STM JOURNALS

International Journal of
Antibiotics

ISSN: 2584-2986
Volume 3, Issue 1, 2026
January–June
DOI (Journal): 10.37591/IJAB

https://journals.stmjournals.com/ijab

Review IJAB

Histological Effects of the Antibiotic Gentamicin on the Ovarian Tissue of Female White Laboratory Rats

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Abstract

This study aimed to evaluate the histological alterations induced by Gentamicin in the ovarian tissue of white laboratory rats. A total of 12 adult female rats were used and randomly divided into two groups, with each group consisting of six animals. The experimental group received Gentamicin at a dose of 120 mg/kg body weight administered an injection once daily, while the control group received an equivalent volume of normal saline under the same conditions. After the treatment period, all animals were euthanized, and ovaries were dissected for histological examination. Histopathological analysis revealed significant ovarian damage in the Gentamicin-treated group, including cytoplasmic vacuolization, nuclear pyknosis, disorganization of granulosa and theca cells, and oocyte shrinkage. Additional findings included vascular congestion, stromal edema, and infiltration of inflammatory cells, indicating both direct cytotoxic effects on follicular cells and indirect effects on stromal microcirculation. These alterations collectively suggest oxidative stess-mediated follicular degeneration, impaired oocyte maturation, and reduced fertility.

Keywords: Gentamicin, antibiotic, ovarian histology, follicular degeneration, white rats, animal, granulosa

INTRODUCTION

The ovary is one of the primary reproductive organs of the female's reproductive system. It is in the pelvic cavity on either side of the uterus, connected to it by the ovarian ligament on one side and to the fallopian tube on the other. The ovary has an oval and flattened shape, measuring approximately 3–5 cm in length, 1.5–3 cm in width, and about 1 cm in thickness [1]. Its size varies according to the woman's age and hormonal status. The ovary is covered by a superficial layer known as the germinal epithelium, composed of simple cuboidal cells, followed by a fibrous capsule called the tunica albuginea [2].

The ovary is divided into two main regions: the cortex, which is the outer area containing ovarian follicles at various developmental stages, and the medulla, which is the inner region composed of blood vessels, nerves, and loose connective tissue [3].

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Received Date: November 05, 2025
Accepted Date: November 12, 2025
Published Date: January 07, 2026

Citation: Saja A. Mazher, Atyaf S. Hussein, Zahraa H. Abdulateef. Histological Effects of the Antibiotic Gentamicin on the Ovarian Tissue of Female White Laboratory Rats. International Journal of Antibiotics. 2026; 3(1): 1–8p.

The ovary performs two essential functions: a reproductive function, which involves the production of oocytes through a process known as oogenesis [2–5]. This process begins during the embryonic stage and continues after puberty throughout the menstrual cycle. The second is a hormonal function, as the ovary secretes several important steroid hormones, including estrogen, which is responsible for the development of secondary sexual characteristics in females, such as breast growth and endometrial proliferation [4]. Progesterone, mainly secreted by the corpus luteum after ovulation, plays a vital role in preparing the endometrium for implantation of the fertilized ovum and maintaining early pregnancy [26].

Additionally, the ovary produces small amounts of androgens and inhibin, which help regulate the secretion of pituitary hormones [5].

From a physiological perspective, the ovary functions in precise coordination with the pituitary gland and the hypothalamus through a regulatory pathway known as the hypothalamic–pituitary–ovarian [HPO] axis [6]. The secretion of gonadotropin-releasing hormone [GnRH] from the hypothalamus stimulates the anterior pituitary gland to release follicle-stimulating hormone [FSH] and luteinizing hormone [LH], which in turn regulate follicular growth, ovulation, and corpus luteum formation. This reciprocal hormonal regulation ensures a balanced interaction between oocyte production and the secretion of female sex hormones [7].

Antibiotics are considered among the most significant medical discoveries of the twentieth century, as they have played a crucial role in reducing mortality rates caused by bacterial infections. Antibiotics are defined as chemical substances produced by microorganisms such as bacteria or fungi, or synthesized artificially, and are used either to kill or inhibit the growth of pathogenic bacteria [8].

