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Histochemical analysis of wild rabbits' ovaries, oviducts, and folliculogenesis

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Article info

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The reproductive system of wild rabbits is highly specific to support their prolific breeding capacity. The histological characteristics of the ovaries and oviducts in wild rabbits, focusing on follicular development, oviductal structure, and functional adaptations are reviewed in this article. Eight wild rabbits were studied in this investigation, using the routine H&E stain and Masson's trichrome staining for detected collagen fibers. The current research studied the histological features in wild rabbits and found that the animals had lobulated ovaries lined by tall cuboidal germinal layer, followed by thin layer of tunica albuginea. The ovaries exhibit distinct cortical and medullary regions with various stages of follicular growth. The primordial follicles are present and located near the tunica albuginea. In addition, the mature follicles are surrounded by a few fibers (theca interna). Primordial follicles, which are composed of a single layer of flattened granulosa cells surrounding an oocyte, appear to be abundant in the present research. The primary follicles also comprise a single layer of cuboidal granulosa cells enveloping the oocyte, whereas the secondary follicles have a variety of granulosa cells forming, and a theca layer that differentiates into theca externa (fibrous) and theca interna. The stroma of the ovaries has a large amount of collagen fibers and their increase in the cortical region with the decrease in medulla. The oviduct is divided into infundibulum, ampulla, and isthmus, each with unique epithelial and glandular features. The histological structure of the oviduct includes mucosa with ciliated and secretory cells aiding ovum transport and nourishment. Beneath lies the lamina propria for support and immunity. Muscularis mucosae enables movement via contractions. The outer serosa, part of the peritoneum, provides structural support with simple squamous epithelium. Hormonal regulation and seasonal variations also influence these structures. Understanding these histological features provides insights into the reproductive potential of wild rabbits.

Keywords: histochemical, wild rabbits, ovary.

Гістохімічний аналіз яєчників, яйцепроводів та фолікулогенезу у диких кролів

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Репродуктивна система диких кролів є достатньо специфічною за будовою, вона пристосована до багатоплідності й інтенсивного розмноження. У наведеному дослідженні розглядається гістологічна характеристика яєчників та яйцепроводів у диких кролів, а також особливості розвитку фолікулів, структури яйцепроводів та їх функціональних адаптацій. Встановлено, що дикі кролі мають часточкові яєчники, вистелені високим кубічним зародковим шаром, за яким розташований тонкий шар білої оболонки (*Tunica albuginea*). Яєчник має чітко виражені кортикальну та мозкову зони з фолікулами, що знаходяться на різних стадіях розвитку. Примордіальні фолікули (0 стадія) розташовані поблизу білої оболонки (*Tunica albuginea*). Встановлено, що зрілі фолікули оточені кількома шарами волокон (*Theca interna*). За кількісним показником примордіальні фолікули виявлені у значній кількості. За своєю морфологічною будовою вони складаються з одного шару сплющених гранульозних клітин, що оточують ооцит. Первинні фолікули, також, складаються з одного шару кубічних гранульозних клітин, які огортають ооцит, тоді як вторинні фолікули мають гранульозні клітини на різних стадіях їх формування, та шар, який складається із зовнішньої (фіброзної) та внутрішньої оболонки. Строма яєчників має велику кількість колагенових волокон і їх кількість збільшується в кірковій зоні, і навпаки – зменшується в ділянці мозкової речовини. Яйцепровід поділяється на воронку (розширена частина, що відкривається біля яєчника), ампулу (середня, найширша частина) та перешийок (прилягає до рога матки), кожен з яких має унікальні епітеліальні та залозисті клітини. Гістологічна структура яйцепроводу включає слизову оболонку з війчастими та секреторними клітинами (відповідно виконують транспортну функцію та живлення й капацитації). Під нею знаходиться власна пластинка, що має провідну роль у підтримці імунітету. М'язова оболонка (*Tunica muscularis*) забезпечує рух за допомогою скорочень. Розуміння цих гістологічних особливостей дає уявлення про репродуктивний потенціал диких кролів.

Ключові слова: гістохімія, дикі кролики, яєчник.

