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Differentiation of Epiglottal Epithelia during Prenatal and Postnatal Human Development

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A B S T R A C T

Differentiation of epiglottal epithelia during human development was for the first time investigated by the light microscopy and documented in celoidine collection of human embryos from the Archive of the Department of Histology and Embryology, School of Medicine University of Zagreb, Croatia. At 6 weeks epiglottal swelling was found to be covered by a simple squamous epithelium consisting of a single layer of cells. At 8 weeks epithelium changed to a two-layered cuboidal epithelium which at the end of the 8th week transformed to multilayered columnar epithelium without cilia and goblet cells. In the one-day-old newborn, the majority of epiglottis was found to be covered by the mature ciliated columnar pseudostratified epithelium with goblet cells while only a minor part of the oral surface next to the tongue by the stratified squamous epithelium. This unexpected finding is in contrast to the domination of the stratified squamous epithelium found at the age of 13 years and in 35-years-old adult. Reversal of proportion covered by different types of epithelia between birth and puberty /adulthood is probably connected to the establishment of the air-flow which could be stimulating for differentiation of stratified squamous epithelium.

Key words: *epiglottis, human, development, epithelium*

Introduction

The epiglottis is lying behind the tongue and in front of the entrance to the larynx. During swallowing, it folds back to cover the entrance to the larynx, preventing food and drink from entering the respiratory system. However, agenesis of the epiglottis was described in an adult without the history of dysphagia, stridor and respiratory infections[1]. Epiglottis was also used as an autologous composite graft in eyelid reconstruction[2].

Epiglottis of the adult consists of the elastic cartilage. Cartilage cell density of the epiglottis seems to be lower in males compared to females[3]. Epithelia and lamina propria containing blood vessels, lymph vessels and tubuloacinar glands form the mucosa covering epiglottic cartilage. At its oral surface and the majority of laryngeal surface epiglottis is covered by stratified squamous epithelium. At the bottom of the laryngeal surface it is covered with ciliated pseudostratified epithelium. A narrow zone of stratified cilindrical epithelium is situated between these two epithelia[4].

Although the embryology of the larynx has been well established, the development of the epiglottis has not been investigated in all details. It is thought that the epiglottis arises as a swelling on the hypobranchial eminence approximately during 32nd day of intrauterine life. During this time, arytenoid swellings and aryepiglottic folds also become apparent. It is interesting to note that the hard palate also develops during this time period. It is thought that interruption of growth at any time before this period results in epiglottic anomalies, ranging from total absence, to hypoplasia, to a bifid epiglottis[1].

The epiglottis develops from the caudal part of the hypopharyngeal eminence, a prominence produced by proliferation of mesenchyme in the ventral ends of the third and fourth pharyngeal arches. The rostral part of this eminence forms the posterior third or pharyngeal part of the tongue. Because the laryngeal muscles develop from myoblasts in the fourth and sixth pairs of pharyngeal arches, they are innervated by the laryngeal

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branches of the vagus nerves (cranial nerve X) that supply these arches[5]. During the fourth and fifth weeks of gestation, a rapid proliferation of the fourth and sixth pharingeal arch mesenchyme around the site of origin of the respiratory bud converts the opening slit from the esophagus into a T-shaped glottis bounded by two lateral arytenoid swellings and a cranial epiglottis[6]. The root of the tongue develops from the 2nd, 3rd and 4th pharyngeal arch. Nervus laryngeus superior inervates the posterior part of the tongue and the epiglottis indicating that those parts develop from the fourth pharyngeal arch[7].

