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Histological Study of the Adrenal Gland Development of the Local Rabbit from One Month to One Year of Age

[1] Diana Mohamed Ragab Mohamed, [2] Ayad Ibrahim Hameed

1, 2 Department of Anatomy & Histology, Veterinary Medicine College, Tikrit University, Iraq

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Corresponding Author:
Name:
Diana Mohamed Ragab
E-mail:
dm230009pve@st.tu.edu.iq
Tel: 07705422823

ABSTRACT

Background and Objective: Two distinct kinds of signaling molecules are found in each of the two embryonic tissues that make up the adrenal gland. One is the steroid hormone-producing outer cortex, which is mesenchymal in origin. The neuroectoderm-derived medulla, which secretes catecholamines, is the other. Therefore, the current study aimed to track the development of the adrenal gland of local rabbits from one month after birth until one year of age using histological studies.

Methods: Using histological studies, the development of the adrenal gland of local rabbits was tracked from one month after birth until one year of age.

Results: The results showed that after one month, the adrenal gland is surrounded by a fibrous capsule composed of elastic yellow-fibers. Spherical epithelial cell clusters, each composed of 6-8 cells with spherical nuclei, form the glomerular zone. Beneath this zone, larger cells with pale cytoplasm and smaller spherical nuclei are found together in the glomerular zone, where they form the fascicular zone. On the other hand, the medulla of the gland contained all the cytoplasmic chromophore cells of the basophilic type, surrounded by large chromophore cells with acidic cytoplasmic pigmentation, with clear cell boundaries.

After three months, the Endocrine cells then divide into bundles or columns of pale pigment, extending toward the medulla of the gland. The reticular zone is classified as a zone containing a number of endocrine cells in a reticular pattern adjacent to the medulla of the gland. The medulla of the adrenal gland in rabbits contained chromaffin aggregates around the central vein with a wide lumen.

After six months, in addition to the presence of clusters of endocrine cells in the form of an arc, forming the arc band. These cells, at their bases, are continuous with the columns of fascicle cells, composed of two rows of adjacent cells, columns in the form of bands, and the presence of nuclei closely packed together.

Conclusions: The histological development of the adrenal gland of local rabbits was tracked from one month after birth until one year of age, demonstrating progressive structural organization of both the cortex and medulla.

Introduction

Rabbits are little animals that are found all over the world and are members of the Leporidae family of the Lagomorpha order. Eight distinct genera are categorized as rabbits in this family, including the Amami rabbit (Pentalagus furnessi), cottontail rabbits (Sylvilagus), and European rabbits (Oryctolagus cunniculus). The female produces consecutive litters, usually three to seven pups, 30 days apart throughout their breeding season, which runs from January to August. Rabbits become sexually mature at four months of age [1]. Mammals typically have two adrenal glands; one located atop each kidney. The cortex and the medulla are the two functionally separate components of the adrenal gland, that has been examined after birth. It is an essential endocrine gland that is crucial to the body's systems for controlling metabolism. Three main parts make up the tissue of the adrenal glands in birds and certain other animals: the vascular sinusoids, the chromaffin tissue, and the cortical tissue [2, 3]. Three, four, or two different epithelial cell zones make up the adrenal cortex. The outermost zone in ruminants, primates, and certain rodents is referred to as the zona glomerulosa. The visceral tissue forms arches just beneath the capsule in the majority of other domestic animals, such as dogs, cats, horses, and pigs [4, 5]. Thus, this part of the cortex in these animals is referred to as the zona arcuata. The next part of the cortex is called the zona fasciculate. The secretory cells of the zona fascicula have a foamy appearance due to the presence of numerous lipid droplets. Because of this morphological characteristic, they are called spongiocytes. This zone is the widest in the adrenal cortex and consists of radially arranged cords of cuboidal or columnar cells. The cells of the zona fasciculate terminate internally adjacent to the innermost region of the cortex, the zona reticularis. As the name suggests, the parenchyma of the zona reticularis appears to anastomose, forming reticular chains of cells [6, 7, 8]. In rabbits, in the adrenal medulla, noradrenaline is found outside the gland, while adrenaline is found only in the chromaffin cells of the medulla [9]. The suprarenal medulla in albino rats consists of clusters of epithelial cells supported by thin connective tissue and separated by blood sinuses [10]. Domestic animals' medulla is primarily made up of modified postganglionic sympathetic neurons, which have a large number of brown granules in their cytoplasm and absorb and stain strongly with chromium salts [11]. Therefore, the current study aimed to track the development of the adrenal gland of local rabbits from one month after birth until one year of age using histological studies.

