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Original Research

Morphological, Histological, and Histochemical Studies on the Adrenal Gland of the Japanese quail (*Coturnix japonica*) During the Post Hatching Period

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Abstract

The adrenal gland of the Japanese quail is a bilateral endocrine organ that is located in the abdominal cavity. The development of the adrenal gland begins in the pre hatching period and continues during the post hatching. The current study aimed to describe the anatomical and histological changes of the adrenal gland in Japanese quail during the post hatching period. The present study was carried on Japanese quail chicks, at ages of day of hatching, two- and four-weeks post-hatching. The dissected adrenal glands were investigated morphologically, histologically, and histochemically. In the current work, the interrenal tissue makes up most the adrenal parenchyma and the chromaffin mass gradually increase with the age. The interrenal tissue at the peripheral zone of the gland arranged into arch-like cords, becomes more prevalent throughout the gland with age, notably at five weeks. They were strongly positive for PAS especially on the day of hatching age but appeared negative by Grimelius argyrophilic stain. At the two weeks of age, chromaffin cells appeared in the form of triangular islets scattered between the interrenal cells. They are smaller and fewer than the interrenal cells, at the age of five weeks the chromaffin islets increased in size and concentrated at the central zone. Two types of chromaffin cells were observed by using Grimelius argyrophilic stain; one of them contain dark brown granules and the other is free from these granules. Finally, distinct morphological changes in the adrenal gland occur during the post-hatching phase.

KEYWORDS
Adrenal, Quail, Interrenal, Chromaffin

INTRODUCTION

The adrenal gland is an important endocrine gland which plays vital role in stress response, immune function and regulates blood pressure (Lucy, 2014). The adrenal glands of birds as in mammals, are paired abdominal organ, located in the abdominal cavity cranio-medially to the anterior pole of the kidneys (Carsia, 2015). The adrenal glands differ in size and shape among different bird species. In adult Japanese quail both glands are flattened dorsoventrally. The right gland is triangular in outline and the base directed cranially while the left one is elongated, longer and narrower than the right one with a narrow caudal part (El-Desoky and Mustafa, 2021). Histologically, the avian adrenal glands are invested by a capsule consisted of dense fibro-elastic connective tissue. The adrenal glands' parenchyma of birds differ from those of mammals in that the cortex does not encapsulate the medulla but is intermingled with it (Al-Jebori *et al.*, 2016). The cortex is renamed the interrenal tissue and the medulla is the chromaffin tissue (Carsia, 2015). The adrenal gland of birds develops from two separate embryological tissues; the interrenal tissue develops from the mesoderm and the chromaffin tissue develops from ectoderm (from the neural crest) (Reavill and Schmidt, 2008). The adrenal gland development starts during the embryonic life and continues in post hatching stages. The current study provided insights on the morphology, histology, and histochemical alterations of the adrenal gland in Japanese quail, allowing researchers to better understand the changes that take place during the post-hatching period.

MATERIALS AND METHODS

Sample collection

The current study was performed on 21 eggs of Japanese quail (*Coturnix Coturnix japonica*) that were incubated at farm that belong to the Faculty of Agriculture, Assiut University, Egypt. The Japanese quail was collected on the day of hatching, and at ages of two- and four-weeks post-hatching. The examination of the quail was performed in the Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Assiut University.

Ethical approval

The Ethics Committee of Assiut University and Veterinary authority, Egypt has approved this study.

Gross examination

Chicks were anaesthetized by chloroform and dissected out as soon as possible. The shape, color and relationship of the ad-

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renal gland with other organs were evaluated after exposing the gland by creating a longitudinal incision in the abdomen.

Histological examination

The adrenal gland specimens were dissected carefully and immersed in a fixative consisting of Bouin's solution. The fixed specimens were dehydrated in ascending grades of ethanol, cleared in methyl benzoate, embedded in paraffin wax. Finally, paraffin blocks of the processed samples were prepared. Thin sections (5-6 µm thick) were cut, dried in an electrical incubator and stained with Harris's hematoxylin and eosin for detection of general structure of the gland, combined Periodic Acid–Schiff (PAS)– Alcian blue (AB) for detection of both neutral and acidic nucopolysaccharides, Crossmon's trichrome for detection of collagenous fibers and the Grimelus silver method (Bancroft, 2002).

