
Gross morphometry, histology and stereological evaluation of the ovaries and oviducts of young chicken pullets given oral sulfaquinoxaline-diaveridine therapy for varied durations before onset of egg production

Mansur Zubairu^{1*}, Muhammad H. Sulaiman¹, Magdalene N. Ali¹, Waziri I. Musa², Tauheed A. Muazu³, Abdullahi Baso³, Sherif O. Ajeigbe⁴ and Ese Abiyere¹.

¹ Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

² Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

³ Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Bayero University, Kano, Nigeria.

⁴ Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Federal University of Agriculture, Zuru, Nigeria.

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Abstract

In this study, young chicken pullets were treated with sulfaquinoxaline-diaveridine (SD) prior to onset of egg production, and its effects on their ovaries and oviducts were investigated. A total of twenty 13-week-old pullets were used for the study. They were randomly assigned to four groups: an untreated control group (Group I) and three treatment groups (Groups II, III and IV) that received SD for varied durations: Group II – received SD for one week; Group III – received SD for two weeks; and Group IV – received SD for three weeks prior to expected onset of egg production. The SD was administered in drinking water (2.4g SD in 4 litres of water). Gross morphological examination revealed that pullets in the all groups possessed underdeveloped oviducts, which is expected at this pre-laying stage. However, birds in the SD-treated groups exhibited visible signs of vascular congestion and mild inflammation of the oviduct. Histological evaluation showed that the general tissue architecture of the oviduct remained intact across all pullet groups, with no marked structural alterations attributable to SD exposure. In contrast, the ovaries of treated birds, particularly in Groups III and IV exhibited varying degrees of follicular degeneration, including disrupted follicular organization and cellular breakdown. Stereological analysis demonstrated a significantly ($p < 0.05$) lower follicular volume in SD-treated groups, when compared to the untreated control, with the most pronounced effects recorded in birds subjected to prolonged exposure to SD. Overall, the results indicate that prolonged SD treatment exerted detrimental effects on ovarian follicles of chicken pullets treated just before point of lay. These findings raise concerns about the potential of SD use in pullets compromising reproductive capacity, and highlight the need for cautious use of SD in growing pullets, and also underscore the importance of further studies to elucidate the long-term reproductive consequences and underlying mechanisms of SD-induced ovarian damage.

Keywords: Sulfaquinoxaline-diaveridine; Chicken pullets; Ovaries; Oviduct; Histology; Stereology; Follicular disruption.

* **Correspondence:** Mansur Zubairu; Email: dr01mansur@gmail.com; Phone: +2347034980639

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Introduction

Poultry production stands as a cornerstone of the livestock sector in Nigeria, and it plays a vital role in both rural livelihoods and national food security (Anosike *et al.*, 2018). With an increasing population, and consequent increased demand for protein-rich foods, the poultry industry serves as a critical source of affordable animal protein for millions of Nigerians (Mottet and Tempio, 2017). Poultry farming in Nigeria covers a wide range of practices, including small-scale backyard set-ups and large commercial ventures (Mottet and Tempio, 2017). It involves several exotic and local poultry species including chickens, turkeys, ducks and guinea fowl as the main breeds (Anosike *et al.*, 2021; Gržinić *et al.*, 2023).

Layer chickens contribute significantly to egg production in Nigeria, and encompass a diverse array of operations that reflects the adaptability and scalability of their production (Ajala *et al.*, 2020). With a growing demand for eggs across urban and rural areas, the production of layer chickens has become a vital component of the agricultural landscape in Nigeria (Mottet and Tempio, 2017). Layer chicken production is faced with challenges such as disease outbreaks, feed availability and infrastructure limitations (Hafez and Youssef, 2020). Despite these challenges, the sector continues to evolve, and this is driven by advancements in breeding techniques, management practices and market dynamics (Castro *et al.*, 2023).

The effective management of coccidiosis in layer chickens involves implementation of preventive measures such as maintenance of clean and dry housing conditions, practicing strict biosecurity protocols, and the use of coccidiostats in feed or water to control the disease (Ahmad *et al.*, 2024). The treatment and prevention of coccidiosis in poultry often involves administering drugs such as sulfa-drugs, notably sulfaquinoxaline-diaveridine

combination, to mitigate the impact of the protozoan infection and promote the recovery of affected birds within the flock (Saleh *et al.*, 2024). Sulfaquinoxaline (4-amino-N-quinolin-1-yl-benzenesulfonamide)-diaveridine (C₁₃H₁₆N₄O₂) is an antibiotic combination commonly used in veterinary medicine to treat bacterial and protozoal infections in poultry and livestock (Cheong *et al.*, 2010).