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Introduction

Wild animals serve as important subjects for histological studies due to their diverse physical features and physical adaptation, providing significant insight into evolutionary biology, disease pathology and protection science [1]. Histological examination of wild animal tissue helps researchers understand the responses to species-specific cellular structures, organ functions and environmental stresses, offering a comparative perspective that is often absent in the study of pets or laboratory animals [2].

Increasing hazards for wildlife, including habitat destruction, climate change, and emerging zoonotic diseases, the importance of histological research of wild population health and disease supervision is outlined [3]. For example, histological analysis plays an important role in identifying neoplastic conditions in infectious diseases, toxic effects, and endangered species, assisting protection strategies [4]. Additionally, wild animals serve as an important model to study tissue regeneration, immune responses and evolutionary adaptations, with biomedical applications [5].

This article deals with the importance of wild animals in histological research, which emphasizes veterinary pathology, ecological health assessment and their contribution to comparative therapy. By integrating histological findings with ecological and physical data, researchers can increase wildlife management and disease prevention efforts. Customizing different mammals to live in diverse settings may include different combinations of cellular, physical, practical and ecological characteristics [6, 7].

The ovaries are an important reproductive organ in the female vertebrae, responsible for the production of gametogenesis (ozensis) and steroid hormone. In wild animals, histological studies of the ovaries provide the insight under the influence of environmental factors on breeding biology, seasonal reproductive patterns and fertility. This article reviews the histological structure of the ovaries in wild animals, its diversity in species and its importance in wildlife conservation and reproductive studies [8, 9].

Rabbits are considered as small mammals that were included in the family of Leporida, which inhabit many parts of the world. A decade ago, rabbits were considered good experimental animal model in research of various morphological anomalies and diseases in both humans and animals. They have been used as an experimental model in inducing many diseases and subsequent studies have focused on many aspects such as toxic science, pharmacology and surgery in various universities [10]. Accordingly, the subtle structure of different organs of this animal requires the study of such organs as the ovaries of the reproductive system. Rabbits belong to certain species, in which ovulation is induced by intercourse, resulting in a separate pregnancy and fetal age a few hours or days after. They have a small breeding cycle and pregnancy lasts for about 31 days, in which female sexual maturity occurs at about 4 to 5 months of age, based on strains or breed, which occurs about 10 to 13 hours after intercourse with ovulation [11, 12].

The aim of the study

The purpose of this study was to highlight the main differences of histological changes in wild animals, conduct histological studies of the ovaries to increase our understanding of reproductive physiology, adaptation and conservation challenges. Further research is required to detect species'specifics.

Materials and methods

Eight female wild rabbits were obtained from different environments and placed into the animal house in a typical condition. Under the supervision of a certified veterinary doctor, the rabbits were given a typical diet consisting of bread and tap water. The proper lighting and dietary requirements were followed in order to create a pleasant and hygienic working environment.

All experimental animals were administered intramuscular injections of ketamine and xylazine solution (dosage of 80–100 mg/kg body weight of ketamine, and between 10–12.5 mg/kg of xylazine) and weighed alive on a digital balance. This was followed by their death at scheduled [13] intervals. The abdominal cavity was then opened and bowel displaced to gain access to the ovary for evaluation of the morphology. Two dimensional digital photographs were taken from the kidneys [14]. From there, the ovaries were immersed in a 10 % formalin solution for about 48 hours, after which the ovaries were sliced into small pieces, kept in 70 % ethyl alcohol till the end and then dehydrated in alcohol series, cleaned up with xylene and finally were embedded in paraffin wax as usual. Sections (7–25 µm thick) were put on clean glass slides [15].

Routine histological scoring was performed with hematoxylin staining as described. Collagen filaments were stained by Masson's trichrome dye [16] to locate collagen fiber 3d distributions in connective tissues.

Results and discussion

Histochemical investigations

The present study demonstrates that the histological characteristics of the left and right ovaries are comparable. They were surrounded by the simple cuboidal layer of cells known as the germinal epithelium. Below the germinal epithelium was a thin layer called the tunica albuginea, which was home to dense irregular collagenous connective tissue (DICT) fibers. This result has been consistent with earlier histological research that characterizes the germinal epithelium as a protective mesothelial layer that is not directly involved in oogenesis. Moreover, the tunica albuginea has been often described as a layer of connective tissue rich in collagen that give the ovarian cortex structural support. Despite the lateralization, the two ovaries' identical histological characteristics point to a homogeneity in functional morphology [17].

