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INFLUENCE OF MONOCHROMATIC GREEN LED LIGHT ON THE DEVELOPMENT OF PINEAL GLAND STRUCTURE IN CHICKENS

INFLUENȚA LUMINII LED VERDE MONOCROMATICĂ ASUPRA DEZVOLTĂRII STRUCTURII GLANDEI PINEALE LA PUI

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ABSTRACT

The chick pineal gland was histologically analysed to observe its development under the influence of monochromatic green LED light. The samples collected were from 50 chickens, divided into 2 groups: control (LM) and experimental (LE). Throughout the experimental period, LM was subjected to white light, and LE was subjected to green LED light. 5 birds from each group were sacrificed at the ages of 14, 21, 28, 35 and 42 days. Samples were fixed with 10% formaldehyde, embedded in paraffin, sectioned at 5 µm, HE stained and evaluated by light microscopy. The pineal gland consists of a series of follicles that are separated by a prominent septum. Each of the follicles has a central cavity into which the apical ends of the pineal cells protrude. The interstitial spaces contain connective tissue, small capillaries and lymphoid cells, present singly or in small groups. Pineal gland-associated lymphoid tissue (PALT) was usually found at the peripheral edge of the gland but also centrally between neighbouring follicles. The major changes that occur histologically with the ageing of the chick are progressive reduction of follicular cavities and the development of the solid appearance of follicles by increasing the number of parafollicular cells. The stromal area increases also, dividing the pineal parenchyma into small calibre areas. The morphostructure of the pineal gland in the two groups showed cellular changes leading to functional changes. Monochromatic green LED light (560 nm) induced proliferation of both types of pinealocytes with the formation of larger follicles than LM and promoted maximal development of PALT at 35 days.

Keywords: pineal gland, pinealocytes, chicken, histology, LED light

REZUMAT

Glanda pineala a puilor a fost analizată histologic pentru a observa evoluția ei sub influența luminii LED verde monocromatică. Probele au fost recoltate de la 50 de pui, împărțiți în 2 grupuri: martor (LM) și experimental (LE). De-a lungul perioadei experimentale, LM a fost supus luminii albe iar LE a fost supus luminii LED verde monocromatică. 5 păsări din fiecare grup au fost sacrificate la vârstele de 14, 21, 28, 35 și 42 de zile. Probele au fost fixate cu formaldehida 10%, incluse în parafină, secționate la 5 µm, colorate HE și evaluate la microscopul optic. Glanda pineală este formată dintr-o serie de foliculi care sunt separați de un sept proeminent. Fiecare dintre foliculi are o cavitate centrală în care proemină extremitățile apicale ale celulelor pineale. Spațiile interstițiale conțin țesut conjunctiv, capilare și celule limfoide, prezente individual sau în grupuri mici. PALT a fost găsită de obicei la marginea periferică a glandei, dar și central, între foliculii învecinați. Modificările majore care apar histologic odată cu avansarea în vârstă a păsărilor sunt reducerea progresivă a cavităților foliculare, apariția cavităților cu aspect solid prin mărirea numărului de celule parafoliculare. Țesutul stromal crește de asemenea, împărțind parenchimul pineal în zone de calibru mic. Morfostructura glandei pineale în cele două grupuri a prezentat modificări celulare care au dus la modificări funcționale. Lumina LED verde monocromatică (560 nm) a determinat proliferarea ambelor tipuri de pinealocite cu formarea unor foliculi mai mari față de LM și a promovat dezvoltarea maximă a lui PALT la 35 de zile.

Cuvinte cheie: glanda pineală, pinealocit, pui, histologie, lumină LED

The avian pineal gland is a photo-endocrine organ that perceives light from the environment, converts it into a hormone signal - melatonin- and transmits it endocrynically to the whole organism. This form of gland is a transition between the photosensory organ of lower vertebrates, which is equipped with photoreceptor cells, and the endocrine gland of mammals, which is influenced by light stimuli detected by cone and rod cells in the retina (7). Its anatomical position is strategic, located on the surface of the brain, between the cerebellum and the two hemispheres of the telencephalon. The shape resembles an inverted triangle, with the base towards the skull and a basal pineal stalk connected (in the first days of life) to the dorsal wall of the third ventricle (30). As the chicken skull bone is a translucent tissue in the first weeks of life, environmental light information reaches the avian pineal gland directly through the rudimentary photoreceptor pinealocytes of the gland (13).