The oviduct of domestic chickens is a highly specialized tubular structure that plays a critical role in the formation and transport of eggs (Khanam *et al.*, 2023). It is approximately 60 – 70 cm long and extends from the ovary to the cloaca (Urban-Chmiel *et al.*, 2022). The oviduct is divided into five distinct regions: the infundibulum, magnum, isthmus, uterus (or shell gland) and vagina (Hosotani *et al.*, 2023). Each segment has specific functions related to the development of the egg (Altaey *et al.*, 2023). The infundibulum is the funnel-shaped opening near the ovary, responsible for capturing the ovulated oocyte. Histologically, it is lined with ciliated and secretory columnar epithelium (Apperson *et al.*, 2017). The magnum is characterized by a thick mucosal layer with deep glandular invaginations (Hosotani *et al.*, 2023). These tubular glands secrete albumen proteins, which accumulate around the egg as it passes through. The epithelium here is primarily simple columnar, with a high density of secretory cells (Rizzi, 2021). The isthmus has a thinner mucosal lining compared to the magnum, with fewer and shorter glands that secrete the inner and outer shell membranes. The epithelium in the uterus is highly glandular and secretes calcium carbonate and proteins essential for shell formation (Gautron *et al.*, 2021). The muscularis in this region is particularly well-developed, facilitating the expulsion of the fully formed egg into the vagina, where it remains briefly before being laid. Each region's distinct histological features are essential for the complex process of egg formation and

laying in domestic chickens (Hosotani *et al.*, 2023).

Pharmaceutical products, such as antibiotics like sulfonamides, reportedly can alter the structure and function of the oviduct, a highly specialized organ in laying birds that is in charge of egg development (Sah and Mishra, 2018). Hens treated with sulfa medications have been reported to exhibit significant morphological abnormalities, such as reduced oviduct weight, luminal distension and oedema, as a result of drug-induced toxicity (Adil *et al.*, 2023). Drug-induced changes in the oviduct show up histologically as inflammatory infiltrations, decreased secretory activity and epithelial desquamation. According to research by Kiran and Kumar (2023), exposure to sulfonamide impairs albumen synthesis by causing ciliated epithelial cells in the infundibulum to degenerate and tubular gland density in the magnum to decrease.

There is paucity of information in available literature on alterations in the oviduct and ovaries of chicken pullets exposed to sulfaquinoxaline-diaveridine combination treatment prior to onset of egg lay. The present study exposed young pullets to oral sulfaquinoxaline-diaveridine treatment for varied treatment periods, and evaluated its effects on their ovaries and oviducts.

Materials and Methods

Location of the study: The research was conducted in the Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. Zaria is located in the Northern Guinea Savannah zone of Nigeria, an altitude of 686 m above sea level, and a mean ($\pm$ standard error) monthly photoperiod of 12.13 ± 0.13 h (Dzenda *et al.*, 2011; Kowal and Knabe, 1972). The zone is characterized by three seasons: rainy season (June-October), harmattan (November- February) and heat season

(March-May) (Ayo *et al.*, 1999; Dzenda *et al.*, 2011).

Animals used for the study: A total of 20 pullets, purchased at twelve weeks of age from a reputable company in Nigeria, were used for the study. The pullets were transported to the Experimental Animal Unit of the Department of Veterinary Anatomy, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. They were kept in deep litter system for a period of two weeks to acclimatize. Feed was given at 115 – 125 g of the feed per day and recalculated as weight of the birds increased, and water was provided *ad libitum*. Approval for the use of the pullet chickens for the study was sought and obtained from the Ahmadu Bello University Committee on Animal Welfare and Use, with Approval Number ABU/CAUC/2024/048.

Drug used for the study: Sulfaquinoxaline-diaveridine, marketed as Embazine Forte® (Turner Wright Ltd., Lagos, Nigeria) was used for the study. It was administered orally at the dose of 30g/50L of water, which is equivalent to 2.4g/4L of water daily as only source of water in the morning all through the duration of the study.

