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RAT COCHLEA CONTINUES TO DEVELOP AFTER BIRTH[1]

RAT KOHLEASI DOĞUMDAN SONRA GELİŞMEYE DEVAM EDER

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ABSTRACT

Introduction: The ear, originates from the ectoderm, exhibits a complex structure that differs from species to species in terms of development.

Objectives: This study was conducted to describe the prenatal and postnatal development process of the organ of Corti in the iner ear using light microscopy.

Methods: In this study, Wistar Albino rats were used. The rats were divided into two groups as prenatal and postnatal groups. Prenatal groups consisting of embryos on day 17, 19, 21 and postnatal groups consisting of pups on days 1, 5, 10 and 15. The rats were sacrificed to investigate the cochleas by light microscopy.

Results: The lumen of the cochlear tunnel was lined with pseudostratified columnar epithelia throughout the prenatal period. The cochlear tunnel was not divided into scala media, scala tympani and scala vestibuli in the embryonic sections. Organ of corti hasn't taken its final form until postnatal day 15.

Conclusion: In rats, formation of organ of Corti is completed in postnatal 15 day. Pups of rats are not able to hear in the embronic and early postnatal life.

Keywords: Organ of corti, Prenatal development, Postnatal development

ÖZET

Giriş: Ektoderm kaynaklı kulak, gelişim açısından türden türe farklılık gösteren kompleks bir yapı sergilemektedir.

Amaç: Bu çalışma iç kulaktaki korti organının doğum öncesi ve doğum sonrası gelişim sürecini ışık mikroskobu kullanarak tanımlamak için yapılmıştır.

Materyal-Metot: Bu çalışmada Wistar Albino cinsi sıçanlar kullanıldı. Sıçanlar prenatal ve postnatal olarak iki gruba ayrıldı. Prenatal gruplar 17.,19., ve 21. günlerden oluşurken; yavrulardan oluşan doğum sonrası gruplar 1,5,10,15. günlerden oluşturuldu. Denekler etik kurallara uygun olarak dekapite edildikten sonra kohleaları çıkarılıp ışık mikroskobunda incelendi.

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Bulgular: Kohlear tünelin lümeni prenatal dönem boyunca yalancı çok katlı epitel ile örtülüydü. Embriyonik dönemde kohlear kanalın scala media, scala timpani ve scala vestibuli bölmelenmesi tamamlanmadı. Korti organı doğum sonrası 15. güne kadar erişkin halini kazanmadı.

Sonuç: Sıçanlarda korti organı oluşumu postnatal 15. günde tamamlanır. Sıçan yavruları embriyonik ve erken doğum sonrası yaşamlarında işitemezler.

Anahtar kelimeler: Korti organı, Postnatal gelişim, Prenatal gelişim.

1. INTRODUCTION

The ear serves as the primary organ of hearing and balance and is located in the temporal bone. It consists of three parts, the outer, middle and inner ears, all with different functions and structures[1]. The inner ear contains the receptors related to hearing and balance (Donaldson - Duckert, 1991:23; Akyıldız, 1998:97; Karasalihoglu, 2003:3) and is located in the deep petrous part of the temporal bone (Kopelman, vd.1998:858; Chen, vd. 2006:820). The first morphological event in all vertebrates is the formation of the embryonic otic plaque, the thickening of the head ectoderm in the developing hindbrain. By interacting with tissue from numerous other embryonic sources and adding tissue, the plaque develops into the differentiated structure of the otic vesicle. Almost all types of cells in the inner ear, including inner and outer hair cells, sensory neurons, secretory cells and support cells, consist of bilateral ectodermal thickening (Noden, vd.1986:15; Torrenza- Giraldez, 1998:5). It is known that human cochlea is fully mature before birth. Histology of the rat cochlea resembles human cochlea. But is their embryology similar as well? Is rat cochlea fully mature before birth? The aim of this study is to describe the prenatal and postnatal development process of the organ of Corti in the inner ear, responsible for hearing function, using light microscopy.

