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Adrenal Gland of Poultry: Anatomy, Microscopy, Morphometry, and Histochemistry

Tetiana Kot[1], Svitlana Tkachuk[2], Svitlana Usenko[2], and Vladislav Prokopenko[1]

[1]*Faculty of Veterinary Medicine, Polissia National University, 10008, Zhytomyr, Ukraine*
[2]*Faculty of Veterinary Medicine, National University of Life and Environmental Sciences of Ukraine, 03041, Kyiv, Ukraine*

Corresponding author's E-mail: tkotvet@ukr.net

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ABSTRACT

The adrenal gland plays a crucial role in poultry's body. Its hormones affect growth, tissue differentiation, and metabolism regulation, as well as the bird body's resistance to infections, intoxication, stress, and low temperature. For poultry farming, veterinary medicine, and ornithology, it is of scientific interest to study the morphological features of the adrenal gland of birds. This review aimed to assess poultry adrenal anatomy, microscopy, morphometry, and histochemistry by summarizing research data from various published articles. The structure of the adrenal gland has been morphologically investigated in clinically healthy chickens, ducks, geese, and quails. Data from the anatomical level of the adrenal gland have indicated that the shape of this organ in poultry of different species is not the same. In most cases, the shape of the adrenal gland of poultry is close to an oval, triangle, or pyramid. The color of the adrenal gland of poultry varies from gray to brown, which depends on the tissue saturation of this organ with carotenoids. The mass of the adrenal glands of poultry correlates with their age. The left adrenal gland has higher mass, volume, and length indicators than the right gland. The microscopic structure of the adrenal gland corresponds to the general laws of the structure and function of endocrine organs. However, the adrenal glands of poultry are characterized by class features of its histoarchitectonics. The adrenal capsule contains ganglia of the autonomic nervous system, the cell strands of cortical and medullary tissues are intertwined, and the configuration of these cell strands determines the formation of two or three zones of the adrenal gland. Studies of the adrenal glands of poultry at the cellular level have indicated that cortical tissue is represented by acidophilic cells and medullary tissue by basophilic cells. Depending on the shape and electron density of secretory granules, medullary tissue cells are divided into epinephrine and norepinephrine. Data on morphometric parameters (capsule thickness, area of cortical and medullary tissues, cortical-medullary ratio) of the adrenal gland are not the same and depend on the type, age, gender, and sexual activity of poultry. In conclusion, morphologists have paid great attention to studying the features of the anatomy, microscopy, morphometry, and histochemistry of the adrenal gland in clinically healthy poultry. Therefore, the presented data can be used to assess deviations in the morphofunctional state of the adrenal gland in poultry under the influence of various factors and pathology.

Keywords: Adrenal gland, Anatomy, Morphological features, Histological and cellular levels, Poultry

INTRODUCTION

Birds are a large class of vertebrates. There are about 10000 bird species from 27 genera and 170 families in the wildlife. A large number of bird species have been recorded in Colombia (1700), Ecuador (1357), and Brazil (1440). Other countries, such as Cameroon (670), the United States of America, and Canada (775), as well as Portugal (315) and Greece (339), are in the second and third places, respectively (Tobias et al., 2022). For many centuries, man has domesticated chicken, turkey, guinea fowl, quail, duck, goose, and pigeon. Canaries, cormorants, and parrots are bred in captivity (Domyan and Shapiro, 2017; Batool et al., 2020; Peng and Broom, 2021). Poultry farming occupies a leading position among the livestock industries (Hafez and Attia, 2020). Over the past half-century, poultry production has increased fivefold and continues to grow (OECD-FAO, 2016). According to the Organization for Economic Cooperation and Development/Food and Agriculture Organization agricultural forecast for 2019-2028, poultry is the most consumed animal protein in the world (OECD-FAO, 2019). When raising poultry, diseases that damage the endocrine system can cause many problems (Rudik et al.,

2021). Organs of the endocrine system are divided into central, peripheral, and mixed, including single endocrinocytes and endocrine organs, forming a dissociated endocrine system (Ritchie and Pilny, 2008; Scanes, 2015).

The adrenal gland is a peripheral organ of the endocrine system. The microscopic structure of the adrenal gland corresponds to the general laws of the structure and function of endocrine organs. It is known that there are no excretory ducts in the endocrine organs. The products of their activity (hormones) are released directly into the lymph and blood. Endocrine organs consist of the connective tissue stroma and parenchyma. The parenchyma forms follicles, strands, and islets, the cells of which are in close contact with the vessels of the microcirculatory bed. There are many blood capillaries between the structural components of the parenchymal adrenal gland, mainly of the sinusoid type (Scanes, 2015; Zakrevska and Tybinka, 2019; Scanes, 2020).