Materials and Methods

Ethical Clearance

Every experimental protocol was authorized by Tikrit University's College of Veterinary Medicine in Iraq, and every experiment was conducted in compliance with established protocols.

Animals

From August to November of 2024, this study was carried out in the Veterinary Medicine College's animal house at Tikrit University. Five (5) healthy male rabbits and fifteen (15) adult female rabbits were acquired from the Veterinary Medicine College's animal home at Tikrit University. Weights of animals aged 1 to 12 months varied from 1300 to 1500 g, with one-month-olds weighing 200 g and three-month-olds weighing 350 g.

Experiment design

Twenty females and males were taken and placed in cages

- group A dissected one month after birth which including (5 animals with 1-month old).
- group B dissected three months after birth (5 animals with 3-months old).
- group C dissected six months after birth (5 animals with 6 months old).
- group D dissected one year after birth (5 animals with at 12 months old).

Dissection protocol

Before they were put to death, each animal was examined. Using a dissecting microscope, the adrenal glands were inspected in detail. Following an intramuscular injection of a ketamine HCl 60 mg/kg and xylazine 6 mg/kg mixture, the abdominal cavity was opened, exposing the left and right adrenal glands. The abdominal aorta received an injection of 10% neutral buffered formalin, which was left for 48 hours to fix the organs.

Histological study

Pieces of the adrenal glands were removed, paraffin-processed, fixed with 10% formalin, sliced to a 7 micrometer thickness using a rotary microtome, and stained with PAS and Hematoxylin and Eosin (H&E) histology stains

[12]. Sections were examined using an Optica microscope (Italy).

Results & Discussion

One Month

The adrenal gland is surrounded by a fibrous capsule composed of bile fibers beneath the capsule. Spherical epithelial cell clusters, each composed of 6-8 cells with spherical nuclei, form the glomerular zone. Beneath this zone, larger cells with pale cytoplasm and smaller spherical nuclei are found together in the glomerular zone, where they form the fascicular zone. These cells are arranged in columns extending into the medulla of the gland (Figure 1). On the other hand, the medulla of the gland contained all the cytoplasmic chromophore cells of the basophilic type, surrounded by large chromophore cells with acidic cytoplasmic pigmentation, with clear cell boundaries. Between the cell groups, blood sinusoids were found (Figure 2).

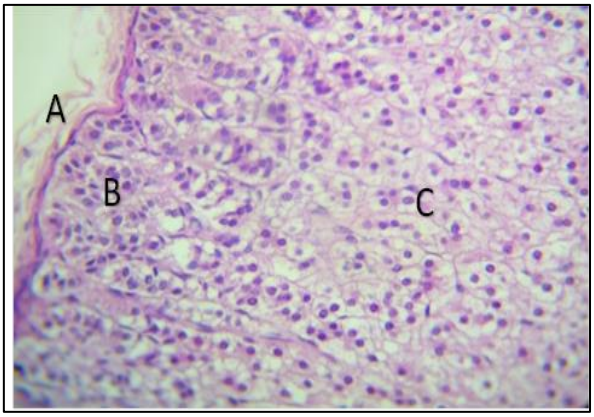

Fig (1): Adrenal cortex, fibrous capsule (A), zona glomerulosa cells (B), large, pale-staining fasciculate cells (C) (H&E 40X).