2.MATERIALS AND METHODS

In the present study, the experiments were performed according to the guidelines (NIH, UCSF) on animal use. The experimental protocol was approved by the Animal Experiment Ethics Committee of xxxxxx University (approval no. 06.11.2019.TS.11.KN.19/202). Wistar Albino rats obtained from the Experimental Research and Application Center of xxxxxx University, were placed into cages containing two females and one male and allowed to mate. The following morning, vaginal smears were taken, and females exhibiting sperm in specimens were regarded as 0.5 days pregnant and were included in the experiment. The rats were equally and randomly divided into two groups. Each group was further divided into subgroups of six rats each:

Group I: Prenatal subjects consisting of embryos on day 17, 19, 21, and

Group II: Postnatal subjects consisting of pups on days 1, 5, 10 and 15.

Embryos were collected under anesthesia on days 17, 19 and 21 days of pregnancy. Postnatal rats were decapitated on days 1, 5, 10 or 15. Examination showed that ossification was incomplete in the prenatal period (days 17, 19, and 21) and at the beginning of the postnatal period (days 1 and 5). The embryo and pup heads were divided into two equal parts and fixed in 10% formaldehyde. Since ossification was complete in the later postnatal periods (days 10 and 15), cerebral tissues were removed through an incision from the foramen magnum to the end of the parietal bones using thin-tipped scissors. The temporal bone was removed from the lower, middle and anterior regions by following the cochlear nerve. The cochlea, protruding towards the middle ear, was fixed, and excess bone tissues around it were removed with scissors. The vestibular connection was thus opened, and the vestibule was removed. Following fixation, the bone tissue was softened in a solution consisting of 80 ml distilled water + 10 ml formaldehyde + 10 ml nitric acid for decalcification. The tissues were washed under running tap water and exposed to routine histological follow-up procedure. Following dehydration with %50, 70, 80, 96 and three times %100 alcohol and clearing with xylene, the cochlea was embedded in paraffin in a horizontal position. Next, 5-µm thick serial sections were taken from the paraffin blocks, deparaffinized and rehydrated, stained with Hematoxylin & Eosin, and examined under the Olympus BX51 microscope.

3. RESULTS

The cochlear tunnel was examined under light microscopy during embryonic development. The lumen of the cochlear tunnel was lined with pseudostratified columnar epithelia throughout the prenatal period (17th and 19th days). The cochlear tunnel was not divided into scala media, scala tympani and scala vestibuli in the embryonic sections (Fig. 1, Fig. 2). Although ossification did not occur, hyaline cartilage structure was preserved in all groups. On day 21, enlarged areas formed in the connective tissue surrounding the cochlear tunnel (Fig. 3).