RESULTS

Gross morphology and topographic location

In Japanese quail, the adrenal glands were creamy to yellow in color and are found in the roof of the body cavity on both sides of the caudal vena cava. They were located craniomedial to both kidneys and just caudally to both lungs. The right adrenal gland was triangular while the left one was elongated and located slightly caudal to the left one. Their ventral surfaces were observed after the removal of the testes (Fig. 1A-C).

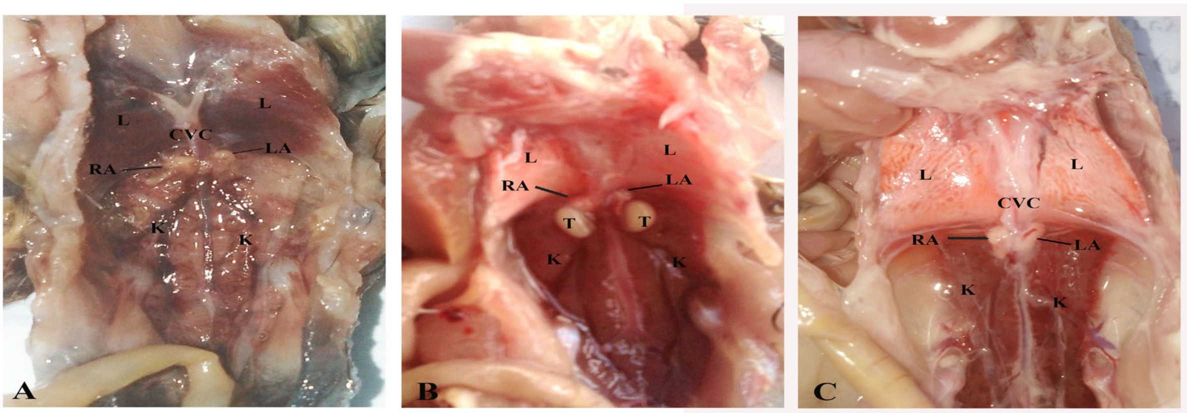

Fig. 1. A Photograph of a Japanese quail's adrenal gland at ages of day of hatching (A), two weeks (B) and five weeks (C) post-hatching. A-C: Two separate adrenal glands (RA & LA) on both sides of the caudal vena cava (CVC), cranio-medially to both kidneys (K) and just caudal to the lungs (L). B: The adrenal glands (arrows) are covered by the testes (T).

Histological observations

At the day of hatching, the adrenal gland was encapsulated with thick connective tissue capsule (Fig. 2A). The parenchyma of the gland was composed of cortical (interrenal) tissue and medullary (chromaffin) tissue that were intermingled with each other with no clear cortex and medulla as well as blood sinusoids. The main tissue of the adrenal parenchyma was the interrenal tissue (Fig. 2B). This parenchyma was subdivided into three distinct zones; subcapsular, peripheral and central zones. The peripheral interrenal cells were arranged into arch- like cord while those at central zone were arranged into cords of two cell lines with a narrow space in between gave it the appearance of acini of the gland. Chromaffin cells were scattered throughout the whole gland, but they increased at the center of the gland. The blood sinusoids were large and surrounded by chromaffin islets., some of these cells bulged into the blood sinusoids (Fig. 2B-C).

At age of the two weeks post-hatching, the differentiation of adrenal parenchyma into three different zones was clearer than the previous age (Fig. 3A). Additionally, the interrenal cords were elongated and became straight or curved at this age. The peripheral interrenal cells were polyhedral with centrally located nuclei or columnar with apically located nuclei. But the central interrenal cells were columnar with apically located nuclei. Their cytoplasm was eosinophilic and lighter than the cytoplasm of the peripheral cells (Fig. 3B-C). The chromaffin islets had an oval, elongated or triangular shape. At this age, their proportion got higher than at the previous one. The mass of these islets increased toward the center of the gland. The blood sinusoids were larger than they had been at previous age (Fig. 3 C).