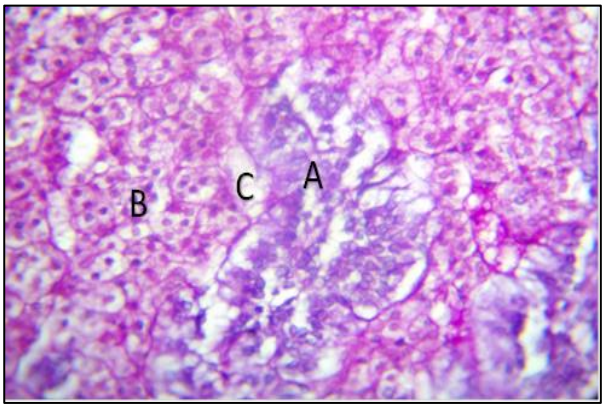

Fig (2): The medulla of the gland contains chromaffin cells with a basic cytoplasmic pigment (A), chromaffin cells with an acidic cytoplasmic pigment (B), and blood sinusoids (C) (PAS ×40).

Three months

The gland is surrounded by a connective tissue capsule composed of bile bundles. The gland contains clusters of glomerular cells beneath the capsule. Endocrine cells then divide into bundles or columns of pale pigment extending toward the medulla of the gland. The reticular zone is classified as a zone containing a number of endocrine cells in a reticular pattern adjacent to the medulla of the gland (Figure 3). The reticular zone deep within the gland's cortex is narrow and composed of a number of small endocrine cells adjacent to the gland's medulla, which contains chromophore-like endocrine cells and blood sinusoids between them (Figure 4). The medulla of the adrenal gland in rabbits contained chromaffin aggregates around the central vein with a wide lumen, thickened basement membrane, and between the chromaffin cells, large ductal sinusoids were found (Figure 5).

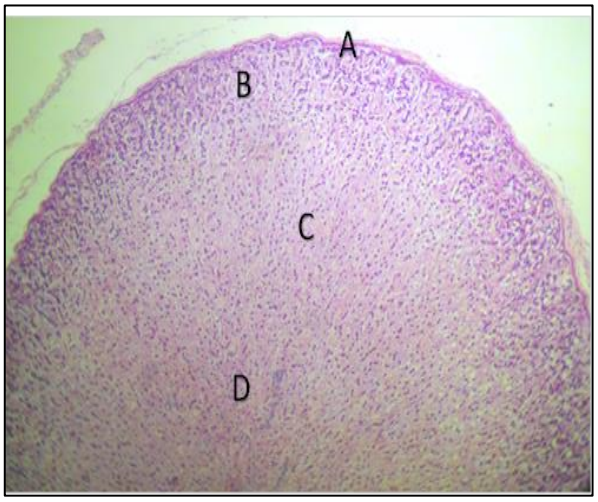

Fig (3) Adrenal gland in rabbits. Gland capsule (A), arcuate band (B), fascicle cell rows (B), reticular band cells (D) (H&E 10X).

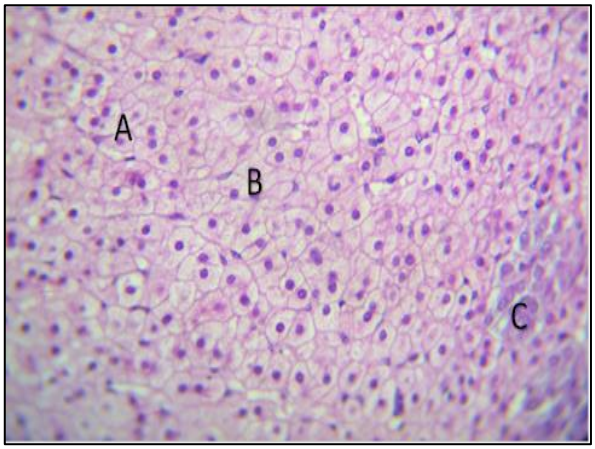

Fig (4): Large fascicular zona pellucida cells with pale cytoplasmic pigment (A) Small zona

reticularis cells (B) Phthalmochrome cells in the pulp (C) (H&E X40).

