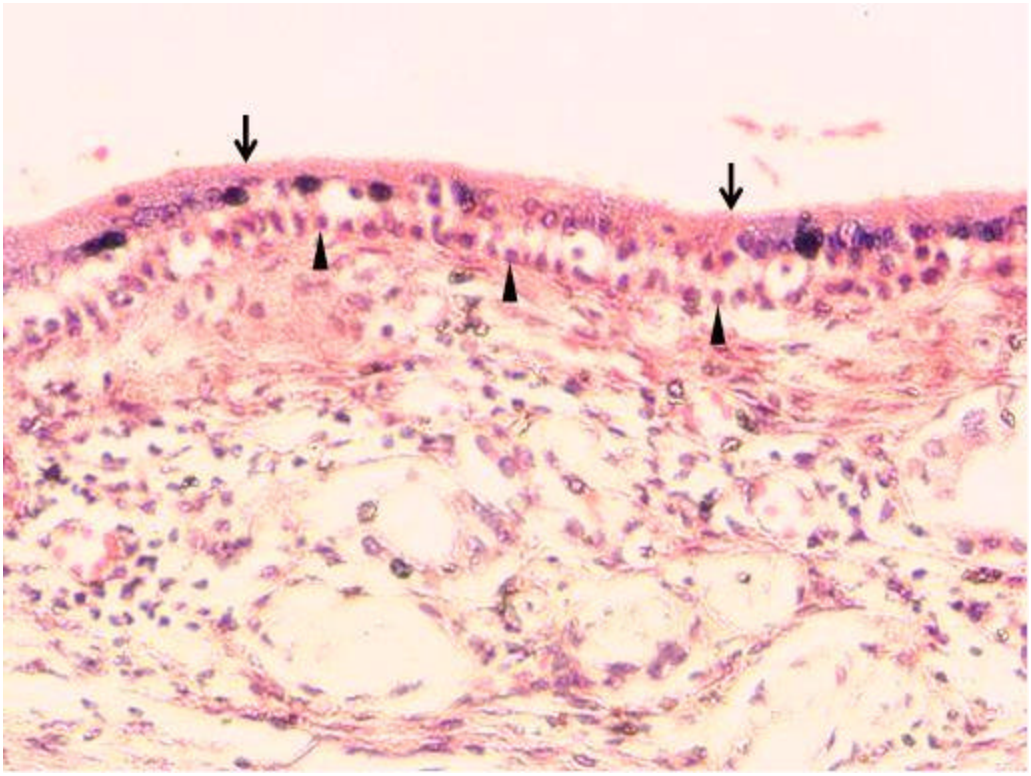

Fig. 25. Photomicrograph of human olfactory mucosa (Gp.II). Disturbance of the zonal distribution of receptor and sustentacular cells in the olfactory epithelium (Arrows). Basal cells (Arrowheads) are seen lying near the basement membrane. Bielschowsky's silver stain. X 200.

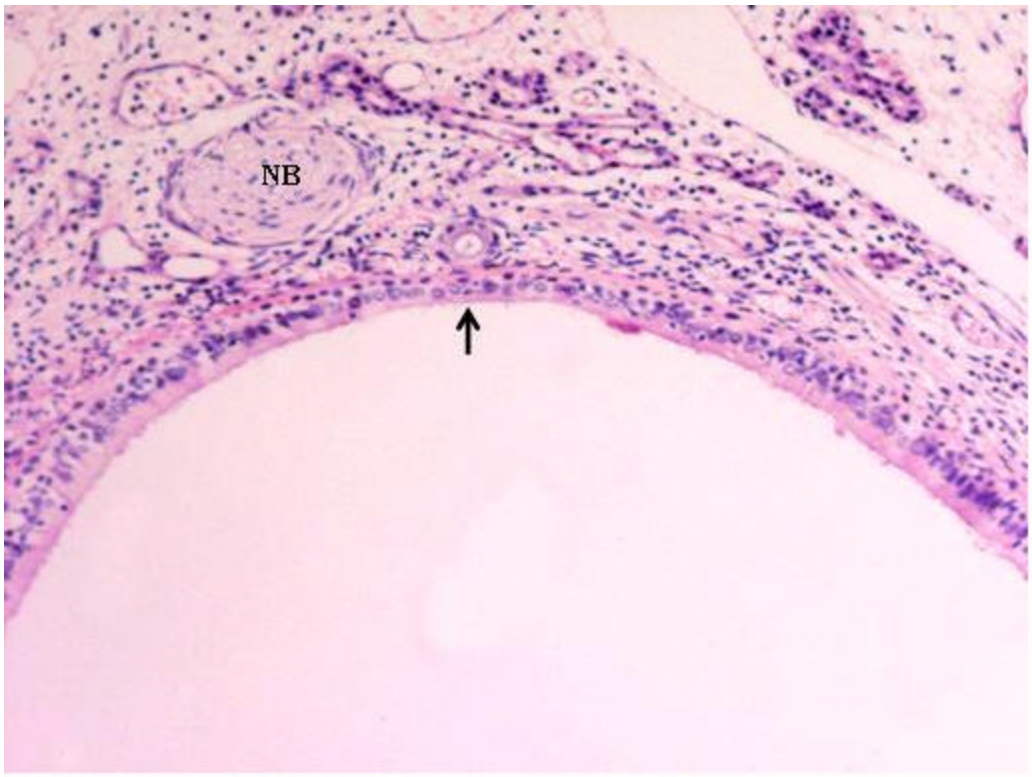

Fig. 26. Photomicrograph of human olfactory mucosa (Gp.III). Age related changes at the roof of the nasal cavity. Thinning (Arrow) of the olfactory epithelium at the roof area of nasal cavity and the loss of normal zonal distribution of sensory and sustentacular cells. A nerve bundle (NB) can be seen in the lamina propria. H and E stain. X 200.