At age of five weeks post-hatching, the most characteristic feature at this age was that the interrenal cell cords at central zone had become longer and curved than they had at the previ-

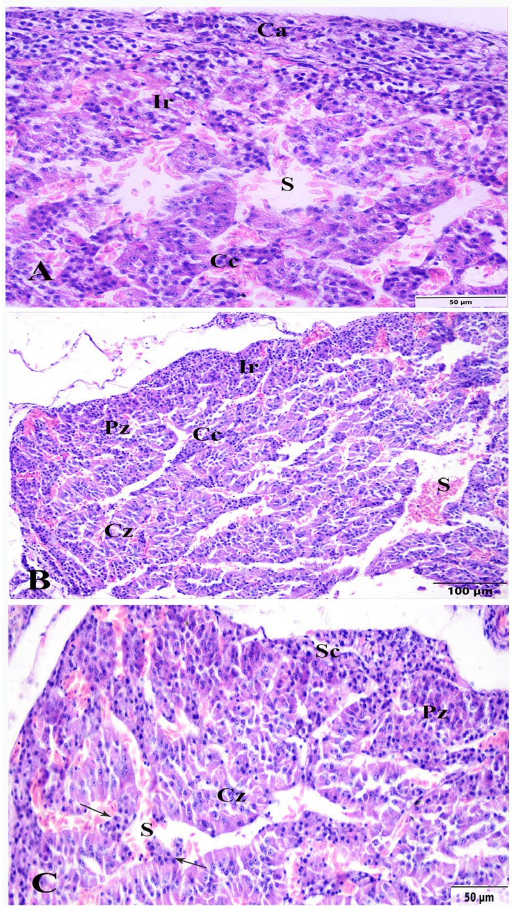

Fig. 2. Paraffin sections stained with H&E of quail adrenal gland at day of hatching show, A-C: The parenchyma of adrenal gland made up of three components; interrenal (Ir) and chromaffin (Cc) tissues as well as blood sinusoids (S). The interrenal tissue differentiated into three zones; subcapsular (Sc), peripheral zone (Pz) and central zone (Cz). The chromaffin tissue (Cc) grouped as islets placed between the interrenal tissue. Some of these chromaffin cells protruded into the blood sinusoid.

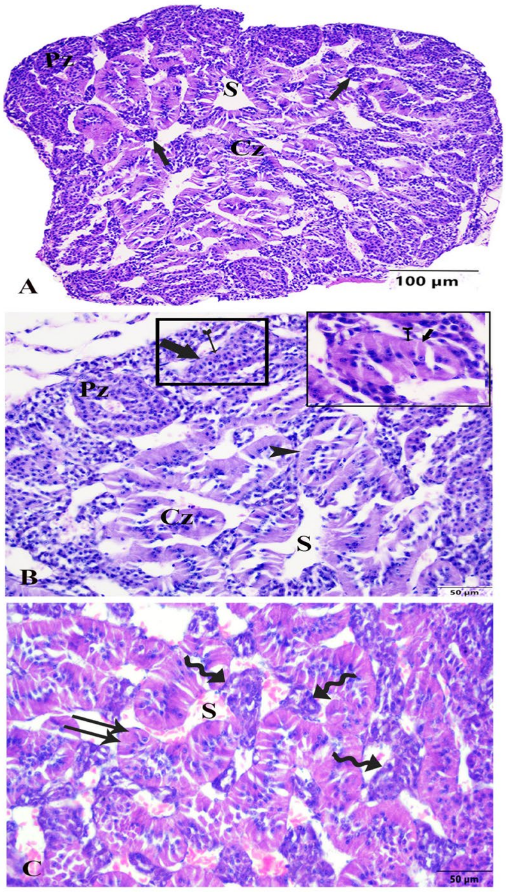

Fig. 3. Paraffin sections stained with H&E of quail adrenal gland at two weeks post-hatching show; A: peripheral zone (Pz), central zone (Cz), chromaffin cells (arrows) and blood sinusoid (S). B: The interrenal cells in the peripheral zone (black square) were polyhedral (forked arrow) or columnar (thick arrow). C: The central interrenal cells were columnar and their cords were straight (arrowhead) or curved (double arrows). Chromaffin islets were oval, elongated or triangular (wavy arrows). Blood sinusoids (S) were wider.