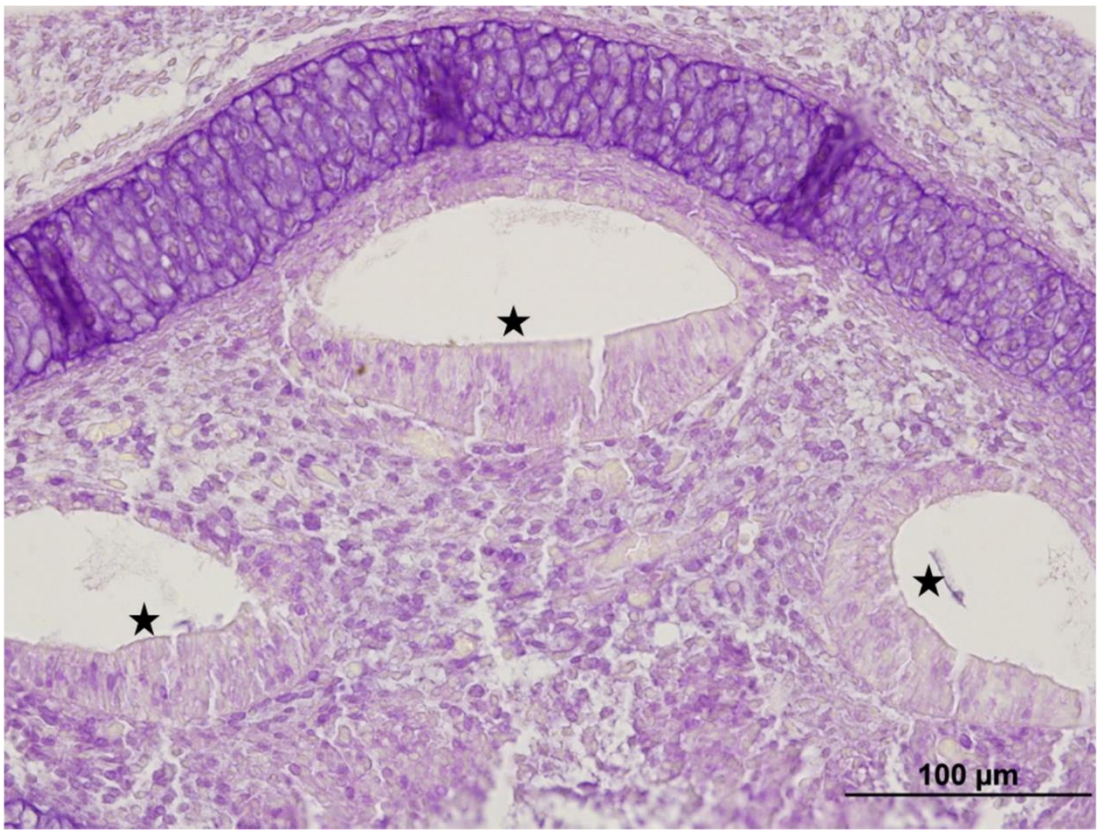

Figure 1: Photomicrograph of ear in the prenatal 17th day, organ of Corti (*), H&EX40.

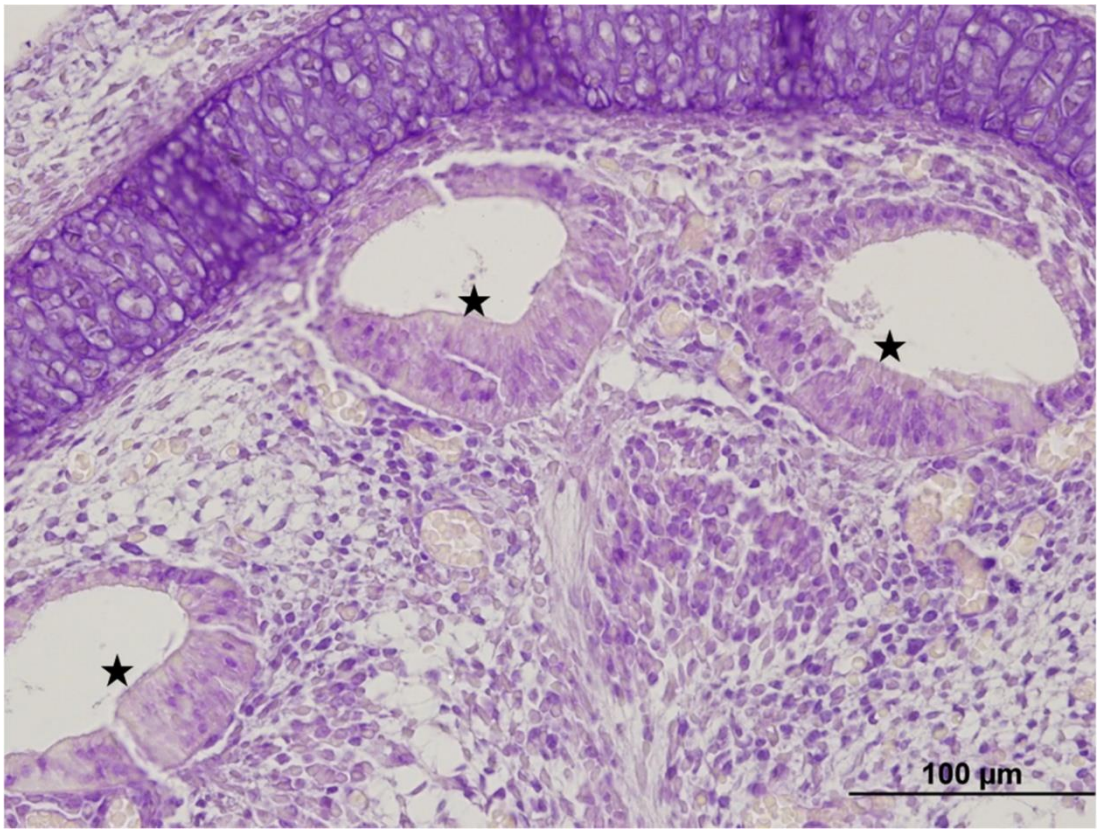

Figure 2: Photomicrograph of ear in the prenatal 19th day, organ of Corti (*), H&EX40.