In the current study it was discovered that the ovaries of wild rabbits were lobulated organs made of e-cortex, which had been packed with corpora lutea, atretic follicles, and primordial, primary, secondary, and tertiary (Graafian) follicles.

There are several phases of follicles that are present in the cortical area next to the tunica albuginea. Meanwhile, the medulla was composed of stromal densely packed irregular collagenous connective tissue that reached the inside of the tissue (***Fig. 1*** and ***Fig. 2***).

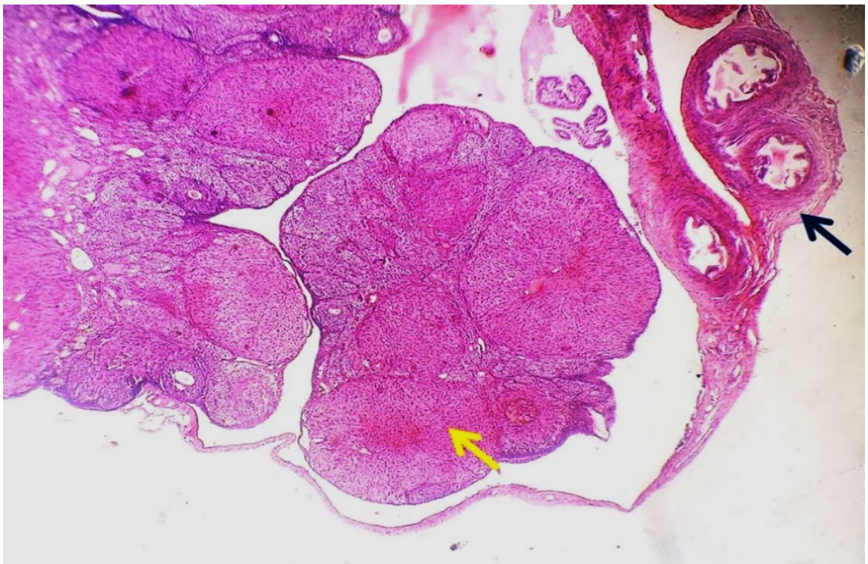

Fig. 1. Photography of lobulated ovary in wild rabbits (the yellow arrow) and oviduct connected with the ovary by isthmus (the black arrow). H&E stain.40×

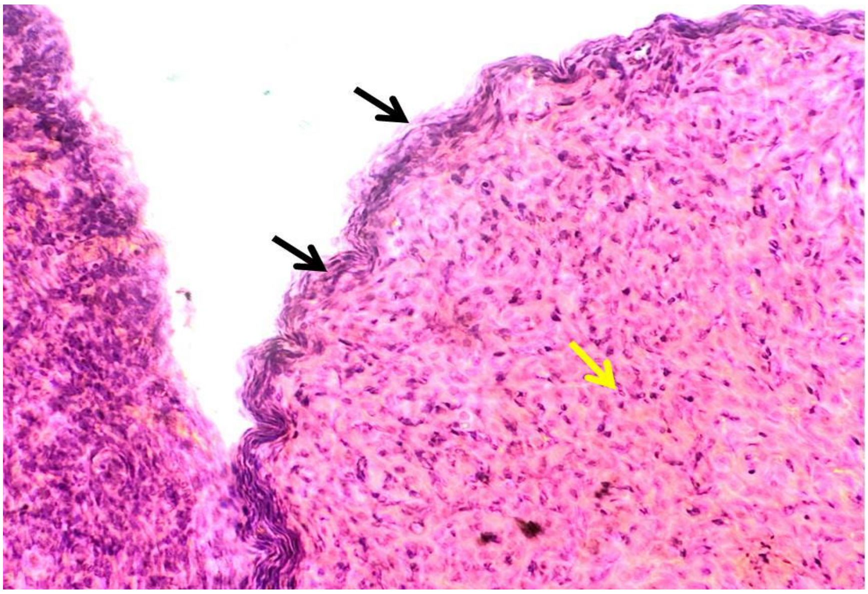

Fig. 2. Photography of the ovary in wild rabbits shows a thin layer of tunica albugenea (the yellow arrow) and the cortex contain highly fibrocytes (black arrow). H&E stain.40×

In general, the detection of atretic follicles, corpus luteum, and many follicular phases suggests active reproductive cycles in wild rabbits, which differ from many other mammals by their short estrous cycles and high reproductive potential. Previous observations on ovarian morphology in lagomorphs and other polytocous species are supported by the structural organization observed in this work [18].