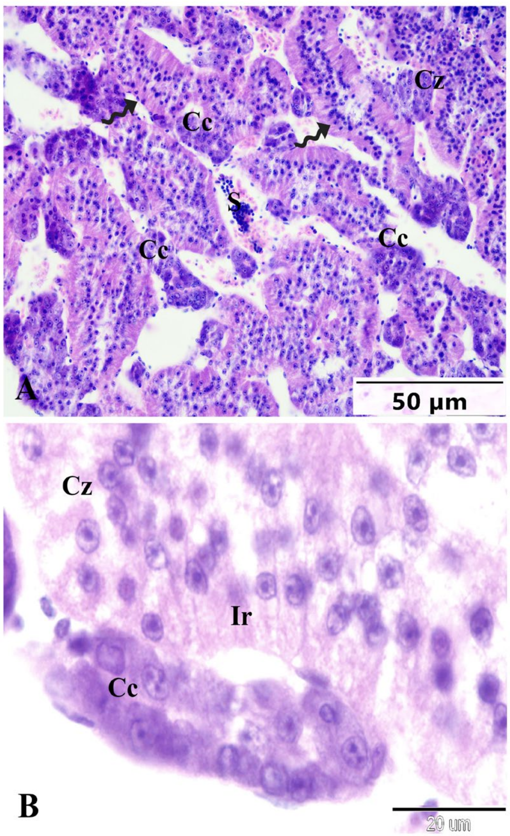

Fig. 4. Paraffin sections stained with H&E of the quail adrenal gland at five weeks post-hatching show, A: Elongated interrenal cords (wavy arrows), chromaffin islets (Cc) and blood sinusoid (S). B: Higher magnification at the central zone (Cz) of the gland show, columnar interrenal cells (Ir) and polyhedral chromaffin cells (Cc).

ous ages. The narrow space in between the two cell lines of these cords disappeared at this age. The gland was highly condensed with chromaffin islets (Fig. 4A). The interrenal cells were columnar with apically located oval to round nuclei and their cytoplasm was lightly stained eosinophilic. The chromaffin cells were polyhedral with large, rounded and centrally located nuclei. Their cytoplasm was deeply stained basophilic (Fig. 4B).

Histochemical observations

At the day of hatching, two weeks, and five weeks post-hatching; both interrenal and chromaffin cells were positive for PAS stain. The positive reaction for PAS was stronger at the peripheral interrenal cells than the central interrenal ones especially on the day of hatching and declined with the age. Chromaffin cells showed stronger reaction for PAS than the interrenal cells. With age the positive reaction became stronger, it was strongest at age of five weeks than the previous ages (Fig. 5A-C). At day of hatching, both interrenal and chromaffin cells were negative for Alcian blue (Fig. 5A). At two- and five-weeks post-hatching, the positive reaction for AB was expressed at the basement membranes of the central interrenal cells and the chromaffin cells were negative for AB (Fig. 5B-C). At age of five weeks post-hatching, the capsule of the gland showed strong positive reaction for PAS and moderate reaction for AB (Fig. 5C).

By using Crossmon's trichrome stain, a green color was observed within collagenous fibers of the capsule. The amount of these fibers at the peripheral zone was few (Fig. 6A&C) but the central zone contained more collagenous fibers (Fig. 6B&D). Furthermore, when Grimelius' silver nitrate was used, the interrenal cells revealed a negative reaction. However, the chromaffin cells had two types of cells with different staining affinities, the first type contained argyrophilic granules of brownish color which showed a strong positive reaction for silver stain and the second type was negative (Fig. 7A-C).