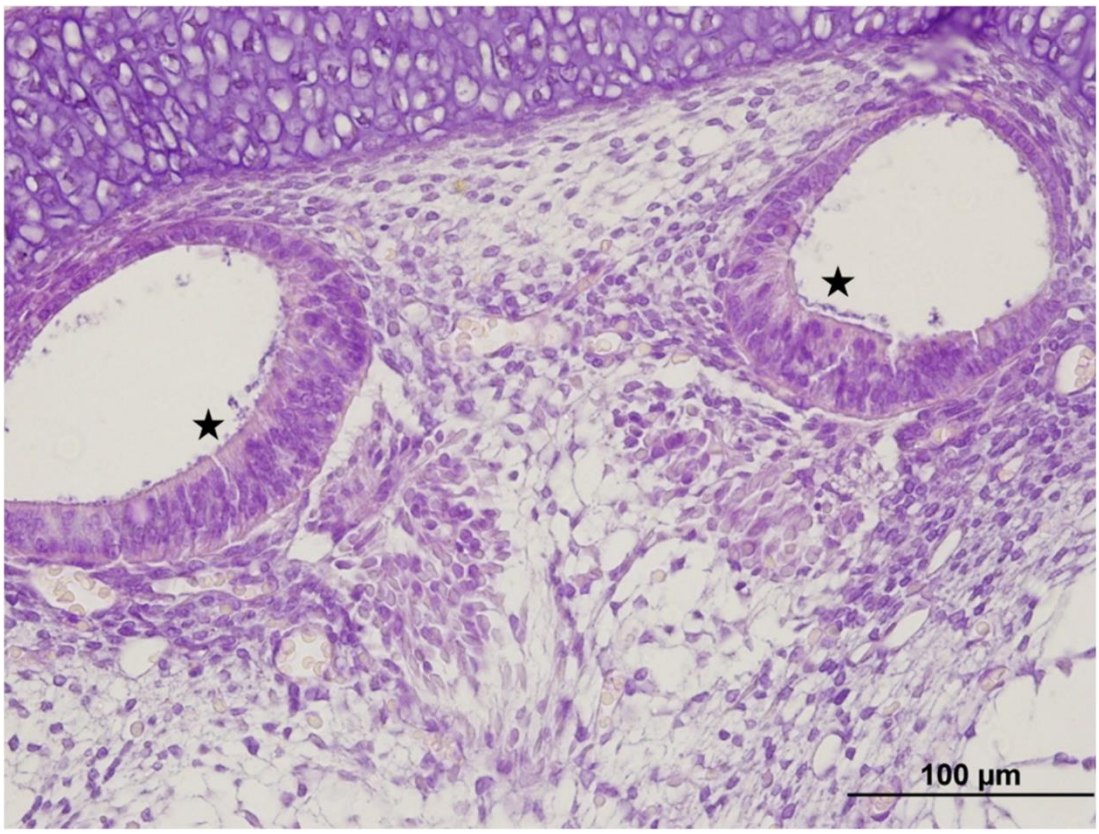

Figure 3: Photomicrograph of ear in the prenatal 21th day, organ of Corti (*), H&EX40.

On postnatal day 1, histological images revealed incomplete division of the cochlear tunnel. The pseudostratified columnar epithelia cells surrounding the cochlear tunnel in the embryonic period underwent changes in the postnatal period, and the organ of Corti in the scala media began becoming visible (Fig. 4). On postnatal day 5; scala media, scala tympani, and scala vestibuli were delimited by Reissner and Basilar membranes in the cochlear canal. At the same time, over the organ of Corti the tectorial membrane, started becoming visible, although the tunnel of Corti was indistinguishable (Fig. 5).