During early infancy the epiglottic cartilage appears to play an important role in separating the upper respiratory tract from the upper digestive tract. This separation is accomplished by approximation of the epiglottis to the palate, providing a continuous airway from the nose through the larynx into the trachea. This structural arrangement, however, is uniquely lost in man during postnatal development. Maturational descent of the epiglottis, found to occur between 4 to 6 months age, was verified by cineradiography. This structural change, requiring a reorganization of respiratory function, represents a discrete developmental event manifesting the potential for oral tidal respiration from a pattern of obligate nasal breathing. This period, four to six months postnatally, interstingly coincides with the pick incidence of sudden infant death syndrome (crib death) which similarly occurs at 3 to 5 month of age[8]. Maturational descent of the epiglottis starts in infancy and continues into adolescence[9]. Growth of the larynx and epiglottis is rapid during the first 3 years after birth. By 3 years of age, the epiglottis has reached its adult form[5].

Little is known about the differentiation of epiglottal epithelia during human development and therefore the aim of this investigation was to describe and document by microphotographs the differentiation of epiglottal epithelia during human embryonic and fetal development and compare it to early postnatal, pubertal and adult epiglottises.

Material and Methods

1. First group of epiglottises investigated belongs to The collection of human embryos and fetuses from the Archive of the Department of Histology and Embryology, School of Medicine University of Zagreb, Croatia. This collection consists of histological slides with serial sections of celoidine embedded material routinely processed and stained with hematoxylin-eosin (HE). This investigation was performed on:
   a) 10 mm embryo (6-weeks-old embryo), Carnegie stage 16
   b) 13 mm embryo (7-weeks-old embryo), Carnegie stage 17
   c) 18 mm embryo (8 weeks-old-embryo), Carnegie stage 19
   d) 19 mm embryo (8 weeks-old-embryo), Carnegie stage 20
   e) 9 weeks-old- fetus

Embryos were staged by Duančić (from 1943 until 1956) and now compared with the Carnegie Classification System proposed by O'Rahilly and Müller based on the Carnegie collection of embryos, Washington DC[10,11].

2. Second group of epiglottises investigated belongs also to the Archive of the Department of Histology and Embryology, School of Medicine University of Zagreb, Croatia. This was The collection of paraffin embedded human epiglottises collected from 1980–1982:
   a) epiglottis from an one-day-old newborn
   b) epiglottis from a 13-years-old
   c) epiglottis from a 35-years-old

Serial sections were made and routinely processed and stained with hematoxylin-eosin (HE), orcein (selective stain for elastic fibers) and Masson trichrome stain (for connective tissue)[12].

Results

In present investigation it was discovered that at the 6th week of development epiglottis has not yet separated from the root of the tongue (Figure 1 a,b,c). Epiglottal swelling is at this time and at 7th week of development (Figure 1. d,e,f) covered by a simple epithelium consisting of a single layer of squamous cells (Figure 1 f). At 8 weeks epiglottis has already separated from the root of the tongue (Figure 2. a,b). At the begining of the 8th week two layers of cuboidal cells were found in the immature epiglottal epithelium (Figure 2 b). At the end of the 8th week (Figure 2, c,d,e) this epithelium transformed to a immature columnar epithelium consisting of several layers of columnar cells without cilia and goblet cells (Figure 2 e,). It was not possible to discern only by light microscopy whether this epithelium belongs to a stratified or pseudostratified type of epithelium. This type of epithelium was present also in the 9-week-old fetus (Figure 2, f). In the one-day-old newborn, the majority of epiglottis was found to be covered by a typical mature pseudostratified ciliated columnar epithelium with goblet cells, while only a minor part of the oral surface by a mature stratified squamous epithelium (Figure 3 a,b,c). At the age of 13 years (Figure 3 d) and in the adult aged 35 years (Figure 3 e,f) the stratified squamous epithelium was covering the majority of the epiglottis and therefore was found to be dominating over the ciliated pseudostratified epithelium.

Discussion

This investigation of embryos from the The collection of human embryos and fetuses from the Archive of the Department of Histology and Embryology, School of Medicine University of Zagreb, Croatia has discovered new data about the development of the human epiglottis, especially at the transition from the intrauterine life to the life exposed to the normal conditions of the external environment.