Melatonin is a neurogenic hormone, synthesised in the dark in the pineal gland and in the light in the retina. Of the total circulating melatonin in the bloodstream, approximately 80% is produced by the pineal gland alone (31). In a 24-hour cycle, all biochemical

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changes occurring in the body, hormone production and metabolism are regulated by circadian oscillators in the pineal gland (10). Light is an important environmental factor that can influence the behaviour, growth and health of birds. LEDs are increasingly used in animal housing because they can provide monochromatic or full-spectrum light that resembles natural light. The positive effect exerted by monochromatic green LED monochromatic light (560 nm) has been emphasised in various scientific papers where it promoted melatonin secretion (23), spleen growth (35) and skeletal muscle growth in broiler chickens (23, 2), and shortened hatching time during embryogenesis (33). Melatonin is currently considered to be a component of the neuroendocrine immunoregulatory system (3). Melatonin modulates a wide range of physiological processes, including immunity. These interactions are actually the result of feedback mechanisms from one system to another via soluble immune factors. The accumulation of lymphocytes in the pineal gland in the form of associated lymphoid tissue is the feedback of the immune system to the pineal gland. The impact of different monochromatic lights on the immune system in broiler chickens has recently been documented (19). The objective of this study was to highlight the effect of monochromatic green LED light (560 nm) on the evolution of the histologic structure of the pineal gland, highlighting the photo-endocrine and neuroimmune appearance of the gland at 14, 21, 28, 35 and 42 days of age in chickens.

MATERIALS AND METHODS

The research was carried out in a broiler rearing farm on a total of 50 broilers, harmonious in age and weight. In the first week of life, the chicks were reared under normal white light for accommodation. After that, the chickens were divided into two equal groups and subjected to a different spectrum light regime for 5 weeks. The control group (LM) remained under the same normal white light, and the experimental group (LE) under monochromatic green LED light (560 nm). Throughout the experimental period, all chickens were provided with optimal environmental and forage conditions and were under constant clinical control. After each week (14, 21,