It seems that there are numerous primordial follicles in this study, which are made up of a single layer of flattened granulosa cells encircling an oocyte. The primary follicles also consist of a single layer of cuboidal granulosa cells enveloping the oocyte, whereas the secondary follicles had a variety of granulosa forming cells, and a theca layer that differentiates into theca externa (fibrous) and theca interna. This result is consistent with [19, 20].

The stimulation of latent follicles is indicated by this change from flattened to cuboidal granulosa cells. This process is fueled by endocrine cues like follicle-stimulating hormone and local growth factors. The development of the theca layer, which differentiates into the theca externa and the theca interna, and several layers of granulosa cells were seen as the follicles reached the secondary stage (***Fig. 3***).

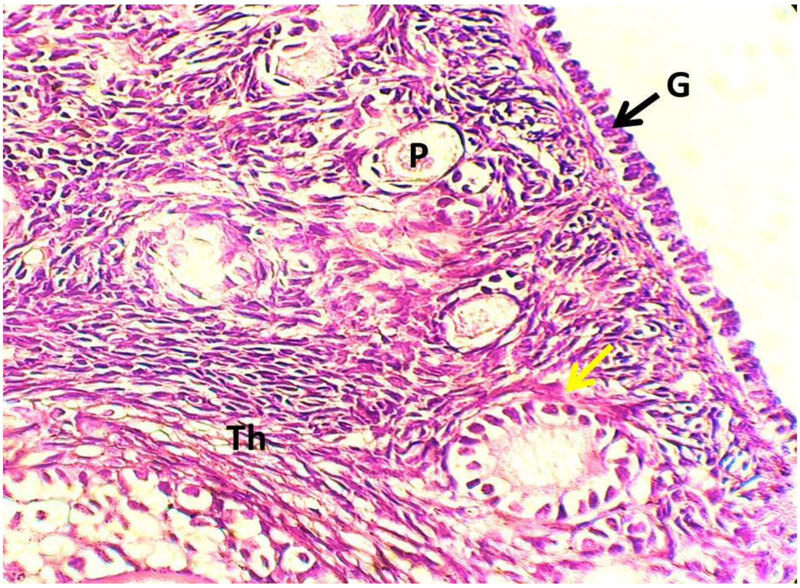

Fig. 3. Photography of the ovary in wild rabbits shows the germinal layers of high cuboidal epithelial cells (G black arrow), with a large primordial (P), primary, secondary (the yellow arrow), and tertiary (Graafian) follicles surrounded by a thin layer of theca interna (Th). H&E stain. 100×

These findings highlight the conserved nature of the ovaries' structure and function across species by indicating that wild rabbits' ovaries not only have a clearly defined hierarchy of follicular phases but also have the traditional histological features of folliculogenesis observed in other animals. A major player in follicular steroidogenesis, the theca interna is well-known for its vascularization and endocrine function, especially the synthesis of androgen. The growing follicle receives structural support from the theca externa, which is mainly made up of fibrous connective tissue. While theca externa had many fibers surrounding the follicles, theca interna had only a small amount. The mature follicles were detected in the middle region of the cortex and were encircled by thin layers of fibers (***Fig. 4***).