Experimental design: The 20 pullets used for the study were randomly grouped into four groups (I, II, III, and IV) of five each, as follows: Group I – Untreated control group; Group II – Pullets given SD for one week (during week 14 of age); Group III – Pullets given SD for two week at weeks 14 and 16, with a seven day period of no SD treatment; and Group IV – Pullets given SD for three weeks, at weeks 14, 16 and 18 of age, with two sets of seven day period of no SD treatment in between the three weeks of SD treatment.

Pullets in Groups II, III and IV were given their group specific SD treatments for the specified duration while the Group I (untreated control) was not given SD. After week 18, the pullets were euthanized, and the carcasses were subjected to a comprehensive gross

examination. Fat was trimmed off from the ovaries and oviducts before they were measured using vernier caliper MG6001DC (General Tools and Instruments Co., New York) and weighed using Mettler balance P1210 (Mettler Instrument AG, Switzerland), with a sensitivity of 0.001g. The two organs were harvested for histological studies.

Histological Studies: The harvested ovaries and oviducts were immersed in labeled bottles containing 10 % neutral buffered formalin for fixation. Post fixation, the tissues were dehydrated in series of graded ethanol solution (50 – 100 %) and embedded in paraffin wax (Bancroft and Gamble, 2008). Paraffin sections of 6 μ m thickness were cut and stained using haematoxylin and eosin. The sections were observed using light microscope (Amscope, T120B California) and photographed using Amscope Digital Camera (Tokyo, Japan) for microscope version 2.0.

Stereology: The absolute volumes of the ovarian follicles were estimated using the Cavalieri estimator of volume according to the method of West *et al.* (1999). To achieve this, the entire ovaries were sectioned sagittally, and were then processed and sectioned serially using a microtome, as described by Gundersen and Jensen (1987) to provide ten sections; each section had a section of the ovary embedded within it. Tissue sections containing the ovaries were selected using a

systematic uniform random sampling method (Makris *et al.*, 2013). The sections derived were then stained using haematoxylin and eosin stain. A transparent counting grid was placed randomly over the cut surface of the sagittal section. The number of points hitting the ovaries were counted and recorded. The volume was then estimated using the Cavalieri's principle (Gundersen and Jensen, 1987).

Statistical analyses: All data obtained were subjected to one way analysis of variance using SPSS version 21.0 software. Variant means were separated using the Tukey post-hoc test. Probability values less than 0.05 were considered significant. Summary of the results were expressed as mean ($\pm$ standard error of the mean).

Results

Grossly, the ovaries of the birds were observed to be compact and not yet fully developed in all the pullet groups. The oviduct of the domestic chicken was observed to be a long, coiled tubular structure located on the left side of the abdominal cavity. It extended from the ovary to the cloaca and is divided into five distinct regions, and there were no obvious size differences in the oviducts of the various pullet groups (Figure 1).

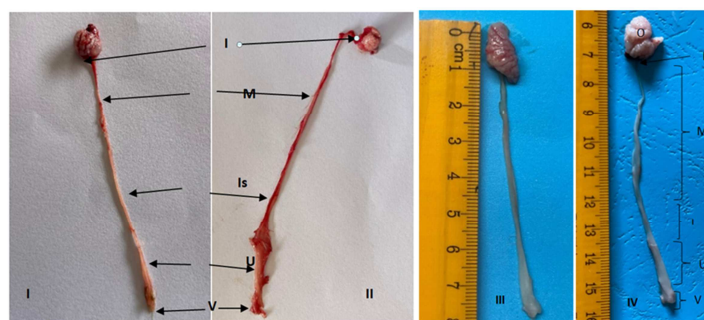


Figure 1. Photographs of the ovaries and oviducts of pullets in the four pullet groups (I, II, III and IV) treated for various durations with sulfaquinoxaline-diaveridine before onset of egg production, showing: I – Infundibulum, M – Magnum, Is – Isthmus, U – Uterus and V – Vagina. The ovaries and oviducts grossly appeared normal in all the pullet groups.