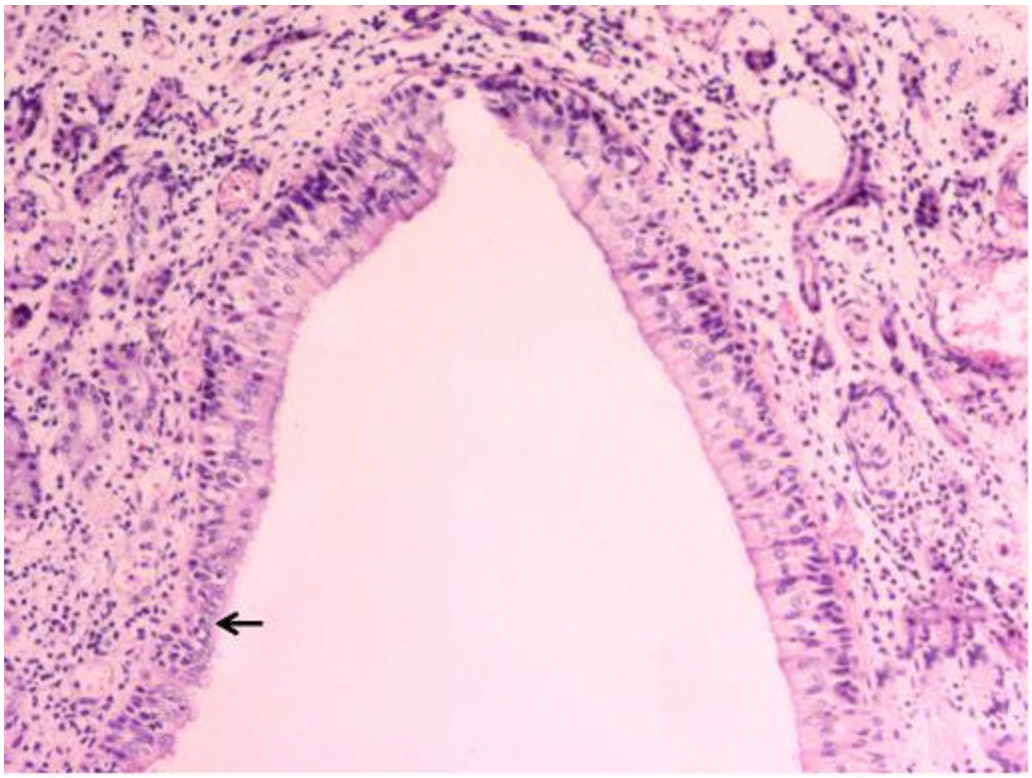

Fig. 27. Photomicrograph of human olfactory mucosa (Gp.III). Morphological changes in the olfactory epithelium. A gradual decrease in the height of epithelium and loss of zonal distribution is evident on the left lateral wall (Arrow). H and E stain. X 200.

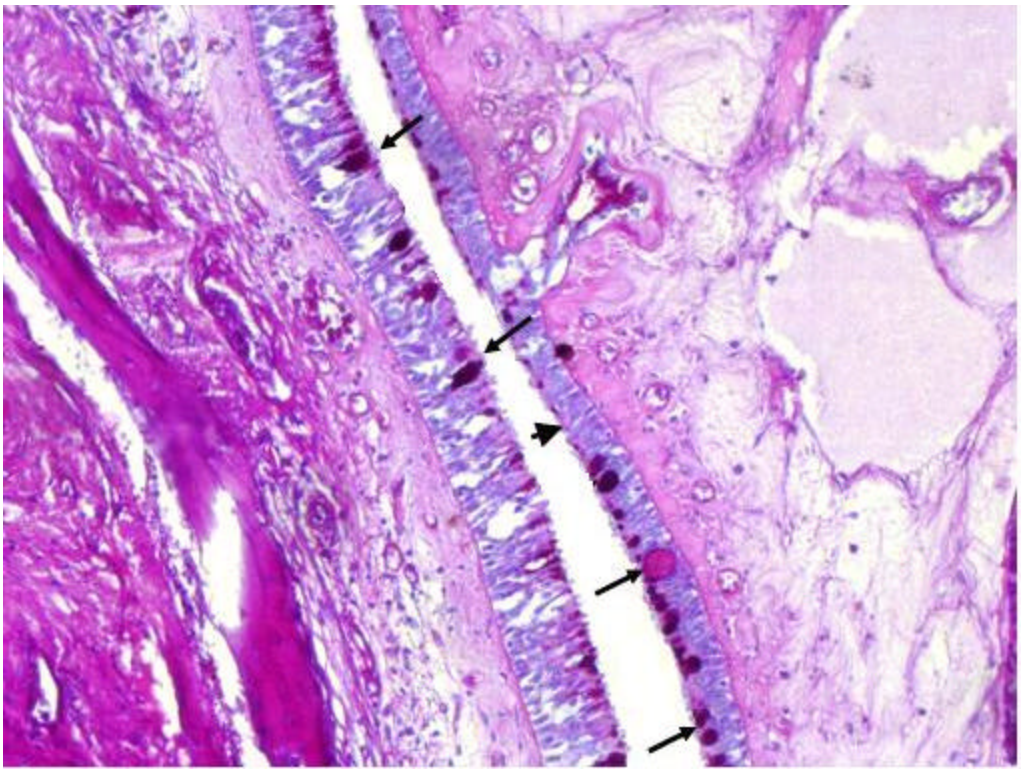

Fig. 28. Photomicrograph of human olfactory mucosa (Gp.VII), showing the olfactory epithelium (Arrowhead) and the respiratory epithelium (arrows) in the same and opposite walls of the nasal cavity. Note the reduced height of the olfactory epithelium. PAS stain. X 200.