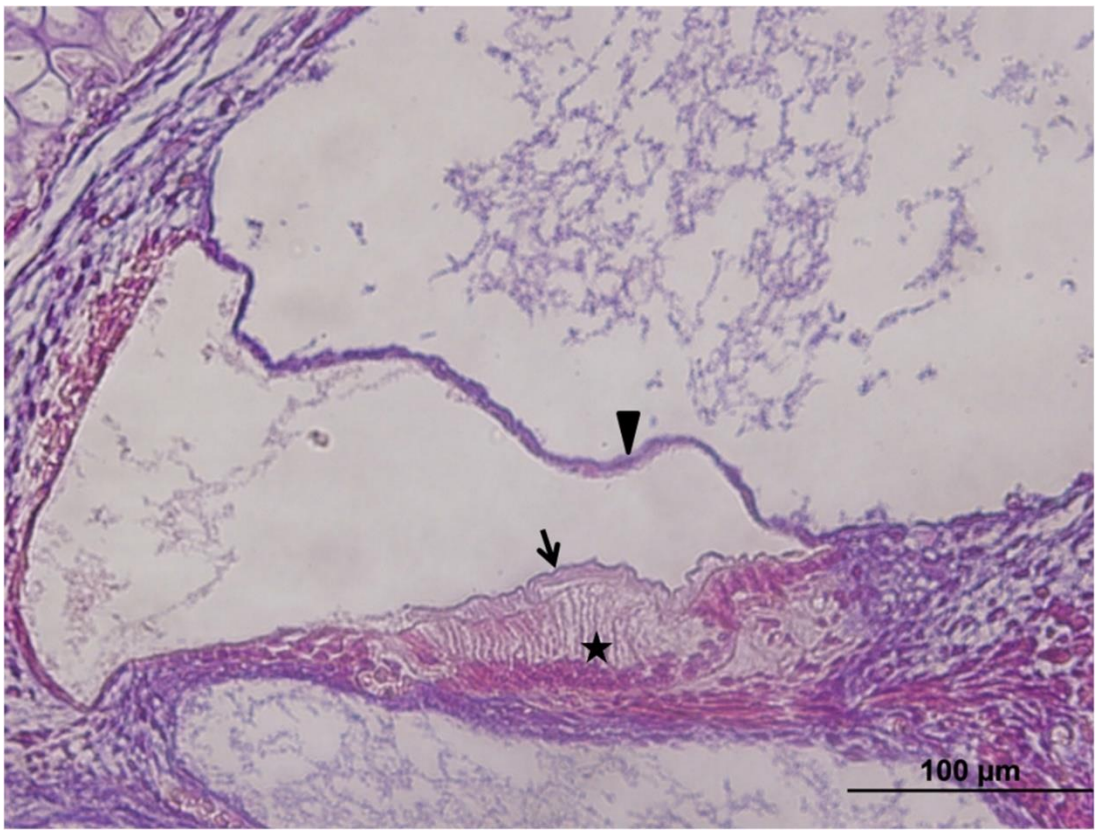

Figure 4: Photomicrograph of ear in the postnatal 1th day, Reissner membranes (arrowhead), Tectorial membrane (thin arrow), organ of Corti (*), H&EX40.

