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COMPARATIVE STUDY ON THE PINEAL GLAND, TESTES AND OVARY IN PUPPIES BEFORE AND AFTER VISION WITH REFERENCE TO PINEAL ROLE

Khalil Abou-Easa[1]; Ehab Tousson[2]; Somaya Shalaby[3] and Maha Abo Gazia[4]

[1]Dept. of Cytology and Histology, Faculty of Vet. Med., Kafr-El-Sheikh University; [2]Zoology Department, Faculty of Science, Tanta University; [3]Zoology Department, Faculty of Science, Minoufiya University; [4]Histology Department, Faculty of Medicine, Al-Fayoum University, Egypt

ABSTRACT

The study was performed on 12 male and female puppies living under natural lighting condition during blindness at the ages of 2 and 10 days and after vision at the ages of 25 and 40 days. The pineal, testes, ovary and pancreas (as a control tissue for detection of pineal tryptophan) were sampled afternoon at beginning of autumn then fixed. Slices stained by H & E, PAS, PAS-Orange G method, and DMAB-nitrite method for tryptophans were prepared. The pinealocytes nuclei deviated from each other after vision indicating the increasing of the cells size. In an attractive feature the pinealocytes in all ages stained negatively by DMAB- nitrite method for tryptophan the building stones of melatonin, while pancreas of the same animal showed an observable distribution of tryptophan in its acini. The testes approximately showed no variation except that the interstitial cells became more differentiated after vision. The oogonia showed a great number of different mitotic stages before vision which dropped at the age of 25 days after vision and not present at the age of 35 days. The results point to a probable that melatonin is not produced from the puppies pineal glands in all studied ages. Therefore the progressive changes of the testes and ovary is not under the control of pineal melatonin. Moreover, the pineal gland, interstitial cells and ovary may follow approximately equal changes which may be accentuated by vision.

Keywords: Pineal gland; Testes; Ovary; Puppies; Pancreas; Vision;

INTRODUCTION

The pineal gland of many mammalian species, particularly rodents, has been extensively studied either histologically or fine structure and physiologically; however relatively few literatures were available on the histophysiology of pineal of domestic animals (Lukaszyk and Reiter, 1975; Middendorff et al., 1996; Abou-Easa et al., 2009). The postnatal changes of the pineal gland and testis, were investigated separately for each organ in different species (Sheridan and Rollag, 1983; Johnson et al., 1996). The relation between the pineal gland and testes was reported (Vaughan et al., 1994). In addition, an evidence for a role of the pineal gland and its major hormonal product, melatonin, in mammalian reproduction was reported by Olcese (1995). A synchronism between the eye vision and the pineal gland was established where photoreceptor-specific proteins was immunocytochemically determined in the mammalian pineal gland (Schomerus et al., 1994; Tong et al., 2003; Engel et al., 2005; El Allali et al., 2008). Moreover, the pineal gland is affected by light- dark cycle (Heinzeller and Tutter, 1991; Ganguly et al., 2002).

Serotonin and melatonin are biologically active derivatives of tryptophan produced in the pineal gland (Devlin, 1986; Lewczuk and Przybylska, 2000a, b; Ganguly et al., 2002; Claustrat et al., 2005; Lahmam et al., 2008). The present work is undertaken to investigate the histological changes of the pineal, testes and ovary in puppies before and after vision.

MATERIALS AND METHODS

Twelve male and female puppies, during blindness at the ages of 2 and 10 days and after vision at the ages of 25 and 40 days were used in this work. The puppies are usually born blind with the lid margins still adherent to one another and separate about 2 weeks postpartum (Evans, 1993). Pregnant bitches were kept under supervision until whelping. The bitches and their puppies were apparently healthy and living under natural lighting condition. From these puppies the materials were obtained by using three puppies in each of the mentioned ages. The puppies were harvested by cutting the jugular vein afternoon at beginning of autumn after inhalation anesthesia using chloroform. The pineal gland, testes, ovaries and pancreas (as a control tissue for detection of pineal tryptophan) were sampled. Bouin's fluid, 10 percent neutral formalin and neutral formol saline were used in fixation.Serial paraffin sections, 3-7 µm thick, were prepared. Slices were stained with Hematoxylin and Eosin (H & E), Periodic Acid-Schiff technique (PAS), PAS-Orange G method, and dimethylaminobenzaldehyde-nitrite method for tryptophan (DMAB-nitrite method) with counter staining by neutral red(Bancroft and Stevens 1990).

