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ORIGINAL ARTICLE

Post-hatching development of the turkey pineal organ: Histological and immunohistochemical studies

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Abstract

OBJECTIVE: The study was performed to analyze structural changes of the turkey pineal during the post-hatching development.
MATERIAL AND METHODS: The pineals were collected from male turkeys at the age of 1 day, 2, 8, 22, 56 weeks and subjected to histological investigations including morphometrical analyses. The pinealocytes were identified immunohistochemically using antiserum against hydroxyinolo-O-metyltransferase (HIOMT).
RESULTS AND CONCLUSIONS: Independently of age, the pineal consisted of a narrow proximal part and a club-shaped top. The narrow part extended into the stalk attached to the diencephalon. The pineal parenchyma was formed by the follicles, surrounded by the connective tissue. The caudal part of the organ contained the pineal lumen, which prolonged into the stalk lumen. Up to the age of two weeks the stalk lumen was open to the third ventricle, later – closed. The proximal part of the stalk showed age-dependent reduction. During the investigated period of life, the pineal increased in size due to creation of new follicles, enlargement of the follicles and development of the stroma. In immature turkeys, the follicular wall was formed by elongated cells bordering the lumen and sparse, peripherally localized, round cells. This pseudostratified organization was transformed during ontogenesis into thick, multilayer structure (characteristic for 22- and 56-week-old turkeys) composed by the layer of elongated cells and several layers of round cells, located peripherally. The rudimentary-receptor and secretory pinealocytes were demonstrated based on HIOMT-immunoreactivity. The secretory pinealocytes were sparse in young birds and predominated in mature turkeys. Intra-pineal calcified concrements occurred in 56-week-old turkeys.

Introduction

The phylogentic transformation results in considerable diversity of the vertebrate pineal organs concerning their histological forms, structure of parenchymal cells and regulation of melatonin secretion [1, 2, 3, 4, 5, 6].

The avian pineal organ is considered as a transitional type between the photosensory pineal organ of lower vertebrates and the endocrine pineal gland of mammals [1, 2, 3, 4, 5, 7, 8]. In consequence of the evolutionary position, the avian pineals present high variability of forms [3, 4, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21]

Based on the histological structure two classifications of the avian pineal organs have been suggested. According to the first classification three principal morphological types are distinguished: saccular, tubulo-follicular and solid [4, 9]. In the second one, the avian pineals are divided into two structural forms: tubulo-follicular and solid-tubular [12, 17, 18]. In many avian species the intermediate forms have been described as well [12, 13, 20].

Parenchyma of the avian pineal organ consists of three types of cells – pinealocytes, supporting cells and nerve cells. Avian pinealocytes, taking the intermediate position between pineal photoreceptory cells of lower vertebrates and secretory pinealocytes of mammals, display a large morphological variability. Remarkable differences in the appearance of receptive and effective poles of avian pinealocytes are observed [4, 11, 12, 21]. Although, there is no generally accepted and used classification of these cells, three main types of avian pinealocytes are distinguished: receptor pinealocytes, rudimentary-receptor pinealocytes and secretory pinealocytes [1, 3, 4, 22, 23, 24, 25]. The rudimentary-receptor pinealocytes (called the follicular cells in the chicken), which distinguished by the presence of apical protrusions projecting into the lumen of follicle, are the most frequently observed. The receptor pinealocytes resembling photoreceptor cells of lower vertebrates are mainly found in saccular organs, whereas the secretory pinealocytes (called the parafollicular cells in the chicken) are typical for the organs with a more solid structure. Functionally, avian pinealocytes are similar to pineal photoreceptory cells in poikilotherms and they possess photoreceptive capability, endogenous circadian oscillator and melatonin synthesizing machinery [26, 27].

Supporting cells are represented by ependymal-like cells localized around the cavities of the pineal follicles and small, round cells present in the outer part of the follicular wall. Ependymal – like cells are slim with microvilli or cilia on their apical surface [4, 10, 15]. The population of the supporting cells localized peripherally in the follicles is poorly known.

The third type of the cells – neurons – includes the acetylocholinesterase–positive afferent ganglion cells [7, 8, 28, 29, 30] and the neurons showing NADPH-diaphorase activity [31]. The number of acetylocholinesterase – positive neurons varies extremely from species to species [8].

The knowledge about the process of the avian pineal organ differentiation during ontogeny is based mostly on the studies performed on the domestic fowl [17, 22, 24, 25, 32, 33, 34, 35, 36, 37, 38, 39, 40]. During the post-hatching development age-dependent increase in organ size, the modification of connection between the pineal organ and the brain as well as and regression of the pineal stalk were observed [4, 7]. The developmental changes were also reported in communication of the pineal lumen with the third ventricle [24, 38]. The parenchyma appearance changed during the post-hatching life from the vesicular structure to the compact one resembling a solid structure of the mammalian pineal [33]. Simultaneously, chicken pinealocyte population underwent the transformation, leading to the increase in the percentage of parafollicular cells [11, 17, 22, 23, 33, 34, 36, 39]. The ontogenetic development of the pineal cells has also been described in some other avian species [18, 30, 41, 42, 43]. The in vitro studies show that embryonic pineal cells can differentiate into various forms of pinealocytes and neuron-like cells [41, 42, 43]. However, little is known about the regulatory mechanisms involved in determination and differentiation of various pineal cell types [42, 43].