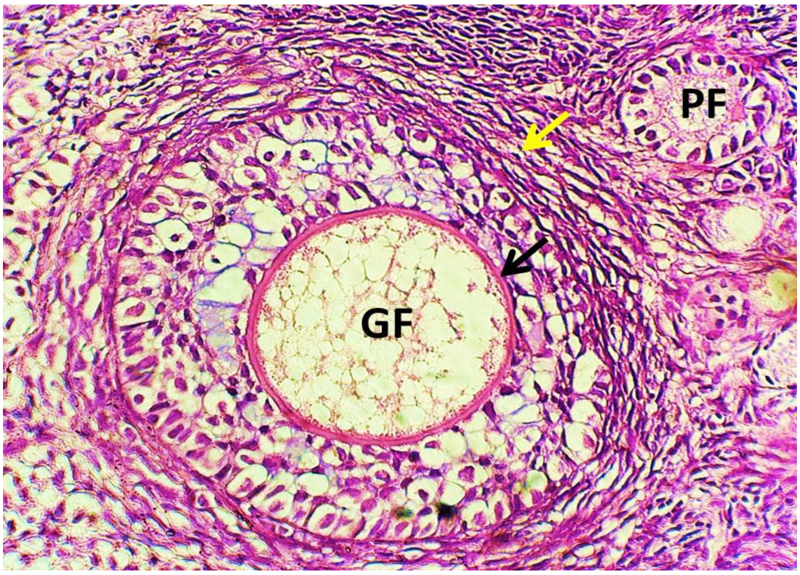

Fig. 4. The photography of the ovary in wild rabbits shows the Graafian follicle surrounded by a thin layer of theca interna (the yellow arrow), include the oocyte surrounded by thick zona pellucida (the black arrow). H&E stain. 400×

This is in line with earlier research in mammalian ovaries, where the theca interna is mainly made up of endocrine cells with a little amount of fibrous material. These cells are specialized in steroidogenesis, specifically the generation of androgens [21, 22].

On the other hand, it was found that the theca externa had a more noticeable connective tissue layer due to a greater distribution of collagenous fibers. Fibroblasts and smooth muscle cells are found in this outer layer, which primarily serves as a supporting structure, aid in follicular stability and may even aid in ovulatory contraction [23, 24].

Ovarian Medulla

According to histology, the ovary's medulla in wild rabbits is a highly vascularized area with mostly loose connective tissue. This area serves as a wide supporting bed for cortical structures and is made up of vascular, lymphatic, and innervation-per webbing. The connective tissue stromal are regular interspersed elastic fibers and a large number of collagen fibers, together with a few fibroblasts. Some specimens exhibit clusters of interstitial endocrine cells, especially those that are close to blood arteries, indicating a remote thecal or luteal source.

The results lend credence to the idea that, in wild rabbits and mammals in general, the ovarian medulla functions as an active, multifunctional compartment that is essential for the ovaries' endocrine and circulatory infrastructure, rather than just as a passive structural core [25, 26].

The medulla is clearly different from the cortex in that it lacks follicular features. The basic shape is similar to the functional architecture that supports the ovaries' hormonally active cortex through neural networks and circulatory supply. Vascular heterogeneity or differences in stromal cellularity may be identified based on the age or reproductive status of the rabbit (***Fig. 5***).

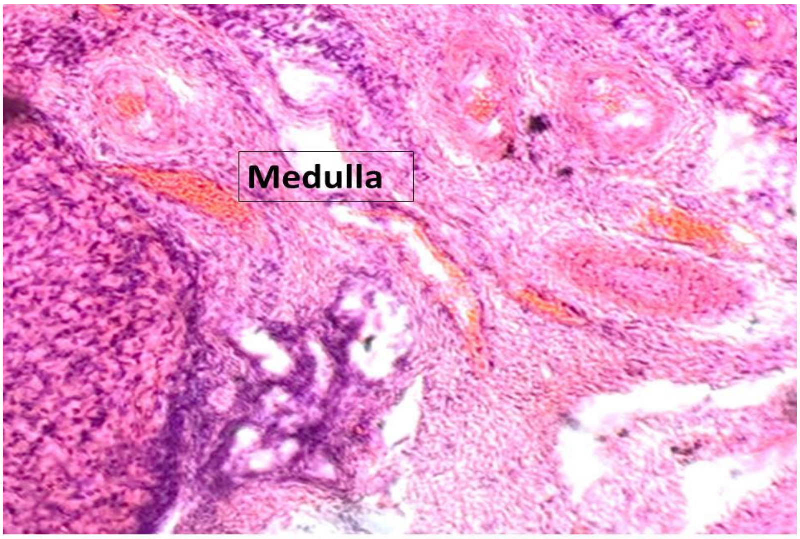

Fig. 5. The photography of the ovary in wild rabbits shows that the medulla appeared highly vascularized.
H&E stain.100×

These results highlight the medulla's extremely useful but non-gametogenic function in ovarian physiology. Its form is designed to support the hormonally and metabolically active cortex rather than folliculogenesis, offering a neurovascular interface that adapts to the ovaries' changing physiological needs over time [27].