Adrenal hormones play a crucial role in the vital activity of the body by affecting the growth and differentiation of tissues, the development of reproductive organs, and the course of the sexual cycle (Kot et al., 2021; Gjuen and Jensen, 2022). Adrenal hormones regulate water, protein, carbohydrate, fat, and mineral metabolism (Manju Madhavan and Varghese, 2016; Lotfi et al., 2018; Di Lorenzo et al., 2020). The adrenal gland is sensitive to changes in the external and internal environment (Muller et al., 2015; Rudik et al., 2021). Morphological studies have indicated that the mass, size, vascularization of the adrenal gland, the ratio of cortical and cerebral zones, and their cytophysiological characteristics change in poultry affected by stress, hypothermia, and diseases (Scanes, 2016; Lotveld et al., 2017; Qureshi et al., 2020). Adrenal morphology in clinically healthy poultry is closely related to the anatomical, histological and cellular levels. The obtained data can be used to form a basis for the normal morphological characteristics of the adrenal gland in poultry. This makes it possible to assess the morphofunctional state of the adrenal glands of poultry in specific periods of their life, which is necessary for the scientific justification of technologies used in raising poultry, as well as for mastering the mechanisms of the development of adrenal diseases. This review aimed to assess features of the anatomy, microscopy, morphometry, and histochemistry of the adrenal glands of poultry by summarizing research data from various published articles.

ANATOMY

Topography, shape, and color

The adrenal gland of poultry is a paired organ. The left and right glands are situated cranio-medially to the kidneys on each side of the dorsal aorta and inferior vena cava (Figure 1A, B, C; Kober et al., 2012; Moawad and Randa, 2017; Prokopenko, 2022). Accessory adrenal glands in the chicken are near the main adrenal gland or in its capsule (Kot and Prokopenko, 2020). Accessory adrenal glands often develop in fish (Gaber and Abdel-maksoud, 2019) and mammals (Zakrevska and Tybinka, 2019). It has been claimed that accessory adrenal glands are the epicenter of neoplasms development (Afuwape et al., 2009). Kassaby et al. (2017) found adrenal tissue in a hernia sac of a human.

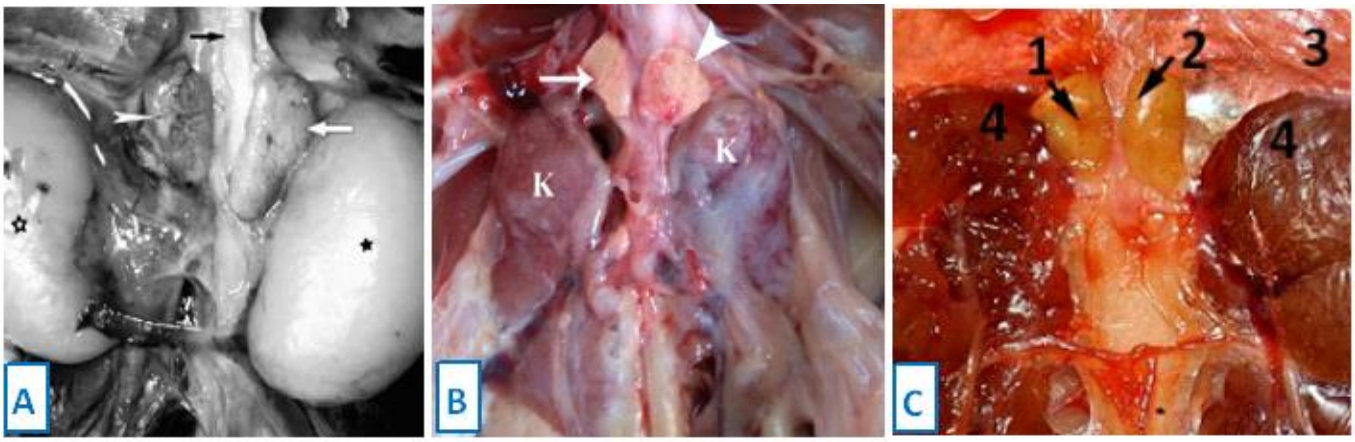

Figure 1. Anatomical position of the adrenal gland in chickens. (**A**): White arrowheads: Right and left adrenal gland, Black-and-white star: Right and left testis, Black arrow: Vena cava (Kober et al., 2012); in chicken (**B**): White arrows: Right and left adrenal gland, K: Kidneys (Moawad and Randa, 2017); in blue rock pigeon (**C**): 1: Right adrenal gland, 2: Left adrenal gland, 3: Left lung, 4: Superior kidney lobe (Prokopenko, 2022).

The shape of the adrenal gland in poultry is different (Figure 2A, B, C; Fathima and Lucy, 2014; Kober et al., 2012; Kot and Prokopenko, 2020). In a one-day-old duck, the adrenal gland has a spherical shape. By the age of 24 weeks, the right adrenal gland becomes pyramidal, and the left adrenal gland becomes oval in shape (Fathima and Lucy, 2014). The right adrenal gland of the chicken has an oval or triangular shape, and the left adrenal gland is elongated-oval in shape (Kober et al., 2012; Sarkar et al., 2014; Kot and Prokopenko, 2020), while in the ostrich they are ellipsoid and oblong in shape, respectively (Tang et al., 2009). The shape of the right gland of Japanese quails is triangular, while the left is elongated in shape (Rudik et al., 2021).

The color of the adrenal glands of poultry varies from gray, cream in pigeons, cream-yellow in quails (El-Desoky and El-Zahraa, 2021; Erdem et al., 2021) to yellow or yellow-red in guinea fowl (Prokopenko and Kot, 2021a). The duck is characterized by a change in the cream or yellow color of the adrenal gland in young animals to brown in adult birds (Fathima and Lucy, 2014). As reported by Scanes (2015), the intensity of the yellow color of the adrenal gland depends on the saturation of its tissues with carotenoids.