The innervation of the avian pineal gland also shows remarkable changes during ontogeny. The most important one includes progressive reduction of the pinealofugal component, clearly visible in the decrease in AChE – positive neurons and the parallel increase in the pinealopetal sympathetic innervation [29, 30, 37]. The post-hatching development is also the time of changes in cells immunoreactive to neuron-specific enolase, what has been interpreted as a sign of increase in neurosecretory – like capacity of the pineal organ [39].

The great morphological variability of the avian pineal organ, known on the basis of studies being carried out so far, suggests an occurrence of important interspecies differences in the pineal development during ontogenesis. Few published reports concerning the developmental changes in the pineal morphology in other avian species than the chicken, including our preliminary data about the turkey pineal gland [44], strongly support this supposition. Therefore, the present study was performed to examine changes in histological structure of the pineal organ of domestic turkeys from the hatching to the age of 1 year. Special attention was paid to the changes in structure of the pineal follicles, which were subjected to morphometrical analysis. In addition to histological and histochemical methods, the presence of hydroxyindolo-O-methyltransferase, the last enzyme in melatonin synthesis pathway, was detected by immunohistochemistry to identify pinealocytes among cells forming the pineal follicles.

The studies on the pineal organ in turkeys may have a practical significance. Recently published data show that the treatment with melatonin, the main pineal hormone, improves the function of the turkey immune system [45]. Melatonin and short photoperiod prevent the formation of ovarian tumors in turkey hens [46]. The positive effects of melatonin on reproductive system in male turkeys have been also reported [47]. Moreover, it can be

suspected, that melatonin may play an important role in antioxidative defense system in birds [48, 49], especially in fast-growing broilers.

Materials and methods

Animals and tissues

The studies were performed on fifty males of the domestic turkey (Meleagris gallopavo). The birds (apart from new-hatching ones) were housed under a cycle of 12 hours light: 12 hours dark (light form 07:00 to 19:00). The turkeys were anesthetized with halothane and killed by decapitation between 08:00 and 09:00 during the first day after hatching and at the age of 2, 8, 22 and 56 weeks (10 animals per each age-group). The pineal glands with adjacent parts of the brain were immediately removed and prepared to the investigations.

Histological and histochemical studies

Five pineal organs (with neighbouring parts of the epithalamus, the cerebrum and the cerebellum) from each age-group were fixed in Solcia's solution, two others – in 70% ethanol. One pineal from each group was cut into small pieces, which were fixed in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in phosphate buffer (pH 7.4).

The tissues were dehydrated and embedded in a paraffin (after fixation in Solcia's solution and ethanol) or in Epon 812 (after fixation in a mixture of glutaraldehyde and paraformaldehyde). The pineals embedded in paraffin were cut in sagittal plane into consecutive 7 μm thick sections using a Reichert microtome and the fragments embedded in Epon – into 1 μm thick sections using a Leica ultramicrotome.

The tissues fixed in Solcia's solution were stained with hematoxylin-eosin and Mallory's methods. The alcohol-fixed pineals were stained in turns with one of two methods for histochemical demonstration of calcium: Alizarin red S procedure and von Kossa's method [50] As a control we used the sections decalcified by incubation in 10% solution of EDTA for 5 hours. Semithin sections of Epon-embedded tissues were stained with toluidine blue.

Immunohistochemical studies

Two pineal organs from each age-group were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 60 minutes. Then, the tissues were rinsed with phosphate buffer and transferred into 25% sucrose solution in the buffer, where they were kept (at 4oC) until sectioning. The organs were frozen at –35 oC and cut into 15 μm thick sections using a Microm cryostat. The sections mounted on gelatinized glass slides were subjected to an immunohistochemical procedure using the rabbit primary antiserum against chicken HIOMT (working dilution 1 : 500), obtained from dr Pierre Voisin (University in Poitiers, France) and characterized in details previously [51]. The primary antibodies bounded to the antigen present in tissue slides were visualized using swine anti-rabbit immunoglobulins, ABComplex and 3,3'-diaminobenzidine (all from DAKO Cytomation) as described earlier [52]. A control procedure was performed with the omission of the primary antiserum.

Morphometrical analyses

The morphometrical analyses were performed on sagittal paraffin sections of the pineal organs fixed in Solcia's solution. The sections were stained with Mallory's method.

The length of the pineal stalk, dimensions of the pineal body and the choroid plexus were measured on 15 middle-sagittal sections of each pineal organ (by a microscope with an eye piece containing a ruler) and expressed as averages. Using the same sections the average number of pineal follicles per sagittal section was calculated.