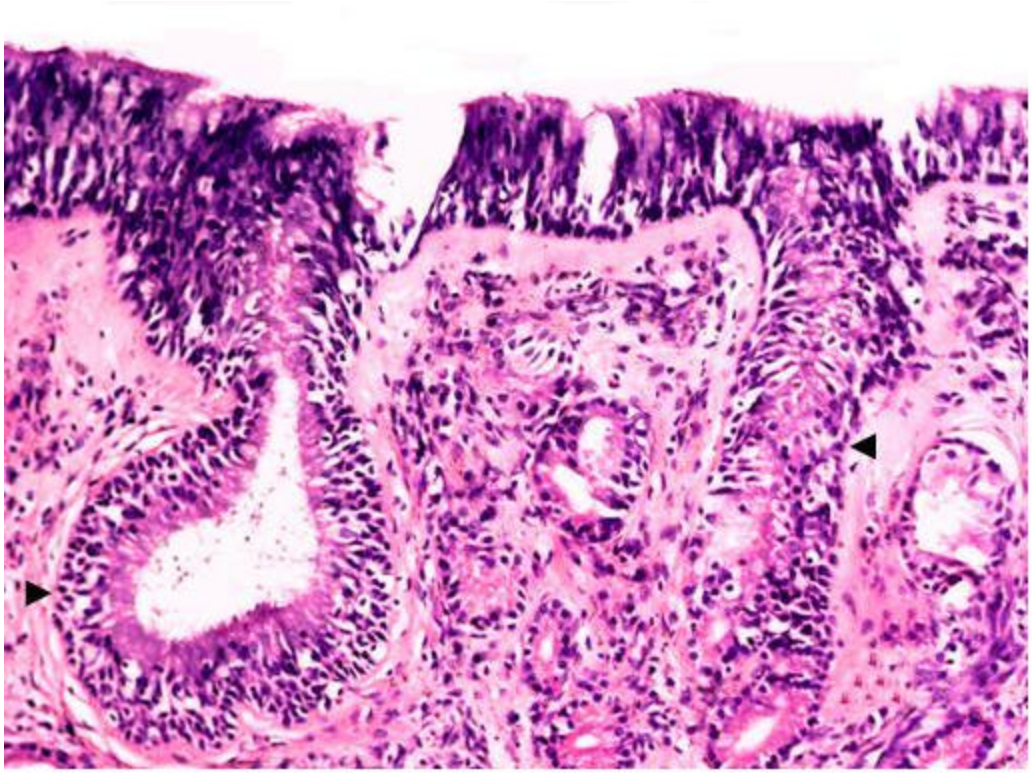

Fig. 29. Photomicrograph of human olfactory mucosa (Gp.III). Olfactory epithelial invaginations (Arrowheads) in the lamina propria. PAS stain. X 200.

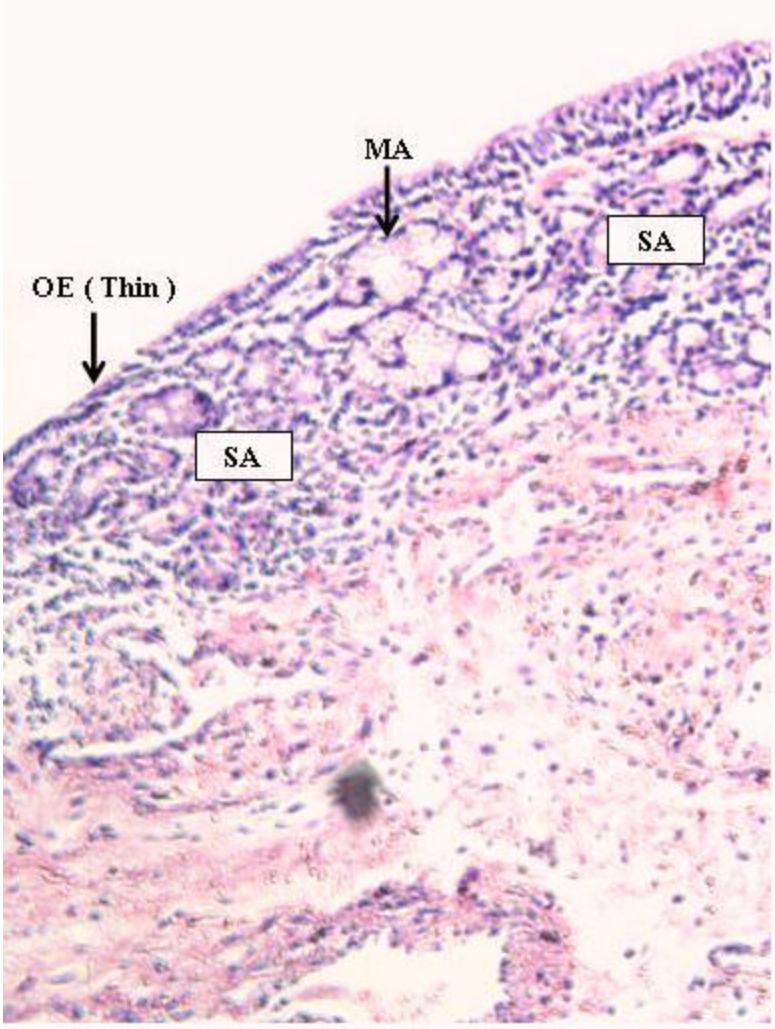

Fig. 30. Photomicrograph of human olfactory mucosa (Gp.IV), showing gradual thinning of the olfactory epithelium (OE). Appearance of mucous acini (MA) among the serous acini (SA). H and E stain. X 200.