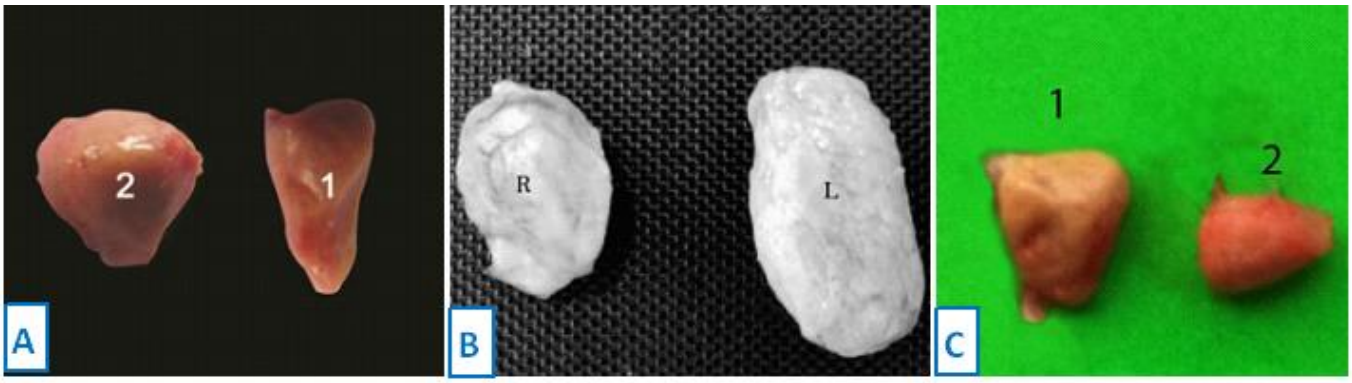

Figure 2. Shape of the adrenal gland in ducks. (**A**): 1: Left adrenal gland, 2: Right adrenal gland (Fathima and Lucy, 2014); in chicken, (**B**): L: Left adrenal gland, R: Right adrenal gland (Kober et al., 2012); in chicken (**C**): 1: Right adrenal gland, 2: Left adrenal gland (Kot and Prokopenko, 2020).

Organometry

The mass of the adrenal glands of poultry correlates with their age. In ducks, the adrenal mass increased from day-old (0.011 ± 0.001 g) to 12 weeks of age (0.093 ± 0.002 g), then decreased to 0.088 ± 0.003 g at week 16 (at the beginning of egg laying) and increased again to 0.137 ± 0.006 g at the age of 24 weeks (Fathima and Lucy, 2014). The relative mass of the duck's adrenal gland decreases intensively after hatching (Fathima and Lucy, 2014). Compared to the right adrenal gland, the left one has higher indicators of mass, volume, and length (Colcimen and Cakmak, 2021; Prokopenko and Kot, 2021a). In chickens, the masses of the left and right adrenal glands are 104.1 mg and 97.2 mg, with a length of 0.9 cm and 0.8 cm, respectively. The width and thickness of the left adrenal gland (0.5 and 0.4 cm) are less than those of the right in chickens (0.6 and 0.5 cm, Kober A et al., 2012). The weight and size of the adrenal gland of poultry change during their sexual activity, violation of the detention conditions, stress, and the use of pharmacological drugs (Vyas and Jacob, 1976; Muller et al., 2015).

MICROSCOPY

Capsule and trabeculae

The adrenal gland of poultry is covered on the outside by a capsule consisting of dense fibrous connective tissue, rich in collagen, reticular fibers, and blood vessels with few elastic elements (Figure 3A, B; Moawad and Randa, 2017; Kot and Prokopenko, 2020). In chickens, the thickness of the adrenal capsule of females (22.09 ± 2.17 µm) is greater than in males (18.14 ± 1.82 µm, Kot and Prokopenko, 2020). The thickness of the pigeon adrenal capsule does not exceed 13.46 ± 0.67 µm (Prokopenko, 2022). Ganglia of the autonomic nervous system and chromaffin cells are recorded in the adrenal capsule of quails (El-Desoky and El-Zahraa, 2021), geese (Prokopenko and Kot, 2021b), chickens (Figure 3B, C; Moawad and Randa, 2017; Kot and Prokopenko, 2020),

and guinea fowls (Moghada and Mohammadpour, 2017). Vascularization and encapsulation of the quail's adrenal glands are completed on day 10 of incubation (Basha et al., 2009). In the adrenal gland of chicken, guinea fowl, and geese, trabeculae are directed from the capsule to enter the parenchymal glandular tissues (Moawad and Randa, 2017; Moghadam and Mohammadpour, 2017; Prokopenko and Kot, 2021b). Capsule duck's adrenal glands consist of connective tissue fibers and flattened cells arranged in 2-3 rows with elongated nuclei. According to morphological features, these structures correspond to different fibroblastic cells (Plakhotniuk et al., 2021).