The measurements of the pineal follicles were performed using the image analysis program Zeiss Axiovision LE ver. 4.1. For this analysis, the images of 40 follicles from the sagittal sections of each pineal organ were randomly taken at an objective magnification of 10 x using the digital camera Olympus DP-12. The following measurements were performed: the area of follicle section, the area of follicular lumen section and the thickness of the follicular wall. Additionally, a ratio of the follicular lumen area to the follicle section area was calculated. The data were analyzed using a one-way analysis of variance followed by a Duncan test as a post hoc procedure. The value of $p \leq 0.05$ was taken as significant.

Results

Histology and histochemistry

New-hatching animals

The pineal organ of one-day-old turkeys consisted of a narrow part located between the cerebrum and the cerebellum as well as a club-shaped top, closely attached to the dura mater. The organ length was 2 mm and the diameter of the distal part ranged from 0.5 to 1 mm. The narrow part of the pineal body prolonged into the pineal stalk that was attached to the intercommissural region of the diencephalon, closer to the habenular commissura than to the posterior commissura. The pineal stalk was composed of the epithelium and the connective tissue. Sporadically, few follicles of the pineal parenchyma were observed in its distal part. The stalk had the central lumen, which contacted with the third ventricle by a narrow cleft of the recessus pinealis. The pineal gland was surrounded rostrally by the well-developed choroids that consisted of elongated cisterns formed by a cuboid epithelium (*Fig.1A,B*).

The pineal body was covered by a connective tissue capsule with numerous blood vessels. The thickness of the capsule differed depending on the region of the organ and it was particularly prominent on the apical surface of the pineal. Thin septa originating from the capsule and penetrating inside the organ created a delicate stroma. They were composed of collagen fibres, connective tissue cells, lymphocytes and numerous blood vessels.

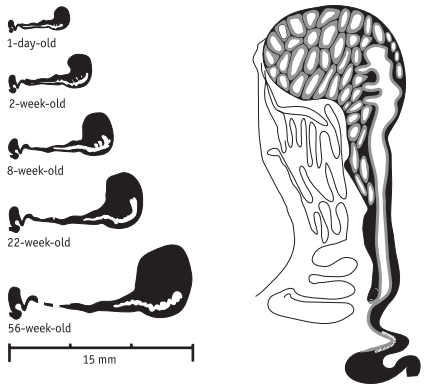

Figure 1. Schematic representation of structural changes occurring in the pineal organ of the domestic turkey (Meleagris gallopavo) during post-hatching development. A. Diagram illustrating size of the pineal body and the pineal stalk at the investigated stages of development. B–F. Diagrams showing the changes in internal structure of the pineal body and pineal stalk in birds at the age: B – 1 day, C – 2 weeks, D – 8 weeks, E – 22 weeks, F – 56 weeks. Note changes in connection between the third ventricle and the central lumen of the pineal stalk, progressive atrophy of the pineal stalk, alternations in the pineal follicles and the connective tissue septa as well as changes in the pineal lumen. HC – the habenular commissure, PC – the posterior commissure

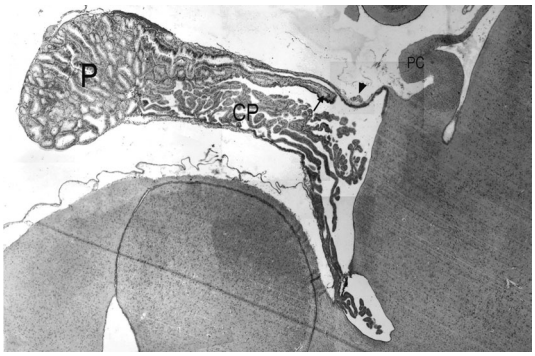

Figure 2. Sagittal section of the pineal organ in two-week-old turkey. Note relations between the pineal body, the pineal stalk and the choroid plexus as well as connection of the pineal with diencephalon, and localization of the accessory pineal tissue. P – the pineal body, CP – the choroid plexus, arrow – the habenular commissure, PC – the posterior commissure, arrow head – the accessory pineal tissue.

The pineal parenchyma was formed by oval or round follicles which number ranged from 60 to 80 on the middle-sagittal section. Oval, usually larger, follicles were situated mostly in the rostral and distal areas of the club-shaped apical part of the organ and round, usually smaller, vesicles the in the remaining areas of the gland. The follicles contained a lumen, which was variable in size and empty. The follicular wall was formed by two types of cells – columnar cells lining the lumen and round, small cells located at the periphery. A part of the columnar cells possessed club-shaped or elongated apical segments extending into the lumen. The number of cells with apical extensions differed among follicles. The peripherally located round cells were infrequently observed. The follicular wall had a regular outline from the connective tissue (*Fig. 3A,B*).

In the caudal part of the gland there was the lumen of the pineal body, about 200 μm in width. It prolonged from the central lumen of the pineal stalk to 2/3 of the pineal body length. The wall of pineal lumen was formed by a columnar epithelium, which shows no differentiation of its adluminal surface. There were also some cells situated basally to the columnar epithelium, but they did not create a continuous stratum. In the club-shaped part of the gland, the lumen had some follicular extensions and derivatives (*Fig. 1B*).

A number number of cells during mitosis was noted in both the follicular walls as well as in the wall of the pineal lumen.