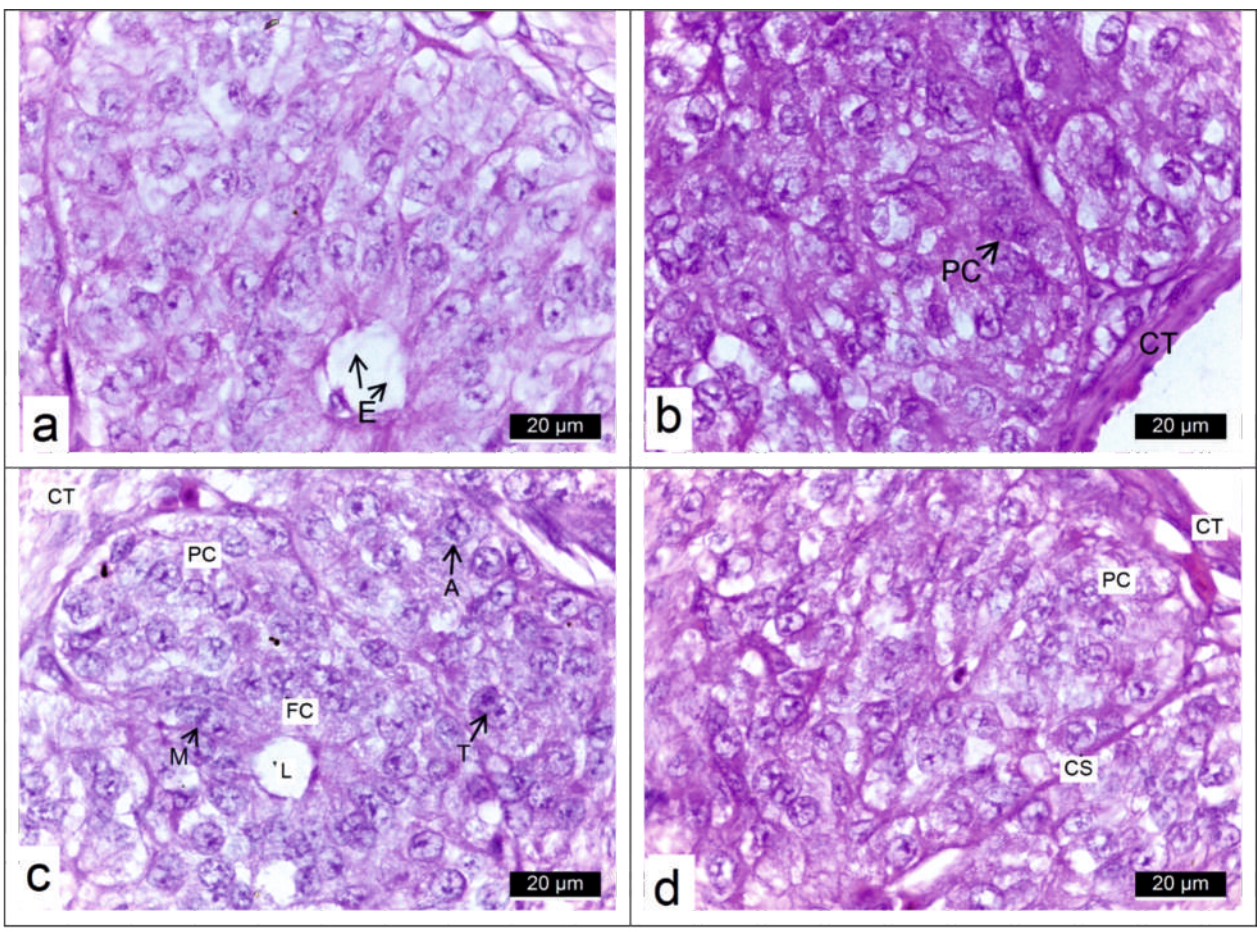

Fig. 1. Chicken pineal gland at 14 days. LM (a) Clear demarcation between the follicular cells delimiting the central lumen and the parafollicular cells distributed at the edge of the follicle. LM (b) Pineal follicle delimited by connective septa originating from the capsule. LE (c) Rudimentary photo-receptor pinealocytes are observed in metaphase and secretory pinealocytes in anaphase and telophase. LE (d) Proliferation of pinealocytes in follicles. (A anaphase; CS connective septa; CT capsule; E extensions; FC follicular cells; L lumen; M mitosis; PC parafollicular cells; T telophase). HE staining (bar = 20 μm)

28, 35, and 42 days in each age category), 10 birds (5 from LM and 5 from LE) were sacrificed and the pineal gland was sampled. The samples were fixed with 10% formaldehyde. After 3 days, samples were dehydrated in successive 70% to absolute ethyl alcohol baths. Xylene was used to clarify the samples. Paraffin embedding involved passing the samples through liquid paraffin baths at 56 and then embedding them in paraffin blocks. Microtomes were used to section the preparations, obtaining 5 μm sections. The obtained sections were stained with haematoxylin and eosin and evaluated under a Leica optical microscope.

RESULTS AND DISCUSSIONS

Accumulation of all current knowledge about the process of development and differentiation of the avian pineal gland during ontogenesis is mainly based on studies in domestic birds (1, 32). After hatching and as a function of age, changes have been observed in the size of the pineal gland, loss of connection with the third ventricle through regression of the pineal stalk (30), and cellular changes occurring in the parenchyma and especially in the pinealocyte population (1).

The pineal gland is covered by a capsule of fibrous connective tissue that emits thin septa invading the parenchyma and dividing it into small pineal lobules, a characteristic found in chickens (18). Septa are rich in connective fibres, connective tissue cells and blood capillaries. The capsule originates from the leptomeninx and is fused with the duramater on the anterior face near the cerebral hemisphere (18). By 14 days of age, each lumen is defined by a layer of follicular cells that are columnar in shape with a basal nucleus and a supranuclear basophilic cytoplasm that is continued by extensions going out into the lu-men. These columnar cells are divided into two types: supporting cells (ependymal) that have microvilli in the apical continuation and rudimentary photo-receptor cells that have a small external light-sensitive extension (Fig. 1).

Surrounding the follicular cells are the parafollicular cells, which are also called secretory pinealocytes