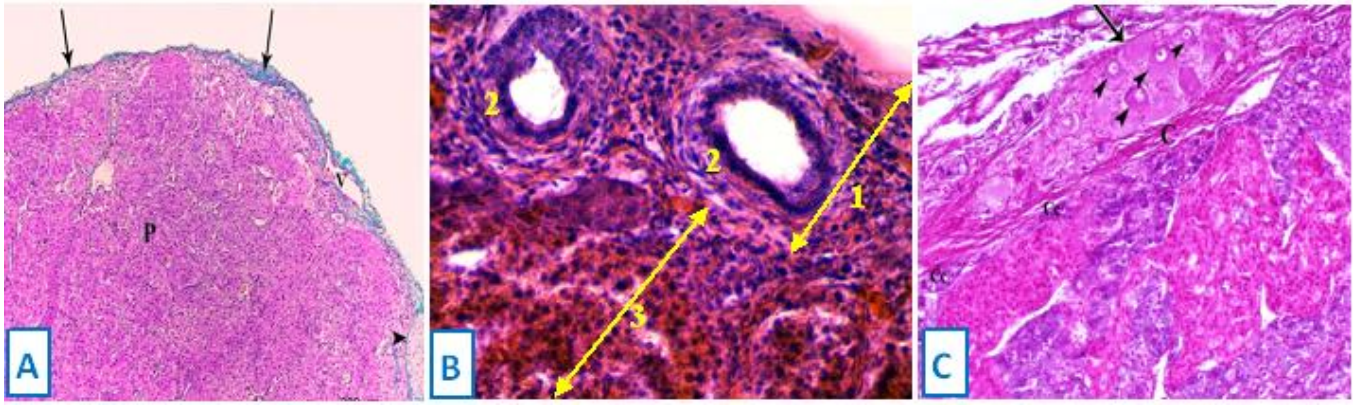

Figure 3. Photomicrographs of the adrenal gland of chickens. (**A**): Black arrows: Collagen fibers in capsule, V: Blood vessels, P: Parenchyma, Arrow head: Septum (Crossmon's trichrome stain, × 100, Moawad and Randa, 2017); (**B**): 1: Capsule, 2: Blood vessels, 3: Parenchyma (H&E, × 400, Kot and Prokopenko, 2020); (**C**): Black arrow: Autonomic ganglia, C: Capsule, Arrow heads: Nerve cells, Cc: Clusters of subcapsular chromaffin cells (H&E, × 200, Moawad and Randa, 2017).

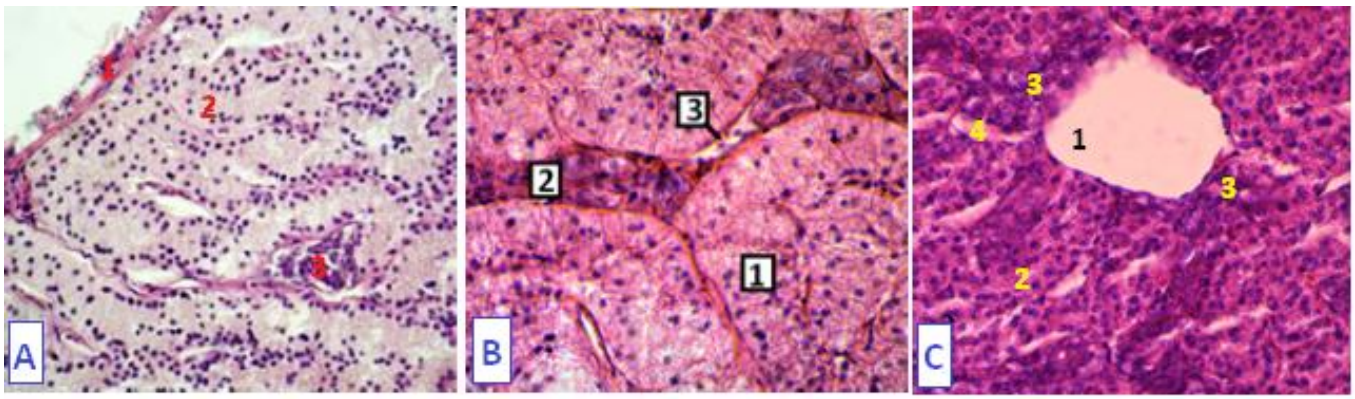

Figure 4. Photomicrographs of adrenal gland in ducks. (**A**): 1: Capsule, 2: Cortical tissues, 3: Medullary tissues (H&E, × 150, Plakhotniuk et al., 2021); in geese (**B**): 1: Cortical tissues, 2: Medullary tissues, 3: Hemocapillary (H&E, × 400, Prokopenko and Kot, 2021b); in chicken (**C**): 1: Lumen of the venous sinus, 2: Cortical tissues, 3: Medullary tissues, 4: Hemocapillary (H&E, × 100, Kot and Prokopenko, 2020).

Parenchima, cortical (interrenal), and medullary (chromaffin) tissue zones

The adrenal parenchyma of poultry consists of cortical and medullary tissues, the cellular strands of which are intertwined throughout the organ (Figures 4A, B; Plakhotniuk et al., 2021; Prokopenko and Kot, 2021b). Narrow gaps between them are filled with layers of loose fibrous connective tissue, in which venous sinuses and hemocapillarie are registered. The wall of the venous sinuses is formed by flat endotheliocytes. In some places, it is intermittent due to sinusoidal hemocapillaries that open into the lumen of the venous sinuses (Figure 4C; Fathima and Lucy, 2014; Moghadam and Mohammadpour, 2017; Kot and Prokopenko, 2020).