Two-week-old animals

In 2-week-old turkeys, the pineal organ increased in size and the pineal stalk was longer comparing to new-hatching birds. The choroids plexus showed no enlargement. There was still a connection between the pineal lumen and the third ventricle by the central lumen of the stalk, although the cleft of the recessus pinealis was very narrow (*Fig.1A,C; 2*).

The pineal capsule as well as the connective tissue septa were thicker and contained more numerous blood vessels than in birds at the age of 1 day (*Fig. 1C; 3C,D*). Lymphatic cells were clearly visible in the stroma. In the apical part of the pineal body, the lumen formed many follicular extensions as well as derivatives. There were small, new-created follicles in the part of the pineal body situated caudally to the pineal lumen. Some follicles were also formed in the part of the pineal stalk nearly to the habenular commisure. Total number of follicles on the sagittal section in 2-week-old birds ranged from 80 to 100.

The thickness of the follicular wall was higher than in one-day-old birds. The wall showed pseudostratified appearance with three rows of cell nuclei: the first one with a small number of round nuclei situated basally, the second one with numerous, ovally shaped nuclei in the middle part of the wall and the third one containing only single apically located nuclei. In the follicular cavity, different types of the cellular prolongations were observed: bulbous prolongations, elongated prolongations with numerous processes creating some reticular structure inside the lumen and elongated prolongations without processes. The apical prolongations were especially numerous in the follicles situated in the rostral part of the pineal body. The cellular debris was noted in the lumen of the follicles, particularly in those localized in the central part of the pineal body (*Fig. 3C,D*).

In one investigated pineal organ, the accessory pineal tissue, formed by few small follicles was found. The accessory pineal tissue was localized close to the proximal part of the pineal stalk (*Fig. 2*).

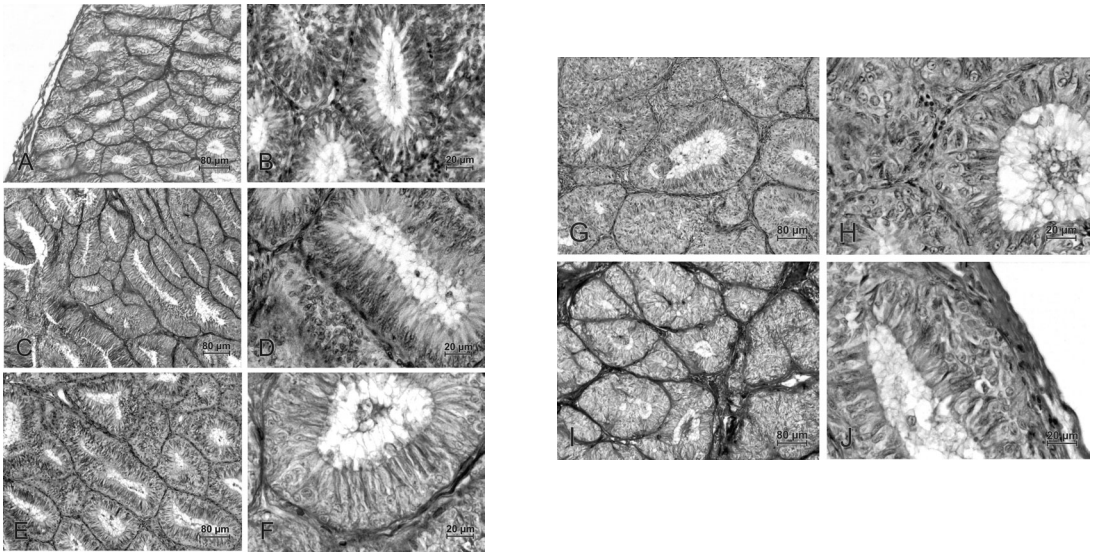

Figure 3. Histological structure of the pineal follicles in turkeys at the age: A, B – 1 day, C, D – 2 weeks, E, F – 8 weeks, G, H – 22 weeks, I, J – 56 weeks. Note changes of the follicular wall arrangement, appearance of the apical protrusions, relation between the wall thickness and the follicular lumen size, changes in the stroma.

Eight-week-old animals

Between the 2nd and 8th week of the turkey post-hatching development the process of morphological changes was continued (*Fig. 1A,D*). There was a clear increase in the pineal organ size. The stalk showed a visible decrease of the thickness nearly to the habenular commissura. The central lumen of the stalk was reduced. In the 8-week-old birds, there was no cleft connecting the pineal recessus with the central lumen, which was completely separated from the third ventricle. The choroid plexus was attached only to the narrow part of the pineal organ and the pineal stalk.

The thickness of the connective tissue capsule and septa was markedly higher than in two-week-old turkeys (*Fig. 1D; 3E,F*). There was an increase in number and size of the blood vessels both in the connective tissue septa and capsule. Numerous blood vessels were also observed in a loose connective tissue located in close vicinity to the pineal organ.