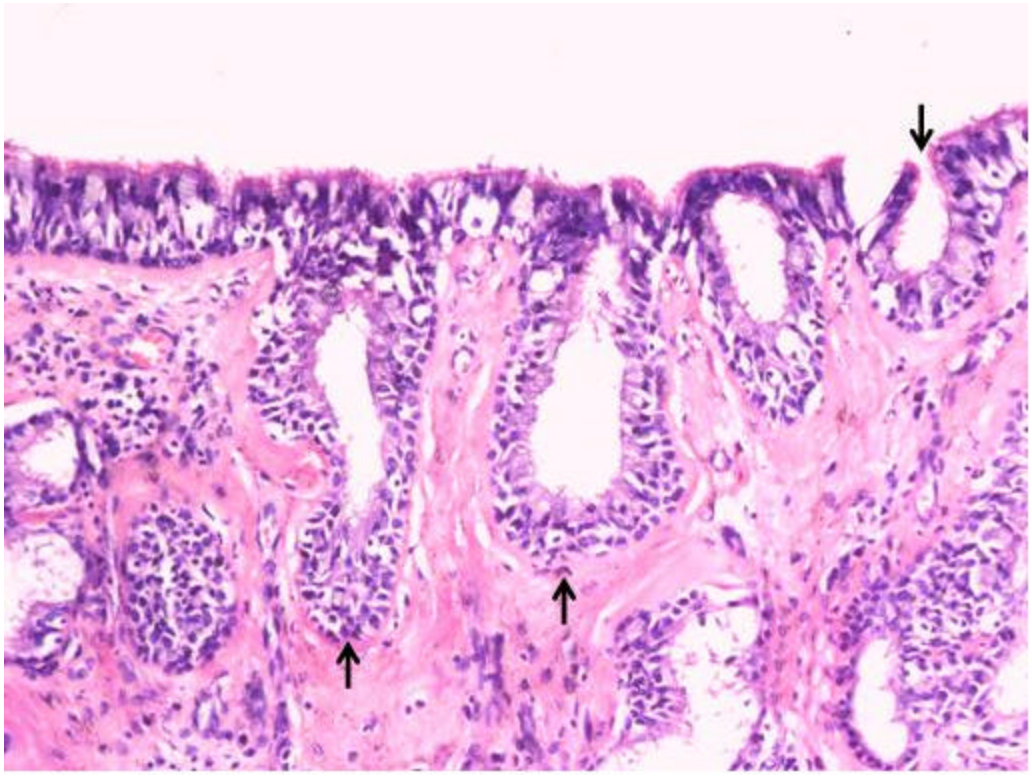

Fig. 31. Photomicrograph of human olfactory mucosa (Gp.VIII). Deep and numerous epithelial invaginations (Arrows) in the lamina propria are evident. PAS stain. X 200.

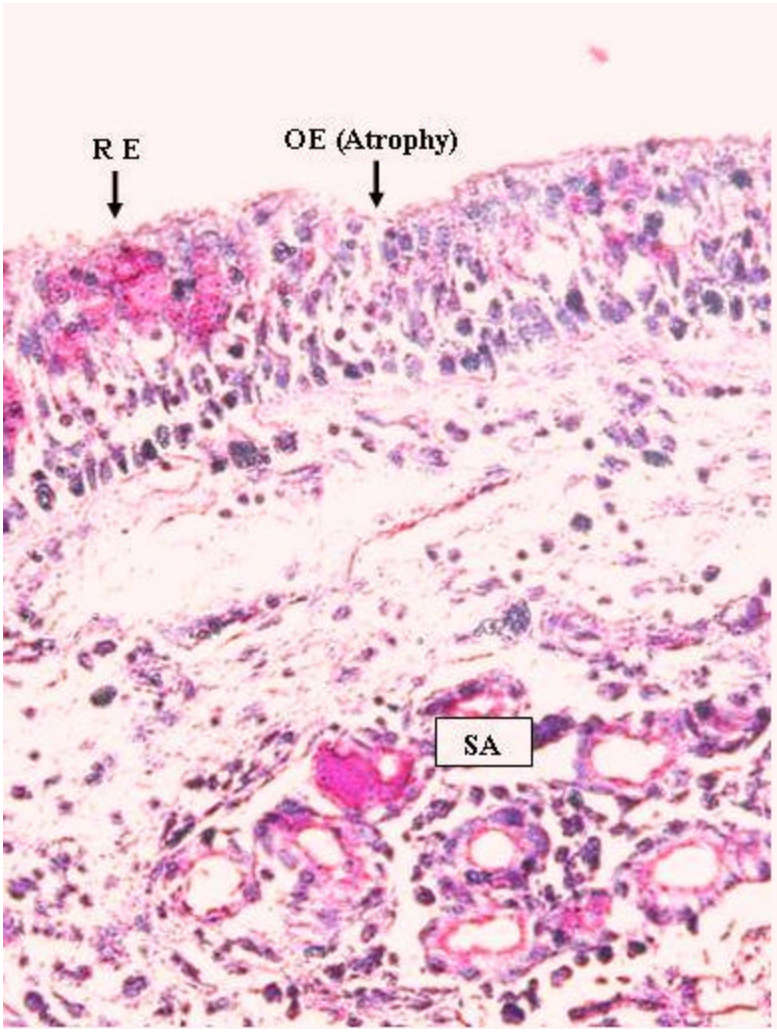

Fig. 32. Photomicrograph of human olfactory mucosa (Gp.IV). Olfactory epithelium (OE) showing atrophy and absence of receptor cells. Deeply stained goblet cells of the respiratory epithelium (RE) are also present. Serous acini (SA) are evident in lamina propria. PAS stain. X 400.