DISCUSSION

The present work investigated the adrenal gland of Japanese quail (Coturnix coturnix) both anatomically and histologically during the post hatching period. In the current investigation, both adrenal glands were seen on either side of the caudal vena cava, craniomedial to the kidneys, and just caudal to both lungs (Dadgar Moghadam and Mohammadpour, 2017). They are appeared as two separate organs as recorded in the majority of birds, in chicken (Humayun *et al*., 2012) and in domestic pigeon (Sadoon, 2018). However, in certain birds, including the crane and white-headed sea eagle, these glands are integrated into a single organ or are entirely united, as in the case of silver gulls (Zhang, 1988). In relation to the gonad (ovaries and testicles) size, the adrenal glands are either entirely or partially covered by the gonads (Mormède *et al*., 2007). In the present study, the adrenal glands are partially covered with the testes at young aged Japanese quail because the testes are too small to cover the whole gland, while in older ages the glands are completely covered with the testes at their ventral surfaces. The color of both adrenal glands is yellow while they differ in shape, the right adrenal gland is triangular and the left one is elongated. These findings are compatible with those in African ostrich chicks (Tang *et al*., 2009) and in duck (Al-Jebori *et al*., 2016). The presence of carotenoids in the lipid droplets may be the cause of the adrenal gland's yellow color (Reavill and Schmidt, 2008). In accordance with the present results, previous studies have demonstrated that, the parenchyma of the adrenal gland in the Japanese quail is made up of two different tissue types that are divided into the cortex and medulla depending on whether they are located centrally or peripherally.

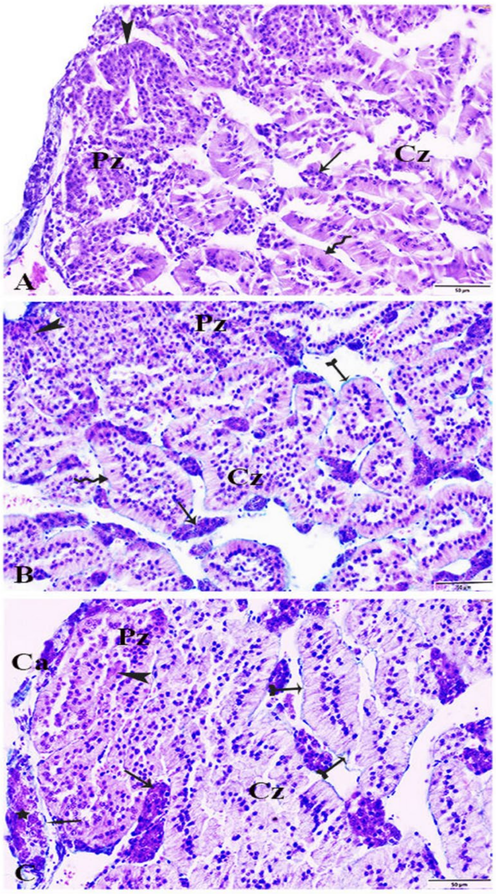

Fig. 5. Paraffin sections of the adrenal gland at the day of hatching (A), two weeks (B) and five weeks (C) post-hatching stained with PAS-AB Combination. A-C: Strong PAS positive reaction at interrenal cells (arrowhead) in the peripheral zone (Pz) and moderate reaction at these cells (wavy arrow) in the central zone (Cz). Chromaffin cells (arrow) showed strong PAS positive reaction. B-C: The capsule (Ca) showed a strong PAS positive reaction (star) and moderate AB reaction (arrow). The interrenal cells' basement membranes showed AB positive reaction (forked arrows).

In contrast to mammals, birds' adrenal glands lack a separate cortex and medulla. Thus, the medulla of the gland is referred to as chromaffin tissue, and its cortex as interrenal (Carsia, 2015). The ratios of interrenal to chromaffin tissues differ significantly amongst the different bird species.; in African ostrich (Ye *et al*., 2018) and in chicken (Moawad and Randa, 2017). According to the findings of the current study, the interrenal tissue makes up most of the adrenal parenchyma and the chromaffin mass gradually increase with the age. However, other birds, such as hawks, had adrenal glands with a higher amount of chromaffin tissue (Zhang, 1988). In the current study, interrenal tissue becomes more prevalent throughout the gland with age, notably at five weeks. This resulted from a greater requirement for the interrenal hormones necessary for ovulation and egg production (Fathima and Lucy, 2012). In Japanese quail, the interrenal tissue is subdivided into three distinct zones: the subcapsular, peripheral and central zones. These findings are similar to those observed in chicken (Humayun *et al*., 2012). However, the interrenal tissue in other birds as Egyptian geese (Elzoghby, 2010), African ostrich chicks (Tang *et al*., 2009) and leghorn fowl (Kondics and Kjaerheim, 1966) is subdivided into two zones; peripheral and central zones. The interrenal tissue made up most of the peripheral zone while chromaffin tissue predominated in the central zone. This result is similar to that observed in pigeon (Sinha and Ghosh, 1961). In the present study, the interrenal tissue at the peripheral zone of the gland arranged into arch-like cords. These cords are arranged perpendicular to the capsule. They are lined by columnar cells with apically located nuclei or polyhedral cells with centrally or eccentrically located nuclei. These results are consistent with those in goose (Anser anser) (Gulmez *et al*., 2004) and Egyptian geese (Elzoghby, 2010). Interrenal cells are grouped into cords of two cell lines in the central zone of the adrenal glands of Japanese quail, giving the appearance of the gland's acini. This come in agreement with the previous study (El-Desoky and Mustafa, 2021). On the day of hatching, the cords are short with low columnar cells, these cords can be straight or curved, and they get longer with advancing the age. The central interrenal cells become high columnar with apically located nuclei. Their cytoplasm is eosinophilic and this finding is similar to that in Wanxi white geese (Wang *et al*., 1999). The cytoplasm of central interrenal cells is basophilic in French goose (Gulmez *et al*., 2004) and this finding is in disagreement with our result. The cytoplasm of the central interrenal cells is lighter than that of peripheral cells.