The number of the follicles per middle-sagittal section was increased to 100–120. Evidently, the follicles were created from the pineal lumen, from which arose numerous follicular extensions and derivatives both to the central as well as to the caudal region of the pineal organ arose. Moreover, the cells of the pineal lumen wall showed mitosis. The new follicles were usually smaller and localized close to the pineal lumen.

The follicular wall still showed a pseudostratified structure (*Fig. 3E,F*). However, some follicles were distinguished form others by the presence of two cell layers: 1) the internal layer of the columnar cells bordering the follicular lumen and 2) the external layer of round or oval cells. A large part of the columnar cells possessed apical prolongations, both bulbous and elongated in shape. There was a large quantity of debris as well as some cells in the follicular lumens.

Twenty-two-old week animals

Between the age of 8th and 22nd week the pineal organ enlarged, however the number of follicles still varied between 100 and 120. The marked increase in thickness of the connective tissue septa has been noted (*Fig. 1A,E; 3G,H*). Some of them created thin branches, which penetrated into the follicular wall and partially separated smaller portions of the follicle. The penetration of connective tissue septa into the pineal lumen has been also observed. The lymphatic cells were as numerous as in younger birds. In one case a lymphoid nodule was found in a central part of the organ (*Fig. 5*). The cartilage tissue was observed in the pineal capsule attached to the dura mater. The accessory pineal tissue, consisting of two or three vesicles, was found in two investigated pineals (*Fig. 6*).

The wall of follicles clearly showed the presence of two parts: the basal part composed by several layers of round and oval cells and the apical part composed by elongated and cone-shaped cells bordering the follicular lumen. In comparison to the younger birds the follicular wall was markedly thicker and the follicular lumen was smaller. The elongated cells contained less apical prolongations (*Fig. 3G,H, 7AB*).

Fifty six-week-old animals

In comparison to younger turkeys, the pineal organ of birds aged fifty six weeks was markedly bigger, but the number of the pineal follicles per the middle-sagittal section was similar to that in 22-week-old animals. The most characteristic features of the pineal organ in adult turkeys were 1) well-developed connective tissue septa with numerous blood vessels around the follicles, 2) very thick follicular wall surrounding the small lumen and 3) the presence of calcium concretions (*Fig. 1A,F; 3I,J*).

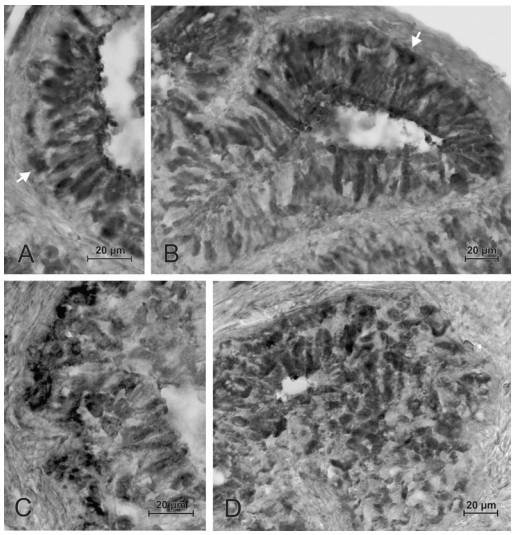

Figure 4. HIOMT – immunoreactivity in the pineal organs of turkeys at the age: A – 1 day, B – 2 weeks, C – 22 weeks, D – 56 weeks. Note presence of numerous positive elongate cells with apical protrusions in the follicular wall of 1-day- and 2-week-old birds. Positive, round or oval cells are sparse in new-hatching and 2-week-old birds (arrows) and numerous in mature turkeys.

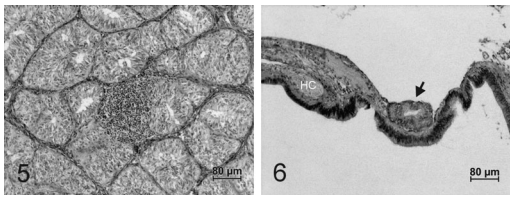

Figure 5. Lymphatic tissue in the pineal parenchyma. 22-week-old turkey.
Figure 6. Accessory pineal tissue (arrow) localized close to the pineal stalk in 22-week-old turkey. HC – the habenular commissure

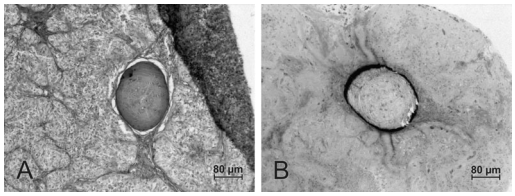

Figure 7. Calcified concretions in pineal parenchyma of 56-weeks old turkey. A – positive staining with alizarin red S method; B – concentric arrangement with osteoblast-like cells inside (semithin sections stained with toluidine blue).

The follicular wall was formed by two well distinguished parts – the basal one consisting of several layers of round or oval cells and the apical one bordering the lumen. The apical part was composed by the cone-shaped and elongated cells. The apical prolongations of the elongated cell were observed sporadically (*Fig. 3J*).