According to Basha et al. (2009), adrenal cortical tissue in quails is formed from the peritoneal epithelium and interrenal mesonephros blastema on the third and fourth days of incubation. On the fourth day, cells of the medullary tissue of the adrenal gland migrate from the prevertebral nerve plexus of the ectoderm of the nerve crest to the cortical tissue. The location of cortical cell strands between sinusoids and cell differentiation begins

from the seventh to the tenth day of incubation. Invasion of medullary tissue cells into the cortical tissue is noted on day 18 of incubation.

In some studies, *Columba livia*, *Passer domesticus*, *Corvus splendens*, *Acridotheres tristis*, *Acridotheres ginginianus*, *Milvus migrans*, *Francolinus pondicerianus*, and *Bubulcus ibis* (Vyas, 1976) are used for cortical tissueand medullary tissue, interrenal tissue, and chromaffin tissue to describe the structure of the adrenal parenchyma in quails (El-Desoky and El-Zahraa, 2021), ducks (Fathima and Lucy, 2014), ostrich (Tang et al., 2009; Ye et al., 2017). The name of the first tissue corresponds to its origin, and the name of the second tissue is based on the ability of its cells to restore chromium, silver, and osmium oxi. The adrenal cortex of quail is represented by acidophilic cells. They are large, polyhedral, or columnar in shape, have acidophilic and vacuolated cytoplasm, and have a rounded or oval nucleus located eccentrically (El-Desoky and El-Zahraa, 2021). Regarding the adrenal gland of quails, the nuclei of acidophilic cells are large with nucleoli and coarse chromatin; the cytoplasm contains many mitochondria, ribosomes, lipid droplets, and endoplasmic reticulum (Basha et al., 2009). Depending on the number of lipid droplets and mitochondria, acidophilic cells are divided into two types. Cells of the first type contain numerous lipid droplets with several slightly larger globular mitochondria (Figure 5A, B; Moawad and Randa, 2017), while cells of the second type entail few lipid droplets (Figure 5C; Moawad and Randa, 2017).

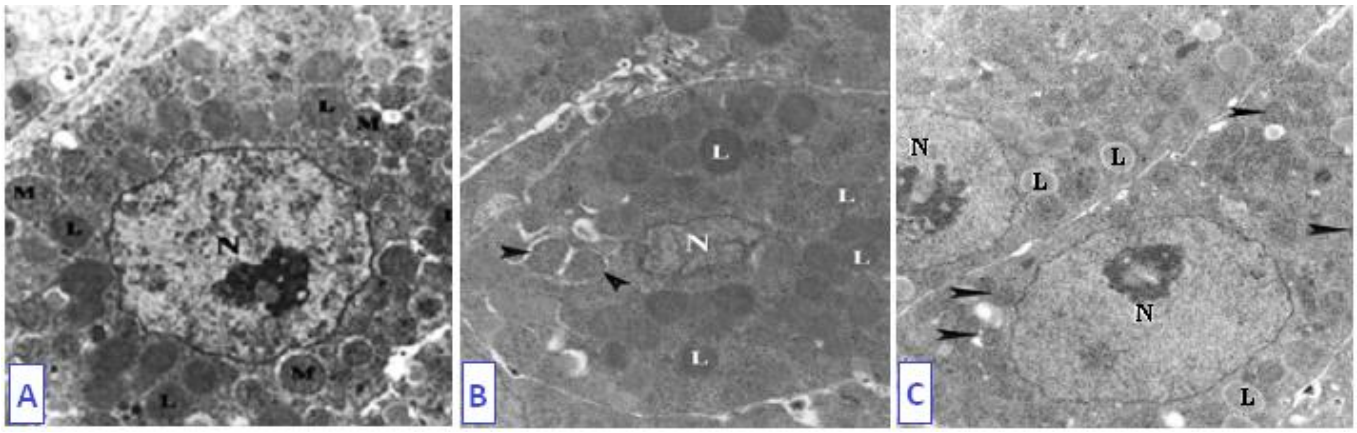

Figure 5. Electron micrographs of the adrenal cortex cells in a chicken. (**A**): N: Spherical basal nucleus, L: Numerous electron dense lipid droplets, M: Few mitochondria (Uranyle acetate and lead citrate stain, × 8000); (**B**): N: Irregular nucleus, L: Many electron dense lipid droplets, Black arrow heads: Few mitochondria (Uranyle acetate and lead citrate stain, × 8000); (**C**): N: Oval basally situated nuclei, L: Few lipid droplets, Black arrow heads: Numerous mitochondria (Uranyle acetate and lead citrate stain, × 8000, Moawad and Randa, 2017).