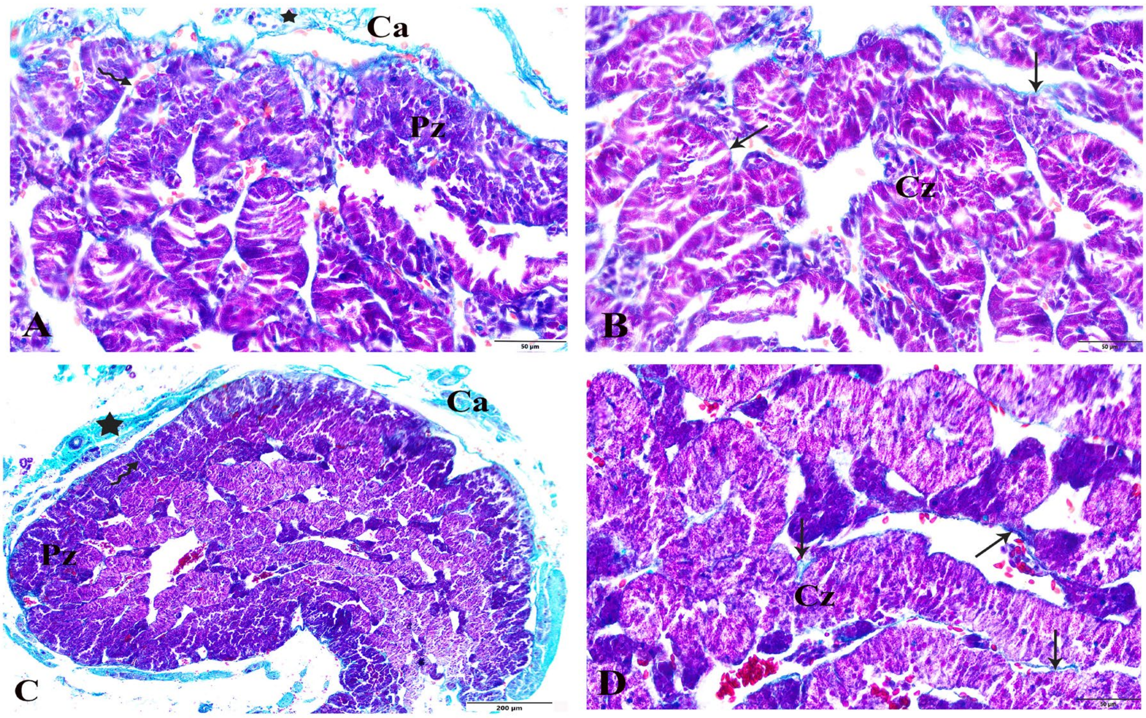

Fig. 6. Paraffin sections of the adrenal gland at the two weeks (A-B) and five weeks (C-D) post-hatching stained with Crossman's Trichrome stain show, abundant collagenous fibers (star) within the capsule (Ca). Few collagenous fibers (wavy arrows) within the peripheral zone and more fibers (arrows) within the central zone (Cz).