Calcified concretions were found in the proximal part of the pineal organ (*Fig. 7A,B*). They were usually large, round or oval in shape with diameters 100–150 µm. Total number of concretions ranged from 3 to 6 per organ. The concretions were stained black using von Kossa's method and red with Alizarin S method. After decalcification by incubation with EDTA the sections showed negative results of alizarin staining. On the semithin section, the concretions showed the presence of osteoblast-like cells. We have never observed concretions located extrapineally, in the connective tissue of the capsule or in the choroid plexus.

Immunohistochemistry

The cells immunoreactivite with the antiserum against HIOMT were exclusively observed in the walls of the follicles. Two types of HIOMT-positive cells were distinguished: 1) elongated cells bordering the follicular cavities and 2) round cells located in the outer layer of the follicular wall and lacking contact with the follicular lumen (*Fig. 4A,B,C,D*).

In the pineal organs of one-day old turkeys, HIOMT immunonorectivity was found almost exclusively in the elongated cells. They were endowed with immunopositive bulbous prolongations projecting into the follicular cavities. The HIOMT-positive round cells were only occasionally observed (*Fig. 4A*).

In the birds aged 2 and 8 weeks, HIOMT-positive cells were slightly more numerous. A clear increase was noted in a number of the HIOMT-positive round cells lacking contact with the follicular lumen. The elongated cells were very well-developed and contained prominent apical prolongations (*Fig. 4B*).

In the follicular walls of 22- and 56-week-old turkeys, the elongated cells were sparse and they usually posses no apical prolongations. In contrast, the round HIOMT-positive cells were numerous and they were predominating components of the basal part of the follicular wall (*Fig. 4C,D*).

Morphometry of the pineal follicles

The mean area of the follicle section was significantly higher in 2- and 8-week-old turkeys than in the new-hatching birds. The significant increase in the area of the follicle section was also noted between 8th and 22nd week of the post-hatching life (*Fig. 8A*). The area of the follicular lumen section showed a progressive increase up to the age of 8 weeks and next the step-wise decrease (*Fig. 8B*). The ratio of the follicular lumen section area to the follicle section area was significantly lower in 22- and 56-week-old turkeys than in the younger birds (*Fig. 8C*). The thickness of the follicular wall increased significantly between the 1st day and the 2nd week as well as between the 8th week and the 22nd week of the post-hatching life (*Fig. 8D*).

Discussion

The results of the present study clearly indicate that the turkey pineal organ undergoes structural alternations during the post-hatching life. The developmental changes affect many aspects of the pineal morphology including the organ size, the attachment of the pineal stalk to the intercommissural region, the structure of

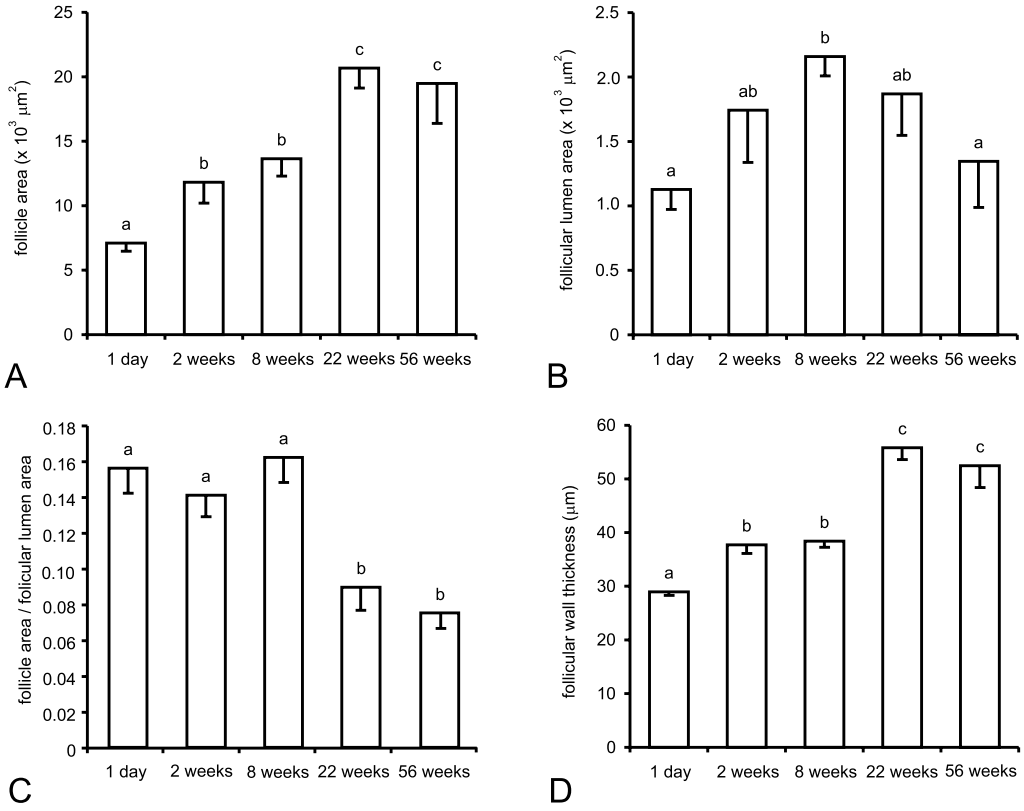

Figure 8. Morphometrical analysis of the pineal follicles at different stages of the turkey post-hatching development. A – the area of the follicle section, B – the area of the follicular lumen section, C – the ratio of the follicular lumen section area to the follicle section area, D – the thickness of the follicular wall. Measurements were done at the age of 1 day, 2 weeks, 8 weeks, 22 weeks and 56 weeks. The values signed with different letters are significantly different $p \leq 0.01$.

pineal parenchyma and stroma as well as the appearance of calcium concrements.