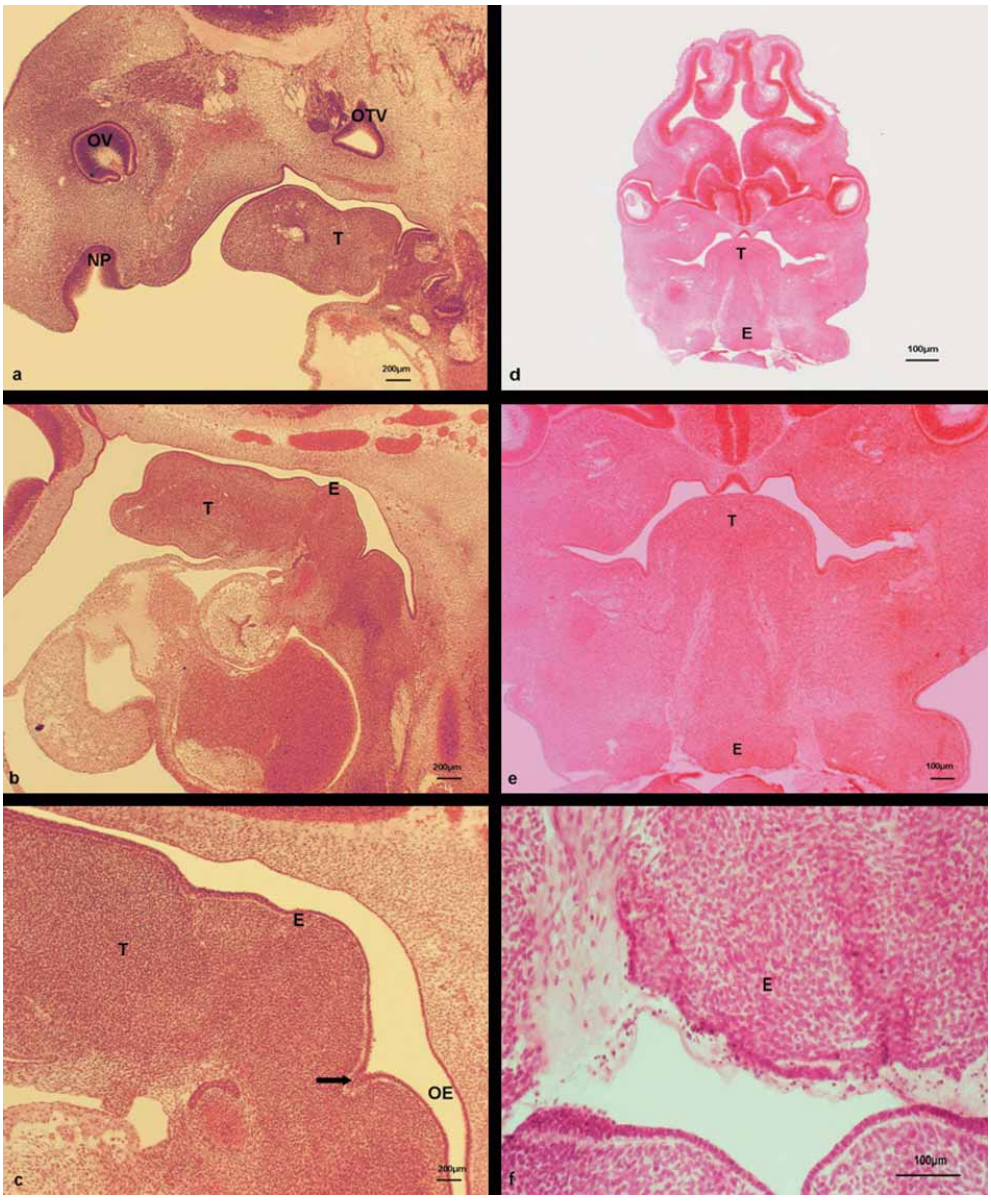

Fig. 1. Embryos at Carnegie stages 16 and 17. Note that future epiglottis has not yet separated from the root of the tongue. a) 6-weeks-old embryo (10mm). Sagittal section, NP = nasal pit, OV = optical vesicle, OTV = otic vesicle, T = tongue, HE × 40; b) 6-weeks-old embryo. Sagittal section, T = tongue, E = epiglottal swelling, HE × 40; c) 6-weeks-old embryo. Sagittal section, T = tongue, E = epiglottal swelling, OE = oesophagus, → entrance to laryinx, HE × 100; d) 7-weeks-old-embryo. Frontal section, T = tongue, E = epiglottis HE × 20; e) 7-weeks-old-embryo. Frontal section, T = tongue, E = epiglottis HE × 40; f) 7-weeks-old-embryo (13 mmm). Frontal section, T = tongue, E = epiglottis, HE × 200. Note that the future epiglottis is covered by a simple squamous epithelium.

During embryonic and early fetal development columnar epithelia that were found to cover the epiglottis after its separation from the tongue could not be easily categorized on archive slides already processed for classical histology. To investigate their contact to the basement membrane and classify them properly as stratified or pseudostratified epithelium finer methods and electron microscopy should be applied. As described by Duančić[4] stratified columnar epithelium persists in the adult although only as a narrow zone between stratified squamous epithelium and columnar ciliated pseudostratified epithelium and therefore it can be speculated that both epithelia might be present at the early stages of development.

The present discovery that the epiglottis of the one-day-newborn is mostly covered by the ciliated pseudostratified epithelium poses questions about the origin of the stratified squamous epithelium covering the majority of epiglottal surfaces of the adult.

Epithelium of rabbit epiglottis was cultivated *in vivo*, using adult rabbits as recipients and rabbit embryos (3–4 weeks) and rabbits (5–6 months) as donors. The epithe-