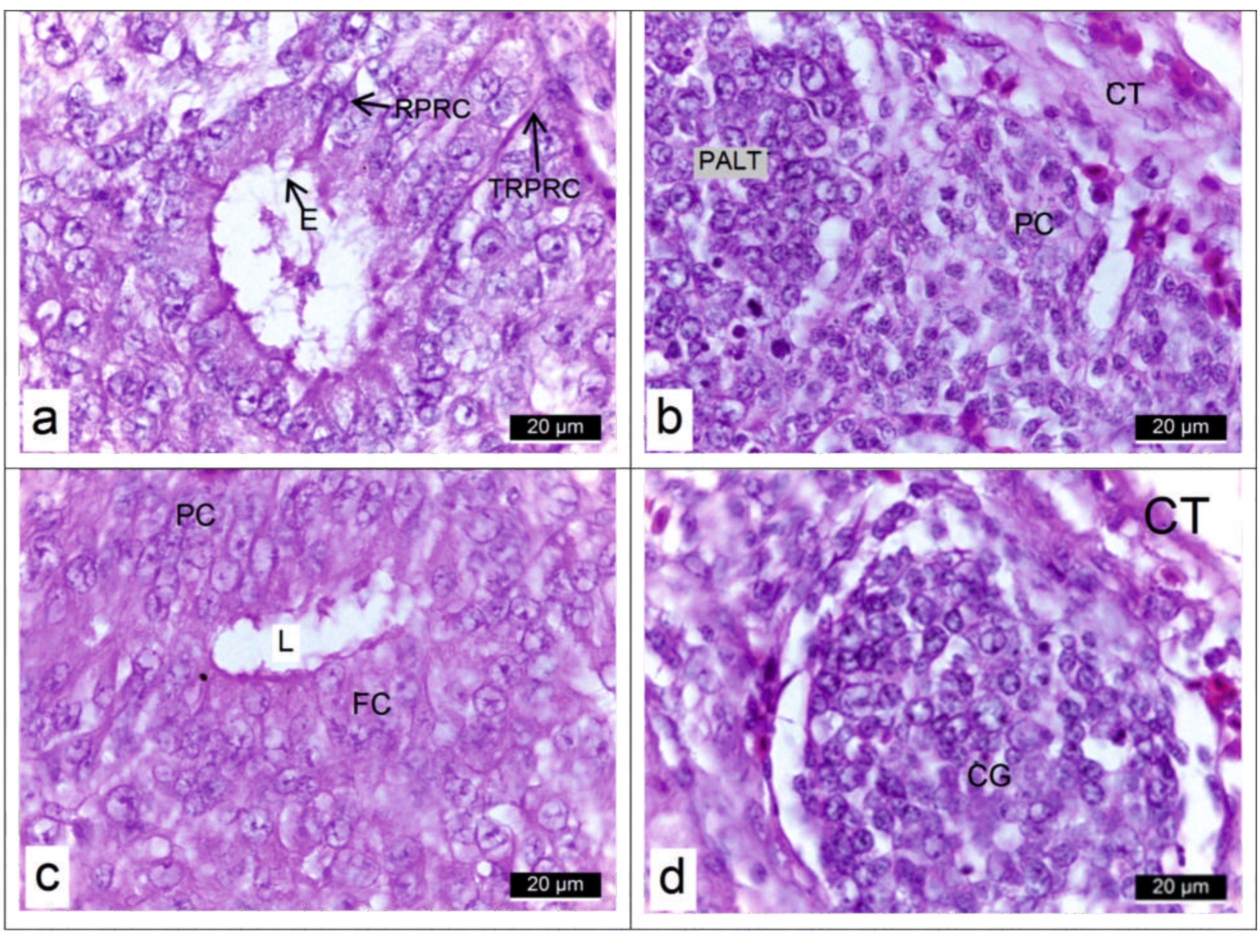

Fig. 2. Chicken pineal gland at 21 days. LM (a) Bipolar cell appearance of rudimentary photo-receptor pineal cells. The light-sensitive extension extends into the lumen, and the basal extension is orientated toward the septate capillaries. LM (b) A monocyte infiltrate is observed immediately beneath the capsule. LE (c) pineal follicle enlarged due to cell proliferation. LE (d) Germinating centres belonging to PALT in the subcapsular space. (CG germinal centres; CS septal; CT capsule; E capsule; E extensions; FC follicular cells; L lumen; PC parafollicular cells; PALT pineal-associated lymphoid tissue; RPRC rudimentary photo-receptor cells; TRPRC rudimentary photoreceptor cell basal extension) HE staining (bar = 20 μm)

and are small with a round nucleus and protruding nucleolus, granular cytoplasm that is weakly basophilic, and star-shaped cytoplasm with fine, short extensions orientated towards the blood vessels in the conjunctival septa. Both cell types are melatonin-secreting cells, except that the rudimentary photo-receptor cells are mediated by the action of light, whereas the secretory pineal cells respond to neurotransmitters. The photo-receptor pinealocyte is an intermediate form between the photo-receptor cell and the secretory pinealocyte. It resembles the photo-receptor cell in that it is composed of the 4 basic segments: cell body with basal nucleus, inner segment, light-sensitive outer segment and basal ending (8). The differences between the two relate to the fact that the photo-receptor cell has the long outer portion and the basal ending in synapsis with a bipolar neurone in the retina, whereas the rudimentary photo-receptor cell is also bipolar in shape but with a shorter outer portion and asynaptic basal ending with vascular polarity typical of the secretory pinealocyte. These basal terminations, near the outer surface of the follicle, widen and, through cell junction structures, form a limiting membrane of the pineal tissue to the vascularisation of the gland. In LE, at 14 days of age, green light induced the proliferation of both rudimentary photo-receptor (captured in metaphase) and secretory pinealocytes (captured in anaphase and telophase) (Fig. 1.). These results suggest that monochromatic green LED light can enhance early pineal gland development through pineal proliferation. From a functional point of view, rudimentary photo-receptor pinealocytes are similar to those of poikilotherms, having light sensitivity, photoreceptor capacity, endogenous circadian oscillator and melatonin synthesising mechanism (11). The outer part of the rudimentary photo-receptor pinealocyte in birds has a bulbar shape and contains opsins similar to those found in the retina. This was first demonstrated by immunoreaction against opsins by Vigh and Vigh-Teichmann in 1981 (34), the immunolabeling being stronger in younger animals. Most of the outer bulbar extensions have shown immunoreactivity to rhodopsin, iodopsin (36), visin, and also to various proteins essential for photochemical transduction in avian