Basophilic cells are part of the chromaffin tissue of the adrenal glands of poultry. They have a polygonal or rounded shape, basophilic cytoplasm, and a large spherical nucleus located in the center and contains two or three nucleoli. In chickens, the cell height and diameter of their adrenal nuclei in males (8.72 ± 0.231 µm and 4.39 ± 0.359 µm, respectively) are higher than in females (8.67 ± 0.218 µm and 3.84 ± 0.326 µm, respectively, Sarkar et al., 2014). The cytoplasm of basophilic cells contains mitochondria with tubular crosses, ribosomes, endoplasmic reticulum, lipid droplets, and secretory granules (Figures 6A, B; El-Zoghby, 2010; Moawad and Randa, 2017). Depending on the shape of the secretory granules, basophilic cells are divided into two types. Epinephrine cells are composed of homogeneous polymorphic electron-dense secretory granules, and norepinephrine cells contain secretory granules with an electron-dense nucleus bounded by a light border (Figure 6C; Tang et al., 2009; El-Zoghby, 2010; Moawad and Randa, 2017). Prabhavathi et al. (2011) identified epinephrine, norepinephrine, and stellate-shaped satellite cells in the chromaffin tissue of the guinea fowl's adrenal glands. Differentiation of quail adrenal chromaffin cells into epinephrine and norepinephrine cells is completed by day 15 of incubation (Basha et al., 2009). In chickens, ganglion cells are registered among the cells of the medullary tissue of the adrenal glands (Moawad and Randa, 2017).

The placement and configuration of cell strands of cortical (interrenal) and medullary (chromaffin) tissues determine the formation of adrenal parenchyma zones in poultry (Ritchie and Pilny, 2008). Peripheral (subcapsular) and internal (central) zones are distinguished on the incision of the adrenal gland in a chicken (Moawad and Randa, 2017).

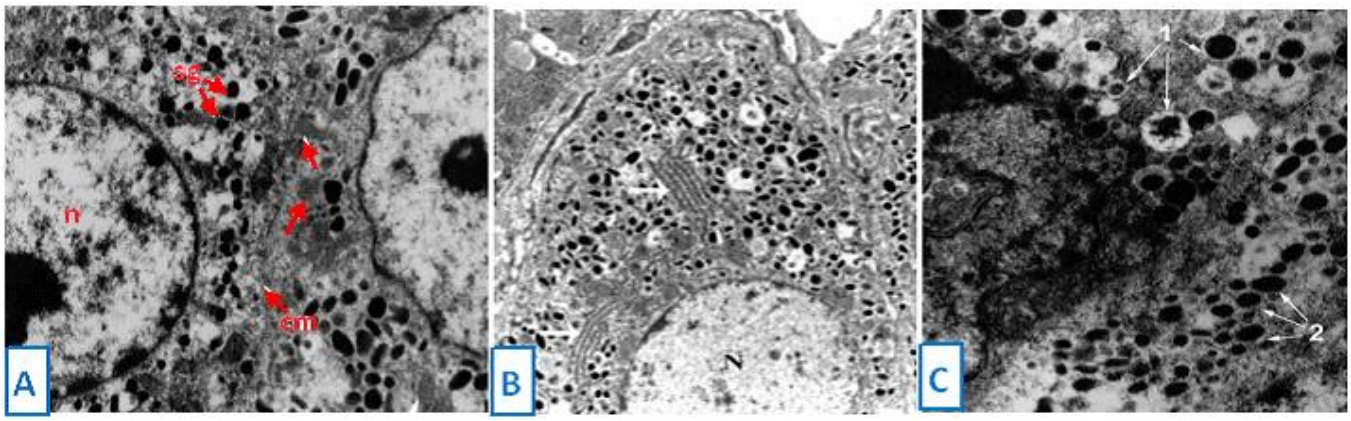

Figure 6. Electron micrographs of chromaffin cells of the adrenal gland in a goose. (**A**): n: nucleus, cm: Cell membrane, Arrows: Mitochondria, sg: Secretory granules (Uranyle acetate and lead citrate stain, × 1000, El-Zoghby, 2010), in chicken (**B**): n: Nucleus, Arrows: Strands of rough endoplasmic reticulum (Uranyle acetate and lead citrate stain, × 8000, Moawad and Randa, 2017), in geese (**C**): 1: Secretory granules of electron dense core surrounded by hollow electron lucent coat of chromaffin cells showing: norepinephrine, 2: Homogenous, polymorphic electron dense secretory granules of chromaffin cells showing: epinephrine (Uranyle acetate and lead citrate stain, × 1200, El-Zoghby, 2010).