RESULTS

Before vision:

Pineal gland :The puppies had a little pineal gland, the cells of which possessing darkly stained spherical and crowded nuclei due to the small size of the cells. These cells are often the early pinealocytes. The dense pineal cells were intermitted by light areas containing fine less developed cell processes and lodged small blood vessels and capillaries, the fine cell processes also extended between the cells (Figs. 1 & 2). Few cells with characteristic large pale nucleus with obvious nucleolus could be difficulty differentiated. The pineal recess extended into the gland. The pale- stained areas were slightly reduced at the age 11 days due to the increasing of the cell number by mitosis (Fig.3). The immature pinealocytes and other pineal tissue stained negatively with DMAB-nitrite method for tryptophan while control tissue, the pancreas of the same animal showed a recognizable distribution of tryptophan in its acini (Figs. 4 & 5).The pigment granules were liked to be seen in the ventral side of the pineal gland toward its base.

Testis: The solid germinal cords (sex cords) of the testes were loosely arranged and consisted of large primordial germ cells with spherical large nuclei and paler cytoplasm, in addition to small primitive Sertoli cells with ovoid nuclei which were more restricted toward the basement membrane. Sometimes the primordial germ cells surrounded by a single layer of primitive Sertoli cells. The interstitial cells were difficult to be demonstrated within the dense interstitial tissue (Fig. 6). The mitotic figures of the germinal cords were absent

Ovary: The oogonia showed a great number of different mitotic stages. These oogonia characterized by darkly stained nuclei with irregular contour and pale cytoplasm. The oogonia arranged in aggregations (Fig.7). The ovarian stroma was cellular.

After vision:

Pineal gland: Within the immature pineal parenchymal cells it was possible to identify mature pinealocytes which were the major cells and glial cells which were mostly astrocytes at the age of 25 days. The mature pinealocytes characterized by their spherical or ovoid nuclei with clumped or granular chromatin and one or two nucleoli while the astrocytes nuclei were identified by their slightly homogeneous chromatin and indistinct nucleoli (Figs. 8 & 11). The latter cells were usually seen in the perivascular spaces. Also the mature pinealocytes were larger than the immature ones; and the cell processes were more developed and showing PAS- positive reaction (Figs.8 &11). Pigment granules were like those before vision. Cells with large pale nucleus and obvious nucleolus were more identified although they remained few (Figs. 8, 10 & 11). The differentiation of mature pinealocytes continued at the age of 35-40 days and dense areas of immature cells were still seen (Figs.10 & 11). There was an increase in the pinealocytes size when indicated by the loose distribution of their nuclei (Fig. 9), The increased cell size as well as mitosis (Fig.11) increased th size of the pineal gland. The pinealocytes were irregularly distributed and rarely follicle arrangement of cells was seen (Fig. 9). Pineal recess extended inside the gland. In an attractive feature the pinealocytes stained negatively by DMAB-nitrite method for tryptophan the building stones of melatonin while pancreas of the same animal showed an observable distribution of tryptophan in its acini (Figs. 12 & 13). These results point to a probable that melatonin is not produced from the puppies pineal glands in all studied ages.

Testes: The testes approximately showed no variation except that the interstitial cells became more differentiated and the interstitial tissue started to be reduced. The sex cords appeared containing primordial germ cells and primitive Sertoli cells without changes from those before vision (Fig. 14).

Ovary: The number of mitotic stages of the oogonia dropped at the age of 25 days (Fig.15) and not present at the age of 35 days where the primary oocytes differentiated, loosely arranged and moderate number of them were surrounded by a single layer of flattened cells (Fig.16). The density of the ovarian stromal cells perhaps not changed although the number increased with the growing of the ovarian size (Fig. 16). The pineal gland, interstitial cells and ovary may follow approximately equal changes which may be accentuated by vision.