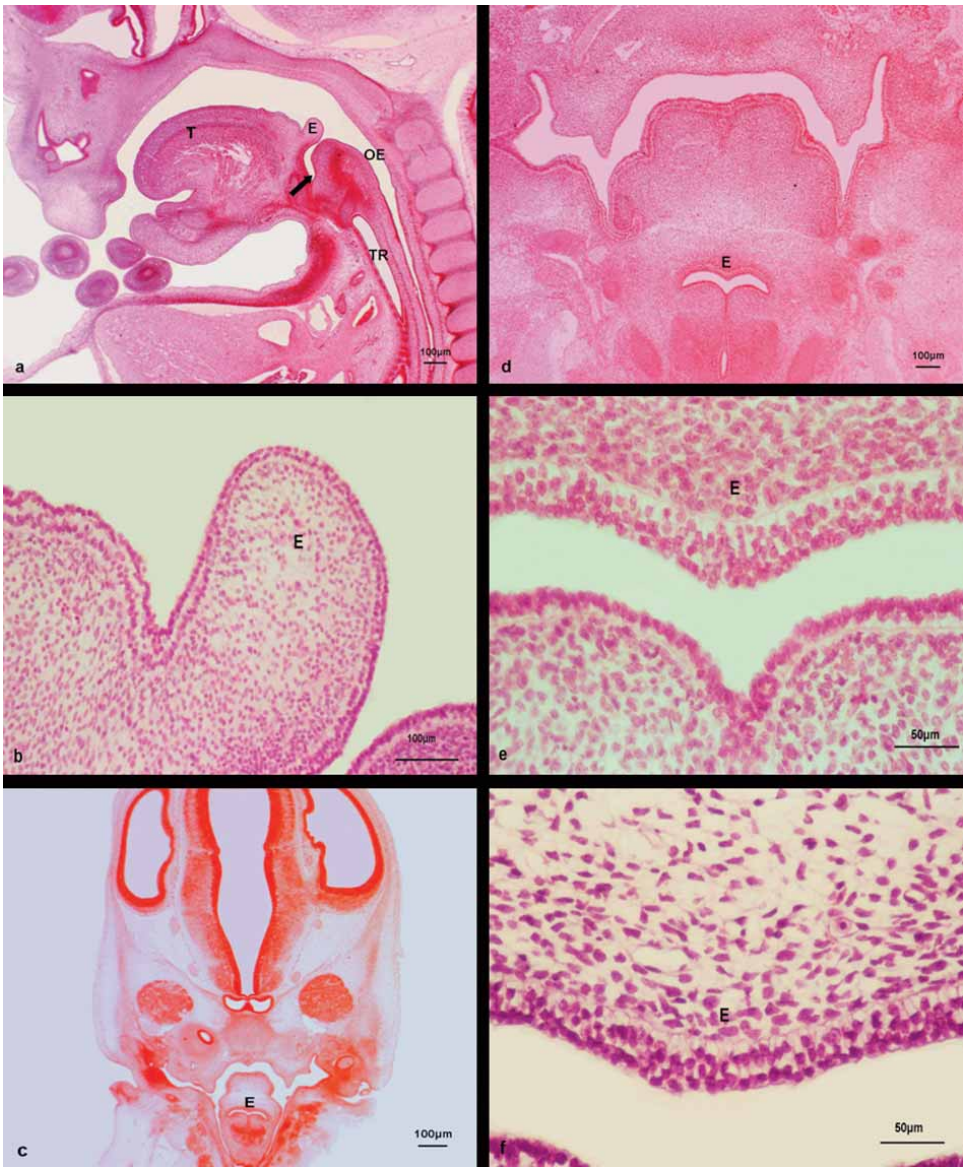

Fig. 2. Embryos (Carnegie stages 19 and 20) and a fetus. Note the separation of epiglottis from the root of the tongue. a) 8-weeks-old embryo (18 mm). Sagittal section, E = epiglottis, T = tongue, OE = oespohagus, TR = trachea, → entrance to laryinx, HE × 15; b) 8-weeks-old embryo (18 mm). Sagittal section, E = epiglottis, HE × 200; c) 8-weeks-old embryo (19 mm). E = epiglottis, HE × 20; d) 8-weeks-old embryo (19 mm). E = epiglottis, HE × 40; e) 8-weeks-old embryo (19 mm). E = epiglottis, HE × 400; f) 9-weeks-old fetus. E = epiglottis, HE × 400. Note the transition in epiglottal epithelia from the two-layered cuboidal (a,b) to multi-layered columnar epithelium (c-f).

lium of the epiglottis revealed high reactivity and plasticity, as shown by active growth followed by the formation of stratified, multi-layered and single-layered sheets and glandular structures. These properties of the epithelium of the epiglottis diminished with age. Because of the caracter of growth of the epithelium of the epiglottis when cultivated *in vivo*, and its high plasticity, similar to that of other organs derived from the foregut, it may be classed as an epithelium derived from a prechordal anlage with a wide range of biological potentialities[13]. Recently it was demonstrated that cilliated cells are able to transdifferentiate to a squamous stratified epithelium during repair of injured respiratory epithelium[14].

In embryonic rat cultures *in vitro* it was shown that differentiation of tissues is in general stimulated by the air-lifting (embryonic explants are not immersed in the liquid medium but are growing on a metal support to be in direct contact with the air) and this holds true also for the epidermal differentiation[15]. It was discovered that, regardless of the medium used *in vitro* and even after spending two-weeks in the simple protein-free medium, embryos cultivated at the air-liquid interface retained the developmental potential for differentiation of skin and its appendages (hair and sebaceous glands) after subsequent transplantation to the ectopic environment *in vivo*[16,17,18]. Recent investigations on human corneal