Histological Structure of the Oviduct

The histological oviducts, commonly referred to as the fallopian tubes, are made up of several layers, each of which has unique characteristics for aiding in the transportation of ovum and early embryos and additionally for providing nourishment. The mucosa, which is the layer with the greatest depth, is extensively folded and covered with tall, simple columnar epithelium, which contains two main cell types: ciliated and secretory cells. These secretory cells can provide trophic material for sperm, ova, and early embryos, and these functioning ciliated cells can sweep the ovum into the uterus with their synchronized beating (***Fig. 6***).

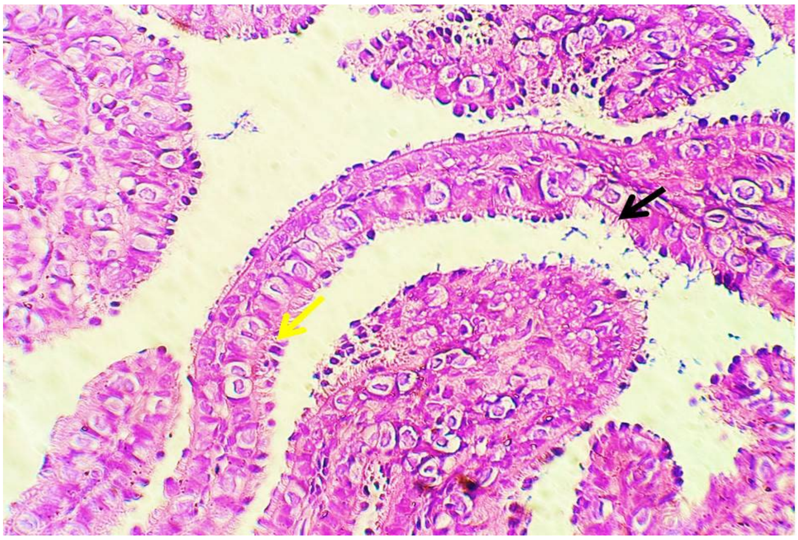

Fig. 6. The photography of the oviduct in wild rabbits shows infundibulum lined by tall columnar ciliated epithelia (the black arrow) with (peg) cells (the yellow arrow).
H&E stain. 400×

These results highlight the oviduct epithelium's specificity in functioning across several segments, illustrating its simultaneous roles in gamete and embryonic metabolic support and mechanical transport. A well-balanced adaptation to the reproductive requirements of mammals is the existence of both secretory and ciliated cells [28].

The lamina propria is a connective tissue layer that lies directly beneath the epithelium. It contains immune cells and blood vessels and maintains the mucosal epithelium while also participating in the genital tract's mucosal immunity. A layer of smooth muscle called the muscularis mucosae is between the mucosa and the serosa. Sperm and ova are propelled in motion by the peristaltic and segmental muscular contractions of these muscle layers. The serosa, which is basically a thin layer of mesenchyme with simple squamous epithelium (mesothel), is the outermost coat. It provides the organ with structural support and is a part of the peritoneum. In order to maximize reproductive success, the arrangement of these layers specifically, the synchronization of the contractions of the muscularis mucosae and the ciliation of the epithelium represents an evolutionarily conserved adaptation that guarantees the appropriate timing and direction of gamete and embryo movement [25].

Conclusions

The ovaries of the female reproductive system of wild rabbits were the subject of the current study, which clearly identified the characteristics of these animals. It was found that the ovaries of this species of animals were lobulated, had unique features of the cortex and medulla; the ovaries had a large number of primary follicles with a small number of fibers in the theca interna; the ovarian stroma contains a large number of collagen fibers and increases in the cortical region with a decrease in the medulla.

Conflict of interest

The author (s) state that there is no conflict of interest.

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