DISCUSSION

The puppies had a little pineal gland. Before vision the cells of the puppies pineal gland possessed darkly stained spherical and crowded nuclei due to the small size of the cells. These cells are often the early pinealocytes.

Also, the cells of the dog pineal gland in the first postnatal stage and those of rabbit in the first 24 hours of postnatal life were described as immature cells (Calvo et al., 1990 a,b; Garcia-Maurino and Boya, 1992). However, these authors did not study the effect of vision. The dense pineal cells were intermitted by light areas containing fine less developed cell processes and lodged small blood vessels and capillaries. This appearance agreed with that of previously published light microscopic and ultrastructural discription (Calvo et al., 1990 a,b). According to the present study, it was possible to identify mature pinealocytes which are the major cells and astrocytes at the age of 25 days after vision. The mature pinealocytes are characterized by their spherical or ovoid nuclei with clumped or granular chromatin and one or two nucleoli while the astrocytes nuclei were identified by their slightly homogeneous chromatin and indestinct nucleoli. The latter cells were usually seen in the perivascular spaces. Similar observations were reported (Calvo et al., 1990 a,b). There was an increase in the pinealocytes size which with mitosis increased the size of the pineal gland after vision. Also the cell processes became more developed. In the adult dog, the length of the pineal gland axes almost quadruplicated that of the pineal gland in neonatal stage (Calvo et al., 1990 a). Similar result was also reported by Garcia-Maurino and Boya (1992) along the postnatal period in rabbit. Although the latter authors observed rosette-like arrangement of pinealocytes which no longer seen beyond the 30th postnatal day; irregularly distribution of pinealocytes and rarely follicle arrangement of cells was seen in the pupies after vision. Not well-defined acinar-like formation made by pinealocytes was observed by Ellsworth et al. (1985) in beagle dogs.Pigmented pineal cells were found from the second postnatal day on wards (Calvo et al., 1990 a). This finding was supported in the present study. However Calvo et al. (1990b) observed the pigmented cells form the fourth postnatal day. Melanin pigments were detected in the pineal glands of mature and immature rabbit without a regular manner in their distribution (El-Baz, 1995), but in the present study the pigment granules were distributed in the ventral aspect of the gland toward its base. These cells were identified by their larger pale nuclei, obvious nucleoli, they were more differentiated after vision.

The solid germinal cords of the testes consisted of primordial germ cells and primitive Sertoli cells; the mitotic figures were absent before and after vision. Similar appearance was reported by Cormack (1987) before adolescence in humanbeing. On the contrary cells were termed gonocytes were said to be disappeared completely from the seminiferous cords while an other small cells were differentiated into spermatogonia and Sertoli cells in young camel at early postnatal period (Ahmed, 1994). The developmental and hormonal regulation of certain enzymes in the rat testis was studied (Jue et al., 1995). The result of Taher et al, (1991) in camel that the differentiation of the mesenchymal cells and/or fibroblast in the direction of the interstitial cells supported the present study that in advanced ages (after vision) the interstitial tissue started to be reduced , while the interstitial cells became more differentiated .

Increase of the share of growing follicles correlates with the increase of the ovarian stroma relative volume; this allows suggested the possibility of participation of the latter in the regulation of follicular growth initiation (Petropavlovskaia, 1994). In the present study, the density of ovarian stromal cell perhaps not changed after vision from that before vision where follicular growth was not achieved yet. Ovarian cellular changes and follicular growth were studied (Banks, 1993; Koering et al., 1994; Hirshfield and DeSanti, 1995). The former author reported that the oogonia differentiate and multiply within the developing fetus before parturition but in carnivores they differentiate in the immediate postnatal life. Therefore the great number of mitotic stages in puppies oogonia before vision was for proliferation while the differentiation of primary oocytes was accentuated after vision. The pineal, interstitial cells and ovary may follow approximately equal changes which may be accentuated by vision.