Fig (5): The medulla of the adrenal gland of a rabbit, including the medullary vein (A), thickening of the basement membrane (B), chromaffin cells (C), vascular channels of the sinusoids (D), (PAS X40).

Six months

The glandular capsule, composed of bundles of colloidal fibers, is surrounded by loose connective tissue containing small blood vessels and nerve fibers, in addition to the presence of clusters of endocrine cells in the form of an arc, forming the arc band. These cells are continuous with the columns of fascicle cells, composed of two rows of adjacent cells, columns in the form of bands, and the presence of nuclei closely packed together (Fig: 6). The reticular zone of the adrenal cortex contained small endocrine cell groups with acidic cytoplasm. Between these cells were found blood capillaries with branching channels towards the medulla, which is composed of groups of chromogenic medullary endocrine cells with basophilic cytoplasm (Fig. 7).

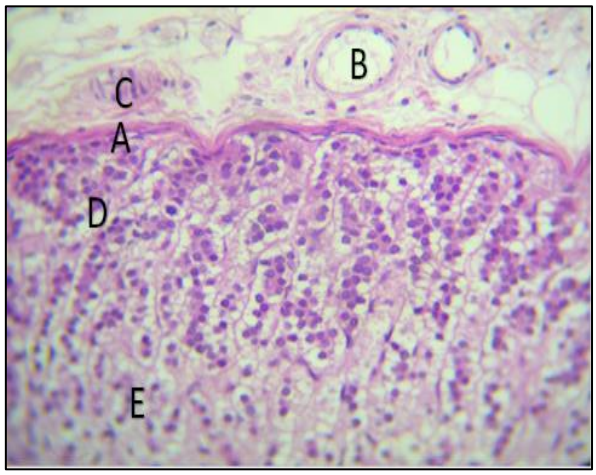

Fig (6): Glandular capsule (A) Blood vessels in the glandular fascia (B) Nerve bundle (C) Subcapsular arcuate band (D) Rows of fascicle cells (E) (H&E X40).

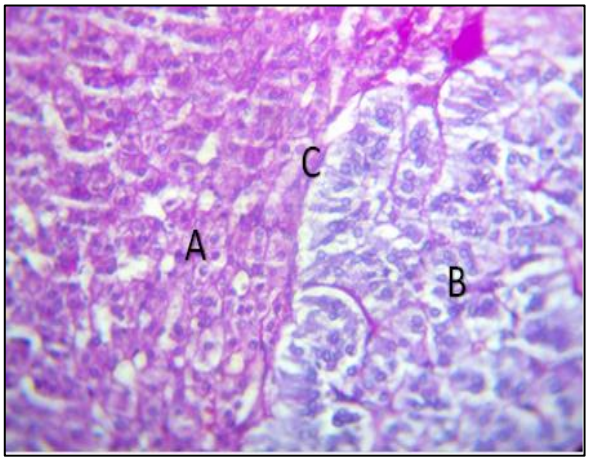

Fig (7): Reticular cells with acidic cytoplasm (A) Basophilic cells (B) Capillaries (C) (PAS X40).

One-year old

Cross sections of the adrenal glands of one-year-old rabbits, using Masson's stain, show that the capsule of the gland in rabbits appears thick, containing bundles of loose colloidal fibers. The subcapsular endocrine cells have cytoplasm, and these cells are found closely packed together. Some cells have brown cytoplasm. The fasciculate cells are enlarged, with cytoplasm free of pigment and small, dark, spherical nuclei (Figure 8).