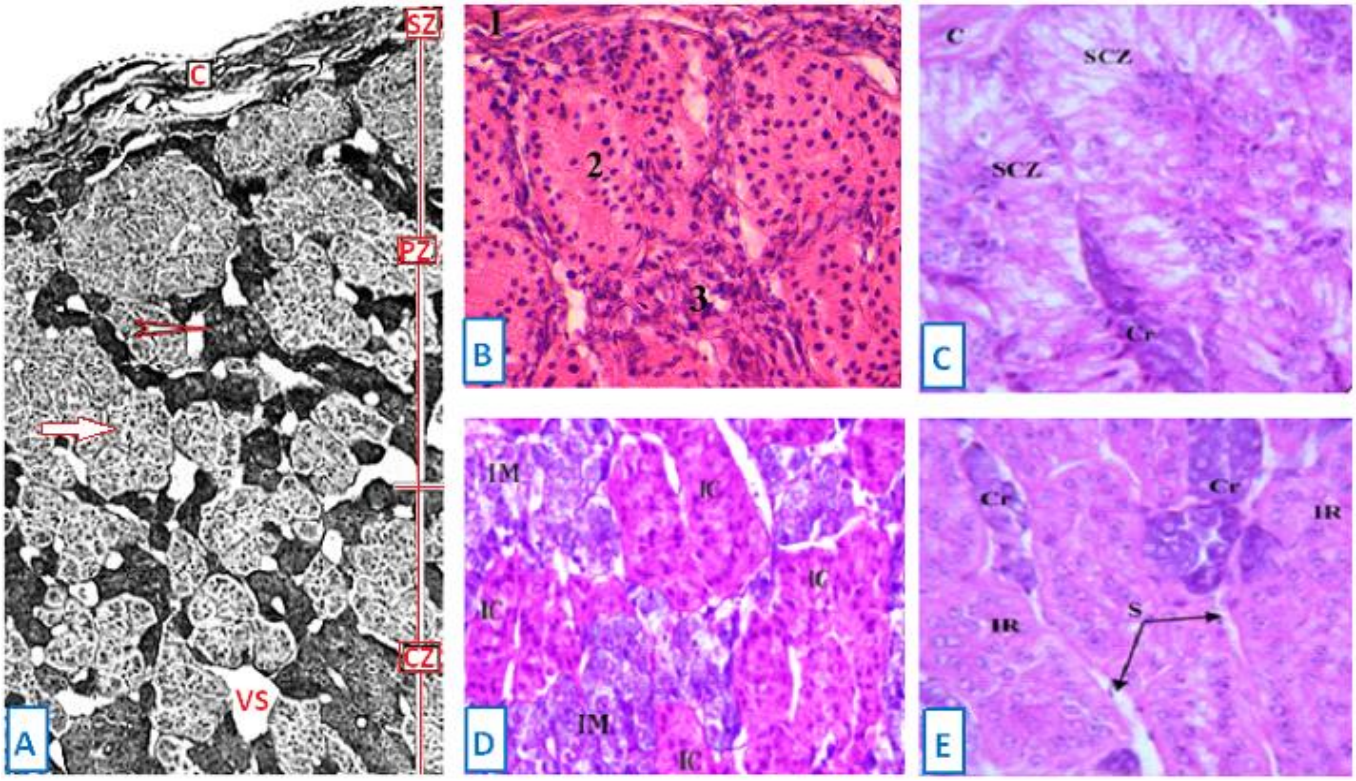

Figure 7. Photomicrographs of adrenal gland in chickens. (**A**): SZ: Subcapsular zone, PZ: Peripheral zone, CZ: Central zone, VS: Venous sinus, C: Capsule, Arrow: Cortical tissue, arrowhead: Medullare tissue (Azan, × 100, Humayun et al., 2012); in chicken (**B**): 1: Capsule, 2: Cortical tissue, 3: Medullare tissue (H&E, × 400, Kot and Prokopenko, 2020); in ostrich (**C**): 1: Capsule, 2: Subcapsular zone, Cr: Chromaffin tissue (H&E, × 400, Tang et al., 2009); in chicken (**D**): IC: Inner cortical cords, IM: Inner medullare cords (H&E, × 400, Moawad and Randa, 2017); in ostrich (**E**): IR: Interrenal tissue, Cr: Chromaffin tissue, S: Venous sinus (H&E, × 400, Tang et al., 2009).

Humayun et al. (2012) recorded a subcapsular layer, peripheral and central zones in the chicken adrenal gland (Figure 7A), which is consistent with the results of studies on guinea fowls (Moghadam and Mohammadpour, 2017) and quails (El-Desoky and El-Zahraa, 2021). The central and peripheral zones are distinguished for the adrenal gland of the ostrich. The latter zone consists of an external part (subcapsular zone) and an internal part (Tang et al.,

2009; Ye et al., 2017). In the subcapsular zone of the adrenal gland, cellular strands of cortical tissue have the appearance of loops in chickens (Figure 7B; Kot and Prokopenko, 2020) and quails (El-Desoky and El-Zahraa, 2021), and a type of bow in an ostrich (Figure 7C; Tang et al., 2009). Islands of medullary tissue are registered between them. In the guinea fowl adrenal gland, the subcapsular zone is represented only by medullary tissue (Moghadam and Mohammadpour, 2017). Cortical tissue cells form straight or arched cords in the central zone of the adrenal gland of chickens (Figure 7D; Moawad and Randa, 2017) and quails (El-Desoky and El-Zahraa, 2021). In the ostrich, the cords of the cortical tissue of the central zone are smooth and located on the right side of the periphery of the adrenal gland (Ye et al., 2017).

Micrometry

In the chicken adrenal gland, the proportion of medullary tissue in the central zone (49.7%) is twice as high as in the peripheral zone (24.8%), and the adrenal cortical-medullary ratio is 1.6:1 (Humayun et al., 2012). According to Sarkar et al. (2014), the adrenal cortical-medullary ratio of chicken in females (1.9:1) and males (1.43:1) is not the same. In duck ontogenesis, the adrenal cortical-medullary ratio changes from 1.15:1 (6 weeks of age) to 2:1 (18 weeks of age) due to an increase in the proportion of cortical tissue. At the beginning of egg laying, the layer hens experience stress, and the adrenal gland releases corticosterone and catecholamines into the blood, which help the adaptation to new conditions (Fathima and Lucy, 2014). Basha et al. (2009) recorded a decrease in the proportion of interrenal tissue and ganglion transformation of adrenal chromaffin tissue during molting in quails.