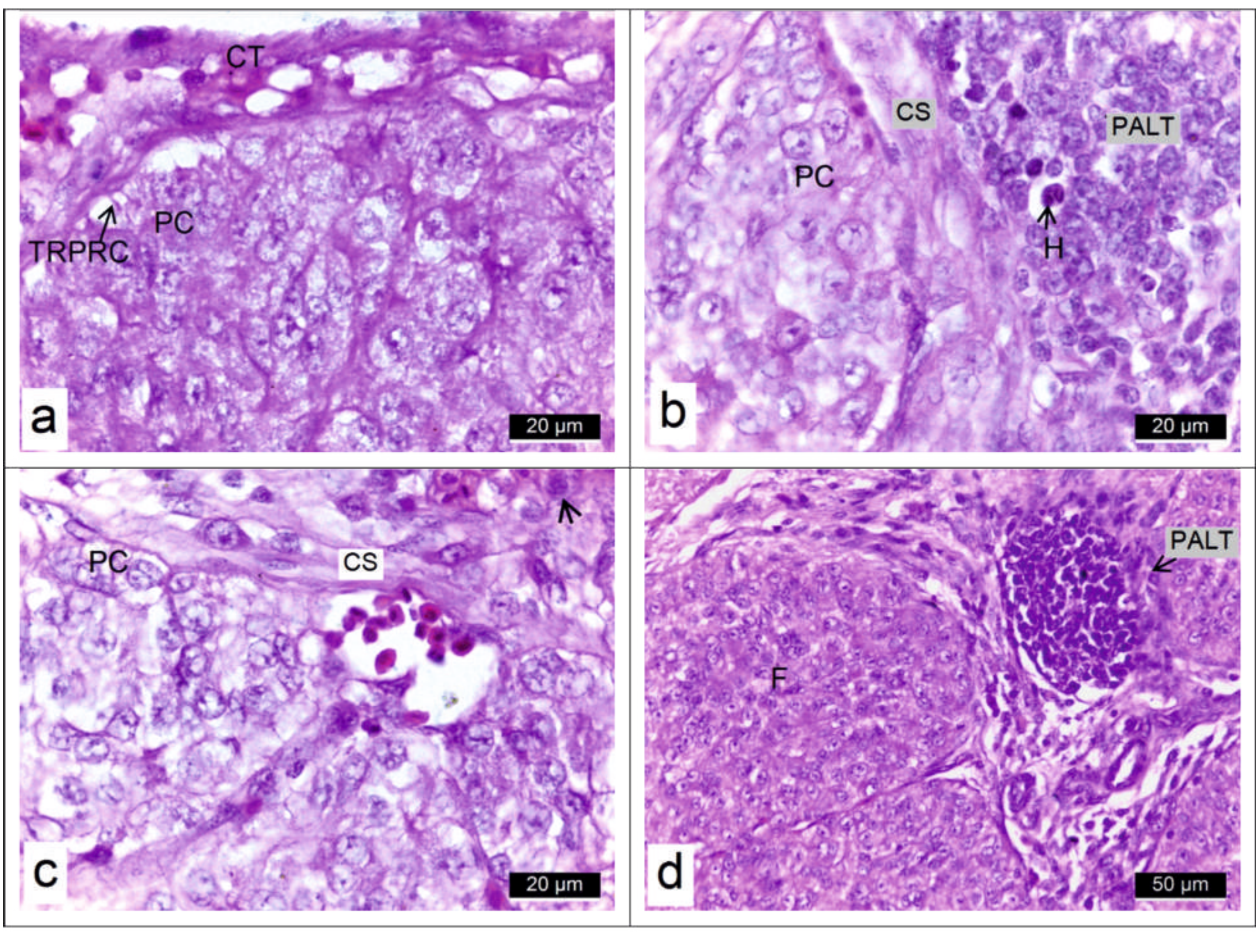

Fig. 3. Chicken pineal gland at 28 days. LM (a) Numerous capillaries in the pineal gland capsule. LM (b) Organisation of PALT without invasion of the glandular parenchyma. LE (c) Proliferation of parafollicular cells and much thicker connective septa. LE (d) Germinal centre localised between pineal follicles deep within the gland. (CS septal septa; CT capsule; E extensions; F follicule; H heterophil; L lumen; PC parafollicular cells; PALT pineal-associated lymphoid tissue; RPRC rudimentary photoreceptor cells; TRPRC rudimentary photoreceptor cell terminals; arrow plasma cell). HE staining (figs a-c: bar = 20 µm; fig d: bar = 50 µm)

pinealocytes (alpha-transducin, S-antigen, recoverin and interstitial retinol-binding protein) (9, 15, 14).

A recent study tracked melatonin secretion in the pineal gland of chickens under lights of different colours, concluding that green light significantly increased melatonin secretion as well as AANAT mRNA (key enzyme in melatonin biosynthesis) expression, in contrast to blue and white lights (20). Moreover, after pinealectomy of the chickens and subjected to lights again, the circadian amplitude of plasma melatonin was markedly reduced. This aspect emphasises the property of the pineal gland in the chick to secrete melatonin due to its sensitivity to light.

At 21 days of age, follicle size and lumen diameter are larger than in the previous age group. In LE, the follicles have larger lumens, and the pluristratified appearance of the parafollicular cells was observed. Immediately beneath the capsule and near the dorsal end, mononuclear cell infiltration occurs, reaching the pineal gland via blood capillaries in the stroma. Lymphocytes predominate, and their accumulation does not alter the morphostructure of the glandular parenchyma. Initially, the infiltration is diffuse, and germinal centres organise, forming the pineal gland-associated lymphoid tissue (PALT). This tissue is delimited by the basement membranes of the pineal follicles and is composed of large reticular cells, capillaries, a mesh of reticulin fibres, lymphocytes, plasma cells, basophilic and eosinophilic granulocytes and neuroglial macrophages (Fig. 2). Lymphoid infiltration in the pineal gland begins around 21 days of age and can increase to 30% of the pineal mass (5). This lymphoid tissue is immunologically active and capable of producing antibodies (6). The PALT lymphoid accumulation is closely associated with the vein at the distal end of the gland, which allows their penetration into the stroma (29).