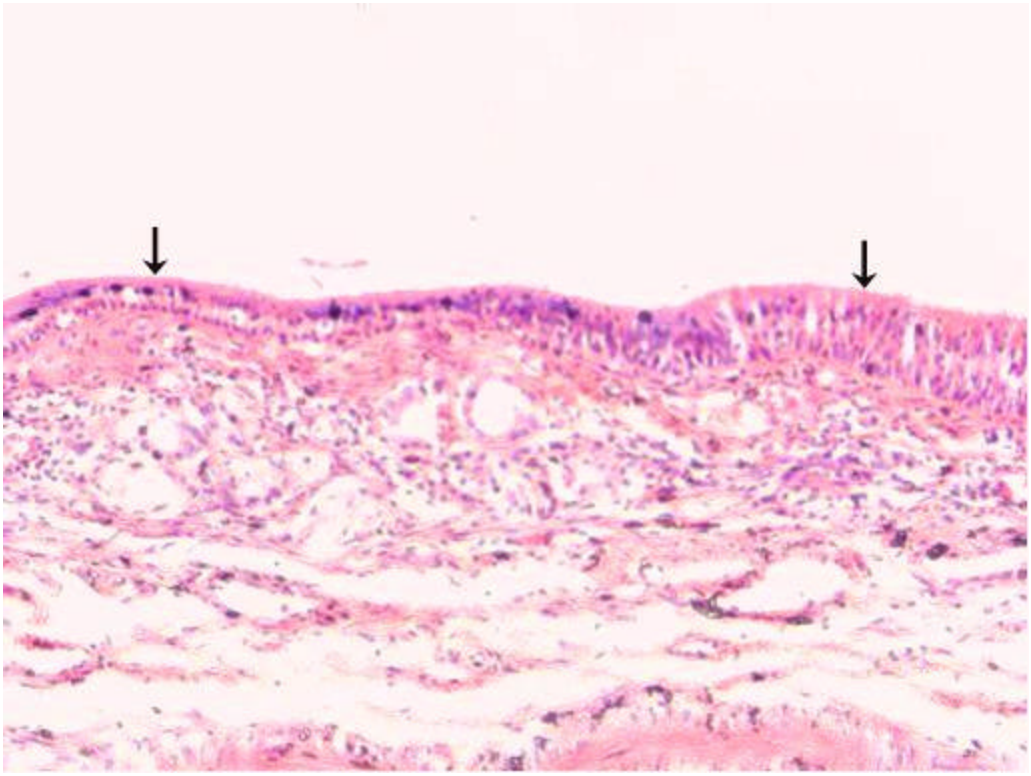

Fig. 33. Photomicrograph of human olfactory mucosa (Gp.IV). Gradual thinning of the olfactory epithelium (Area between the arrows) due to reduction in height and substantial decrease in the number of nuclei. H and E stain. X 200.

DISCUSSION

Although most of the previous light microscopic studies on the human olfactory mucosa focused on the morphology of the olfactory epithelium, little attention has been directed at age-related changes during adult life span. However, some evidence does exist which shows that as we get old, our sense of smell declines (Larsson *et al*., 2000; Ship *et al*., 1996; Farbman, 1994; Murphy *et al*., 2000; Stevens *et al*., 1982). Such perceptual alterations are accompanied by changes in the anatomy and physiology of the olfactory mucosa (Rombaux *et al*., 2005; Feng *et al*., 1997; Stevens *et al*., 1989; Elsener, 2001). Histological studies show that there is an age-related tendency to lose olfactory receptor cells, the sensory epithelial surface being replaced by respiratory epithelium (Legrier *et al*., 2001; Nakashima *et al*., 1991; Paik *et al*., 1992; Feron *et al*., 1998). There is also evidence that women, while losing smell sensitivity with age, perform better than men at all ages (Jacob, 2006; Murphy *et al*., 2002). In the present study, the morphology of the human olfactory mucosa was explored in male and female subjects ranging in age from 30 to 82 years in a Pakistani population. In this respect, this is a pioneer study from this part of the world regarding the histology of human olfactory mucosa, its regional distribution and age and sex related changes.

The olfactory epithelium of vertebrate species including humans has been extensively studied using different morphological approaches (Cuschieri and Bannister, 1975; Graziadei and DeHan, 1973; Hinds *et al*., 1984; Belangar *et al*., 2003; Mandal *et al*., 2005; Moran *et al*., 1992; McBride *et al*., 2003; Nomura *et al*., 2004; Kocianova *et al*., 2003; Datta and Bandyopadhyay, 1997). Light and transmission electron microscopic studies have shown that the human olfactory epithelium has morphological characteristics similar to those in other vertebrates (Engström and Bloom, 1953; Naessen, 1970, 1971a,b; Nakashima *et al*., 1984; Naguro and Iwashita, 1992; Morrison and Costanzo, 1992; Kocianova *et al*., 2001). All of these studies agree that the olfactory epithelium is pseudostratified and consists of olfactory receptor cells, sustentacular cells and basal cells. In the present study, morphologically pseudostratified columnar epithelium resting upon a uniform basement membrane was identified in the area of roof, medial and lateral

walls of right and left nasal cavities as has been shown by workers in the past. Four cell types (olfactory receptor cells, sustentacular cells, basal cells and microvillar cells) in the olfactory epithelium could be identified. Prior to 1982, most morphological studies of human olfactory material (Smith, 1938; Bloom and Engström, 1952; Polyzonis *et al*., 1979; Ohno *et al*., 1981) have described only three types of cells in the olfactory epithelium. However, Moran *et al*. (1982a,b) consistently observed a fourth cell type in electron microscopic studies on biopsies of normal human olfactory epithelium. They named it as 'microvillar cell'. Prior to the observations of Moran *et al*. (1982a), there was no definitive description or identification of a mammalian microvillar cell. Monti Graziadei and Graziadei (1979) had seen neurons located near the epithelial surface showing a peculiar type of "degeneration", characterized by a clear and swollen cytoplasm but no definitive identity could be ascribed to these cells. Okano *et al*. (1967) observed a fourth cell type with microvilli, having electron lucent cytoplasm, and Loo (1977) observed microvillar cells in one of the four primates he investigated (slow loris), but he called these cells, non-cilliated receptor cells. The observations of the microvillar cell type by Moran and coworkers (1982a,b) received clear support for the first time from more recent studies of Morrison and Costanzo (1990), Miller *et al*. (1995) and Jafek *et al*. (2002) who also observed microvillar cells near the apical surface in the human olfactory epithelium. Kwon *et al*. (2005) were able to note even age-related changes in the microvillar cells of the rat olfactory epithelium on the basis of histological and immunohistochemical methods. They observed that the microvillar cells in the olfactory epithelium of aged rats were markedly hypertrophied with swollen end-feet reaching the basement membrane. They did not observe such features in young cells. They noticed that the ratio of the number of microvillar cells to that of the sustentacular cells increased with aging and the total cell population decreased in the aged olfactory epithelium. They suggested that microvillar cells are non-neuronal and are more resistant to aging compared to the olfactory receptor cells and the sustentacular cells. The microvillar cells are the only type of receptor cells found in the vomeronasal organ; this accessory olfactory organ plays a significant role in olfaction in animals (Aujard, 1997; Keverne *et al*., 1999; Smith *et al*., 2001; Smith *et al*., 2003; Dennis *et al*., 2003). The identity of the

cells designated as microvillar, in the present study, is in agreement with the above mentioned studies.