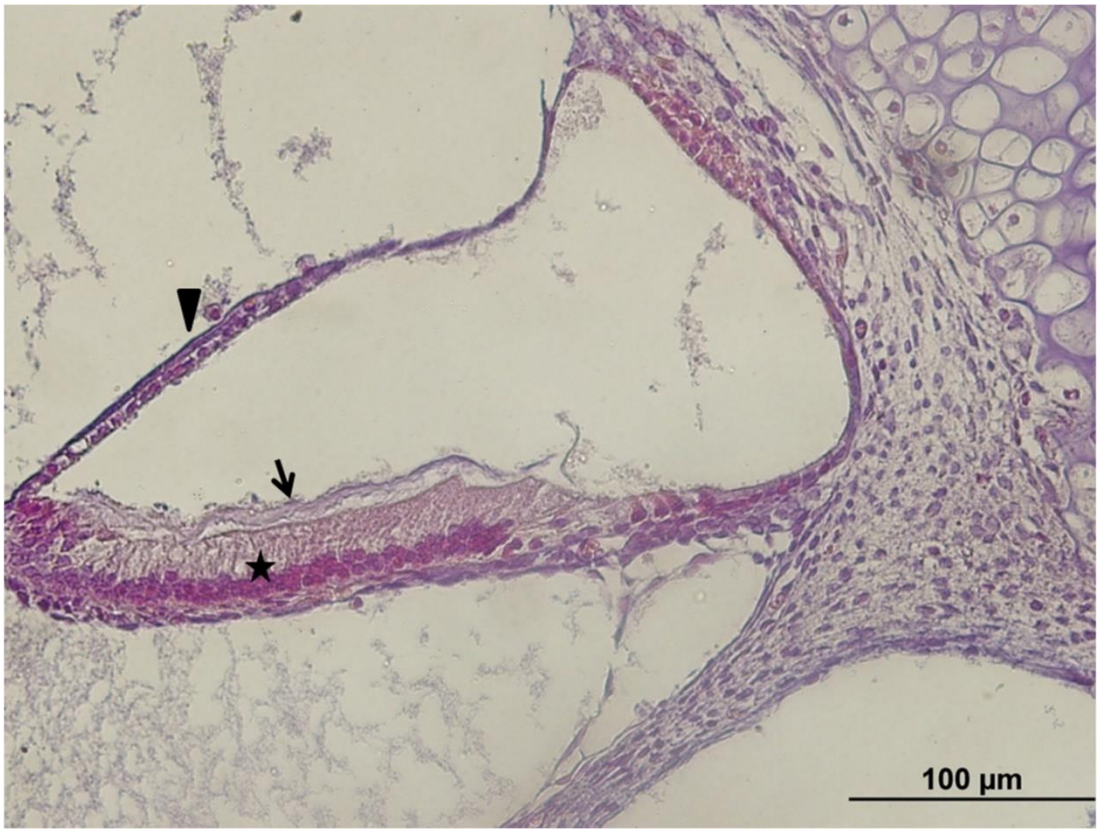

Figure 5: Photomicrograph of ear in the postnatal 5th day, Reissner membranes (arrowhead), Tectorial membrane (thin arrow), organ of Corti (*), H&EX40.

On postnatal day 10, although the tectorial membrane was completely separated from the cells, still the organ of Corti located in the scala media region continued to develop. The tunnel of Corti was still not fully formed even on day 10 (Fig. 6). The organ of corti took its final form on the 15 th day after birth. The tunnel of Corti became visible following the division of the tunnel and the complete formation of the tectorial membrane. On postnatal day 15, the outer and inner hair cells, playing a significant role in hearing, were located in one internal row and three external rows, respectively and the supporting Deiters cells started to become visible following the complete formation of the scale media (Fig. 7).