These results are contrary that observed in African ostrich chicks (Tang *et al*., 2009), in Egyptian geese (Elzoghby, 2010) and adult Egyptian chicken (Moawad and Randa, 2017). Because of their greater affinity for chromium salts, medullary cells are known as chromaffin cells.(Lucy, 2014). At the two weeks of age, chromaffin cells appeared in the form of elongated or triangular islets scattered between the interrenal cells. They are smaller and fewer than the interrenal cells. The cytoplasm of chromaffin cells is basophilic due to presence of numerous basophilic granules (Humayun *et al*., 2012). At the age of five weeks, the chromaffin islets increased in size and concentrated at the central zone (BASHA *et al*., 2014).

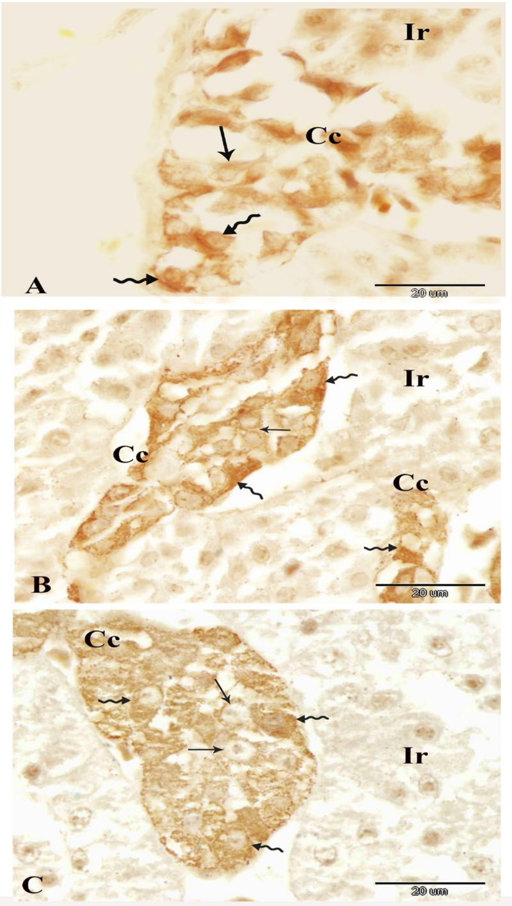

Fig. 7. Paraffin sections of the adrenal gland at the day of hatching (A), two weeks (B) and five weeks (C) post-hatching stained with Grimelius' silver nitrate stain show, negative reaction for silver stain in the interrenal cells (Ir). Two types of chromaffin cells (Cc) appeared. One of them showed positive reaction and contained dark brown granules (wavy arrows) and the other type was negative for silver stain (arrows).

In the current work, we used a PAS-Alcian blue to determine the presence of acidic and neutral mucopolysaccharide. The interrenal cells are strongly positive for PAS (Basha *et al*., 2008; Gaber and Abdel-Maksoud, 2019), especially on the day of hatching age and this reactivity declines with age. This is attributed to high secretory products of cortisol during the embryonic and early post hatching life (Carsia, 2015). The adrenocortical hormones play an important role in the regulation of the mucopolysaccharide metabolism of cells (Karzel and Domenjoz, 1969). The PAS positive reaction is stronger in the interrenal cells of the peripheral zone than in central one. This indicates the higher activity in the cells of the peripheral zone than central one (Basha *et al*., 2008). On other hand, the chromaffin cells showed PAS-AB strong reaction which increase with the age. Using the Grimelius argyrophilic stain revealed that, two types of chromaffin cells; one of them contain dark brown granules and the other is free from these granules. According to Spagnoli *et al*. (1987), cells that contained dark brown granules are norepinephrine containing cells and those free from dark brown granules are epinephrine containing cells. According to the results of the current investigation, norepinephrine cells predominate over epinephrine cells in population. This is consistent with a previous finding in chickens. (Moawad and Randa, 2017).

CONCLUSION

Histologically, the adrenal gland is composed of three distinct zones: subcapsular, peripheral, and central zones. The medullary (chromaffin) tissue increased with the age; this suggests that the demand of bird to medullary hormones increased with age.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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