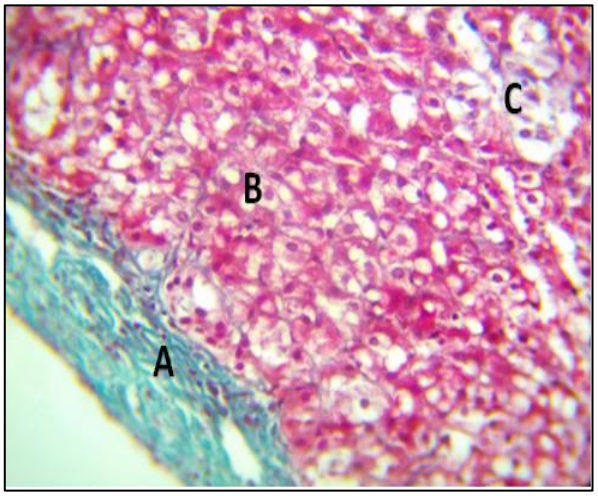

Figure (8): Transverse section of the gland of a one-year-old rabbit showing the gland capsule composed of disjointed colloidal fiber bundles (A), subcapsular endocrine cells with peripheral cytoplasm (B), and enlarged fasciculo-zonular cells (C), (MTC X40).

Discussion

There are two, three, or four different regions of epithelial cells that make up the adrenal cortex [13, 14]. In ruminants, primates, and some rodents, the outermost is known as zonae glomerulosa [15]. The parenchyma develops archades right below the capsule in the majority of other domestic animals, such as dogs, cats, horses, and pigs. Similar results were previously observed in rabbits [16] and Rattus norvegicus [17]. The expanse of the other cortical zones beneath it experienced an increase in thickness. These findings aligned with earlier research on the adrenal glands of rats [18], goats [19], and rabbits [16]. The capsule progressively split into two layers: inner cellular and outside (fibrous). Both the goat's [19] and rabbit's [16] adrenals showed these findings. Little polyhedral cells grouped together to form arches beneath the capsule in the Zona glomerulosa of both ages. Prior research by [20] in the adrenal of male albino rats and [16] in the adrenal of rabbits also noted and recorded this histological outcome. With its development, Zona fasciculate demonstrated a significant aspect. It was longer and wider than the other two brain zones, taking up a significant portion of the cortex. The lower zona reticularis and the upper zona glomerulosa were separated by it. In the zona reticularis, zona fasciculate is also present in the one-day undifferentiated zone before differentiating at the 30-day mark. Because of the vacuoles in their cytoplasm, the cells in this zone had a frothy appearance. Through the histological process of slide preparation, lipid droplets were dissolved with xylene, resulting in the presence of these vacuoles. The cells have spherical or oval, dark-stained basophilic nuclei that are grouped in low columnar or cuboidal cords. According to [21], [20] reported these histological findings in the albino rat's adrenal gland, in mice [22], and in the rat and porcupine [23], respectively. The innermost zone of the adrenal cortex, the zona reticularis, has morphological alterations comparable to those that occur in the zona fasciculate during a one-day undifferentiated zone. A sizable central nucleus was present in the network-like zona reticularis cells. This finding was also noted by [20] in the albino rat's adrenal and [16] in the rabbit's adrenal reticularis. This finding is consistent with those of [18] in the albino rat and [24] in the rat. The Medulla It is made up of medullary cells known as chromaffin cells and resides in the center of the adrenal gland. Its distinctive property, which was also noted by [23] in the porcupine, was the existence of the central vein; numerous other researchers, including [11], have noted this histological feature. Both kinds of chromaffin cells are found in the adrenal medulla, according to earlier research. Additionally, cortical cells and chromaffin cells were present at the cortico-medullary boundary [9], which is consistent with the findings of the present investigation.

Conclusions

Based on the results of the current study, it was found that the adrenal gland in rabbits consists of three regions arranged in the form of a glomerular zone, a molecular zone, and a reticular zone surrounded externally by a fibrous capsule. At one year of age in rabbits, part of the glomerular zone transforms into the molecular zone. Chromocytes composed of preadipocytes were found in the middle of the gland. Nerve bundles outside the gland, consisting of connective tissue, were found between the gland and the kidney.