The topographic relationship observed between lymphocytes and pinealocytes is similar to that in lymphoepithelial tissue. (27). Some lymphocytes can cross the basement membrane and can be found between secretory pinealocytes, between follicular cells or even in the central lumen. These results are proof that lymphocytes regularly traffic between PALT and pineal follicles. Lymphocytes within the pineal parenchyma express twice as much thymidine as lymphocytes in pineal connective tissue. This high rate of

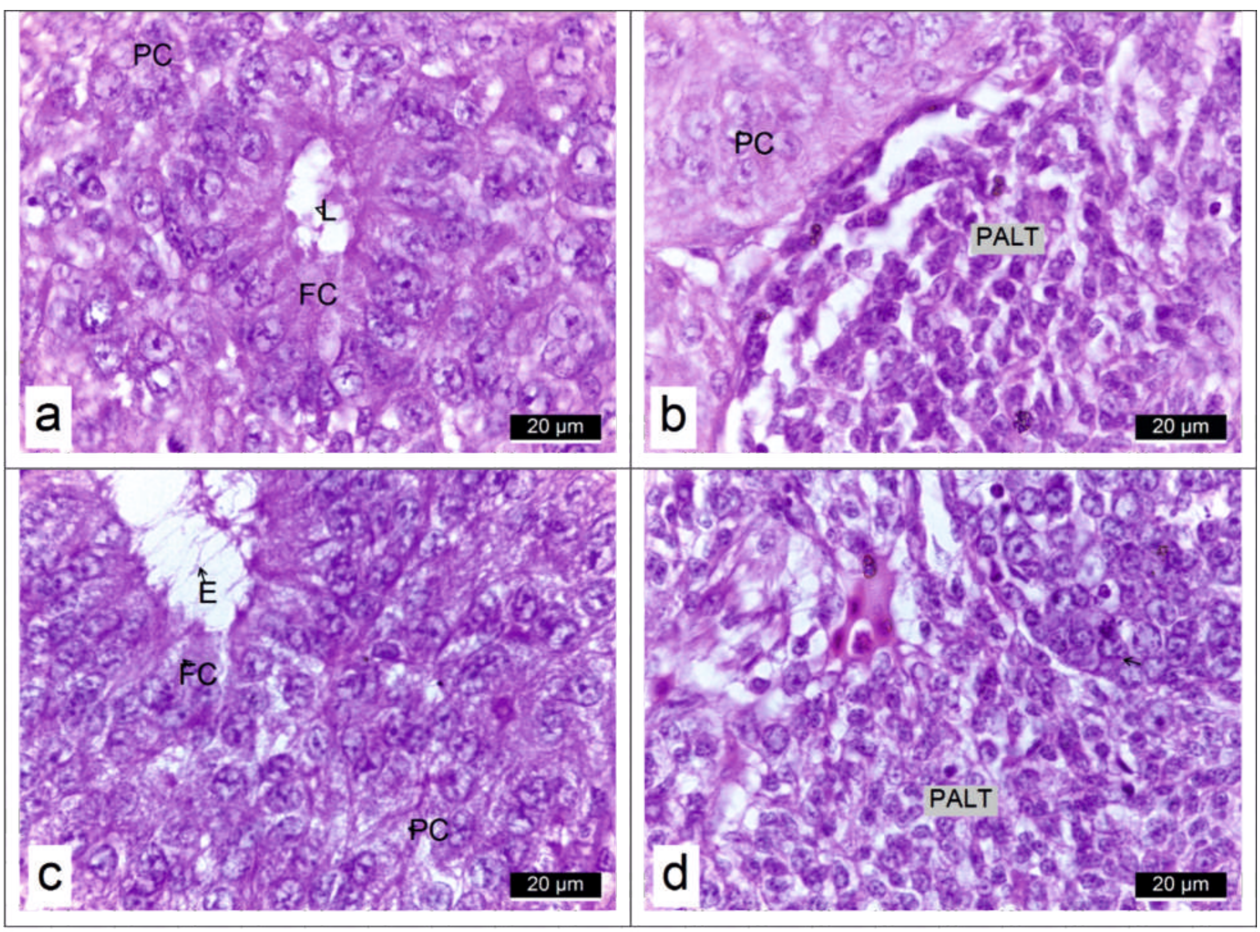

Fig. 4. Chicken pineal gland at 35 days. LM (a) Plurystified appearance of the follicular wall. LM (b) The PALT is delimited from the gland parenchyma by the basement membrane of the follicle. LE (c) The lumen of the follicle has a larger diameter and is occupied by follicular cell extensions. LE (d) Cell population in PALT. (E extensions; FC follicular cells; L lumen; PC parafollicular cells; PALT pineal-associated lymphoid tissue). HE staining (bar = 20 µm)