The present study agreed that the pineal gland showed no staining character for tryptophan. The gland's circulating tryptophan is transformed into serotonin; this serotonin is transformed into melatonin (Rafael, 1994). Therefore the present study point to a probable that melatonin is not produced from the puppies pineal gland. Similar result was achieved in mouse (Ebihara et al., 1986). Moreover synaptic ribbons were not discussed in the ultrastructural study of the postnatal development of the dog pineal (Calvo et al., 1990 b). The synaptic ribbons as cytoplasmic organelles in the pinealocytes (Bhatnagar, 1992; Rafael, 1994) were found to be affected by light and dark period like melatonin (Fielke et al., 1994). Either or the function of these structures remains a mystery (Fawcett, 1994). Also the immature cells of the present study and those showed by the dog pineal parenchyma (Calvo et al., 1990 a) refer to late development and functionally inactivity of the dog pineal in the first postnatal stage. In agreement, there was no effect of time of day on plasma melatonin in lumbs during the first 2 weeks after birth but between 3 and 10 weeks of age there was a significant effect of light and dark and time of day on plasma melatonin concentration (Fielke et at., 1994). Moreover, Evans (1993) succeeded that postnatal growth and differentiation of the dog pineal gland continues after birth. Therefore the developmental changes of the testes, and ovary were not under the effect of pupies pineal melatonin. On the other side, melatonin or the pineal gland were found to affect testes (Vermouth et al., 1995; Edmonds and Stetson, 1995; EL-Baz, 1995). Presumptive secretory material did occur in the cytoplasm of bovine pineal gland cells (Lukaszyk and Reiter, 1975). This agreed with the negative staining exhibited by the puppies pineal with PAS-Orange G. Generally the pineal gland shows variations not only between the different animal species but also between the breeds of the same species.

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LEGENDS OF FIGURES:

Fig. 1: Pineal gland at the age of 2 days before vision; Dense masses of parenchymal cells showing no stained material in their cytoplasm (arrows head); Pale-stained areas (stars); Blood vessels (arrows), (PAS-Orange G, X400).

Fig. 2: Pineal gland at the age of 10 days before vision; Dense masses of most early pinealocytes (arrows head); Light area (stars), (PAS, X1000).

Fig. 3: Pineal gland at the age of 11 days before vision; Immature pinealocytes (stars); PAS-positive blood vessel wall (arrow head); Faint

PAS-stained cell processes in the slightly reduced pale areas (arrows), (PAS, X1000).

Fig. 4: Pineal gland at the age of 11 days before vision stained negatively for tryptophan,(DMAB-nitrite method counterstained with neutral red, X400).

Fig. 5: Pancreas at the age of 11 days before vision showing blue reaction of tryptophan in its acini, (DMAB-nitrite method, X400).

Fig. 6: Testis at the age of 10 days before vision. Sex cords (stars); Primordial germ cells (black arrows); Primitive Sertoli cells (black arrows head) which sometimes surrounding the primordial germ cells; Dense interstitial tissue (D), (PAS,X 400).

Fig. 7: Ovary at the age of 11 days before vision; The oogonia aggregated and showing a great number of mitotic figures (arrows), (PAS, X400).

Fig. 8: Pineal gland at the age of 25 days after vision; Pinealocytes had spherical or ovoid nuclei with clumped or granular chromatin and with one or two nucleoli (arrows head); Astrocyte had spherical nucleus with homogeneous chromatin and indestinct nucleolus (black arrows), Cells with large pale nucleus (white arrow), (H & E, X1000).

Fig. 9: Pineal gland at the age of 35 days after vision. Pinealocytes nuclei are loosely distributed (arrows head); Follicle arrangement of cells (black star); Dense areas of immature cells (white stars), (H&E, X400).

Fig. 10: Pineal gland at the age of 35 days after vision. Mitosis (black arrow); Dense areas of immature cells (star); Pinealocytes (white arrows); Astrocyte (black arrow head); Cells with pale large nucleus (white arrow head), (H&E, X1000).