The infundibulum was seen to be funnel-shaped, thin-walled anterior portion. The magnum was observed to be the longest segment of the oviduct; the glandular wall was thin and less vascularized in all the pullet groups. Grossly, oviducts of Group I pullets were observed to have normal consistency and size, while oviducts of Groups II, III, IV the oviducts were seen to be congested and inflamed (Figure 1). The isthmus was observed to be a narrow portion and shorter than the magnum. The isthmus in Group I pullets was observed to have normal consistency and size but those of pullets in Groups II, III and IV were observed to be congested (Figure 1). The vaginal muscles were poorly developed in all the pullet groups, and was congested in the Groups II, III and IV pullets.

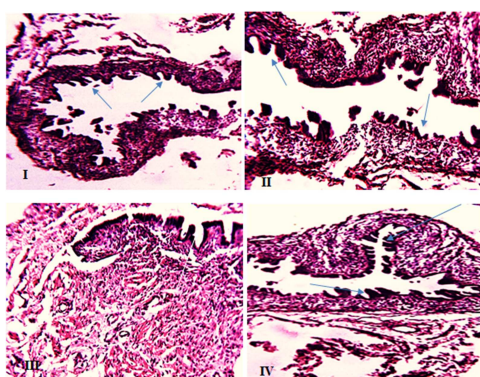


Figure 2. Photomicrographs of infundibulum of pullet chickens in the four groups (I, II, III and IV) treated for various durations with sulfaquinoxaline-diaveridine before onset of egg production, showing normal tissue architectures with developing and less prominent primary folds. [H & E, ×40]

Histologically, the infundibulum of all the pullet groups showed normal tissue architecture with developing and less prominent primary folds and the tunica muscularis appeared to be normal with normal mucosal linings (Figure 2). Photomicrographs of the magnum of the pullets showed normal tissue architecture in Groups I, II, III and IV where the folds, mucosal and muscular layers appeared normal (Figure

3). Also, photomicrographs of isthmus, uterus and vagina of all the pullet groups showed no abnormalities (Figures 4, 5 and 6). However, for the ovaries, photomicrographs of the ovaries of pullets in Groups I and II showed normal tissue architectures, with primordial follicles and primary follicles, but the photomicrographs of ovaries of pullets in Groups III and IV exhibited disruption of follicles (Figure 7). The number of ovarian follicles per millimetre of ovarian tissue was significantly ($p < 0.05$) lower in all the SD treated groups (Figure 8).

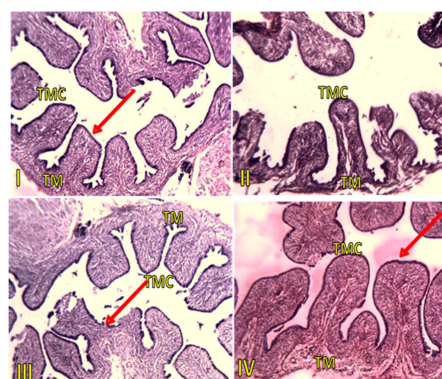


Figure 3. Photomicrographs of the magnum of pullet chickens in the four groups (I, II, III and IV) treated for various durations with sulfaquinoxaline-diaveridine before onset of egg production, showing normal tunica mucosa (TM) and tunica muscularis (TMC) with little villi (red arrows) [H & E, ×40]

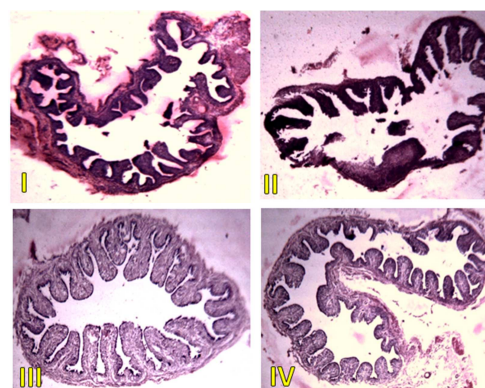


Figure 4. Photomicrographs of the isthmus of pullet chickens in the four groups (I, II, III and IV) treated for various durations with sulfaquinoxaline-diaveridine before onset of egg production, showing normal tissue architecture. [H & E, ×40]