They are employed to treat a wide range of infections, including skin infections, pharyngitis, urinary tract infections, pelvic inflammatory disease [9]. Antibiotics can be classified in several ways. One common method is based on their mechanism of action, which includes: cell wall synthesis inhibitors, protein synthesis inhibitors, nucleic acid synthesis inhibitors and metabolic pathway inhibitors [10].

They may also be classified according to their antibacterial spectrum: broad-spectrum antibiotics, which are effective against a wide range of both Gram-positive and Gram-negative bacteria. Narrow-spectrum antibiotics, which are effective against specific types of bacteria [11].

Furthermore, adherence to the principles of rational antibiotic use is strongly recommended to limit the spread of resistance. This includes avoiding the prescription of antibiotics without accurate diagnosis and ensuring compliance with the appropriate dosage and treatment duration [12].

Gentamicin is a broad-spectrum antibiotic belonging to the aminoglycoside family and is among the most widely used agents for the treatment of severe bacterial infections [13]. It was first discovered in 1963 from the bacterium Micromonospora purpurea and is characterized by its strong activity against a wide range of aerobic Gram-negative bacteria, as well as certain Gram-positive species [14].

Gentamicin belongs to the group of aminoglycosides, which are composed of amino-modified sugars linked by glycosidic bonds to amino acid residues [15]. Pharmaceutical formulations of gentamicin typically consist of a mixture of closely related structural components – Gentamicin C1, C1a, and C2. The drug is commonly used in the form of gentamicin sulfate, which represents the standard formulation for both clinical and laboratory applications [16].

Gentamicin is often administered in combination with other antibiotics, such as penicillins or cephalosporins, to achieve a synergistic effect in the treatment of complex infections. It is used to manage a wide range of severe bacterial infections, including urinary tract infections UTIs], pelvic inflammatory disease [PID], septicemia, lower respiratory tract infections, intra-abdominal and biliary tract infections [17]. Additionally, gentamicin is applied topically in the form of ointment or eye drops for the treatment of skin and ocular infections caused by susceptible bacterial strains [18].

Despite its high therapeutic efficacy, gentamicin possesses potential toxic effects that necessitate careful use. These include nephrotoxicity, ototoxicity, and neurotoxicity, particularly when administered at high doses or for prolonged periods. Therefore, monitoring serum drug concentrations is strongly recommended, especially in patients with renal impairment, to ensure therapeutic effectiveness while minimizing toxicity risks [19].

MATERIALS AND METHODS

In this study, twelve adult white laboratory rats, one month of age and weighing approximately 0.55 kg, were used. The animals were housed in wooden cages lined with wood shavings, and the cages were cleaned regularly to maintain hygiene [20]. Six rats were placed in each cage. Appropriate environmental conditions were provided, including a temperature of 24 ± 4°C and a 12-hour light/12-hour dark cycle. Food and water were provided ad libitum throughout the duration of the experiment. The rats were allowed a two-week acclimatization period before the start of the study [21].

RESULTS

The results of histological sections of the ovary treatment with gentamicin revealed marked alterations in the ovarian tissue architecture. Evident degenerative changes were observed in the ovarian follicles at various developmental stages. The granulosa cells surrounding the follicular cavity exhibited a loss of structural organization, with the presence of cytoplasmic vacuoles resulting from cellular membrane damage. Additionally, edema and tissue disintegration were observed. The cells appeared shrunken with darkly stained nuclei, indicating degenerative cell death [necrosis or apoptosis], as illustrated in Figure 1.

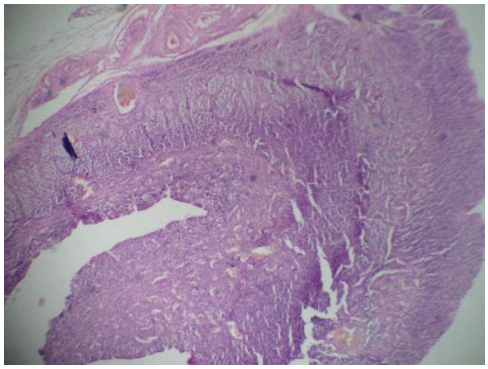

Figure 1. A transverse section of the ovary tissue shows an Evident degenerative changes were observed in the ovarian follicles at various developmental stages. Stain: Hematoxylin and Eosin [H&E]; Magnification: [10x].