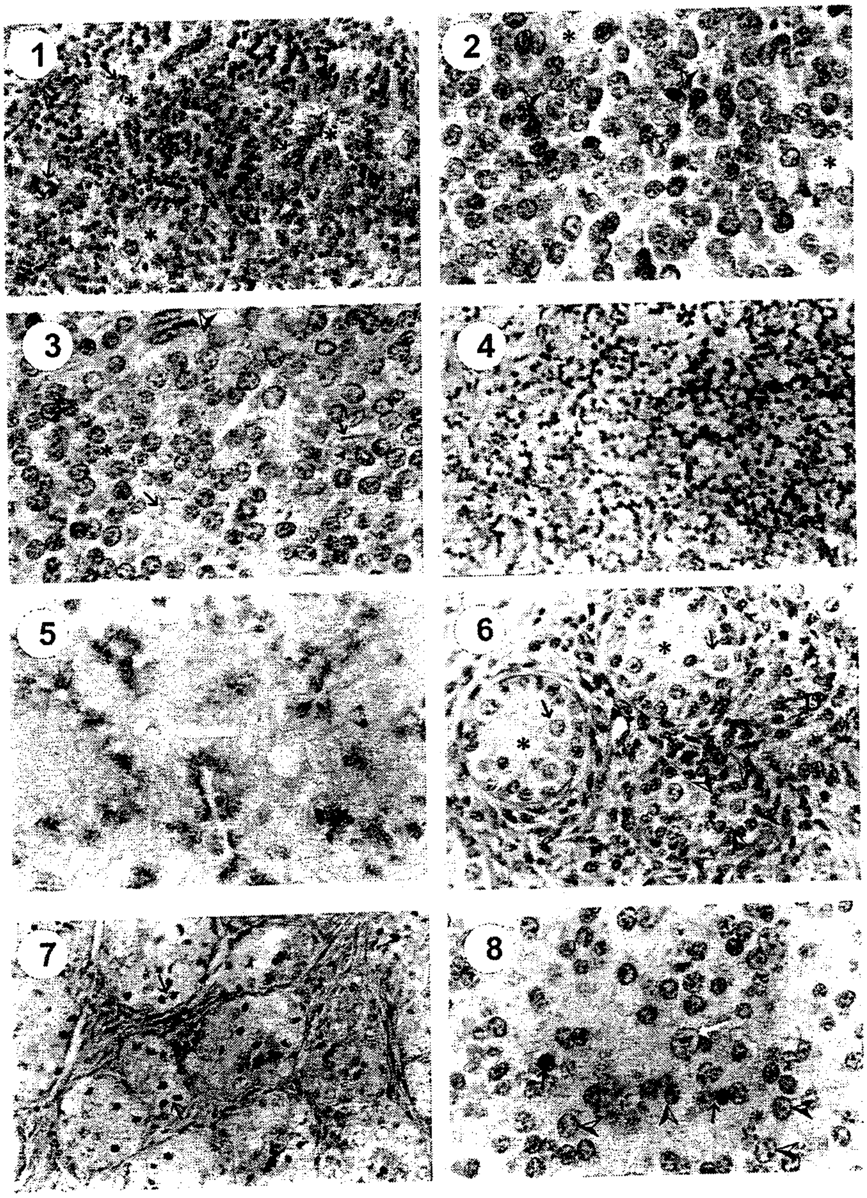
Fig. 11. Pineal gland at the age of 40 days after vision. Mature pinealocytes (white arrows), Immature pinealocytes (black arrow). Astrocytes (white arrows head); Cell processes (black arrows head). Cells with large pale nucleus and clear nucleolus (triangle); The cytoplasm of pinealcytes showing no stained material, (PAS-Orange G, X1000).

Fig. 12 & 13: Photomicrographs of Pineal gland and pancreas of the same animal at the age of 25 days after vision showing negative staining for tryptophan in the pinealocytes **(Fig. 12)**,and blue coloration for tryptophan in the pancreatic acini **(Fig. 13)**, (DMAB-nitrite method counterstained with neutral red, X400).