HISTOCHEMISTRY

Most histochemical reactions by which adrenal medulla hormones are released and detected are based on the property of these hormones to vigorously reduce chromium oxide and osmium silver (Moawad and Randa, 2017). Chromaffin granules of medullary cells of the chicken's adrenal gland acquire a greenish-yellow color in sections prefixed in Ortha's fluid and stained by Giemsa (Figure 8A; Moawad and Randa, 2017).

The adrenal histochemistry of the wild birds (*Columba livia*, *Passer domesticus*, *Corvus splendens*, *Acridotheres tristis*, *Acridotheres ginginianus*, *Milvus migrans*, *Francolinus pondicerianus*, and *Bubulcus ibis*) determined by their sexual activity. During the mating season of wild birds, the interrenal tissue of the central adrenal gland is characterized by the highest content of alkaline phosphatase, glycogen, acidic mucopoly-saccharides, and gross lipids. In the interrenal tissue of the subcapsular layer, moderate content of ascorbic acid is observed. The histochemical characteristics of chromaffin tissue remain unchanged throughout the year, except for acid phosphatase, the amount of which increases with the sexual activity of birds (Vyas., 1976).

In quails, the interrenal tissue is rich in glycogen, lipids, and cholesterol, and the chromaffin adrenal tissue is loaded with acidic mucopolysaccharides and ascorbic acid (Basha et al., 2009). Regarding the staining of histological sections of chicken adrenal glands with Sudan black B and Sudan III, the maximum amount of general and neutral lipids is recorded in cells of the interrenal tissue of the subcapsular zone, slightly less in such cells of the central zone (Figure 8B, C; Moawad and Randa, 2017).

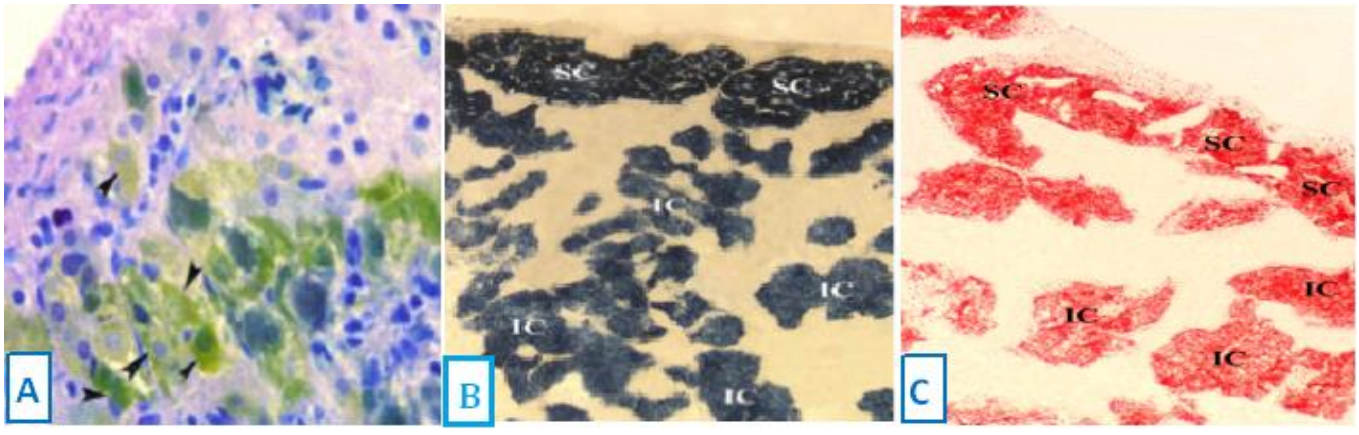

Figure 8. Photomicrographs of the adrenal gland of chickens. (**A**): Black arrow heads: Strong and moderate chromaffin reaction of greenish yellow color in medullary (Giemsa, × 1000), (**B**): SC: Intensive sudanophilic substances fill the subcapsular cortical cells, IC: Less intensive sudanophilic substances fill the inner cortical cells (Sudan black B, × 100), (**C**): SC: Strong positive reactions in subcapsular cortical cords, IC: Moderate reactions in inner cortical cords (Sudan III, × 200, Moawad and Randa, 2017).

CONCLUSION

This review indicated anatomical, histological, ultramicroscopic, histochemical, and morphometric methods of studying the adrenal gland of poultry are a matter of concern. The morphology of the adrenal gland in clinically healthy chickens, ducks, geese, and quails should be studied in depth. The obtained data can be used to assess differences in the morphofunctional state of the adrenal gland in poultry under the effect of various factors and pathological aspects.

DECLARATIONS

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Authors' contributions

Tatyana Kot contributed to data collection, database creation, and preparation of the manuscript. Svitlana Tkachuk was also involved in preparing the manuscript and data analysis. Svitlana Usenko, and Vladislav Prokopenko guided the research, photomicrographs and electron micrographs analysis, and manuscript preparation. All authors checked and approved the final version of the manuscript for publishing in the present journal.

Competing interests

The authors have declared that no competing interest exists.

Ethical consideration

All authors have checked the ethical issue, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication, and/or submission, and redundancy.

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