The problem of age-dependent changes in size and shape of the avian pineal organ is still under discussion. Variations between species and a limited number of data concerning developmental alternations make the analysis very difficult. The increase in the pineal organ size to the age of three years has been observed in the domestic fowl [38]. However, in the house sparrow, larger pineals have been found in young, immature birds than in mature ones [19].

We have found that the turkey pineal organ increases in size during the investigated period of life (up to the age of one-year). The pineal enlargement is very intensive during the first eight weeks of the post-hatching development. Then, the growth is slower, but it is continued even after sexual maturation (16 – 22 weeks). Despite the enlargement of the pineal, no important changes in the organ shape have been observed during the examined period of life.

The different structural composition of the pineal stalk and its variable attachment to the intercommissural region have been observed in the avian. The central lumen of the pineal stalk is commonly considered to have continuity with the third ventricle by a way of the recessus pinealis [9]. However, this structural condition of the pineal stalk is characteristic for adults of few species, such as the pied-billed grebe, but it is not typical for the adults of more advanced groups, such as passerine birds. The most important and frequent modifications of the pineal stalk composition in birds are: 1. reduction in number or loss of parenchymal cells, leading sometimes to apparent isolation of the distal parenchymal body of the organ from the brain; 2. reduction of the central lumen of the stalk, so that the pineal lumen located in the distal part of the pineal body is isolated from the third ventricle; 3. development of and invasion by nerve cells and fibers, forming at first an outer neural layer of the stalk and next remaining as the major stalk components [4, 9]. However, there is little contradictory information concerning the pineal stalk differentiation during ontogeny. In the domestic fowl, the pineal lumen is in an open communication with the third ventricle in 3-day-old chicken and it lacks this communication in 40-day-old chickens [38]. However Spiroff [24] observed the continuity of the pineal lumen with the third ventricle as late as three months after hatching. According to Sato and Wake [38], the pineal organ of 3-year-old domestic fowl is an anatomical entity fully separated from the brain.

Our present findings show that the morphology of the pineal region in the turkey differentiates due to changes in the proportion between the pineal body, the stalk and the choroid plexus as well as the connection of the pineal with the intercommissural area. The continuity of the pineal lumen with the third ventricle is recognizable during two weeks after hatching. In the eight-week-old turkeys, we observe no communication

of the lumen with the third ventricle. Moreover, the attachment of the pineal stalk to the intercommissural region was absent in birds aged 56 weeks. We postulate that these morphological transformations are a consequence of the pineal stalk regression. Therefore, it could be concluded that during the ontogeny the turkey pineal organ undergoes a clear transformation from the type I of the avian pineals (collecting organs connected with the brain via the stalk) to the type II (collecting organs separated from the brain, for details of the classification see 4).

There are sparse histological and morphometrical descriptions of the avian pineal development during the ontogeny [11, 22, 35, 40]. The pineal histogenesis has been studied mainly in the domestic fowl, but the data on this process are partially contradictory. According to Jove and co-workers [40] the chicken embryos showed two forms of pineal cells aggregates – rosettes or vesicles. The rosettes were found at 5th day, but after 15th day their presence was occasional. The number of the follicles was constant after 10th or 15th day [40]. On the other hand, the opposite results concerning the embryonic development were noted by Moller and Moller [35]. The continuous formation of new tubules and follicles connected with differentiation of sensory structures was observed during the second half of the incubation period and the first two weeks after hatching. During the post-hatching development the typical follicular structure was found in the young chickens whereas the parenchyma of the adult hens presented a compact aspect [7, 17, 35]. The follicular cavities, typical for young birds were not found here. In the pineal organs of adult hens, the parenchymal cells showed an apparently homogenous arrangement with uniform appearance [22]. Transformation from the follicular to solid structure of the chicken pineal gland was closely connected with evolution of the pinealocytes: reduction of the follicular cells and development of the parafolicular cells [17, 22, 23, 33, 36,].

The present study shows that during the period of post-hatching development (from 1 day to 56 weeks of age) the pineal parenchyma exhibits marked changes concerning the number of follicles, the size of follicles and their cavities as well as the architecture of the follicular wall and the presence of cell debris inside the follicles. The formation of new follicles derived from the pineal lumen occurs up to the age of two months. The size of the follicles increases significantly during the first two weeks of the post-hatching development and then remains relatively constant between 2nd and 8th week of the life. Next, the marked growth of the follicles takes place at the time of sexual maturation. Similar changes occur in thickness of the follicular wall, whereas the follicular lumen enlarges up the age of eight weeks and then slightly decreases. In consequence, the proportion of the lumen size to the follicle size is significantly lower in turkeys aged 22 – 56 weeks than in younger birds.