Fig. 14: Testis at the age of 40 days after vision. Sex cords (stars); Primordial germ cells (black arrows); Primitive Sertoli cells; (white arrows); More differentiated interstitial cells (arrow head), (PAS, X400).

Fig. 15: Ovary at the age of 25 days after vision showing few mitotic figures (arrow) among the oogonia, (PAS, X400).

Fig. 16: Ovary at the age of 35 days after vision showing loose arrangement of primary oocytes which surrounded by a single layer of flattened cells (arrows); Note the cellularity of ovarian stroma (stars), (PAS, X400).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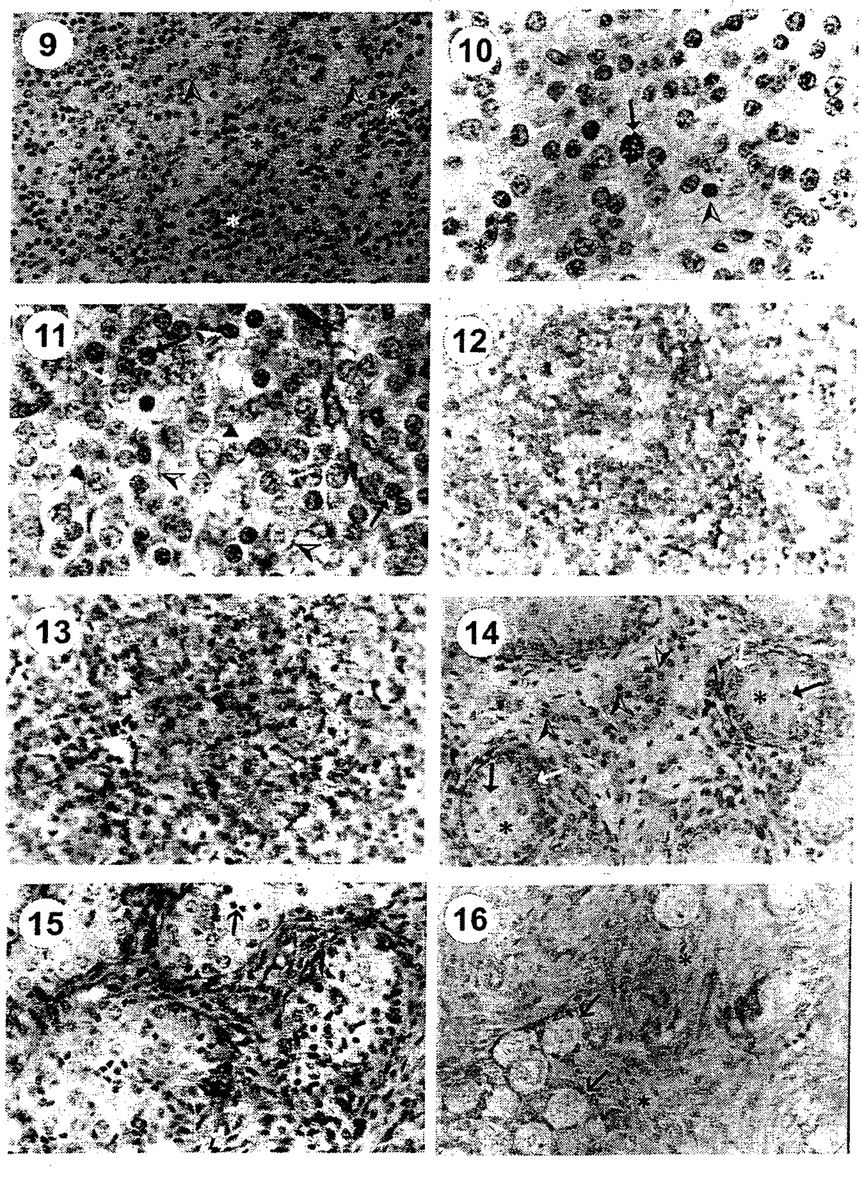
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