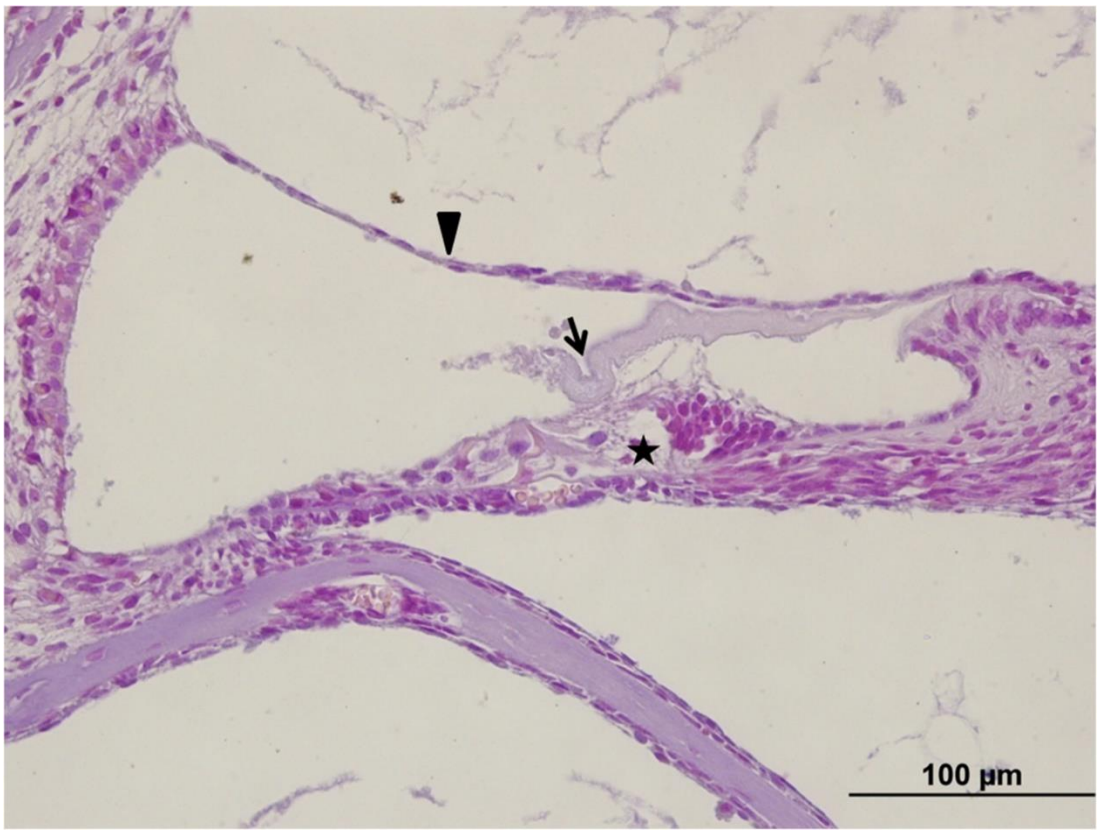

Figure 6: Photomicrograph of ear in the postnatal 10th day, Reissner membranes (arrowhead), Tectorial membrane (thin arrow), organ of Corti (*), H&EX40.

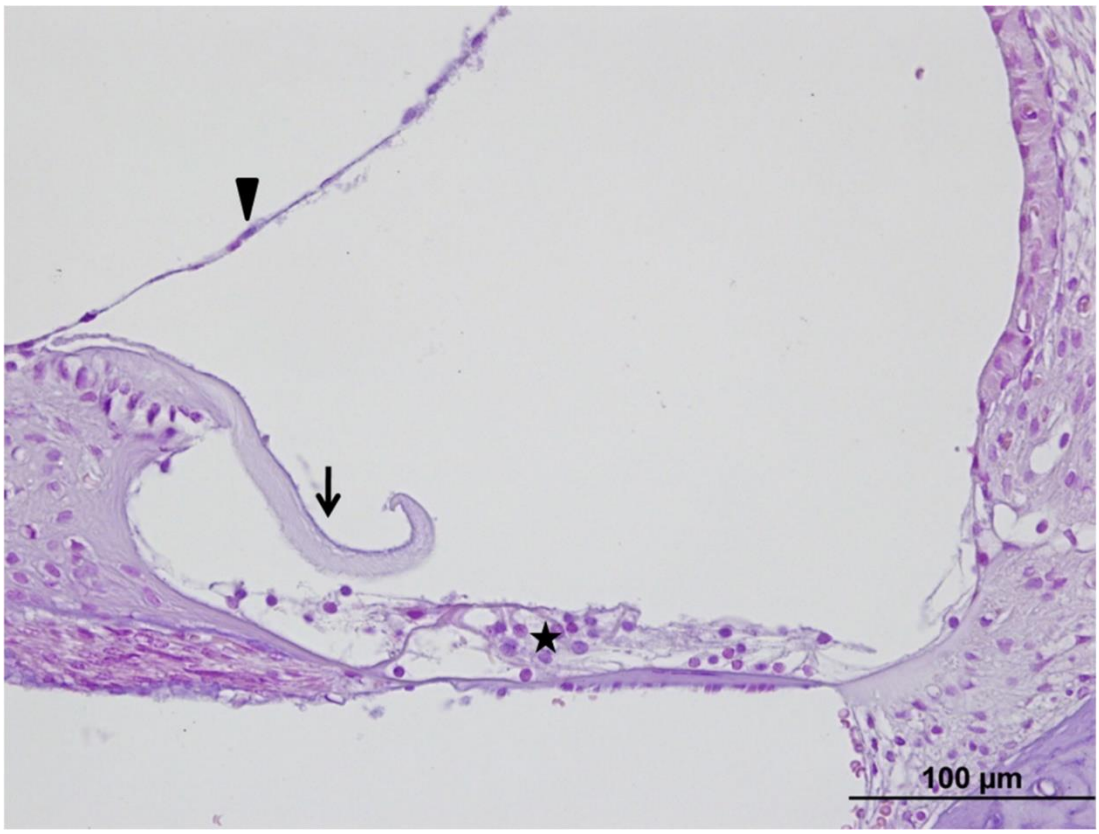

Figure 7: Photomicrograph of ear in the postnatal 15th day, Reissner membranes (arrowhead), Tectorial membrane (thin arrow), organ of Corti (*), H&EX40.