The most essential histological transformations occurring with age concern the follicular wall architecture. Our findings show that the follicles exhibit two main arrangements of the wall structure. In sexually immature turkeys, the follicular wall is formed by elongated cells bordering the lumen and sparse, peripherally localized, round or oval cells. The number of round cells composing the outer part of the follicular lumen increases with age, but even in 8-week-old animals they do not form a prominent layer. Among the elongated cells, HIOMT-positive cells with apical protrusions and basally located nuclei as well as HIOMT-negative cells. HIOMT-immunoreactivity has also been found in some round cells located at the follicular periphery. They were sparse in new-hatching birds and more numerous in birds at the age of 8 weeks. This pseudostratified organization of the follicular wall is transformed during the ontogenesis into the multilayer structure, composed by the layer of elongated cells limiting the lumen and several layers of round or oval cells, located peripherally. The thick, multilayer follicular wall is characteristic for 22- and 56-week-old turkeys. The immunoreactivity against HIOMT has been demonstrated both in a subpopulation of elongated cells and in the majority of round cells located peripherally. Simultaneously with the development of the outer layer formed by numerous oval cells, the elongated cells bordering the follicular lumen undergo regression. They lose apical protrusions and become shorter.

The use of antibodies against HIOMT enabled us identification of pinealocytes based on the presence of the last enzyme in the melatonin synthesis pathway [51]. Two types of pinealocytes have been distinguished: the elongated pinealocytes with apical prolongations, probably representing the rudimentary-photoreceptor type and the secretory pinealocytes, represented by round cells located at the follicular periphery. The last type is sparsely represented in young bird but it predominates in sexually mature turkeys. The secretory pinealocytes are also present in the distal part of the pineal stalk. Similar changes in localization of HIOMT-immunoreactivity have been observed during ontogenesis in the chicken [53].

It should be emphasized, that despite several modifications of the follicles leading to the increase in the follicular wall thickness and reduction of the lumen, the follicular arrangement is conspicuous even in one-year old turkeys. In this aspect of the pineal ontogenesis the turkey pineal organ differs markedly from the chicken pineal, which structure transforms from the follicular form to the solid one.

The presence of the accessory pineal tissue is a peculiar attribute of the avian pineal organ and it has been noted in several species [4, 9]. Forms, size and morphology of this structure differ among species. The attachment of the accessory pineal tissue is usually anterior to the habenular commissure, with only one known exception in a pigeon, in which it is localized in the vicinity of the primary pineal body. In spite of the investigation of serial sections we have found a prevalence of the accessory pineal tissue only in three turkeys. Moreover, in all cases the accessory pineal tissue was poorly developed. It can be interpreted that this structure is not a common attribute of the turkey pineal organ.

In addition to the changes in structure of the follicles, we have found a considerable age-dependent increase in the connective tissue septa subdividing the parenchyma and separating the follicles. The development of the connective tissue is associated with enhanced vascularization by arteries, venues and capillaries surrounding the follicles. The increase is particular intensive between 8th week and 22nd week of age and it seems to be synchronized with changes in the follicular wall arrangement.

The presence of lymphoid tissue is regarded as a typical feature of the avian pineal organ; however, it shows species-dependent variability [4]. In the domestic fowl, accumulations of lymphocytes in the pineal stroma are observed as early as on 4th day or between 10th and 15th day after hatching and they are still present in 34-month-old chickens [24, 25]. In contrast, Campbell and Gibson [54] reported complete disappearance of the lymph nodes in one-year-old hens. The foci of heterophilic granulocytes in the chicken pineal have been found in birds at the age from 24 hours to 18 weeks after hatching [55].

In the present study, moderate number of lymphatic cells within the connective tissue of the turkey pineal was observed independently of the age. The accumulation of lymphocytes in a form of lymphatic node was noted only in one case in spite of investigation of the consecutive sections.

The presence of calcified concretions in the pineal gland of mammalian species is a well know phenomenon. They have been described among others in the bovine [56], the pig [57], the rat [58], but the concrements are extremely frequent in the gerbil [59] and the human [60]. The biological significance of the concretions is still unknown [61]. There is a correlation between the age of the subject and the number of the acervuli. Among bird species, the calcium concrements located in the choroid plexus have been found in the duck [10, 61].

Our results show the regular presence of calcium concrements in the pineal organs of 56-week-old turkeys and their lack in younger birds, which suggests a relationship between age and calcification.

Summing up, the histological structure of the turkey pineal gland undergoes several modifications during the period of post-hatching development. Probably, many of these alternations affect the pineal secretory process and its regulatory mechanisms. Both bi-polar, elongated cells with prolongations (presumably the rudimentary-photoreceptor pinealocytes) and round cells classified as the secretory pinealocytes seem to be responsible for the secretory activity of the turkey pineal organ, but their significance may change significantly during ontogeny.

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