The mature follicles exhibited a clear disruption of the basement membrane separating the granulosa and theca cell layers, accompanied by evident cytoplasmic degeneration within the follicular cells and the presence of intercellular vacuolation and cell dissociation. The granulosa cells surrounding the oocyte showed noticeable variation in size and staining intensity. In some follicles, the oocyte appeared irregular in shape, with a loss of clarity in the zona pellucida, indicating the onset of shrinkage or atrophy. These findings suggest a direct toxic effect of gentamicin on the oocyte, as shown in Figure 2.

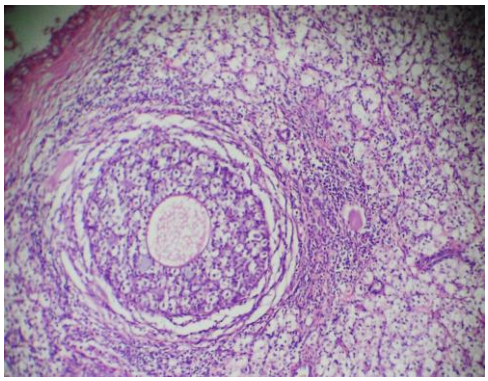

Figure 2. A transverse section of the mature follicles exhibited a clear disruption of the basement membrane separating the granulosa and theca cell layers. [Stain: Hematoxylin and Eosin [H&E]; Magnification: [10x].

Furthermore, prominent vacuolation was observed within the cytoplasm of the follicular cells and in the stromal tissue, accompanied by an infiltration of inflammatory cells, reflecting a toxic effect on mitochondria induced by oxidative stress and the intracellular accumulation of gentamicin. In addition, marked congestion of the microvasculature was evident. This histopathological image indicates a reduction in the metabolic activity of the ovary and a decline in follicular function, as illustrated in Figure 3.

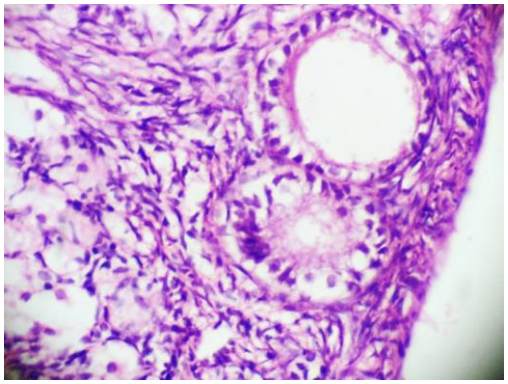

Figure 3. A transverse section of follicular cells shows prominent vacuolation within the cytoplasm and in the stromal tissue and in the stromal tissue. [Stain: Hematoxylin and Eosin [H&E]; Magnification: [10x].

The transverse histological sections of the ovary revealed evident degenerative changes, characterized by nuclear disintegration and separation, along with the formation of cytoplasmic vacuoles within the follicular cells, the central oocyte appeared shrunken, with a loss of internal cytoplasmic cohesion, indicating functional impairment. Additionally, there was marked vascular congestion in the surrounding ovarian tissue, suggesting an inflammatory reaction or tissue stress response. The stromal alterations included edema, fibrous separation, and a reduction in connective tissue density, all of which indicate an indirect toxic effect of gentamicin on the ovarian structure, as demonstrated in Figure 4.

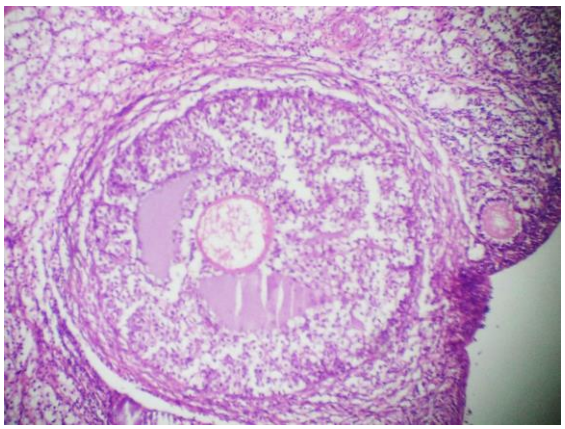

Figure 4. A transverse section of the ovary revealed evident degenerative changes, characterized by nuclear disintegration and separation, [Stain: Hematoxylin and Eosin [H&E]; Magnification: [10x].