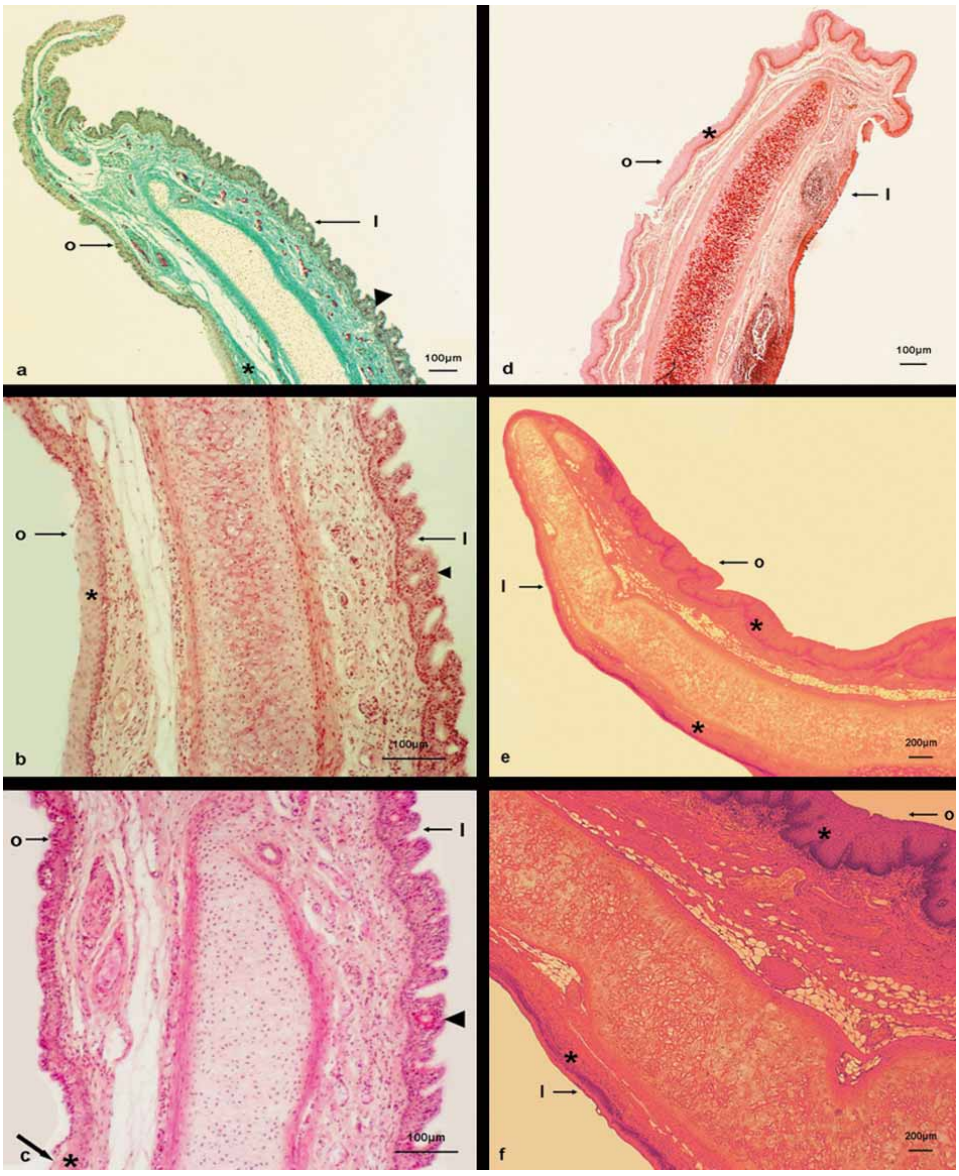

Figure 3. Epiglottis at birth, puberty and adulthood. Ciliated pseudostratified columnar epithelium (arrowhead), stratified squamous epithelium (asterix). a) Epiglottis of an one-day-old newborn, o = oral surface, l = laryngeal surface, Masson trichrome stain × 40. Note that ciliated pseudostratified columnar epithelium covers the major portion of both epiglottal surfaces. b) Epiglottis of an one-day-old newborn, o = oral surface, l = laryngeal surface, orcein × 40. Stratified squamous epithelium is visible at the oral surface of the basal part of the epiglottis. c) Epiglottis of an one-day-old newborn, o = oral surface, l = laryngeal surface, HE × 40. Note the transition between two types of epithelia. d) Epiglottis of a 13-years-old, o = oral surface, l = laryngeal surface, orcein × 20. Note that the majority of epiglottal surfaces is covered by stratified squamous epithelium. e) Epiglottis of a 35-years-old, o = oral surface, l = laryngeal surface, HE × 15. Note that the majority of epiglottal surfaces is covered by stratified squamous epithelium f) Epiglottis of a 35-years-old, o = oral surface, l = laryngeal surface, HE × 40. Note the stratified squamous epithelium.

epithelial cells, umbilical cord epithelial cells and conjunctival epithelial cells cultivated *in vitro* by the air-lifting method also showed that the contact with air is optimal for differentiation of stratified squamous epithelium[19,20,21]. Indeed, it is known that stratified squamous epithelium covering various anatomical structures of the human body is always found at sites contacting directly with the air (e.g. nasal vestibule, oral cavity, oesophagus, true vocal cords etc)[22].

Experiments with direct transplantation of the fetal rat epiglottis *in vivo* to the ectopic site under the kidney capsule revealed that ciliated pseudostratified epithelium differentiates and dominates in the transplant[23] meaning possibly that the experimental conditions lacking direct contact with the air were not optimal for differentiation of the stratified squamous epithelium. Having in mind this fact and the previously mentioned plasticity of the foregut epithelia it can also be hypothesised that