lymphocyte proliferation suggests that the pineal produces lymphocyte cytokines (28). Furthermore, it has been observed that following bursectomy or thymectomy, lymphoid depletion in the pineal gland has occurred, and the influx of lymphocytes reaching the gland in the first week of life is stopped (5). This phenomenon is also found in the secondary lymphoid organs, with the result that the PALT begins to form at the same time as other secondary lymphoid organs, being under the influence of the proper functioning of the primary lymphoid organs (the bursa of Fabricius and the thymus). These links between lymphocytes and pinelocytes produce a unique interaction between the immune system and the central nervous system.

In chicks exposed to monochromatic green LED light (LE), more organised lymph nodes are observed in the pineal stroma. There are few studies related to the influence of different light spectra on lymphocyte proliferation. Green light promoted the proliferation of B lymphocytes in the Bursa of Fabricius (21), T lymphocytes in the thymus (4) and spleen in chickens (16). At the age of 28 days, a clear increase in the size of the pineal gland was observed in both LM and LE. This increase is characterised by a higher number of cells in the follicles and their organisation in a compact structure. Along with the development of the parenchyma, there are also morphologic changes in the stroma. Larger thicknesses are observed for the gland capsule but also for the conjunctival septa determined by the presence of more connective fibres and blood vessels. The capillaries do not penetrate the pineal tissue but only pass through the conjunctival septa. In LE, new lymph nodes formed in the conjunctival septa are seen reaching to the centre of the gland. Small lymphocytes remain in the septa and do not penetrate the parenchyma. Large lymphocytes are found among the pinealocytes and in the lymph nodes. Plasma cells and heterophils are common elements of connective tissue in the stroma, although some also occur in the parenchyma. Microglia-macrophages are found in the periphery of the PALT (Fig. 3).

The greatest accumulation of lymphoid tissue in the pineal gland is observed at 35 days of age in LE. Through connective tissue in the septa, PALT is also