DISCUSSION

The histopathological findings of ovarian sections in this study revealed that treatment with Gentamicin at a dose of 120 mg/kg induced marked structural alterations in follicles at various developmental stages, including disorganization of granulosa cells, cytoplasmic vacuolations, and darkly stained nuclei-features indicative of cellular degeneration caused by oxidative stress. Gentamicin promotes excessive production of reactive oxygen species [ROS], leading to lipid peroxidation,

mitochondrial damage, and disruption of cellular function. These results correlate with the data reported (Figure 1) [22, 23].

The presence of edema and tissue disintegration further reflects an inflammatory response that disturbs the ovarian architecture and ovulatory efficiency and fertility [24].

In addition, the ovarian cortex exhibited significant alterations, including damage to the structure of mature follicles, characterized by disruption of the basement membrane separating the granulosa and theca cells, along with cytoplasmic degeneration and intercellular vacuolations suggestive of impaired cellular communication and loss of structural integrity. These results are aligned with those reported in another research (Figure 2) [25, 26]. This disturbance represents an early marker of toxic effects on granulosa cells, which play a critical role in nourishing the oocyte and regulating its growth; therefore, their damage directly affects oocyte viability and function [27].

Furthermore, the variation in the size, shape, and staining intensity of granulosa cells indicates alterations in their mitotic and metabolic activity [28]. The irregular shape of the oocyte and the loss of clarity of the zona pellucida are considered indicative of oocyte shrinkage or the onset of atresia, suggesting a direct degenerative effect on the oocyte itself [29].

Histopathological analysis of the current study revealed extensive cellular degeneration in ovarian tissue following Gentamicin administration, characterized by prominent cytoplasmic vacuolations in both follicular and surrounding stromal cells, these results agree with previous research (Figure 3) [30, 31]. The presence of such vacuolations indicates mitochondrial degeneration and disruption of cellular homeostasis, suggesting the involvement of oxidative stress as the primary mechanism of gentamicin-induced cytotoxicity [32].

Furthermore, marked inflammatory cell infiltration and severe vascular congestion were observed within the ovarian stroma, signifying an acute inflammatory response [33]. This reaction, while initially protective, contributes to impaired tissue perfusion and exacerbates the degenerative changes within the follicles [34]. Therefore, these pathological alterations can be interpreted as the combined effects of oxidative cytotoxicity and vascular impairment, ultimately leading to decreased ovarian activity and reduced follicular performance [35].

Transverse histopathological sections of the ovarian tissue demonstrated distinct degenerative changes following Gentamicin exposure, including cytoplasmic vacuolization and nuclear degradation within the follicular cells, which correlates with the findings (Figure 4) [36–38]. Such features are indicative of follicular degeneration; a pathological condition frequently associated with metabolic imbalance and oxidative stress resulting from the accumulation of the aminoglycoside antibiotic within the cells [39].

The shrinkage of the central oocyte and loss of cytoplasmic cohesion further reflect damage to the plasma membrane and intracellular organelles, leading to impaired oocyte maturation and functional decline [40]. Additionally, the presence of blood-filled congested vessels and capillary dilation in the ovarian stroma suggests localized vascular congestion, a sign of tissue stress or inflammation caused by disrupted microcirculation and endothelial injury [41].

Moreover, stromal alterations – such as edema, fibrous separation, and decreased connective tissue density – indicate an indirect toxic effect of Gentamicin on the ovarian supportive matrix [42–48].

CONCLUSION

- Gentamicin induces marked cytotoxicity and degenerative changes in ovarian tissue.
- Observed alterations include cytoplasmic vacuolization, nuclear degradation, and oocyte shrinkage.

- The damage is mainly caused by oxidative stress and mitochondrial dysfunction.
- Vascular congestion, edema, and inflammatory infiltration indicate indirect toxic effects on the ovarian stroma.
- These structural changes lead to impaired follicular development and reduced ovarian function.
- Prolonged or high-dose exposure to Gentamicin may result in follicular atresia and decreased fertility potential.

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