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Declaration of interests

The authors declare that there are no conflicts of interest.

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Publication consent

All authors have read and approved the final version of the manuscript and consent to its publication

Data and material availability

All data analyzed and generated in this study are included in this published research.

Author contribution

All authors participated in the study design and conception. Data analysis, data collection, performance of the results, and assent to the final version.

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دراسة نسيجية لتطور الغدة الكظرية عند الأرنب المحلي من عمر شهر إلى سنة

ديانا محمد رجب [1], اياد حميد ابراهيم [2]

2,1 فرع التشريح والأنسجة، كلية الطب البيطري، جامعة تكريت، العراق

الملخص

الخلفية والهدف :تتكون الغدة الكظرية جنينيًا من نسيجين، يحتوي كل منهما على نوعين متميزين من جزيئات الإشارة. يتمثل الأول في القشرة الخارجية المنتجة للهرمونات الستيرويدية ذات الأصل الميزنشيمي، بينما يتمثل الثاني في اللب ذي الأصل الأديمي العصبي، المسؤول عن إفراز الكاتيكولامينات. لذلك هدفت الدراسة الحالية إلى تتبع التطور النسيجي للغدة الكظرية في الأرانب المحلية ابتداءً من عمر شهر واحد بعد الولادة وحتى عمر سنة واحدة.

المواد وطرائق العمل :تم تتبع تطور الغدة الكظرية في الأرانب المحلية من عمر شهر واحد بعد الولادة وحتى عمر سنة واحدة باستخدام الدراسات النسيجية.

النتائج :أظهرت النتائج أنه بعد شهر واحد تكون الغدة الكظرية محاطة بمحفظة ليفية مكونة من ألياف مرنة صفراء. تتكون المنطقة الكبيبية من تجمعات كروية للخلايا الظهارية، يضم كل تجمع منها (6–8) خلايا ذات أنوية كروية. وتحت هذه المنطقة توجد خلايا أكبر حجمًا ذات سيتوبلازم شاحب وأنوية كروية أصغر، مرتبة لتكوّن المنطقة الحزمية. أما لب الغدة فقد احتوى على جميع الخلايا الكرومافينية ذات السيتوبلازم القاعدي، والتي كانت محاطة بخلايا كرومافينية كبيرة ذات سيتوبلازم حمضي، مع حدود خلوية واضحة.

بعد عمر ثلاثة أشهر، انتظمت الخلايا الصماء في حزم أو أعمدة ذات صبغة شاحبة امتدت باتجاه لب الغدة. كما تميزت المنطقة الشبكية بوجود عدد من الخلايا الصماء المرتبة بنمط شبكي والمجاورة للب الغدة. واحتوى لب الغدة الكظرية في الأرانب على تجمعات من الخلايا الكرومافينية حول الوريد المركزي ذي التجويف الواسع.

بعد عمر ستة أشهر، إضافةً إلى ذلك، ظهرت تجمعات من الخلايا الصماء على هيئة أقواس مكوّنةً المنطقة الكبيبية، وكانت هذه الخلايا متصلة عند قواعدها بأعمدة خلايا المنطقة الحزمية، والتي تألفت من صفين من الخلايا المتجاورة مرتبة في هيئة أعمدة أو حزم، مع تقارب واضح بين الأنوية.

الاستنتاجات :تم تتبع التطور النسيجي للغدة الكظرية في الأرانب المحلية منذ عمر شهر واحد بعد الولادة وحتى عمر سنة واحدة، وأظهرت النتائج تطورًا تدريجيًا في التنظيم البنيوي لكلٍّ من القشرة واللب.

الكلمات المفتاحية: تركيب الكظرية، الأنسجة الجنينية، المنطقة الكبيبية، الهرمونات الستيرويدية.