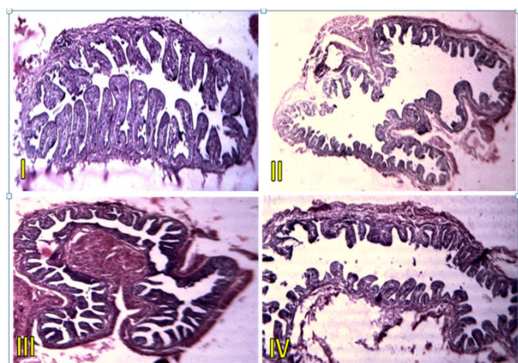


Figure 5. Photomicrographs of the uterus of pullet chickens in the four groups (I, II, III and IV) treated for various durations with sulfaquinoxaline-diaveridine before onset of egg production, showing normal tissue architecture. [H & E, ×40]

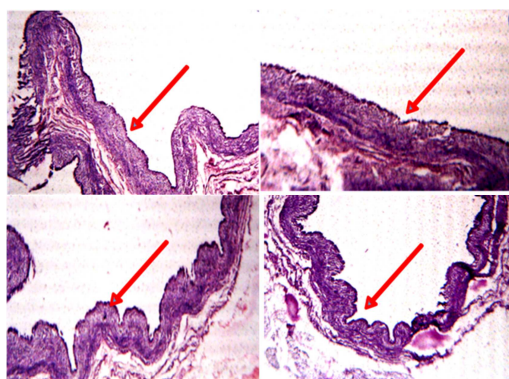


Figure 6. Photomicrographs of the vagina of pullet chickens in the four groups (I, II, III and IV) treated for various durations with sulfaquinoxaline-diaveridine before onset of egg production, showing normal tissue architecture, with epithelial lining (red arrow). [H & E, ×40]

Discussion

The gross congestion and inflammation recorded in the oviduct of the SD treated group is believed to be as a result of drug toxicity, and the greater severity of lesions recorded in the Group III and IV given SD for longer periods suggestively confirms this.

The absence of obvious histological lesions in the oviduct of the SD treated pullets implies that SD treatment did not cause any adverse microscopic structural abnormalities in the oviduct; this concurs with earlier reports by Carrasco and González (2017) and Ritchison

(2023). The normal oviduct tissue architecture recorded for the treated groups despite the treatment is thought to be due to their relatively undeveloped and inactive state during the pre-laying period.

In contrast, the ovaries of Groups III and IV showed histological alterations following prolonged SD treatment, which suggests that the degenerative changes in their ovaries were more associated with prolonged exposure to SD. These findings suggest that prolonged SD administration may adversely affect ovarian tissue during the pre-laying period. The histological findings were consistent with the stereological results, which showed a significantly lower mean number of follicles in the affected groups, further supporting the observed ovarian alterations.

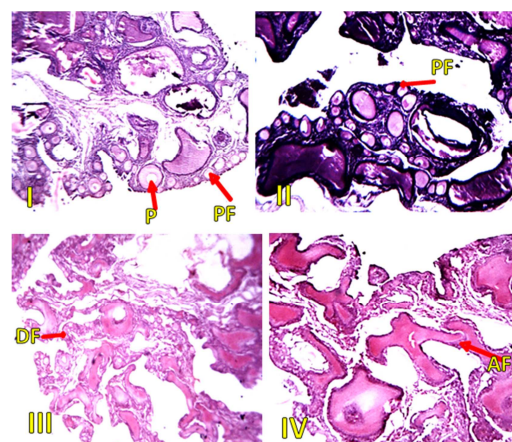


Figure 7. Photomicrographs of the ovaries of pullet chickens in the four groups (I, II, III and IV) treated for various durations with sulfaquinoxaline-diaveridine before onset of egg production, showing normal ovarian tissue architectures in Groups I and II pullets having primordial follicles (PF), and primary follicles (P), while ovaries of group III and IV pullets show degenerated follicles (DF). [H & E, ×40]