4. DISCUSSION

This study focused on the development of the rat ear in the late embryonic and early postnatal periods. In contrast to humans, the development of the rat ear was observed to continue until postnatal day 15. During the early morphogenesis of the inner ear, the bilateral otic plaques initially begin being vaginalized by the otic cups. Then, they migrate to the neural tube to form hollow epithelial spherical structures known as otic vesicles or autocysts. Their separation from the surface ectoderm and the inner ear has been reported to originate from this first transient embryonic structure (week 4 of gestation in humans, embryonic age E8.5 (8.5-9.25 dpc; 8-12 somite pairs) in mice, and (9.5-10.25 dpc) in rats (Torrenza-Giraldez, 1998:5; Whitfield, 2015:112). The otic vesicle contains all the genetic information required for autonomous improvement of the diversity of specialized cells in the mature inner ear. However, mutual tissue inductions have been described as necessary for ensuring that different cells develop in the correct regions along with the architecture of the membranous and bony labyrinth. The formation of the otic vesicle is reported to be due to induction of endoderm, mesoderm and neuroectoderm-induced signals (Wu-Kelly, 2012:1).

In humans; the long, columnar epithelial cells of the growing cochlea channel differentiate into the organ of Corti with multiple support and secretory cells in that organ at between 7 and 9 weeks. Receptor cells on the basilar membrane are arranged in three rows of outer and one row of internal hair cells and are located regularly along the longitudinal axis of the cochlear duct. The organ of Corti is innervated by the spiral ganglia emerging through the center of the modiolus, the nerve fibers arising from the otic ganglia (Powles-Maconochie, 2018:228).

In the final stages of cellular differentiation, terminal mitoses represent the last part of the cell's development and mark the beginning of a completely differentiated cell population (until damaged). The hair cells at the apical end of the mouse cochlea undergo terminal mitosis on embryonic day 12 (E12) (Powles-Maconochie, 2018:228). In our study, cell differentiation occurred in the region where the organ of Corti would subsequently develop when the cochlear channel initially consisted of pseudo-multiple epithelium.

The terminal mitosis of the hair cells in the cochlea begins in the mid-stage of embryonic development. In rodents, similarly to other mammals, the differentiation of hair cells begins during terminal mitosis during embryonic development (approximately E18), but continues for 2 weeks after birth (Lou, vd. 2007:28; Marcotti, 2012:438). In the present study, the cells in the region where the cochlea would subsequently develop in the embryonic period continued until postnatal day 15, and the development of the cortical tunnel was completed with the formation of a tectorial membrane on the epithelial cells.

The developing human cochlea is essentially functional at birth, but neuronal connections continue to develop postnatally by refining both stimulant and inhibitory inputs. Many stages of development, including maturation of axonal and dendritic synaptic connections with sensory hair cells and maturation of highly specialized strip synapses with sensory cells, continue to until late childhood in humans (Moore-Linthicum, 2007:460). In this study, similar results were obtained.

During the embryonic period (days 17, 19, and 21), the cochlea was in a tunnel structure surrounded by pseudostratified columnar epithelia. The scala media, scale tympani, and scala vestibuli were delimited by the formation of Reissner and Basilar membranes in the cochlear canal from the first postnatal day. By postnatal day 15, with the formation of the tunnel of Corti, the inner and outer hair cells and support cells, had acquired similar features to those of the adult structure.

5. CONCLUSION

Our examination of prenatal and postnatal development stages revealed that embryonic development in rats corresponded to later stages than in humans and was a gradual process. This means that, contrary to the human, rats cannot hear before birth since cochlea reaches to full maturity after birth.

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