ciliated pseudostratified epithelium of the newborn changes by transdifferentiation to the stratified squamous epithelium because of the air-exposure that begins after birth. The other possibility is that the stratified squamous epithelium found at the bottom of the epiglottis at the oral surface simply spreads towards the favourable environment so that in puberty and in adult it was found to cover the majority of the epiglottis. To discern between these two possibilities in this particular case developmental in vitro studies including a follow up of differentation markers can be proposed similarly as was done in case of oesophageal epithelium development[24].

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DIFERENCIJACIJA EPITELA EPIGLOTISA TIJEKOM PRENATALNOG I POSTNATALNOG RAZVOJA U ČOVJEKA

SAŽETAK

Diferencijacija epitela epiglotisa tijekom razvoja u čovjeka po prvi puta je istražena svjetlosnom mikroskopijom i dokumentirana u celoidinskoj zbirci humanih zametaka iz Arhiva Zavoda za histologiju i embriologiju Medicinskog fakulteta Sveučilišta u Zagrebu, Hrvatska. U šestom tjednu intrauterinog razvoja pronašlo se da je osnova epiglotisa prekrivena jednoslojnim pločastim epitelom (epithelium squamosum simplex). Tijekom osmog tjedna epitel se promijenio te su u njemu prisutna dva sloja kubičnih stanica. Krajem osmog tjedna epitel prelazi u cilindrični višeslojni ili višeredni epitel bez trepetljika i vrčastih stanica. Veći dio epiglotisa u novorođenčeta starog jedan dan prekriven je zrelim višerednim cilindričnim epitelom s trepetljikama i vrčastim stanicama (epithelium pseudostratificatum columnare), dok je samo manji dio oralne strane uz jezik prekriven višeslojnim pločastim epitelom (epithelium squamosum stratificatum) što predstavlja posve novi, neočekivani nalaz. Naime, u dobi od trinaest i trideset pet godina gotovo cijeli epiglotis prekriva višeslojni pločasti epitel. Ova promjena omjera dvaju epitela između rođenja i puberteta/odrasle dobi je vjerojatno povezana sa uspostavom disanja i protoka zraka koji po svoj prilici potiče diferencijaciju višeslojnog pločastog epitela.