The present study clearly shows age-related decrease in the number of olfactory cells in both male and female subjects. The difference is statistically significant among the various male groups and among the female groups. The decrease in the number of olfactory cells occurs gradually with advancing age. However, the greatest difference in the number of olfactory cells is between the male groups of ages 40-49 years and 50-59 years (Mean Difference = 29.31) as compared to age groups 30-39 years and 40-49 years (Mean Difference = 10.11) and age groups 50-59 years and 60 years onwards (Mean Difference = 4.78). These results suggest that the loss of olfactory receptors becomes pronounced with advancing age of individuals of both sexes. Similarly there is significant age-related decrease in the number of sustentacular cells among the groups of male and female beyond the age of 50, with groups 60+ years showing the most distinct decrease. The basal cells remain unaffected in groups of all ages. In the elderly human subjects, Paik *et al*. (1992) observed a few olfactory receptor cells with tortuous dendrites, having irregular nuclear margins and highly vacuolated cytoplasm suggesting age-related change in the olfactory receptor population. Even degenerating cells were also seen and in a 76-year old male, the respiratory epithelium seemed to have replaced the olfactory epithelium. Nakashima *et al*. (1991) studied the characteristics of olfactory mucosa in human fetuses and 40 subjects ranging in age from 31 years to 80 years. They observed regular zonal distribution of the sustentacular cells, olfactory cells and basal cells in the olfactory mucosa of the human fetus but in adults saw degenerated mucosa and decrease or disappearance of the olfactory receptor cells. Rosli *et al*. (1999) has also shown age-dependant reduction in both the olfactory receptor cells and the sustentacular cells. Changes in both the number and size of the olfactory knobs at the surface and in the density of olfactory cilia could be discerned. Weiler and Farbman (1997) have provided evidence of dramatic decrease in the proliferative density of the olfactory epithelium in rats from postnatal day 1 to post natal day 333. On postnatal day 1, the proliferative density was 151 cells/mm, and on post natal day 333 it was only 8 cells/mm. Hinds and McNelly (1981) observed that the number of olfactory receptors in the septal region of

rats, showed a marked increase from the age of 2 to 3 months, a steady increase at somewhat lesser rate from three to eighteen months and then a decrease in the number of receptor cells at subsequent ages, especially from twenty nine to thirty three months.

Information on the prevalence of disorders of the chemical senses has been rather limited. A survey by the National Institute on Deafness and Other Communication Disorders (NIDCD) in 1994 based on approximately 42,000 randomly-selected households 1994 in the United States of America reported olfactory disorders in 2.7 million (1.4%) adults. This rate increased exponentially with age. It was observed that almost 40% individuals with a chemosensory problem happened to be 65 years or older (Hoffman *et al*., 1998). While gradual loss of olfactory capability with age is a common phenomenon, mechanisms underlying this loss are not fully understood. Deficits occur with age in both identification and detection thresholds for a variety of odours, with changes becoming particularly evident after the fifth decade (Deems and Doty, 1987; Schiffman and Pasternak, 1979; Schemper *et al*., 1981; Özdener and Rawson, 2004). According to Cain and Stevens (1989) chronological age is strongly associated with impairment of the sense of smell. On the basis of a special odour identification procedure, Eskenazi *et al*. (1986) found that normal adults from 20 to 50 years of age could identify 85-100% of the odours presented but deterioration of odour identification ensued as age advanced and at around 50 to 60 years, adult individuals could identify only 65-75% of these odours. Murphy *et al*. (2002) examined odour perception in a population-based study of 2,491 residents of Beaver Dam, Wisconsin, who ranged in age from 53 to 97 years. It was empirically determined that mean prevalence of olfactory impairment was 24.5% which increased with age to 62.5% among 80 to 97 year old individuals. In an earlier study on 1955 individuals ranging from 5 to 99 years of age, it was found that peak ability to identify odours was evident in the 3rd to the 5th decades but it declined markedly after the 7th decade (Doty *et al*., 1984).

The present study also revealed random distribution of respiratory mucosa in the olfactory area of the nasal cavity. These islands of respiratory mucosa in the olfactory area could be identified by the typical respiratory nature of the epithelium and presence

of mucous glands in the lamina propria, confirmed by PAS staining. Such patches were occasional in the age group 30-39 years, but increased gradually as age advanced from 40-49, through 50-59 year groups to 60 years onwards. In the 60+ year group, the patches of respiratory epithelium could be seen in the roof, medial and lateral walls of both nasal cavities; the boundary between the two types of epithelia being less defined. Naessen (1970, 1971a) has described presence of respiratory epithelium in the olfactory area and has suggested that these changes occur with aging. He studied the topographical localization of the olfactory epithelium in human subjects and observed that the olfactory margin displays a characteristic irregularity often associated with atrophy of the olfactory epithelium that commonly occurs with age. The boundary line between the olfactory and respiratory areas appeared clearly defined. The biological basis for changes in olfactory function with age has been investigated in both animal and human models. There is histological evidence that the neuroepithelium becomes patchy with age, with increased infiltration by respiratory epithelium (Paik *et al*., 1992; Loo *et al*., 1996; Hirai *et al*., 1996). These changes imply that the ability of the olfactory epithelium to regenerate deteriorates with age (Walker *et al*., 1990; Doucette *et al*., 1983). In addition, axonal degeneration and changes in the olfactory bulbs of elderly humans have been reported, including loss of glomeruli (Meisami *et al*., 1998; Smith, 1942) and increase in glial cells (Liss and Gomez, 1958). Collectively, these studies suggest that this loss of olfactory receptor cells may explain the commonly reported age-related loss of olfactory sensitivity. Paik *et al*. (1992) has also reported presence of various sized patches of respiratory mucosa in the domain of the olfactory mucosa, with the transition sharply defined between the olfactory and the respiratory mucosa over the septal and the conchal mucosa as well as underneath the cribriform plate of the ethmoid bone. In old individuals, they observed increase in respiratory patches, which were distributed throughout the olfactory area, including the roof of the nasal cavity just underneath the cribriform plate and along the upper 8 mm of the nasal septum. They observed that the probability of obtaining olfactory specimens with the biopsy instrument decreased with advanced age, reflecting loss of olfactory mucosa in aged subjects. Morrison and Costanzo (1990) observed patches of respiratory epithelium in the superior nasal cavity, which is considered a purely olfactory area. Nakashima *et al*. (1984) has also reported that