Conclusion: Grossly, pullets in all groups (I – IV) had undeveloped oviducts, which is consistent with the pre-laying stage. In contrast to the control (Group I), Groups II, III, and IV showed indications of congestion and

inflammation in the oviductal sections, which may indicate substantial morphological alterations brought about by SD. Histologically, all groups displayed normal tissue architecture in the majority of oviduct areas (infundibulum, magnum, isthmus, uterus, and vagina), suggesting that SD exposure did not substantially alter the histology of the oviduct. Nonetheless, the ovaries in Groups III and IV showed deteriorated follicles and disturbed follicular development, suggesting a possible negative impact of repeated SD administration on ovarian follicles. Also, the SD-treated groups (II – IV) had a significantly lower ovarian follicular volume, with Groups III and IV showing the

most noticeable effects. This was consistent with histology results, which indicate that exposure to SD may adversely affect follicular growth and potentially negatively affect egg production. Overall, SD had a negative impact on ovarian follicles, especially when administered repeatedly or over an extended period of time, although it did not significantly change the histology of the oviduct prior to egg production. These results highlight the necessity of caution in using SD in pullets in order to minimize any potential harm to the reproductive system. Further research is recommended, to investigate the possible long-term impacts and mechanisms of SD-induced ovarian follicular disruption.

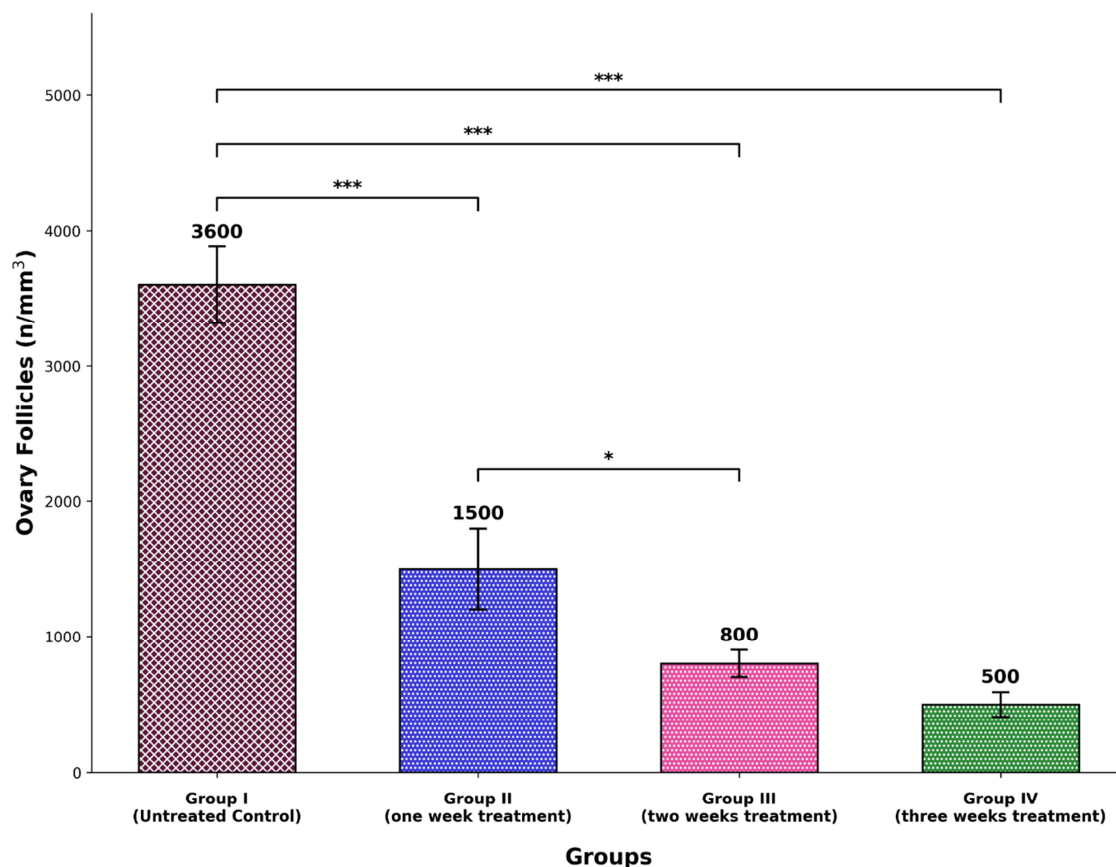


Figure 8. Mean number of ovarian follicles in pullet chickens in the four groups (I, II, III and IV) treated for various durations with sulfaquinoxaline-diaveridine before onset of egg production. [n=4, mean $\pm$ SEM, one way ANOVA, Tukey post hoc test ***=p<0.001 when Group II, III, and IV pullets were compared to the Group I (Untreated control group), *=p<0.05 when first dose was compared to third dose group.

Conflict of interest

The authors declare no conflict of interest in the research and its publication.

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