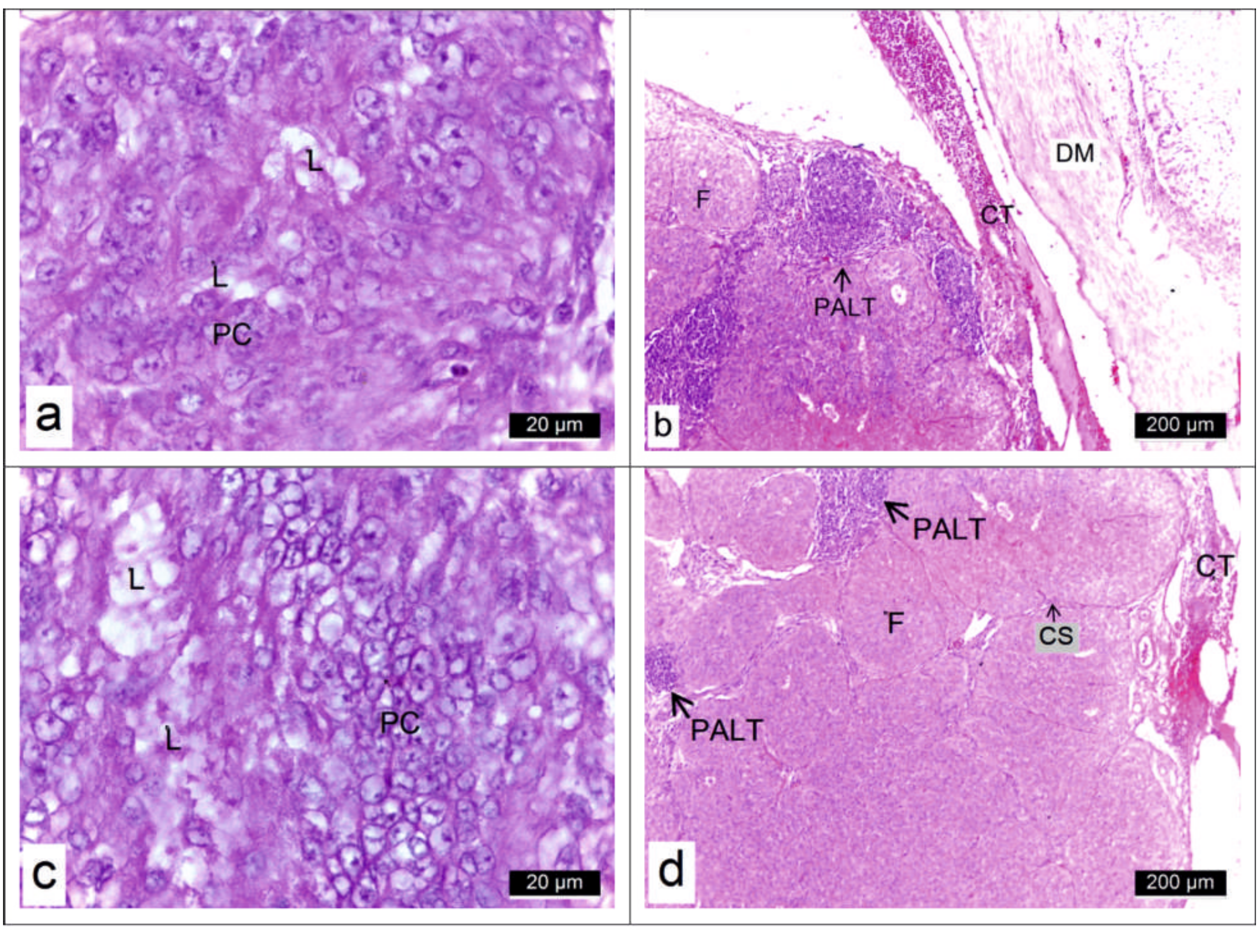

Fig. 5. Chicken pineal gland at 42 days. LM (a) Loss of demarcation between follicular and parafollicular cells. The lumen is almost occupied by cells and prelimbs. LM (b) PALT localisation at the periphery of the gland. LE (c and d) Compact appearance of the gland by proliferation of pinealocytes. (CS connective septa; CT connective tissue; E extensions; F follicule; L lume; PC parafollicular cells; PALT pineal-associated lymphoid tissue) (a and c) HE staining (barr=20); (b and d) HE staining (figs a and c: bar = 20 μm; figs b and d: bar = 200 μm)

formed within the pineal gland. The follicular lumen begins to be occupied by numerous apical extensions from follicular cells in both LM and LE. Some of the rudimentary photo-receptor cells begin to change their structure from a bipolar to the multipolar form found in secretory pineal cells. The multilayered appearance of the follicular wall is also more evident at 35 days of age of the chick (Fig. 4).

In the mammalian pineal gland, pinealocytes have no outer segments, and there are no photoreceptor-like cells (24). Evolution has produced a mammalian pineal gland that is no longer photosensitive but merely neuroendocrine (25). It has been suggested that pineal outer segments are better developed in lower vertebrates than in mammals (24). Reduced photosensory function and regressed outer segments have been reported in bird pineal glands (12). In fact, ultrastructural observations have shown rudimentary photoreceptor cells in degeneration, with outer segments regressed and cytosolic components lost in the adult chick pineal gland (17). It could therefore be suggested that the rudimentary photoreceptor cells in the adult chick pineal gland (5 weeks) progressively lose their light-sensing capability. A previous study, however, reported that cells without outer segments can function as photoreceptors if their plasma membrane is charged with opsin (26). Further studies are needed to investigate whether these rudimentary photoreceptor cells with diminished outer segments can act as photoreceptors. At 42 days, the gland has a compact appearance at a smaller objective. The follicular cavities specific to the first days of the chick's life are missing. Gland compaction is a morphologic change that occurs with age in birds, both by the development of cytoplasmic extensions of the follicular cells that almost close the follicular lumen and by the increase in the number of parafollicular cells. The once well-defined follicular lumen is now fragmented into cavities of smaller diameters, demarcated by newly formed cell extensions. New cells that have invaded the lumen are also seen and the clear demarcation between follicular and parafollicular cells is lost. Most of the time, the shape of the follicles changes from a round to an unregulated shape. The stroma of the pineal gland at 42 days in both LM and LE is more fibrous than in the young chick, with progressive penetration of the parenchyma. Via connective tissue septa, more blood vessels are brought near the pineal follicles. The increase in the number of parafollicular cells (secretory pineal cells) and blood capillaries are morphologic changes that indicate a gradual transition to an endocrine organ (Fig. 5).

In conclusion, monochromatic green LED light was used to see changes in the pineal gland. To the best of our knowledge, this article is the only one in the country addressing this topic. Knowing the positive influences of monochromatic LED lights in the production, metabolism and health of birds, this article is the promoter of future research that will focus on the modulation of immune and endocrine response through melatonin secretion.

CONCLUSIONS

The chick pineal gland underwent a development of histologic structure from a follicular parenchyma (14 days) to a generally compact structure (42 days). Sensitivity to monochromatic green LED light (560 nm) was higher at 14, 21 and 28 days due to the presence of photosensitive outer extensions of rudimentary photo-responsive pinealocytes but also to the proliferation of both types of pinealocytes with the formation of larger follicles compared to LM. Monochromatic green LED light (560 nm) influenced a higher accumulation of lymphoid tissue in the pineal gland, with germinal centres being observed in the depth of the gland. Maximum PALT development was observed in LE at 35 days. The compact appearance observed at 42 days is due to the proliferation of parafollicular cells resulting in loss of intrafollicular delimitation between follicular and parafollicular cells, occupation of the lumen by new cells and cytoplasmic extensions, and a more fibrous structure of the stroma compared to that of the young chick.

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