invasion by respiratory epithelium in the region of olfactory mucosa was more prominent in the roof of the nasal cavity as compared with other regions and deduced that it is characteristic of adult humans. Rosli *et al*. (1999) studied age related changes in the mouse olfactory epithelium under transmission and scanning electron microscope. They observed that the boundary between the respiratory and olfactory epithelium was sharp and clearly delineated with an absence of intermixing of cells in the young and unaffected aged mice. However, in the area of the septum of the aged mice the transition line became interdigitated and tortuous. These findings by various researchers imply that certain morphologic and physiologic changes may occur in the olfactory system as part of the aging process. In the present study, this relates to the observation of the respiratory mucosa in the region of the olfactory mucosa in cases of advanced age and together with earlier studies suggest that age is an important factor leading to decline in the olfactory function in humans. However, some studies have not reported any significant decline in the ability of humans to smell odours. Elsner (2001) studied odour threshold, recognition, discrimination and identification in centenarians. He tested the odour threshold in 21 centenarians (mean = 105.1 years) for phenylethyl alcohol (PEA) and menthol, recognition and discrimination of lexically challenging odours, and identification of common odours. Chronological age was not found to be a significant predictor of abilities for any of the tasks performed by the centenarians in his study.

Age-related changes were also observed in the zonal distribution of cell nuclei in the present study. The changes were quite drastic. Whereas the distribution of the cells was normal in the age group 30-39 years, occasional disturbance of the position of olfactory receptor cells and sustentacular cells was evident in age group 40-49 years which became quite substantial in the age group 50-59 years, this resulted in decreased height of the epithelium. In the age group 60 years onwards, reduction in thickness of the olfactory epithelium was very significant with a concomitant loss of the normal zonal distribution of sensory and sustentacular cell nuclei. These changes were evident in the roof, medial and lateral walls of right and left nasal cavities. Naessen (1971a) and Nakashima *et al*. (1984) have reported disturbance of the zonal distribution of the olfactory receptor cells, sustentacular cells and epithelial degeneration, with the basal

cells remain relatively unaffected. The latter workers found normal distribution of cells with thick neuroepithelium in the fetus but there was loss of such arrangement in the adult olfactory epithelium. Such age-related changes in the distribution of epithelial cells are an accompaniment of degenerative changes which ultimately result in decrease in the height of the epithelium. In the present study, this reduction in epithelial thickness was evident in both the male and female groups. The greatest decrease in the height of the epithelium was in the age groups 40 to 60 years onwards. The olfactory epithelium was much thicker than the respiratory epithelium in the younger age group. In advanced age groups, the height of the epithelium decreased and was thinner than the respiratory epithelium. In their study on rats Weiler and Farbman (1997) reported a 40% increase in neuroepithelail thickness from birth to postnatal day 40, whereas afterwards the thickness gradually decreased. They noted a direct correlation between the epithelial thickness and the number of neurons per unit length. In newborn animals they recorded 190 neurons in 200 µm length of the epithelium. This density increased to a peak of 202 at post natal day 21 but in old age (Post natal day 105) the density decreased reaching a plateau value of 160 neurons. They also observed that the thickness of the epithelium lining the septum and turbinate edges (convex) was higher than the thickness in the vicinity where the turbinates were connected to the lateral wall (concave) or to other concave parts of the turbinates. Rosli *et al*. (1999) have also reported age related changes in the mouse olfactory epithelium under transmission and scanning electron microscope and found that the epithelial thickness decreased with age. Meisami (1989) measured the number of olfactory knobs in rat and showed a rapid proliferation of the olfactory neurons in the first post-natal month, with slower growth between 2 and 18 months and a decline in the number of receptors from 18 to 33 months. Apfelbach *et al*. (1991) showed that the density of the olfactory receptor cells increased rapidly in the first 20 days, a lesser increase until day 220 and decrease in older (>400 days) animals. They also observed the changes in the olfactory sensitivity relating to changes in receptor cell density with maximal sensitivity occurring at 200 days. That reduction in epithelial thickness as a consequence of cell degeneration is cause of anosmic state has been demonstrated in a study by Lee (2000) on olfactory mucosa of 15 patients with persistent anosmia. He compared his observations with 6 patients with normosmia after sinus surgery. The

normosmic patients had sufficiently thick olfactory epithelium and normal nuclear arrangement of the sustentacular cells, the olfactory receptor cells and the basal cells. Contrary to this the anosmic patients entirely lacked olfactory epithelium. In 15 out of 27 samples only respiratory epithelium with or without goblet cells was identified confirming the fact that in anosmia the olfactory receptor cells decrease in number or are entirely lost. The results of the present study also match with these observations suggesting that reduction in the height of neuroepithelium with advancing age may lead to impairment of olfactory sensibility.

In the present study, there was no evidence of significant sex-related differences in the olfactory mucosa. The gender related differences were calculated between the corresponding male and female groups. Comparison of age groups 30-59 years revealed that the females had greater number of olfactory cells as compared to the males. In the age group 60 years and above, the males had greater number of olfactory cells. None of these differences turned out to be significant. Similar results were obtained for the sustentacular cells, basal cells and thickness of the neuroepithelium. These observations are contrary to what has been reported by Diamond *et al*. (2005). They repeatedly measured olfactory thresholds to benzaldehyde in males and females between the ages of 20 and 30 years, and showed that benzaldehyde thresholds decreased among females but not among males. The ability of women to show dramatic increase in olfactory sensitivity following repeated test exposures can explain why females appear to be more sensitive and reactive than males. They demonstrated that induced olfactory sensitivity is both a more general and more restricted effect, with women of reproductive age showing marked sensitization to multiple odourants after repeated threshold testing. Saini and Breipohl (1976) studied the olfactory epithelium of male and female rhesus monkeys. They observed differences in the epithelial surface between male and female animals. These structural differences were more prominent between male and pre-ovulatory females. They postulated a structural basis for changes in olfactory threshold in relation to sex hormones. Doty *et al*. (1984) studied the smell identification ability in 1955 individuals ranging in age from 5 to 99 years and found that women performed better than men, and nonsmokers performed better than smokers. Doty *et al*. (1985)

administered the University of Pennsylvania Smell Identification Test (UPSIT) to four groups belonging to different cultures. The aim was to ascertain the generality of a sex difference noted in odour identification ability. The women of all four groups out-performed men to the same relative degree. The analysis of the proportions of subjects correctly answering each of the test item revealed considerable similarity of relative item difficulty among the subject groups and ethnic or cultural factors did not influence the sex differences in odour identification abilities. Öberg *et al*. (2002) have reported that women have higher proficiency in odour identification. Weiler and Farbman (1997) observed the sex differences in morphology of olfactory epithelium in male and female rats. No differences in the proliferative density or distribution pattern at any investigated age were detected. Murphy *et al*. (1998) studied the underlying central nervous system activity associated with age-related changes in olfactory functioning. They used the olfactory event-related potential (OERP) to study the olfactory decline with age and gender differences and found that the early component of the OERP showed reduced amplitude and longer latency in elderly subjects with larger effects in males. A study conducted by Murphy *et al*. (2002) on 2491 Beaver Dam, Wisconsin residents showed, olfactory impairment was more prevalent among men.

The current study design demonstrated the morphological features of human olfactory mucosa in relation to its regional distribution in males and females of different age groups. On detailed histological study, four types of cells were identified in the olfactory epithelium. Age related variations including, slight epithelial invaginations and irregular zonal distribution of olfactory receptor and supporting cells, were observed in the group comprising of 40-49 years of individuals. However, diminished number of nuclei resulting in disturbance of zonal distribution, reduced height of epithelium, and epithelial invaginations were regarded as significant changes in the olfactory epithelium of the group of 50-59 years of individuals. Moreover, age group of 60 and onwards revealed gradual thinning of the epithelium, relative increase in depth and number of epithelial invaginations, and patchy atrophy of olfactory epithelium at places. There was significant age-related decrease in the number of olfactory and sustentacular cells among

male and female groups, more pronounced in individuals of 50 years and above without significant gender differences.

The analysis of these observations clearly pointed out decline in the sense of olfaction with age possibly due to the mechanism of reduction in the number of olfactory receptors; in addition no evident sex related differences were seen in these groups. A number of age related disorders have been reported with alterations in olfactory function. The most accepted evidence, of smell loss in age related diseases, is based on studies of patients suffering from Alzheimer's disease and Parkinsonism. Although advances have been made in documenting the nature of age related changes in smell perception; causal relations between the changes in the morphology of olfactory mucosa and the underlying pathologic mechanism, are yet to be established. This study presents a perspective for future research endeavors in the emerging area of "geriatric otolaryngology". The findings of this study have implications for understanding the association between aging and morphology of olfactory mucosa.

APPENDIX

Characteristics of autopsy cases

Case No.	Sex	Age (years)	Septal position	Time after death (Hours)
1	M	30	N	2
2	F	48	N	2
3	M	46	N	4
4	M	59	N	2
5	F	52	N	4
6	M	52	N	3
7	M	69	N	4
8	M	78	N	5
9	F	65	N	5
10	M	39	N	2
11	M	36	N	4
12	F	62	N	4
13	F	38	N	4
14	M	47	N	3
15	M	31	N	1
16	M	41	N	5
17	M	55	N	4
18	F	54	N	3
19	M	36	N	4
20	F	39	N	3
21	F	68	N	4
22	M	80	N	2
23	M	31	N	3
24	M	44	N	2
25	M	56	N	2
26	F	40	N	2
27	M	52	N	3
28	F	47	N	2
29	F	31	N	2
30	M	78	N	3
31	M	34	N	3
32	M	48	N	5
33	F	44	N	2
34	F	59	N	4
35	M	58	N	4
36	M	35	N	2
37	F	34	N	3
38	M	51	N	2
39	F	30	N	5
40	M	65	N	4

Case No.	Sex	Age (Years)	Septal position	Time after death (Hours)
41	M	38	N	2
42	M	54	N	4
43	F	30	N	4
44	F	48	N	4
45	F	76	N	3
46	M	71	N	2
47	M	33	N	2
48	F	52	N	3
49	F	35	N	3
50	F	66	N	4
51	M	41	N	3
52	F	60	N	3
53	M	68	N	3
54	F	57	N	3
55	F	40	N	3
56	M	46	N	4
57	M	82	N	4
58	F	37	N	2
59	F	78	N	3
60	M	66	N	4
61	F	43	N	3
62	M	49	N	4
63	F	55	N	2
64	F	32	N	2
65	M	50	N	3
66	F	51	N	4
67	M	60	N	3
68	F	49	N	3
69	M	42	N	3
70	M	43	N	3
71	M	52	N	3
72	F	43	N	3
73	F	72	N	3
74	F	52	N	2
75	F	50	N	4
76	F	69	N	3
77	F	32	N	2
78	F	80	N	4
79	F	41	N	3
80	F	57	N	3

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