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Histopathological Study of Testes in Three Genetic Lines of Japanese Quail (*Coturnix japonica*) Exposed to Different Doses of L-Arginine

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Abstract

The purpose of the study was to investigate the histological effect of dietary L-arginine supplementation at 0%, 5%, and 10% above National Research Council recommendations on the testes of 225 male Japanese quail from three genetic lines (white, Brown, and gray). The current study was conducted from December 6, 2022, to July 1, 2023, at the poultry farm of Kirkuk University's animal production department, College of Agriculture. Initially, the Directorate of Agricultural Research-Baghdad. Birds were divided by color into three groups and fed diets containing different arginine levels from age 30 to 90 days. Testes from selected birds were examined histologically after H&E staining. Results showed that 5% arginine (S2) induced significant spermatogenic activity in all groups, with the most notable effect in gray quail (thickened basement membranes, increased spermatogenesis, and fat infiltration). The Brown line showed moderate changes, while the white line exhibited only mild stimulation with no fat infiltration. At 10% arginine (S3), the gray line displayed severe histopathological changes,

including basement membrane thickening, Sertoli cell disruption, fat infiltration, and signs of early lipoma formation. The Brown line exhibited signs of hyperplasia, while the white line showed enhanced spermatogenesis and sperm maturation with minimal adverse effects. In conclusion, gray quail were highly sensitive to arginine supplementation, Brown quail showed a moderate response, and white quail demonstrated increased spermatogenic activity with minimal histological damage.
Keywords: Arginine, Histopathology, Quail, Spermatogenesis, Testes.

Introduction

Poultry require the important amino acid L-arginine for a number of physiological functions, such as growth and feathering. Proteins, creatine, polyamines, L-proline, hormones, and nitric oxides are all made possible by it (1). Nitric oxide, an Arginine metabolite, inhibits the release of Adrenocorticotropic hormone and corticosterone, which is the primary mechanism underlying Arginine's anti-stress effects (2). In the reactions that produce nitric oxide, which is essential for many physiological functions, including reproduction, Arginine is the only physiological nitrogen donor (3). Moreover, Arginine is immunological, and healthy meals are necessary to give poultry immunity (4). The immune-competence of chicks is influenced by the nutrition of their hens (5). The Arginine requirement set by the National Research Council (NRC) is for laying quails, which might not promote breeder quail hatchability and reproductive performance. It has been demonstrated that giving birds more Arginine in their food than the National Research Council (NRC) recommends will improve their cellular immunity and possibly increase the transmission of antibodies to their progeny (6). On the other hand, no studies on Arginine supplementation in highly stressed breeder quails have been published (7).

During the production phase, male and female Japanese quails can be distinguished from one another starting at 35 days of age (8)The peak levels of estrogen and testosterone are observed between 8 and 10 weeks of age, signifying the full maturation of a seminiferous tubule (9). All male quail reproductive parameters were determined by (10), with the exception of semen ejaculate volume, and cloacal gland area. When compared to a control diet, cloacal gland area and ejaculate volume showed a substantial improvement of 18% in crude protein level (11).In order to create diets for quails that are based on the optimum protein concept, it is critical to understand the sufficient nutritional needs of crude protein (12). The aim of the study was to evaluate the effect of adding different levels of L-arginine in the diet and study the impact of those percentages on the testicles in quail.

Materials and Methods

Management and Feeding

All experimental procedures involving the care and use of animals in this study were approved by the appropriate authority. The current study was conducted from December 6, 2022, to July 1, 2023, at the poultry farm of Kirkuk University's animal production department, College of Agriculture. Initially, the Directorate of Agricultural Research-Baghdad provided (225) male Japanese quail at 30 days of age (white=75, Brown=75, and

gray= 75). The chicks were grown in battery cages with one male per cage and water tanks. The birds received 15 hrs. of light per day, and diet and fresh water were made available ad libitum. Birds were fed production feed that included (CP=24%, ME=2900) (5). All experimental birds received the same diet formulated with different amounts of L-arginine until they reached 90 days old. The purpose of the study was to ascertain the impact of supplementing the Japanese quail's diet with varying amounts of L-arginine.

Test groups and histopathological techniques

Birds were classified into three basic groups based on their genetic lines: white, Brown, and gray. Each line was divided into three groups, with 25 birds for each color category. And supplemented with arginine concentrations 0%, 5%, and 10% times the quail requirements per National Research Council recommendation. Supplement group 1 (S1) was the control group, receiving 0 percent of arginine. Supplement group 2 (S2) was given 5 percent arginine, and Supplement group 3 (S3) received 10 percent arginine. At the end of the study, when the birds reached 90 days of age, ten male birds from each group were randomly selected and slaughtered. Necropsy was performed to extract the testes. Small sections were dissected and immediately preserved in 10% formalin. All samples were then submitted to traditional histological processing techniques. Histological slides were prepared and stained by Hematoxylin and Eosin stain (13). Using a micro camera mounted on an Olympus BX-51 microscope, photomicrographs were taken, and KS 400.3 software was used to digitize the pictures.

Result

In this experiment, we tested the effect of three different supplement doses of arginine (S1, S2, S3). S1(Control group 0%), S2 (5%) ,and S3 (10%) on the testes of three genetic lines of quail birds white, Brown, and grey. Testes slides showed a blunt contrast between the experimental groups and control groups.

The control groups (S1) show the normal architectures of the testes for all line breeds (Figures 1,2,3) normal group-1-white, normal group-2-Brown, and normal group-3-Ggray, all of the figures revealing the normal histological architecture of seminiferous tubule. Which (H&E) staining, 400X, normal basement membrane, Sertoli cells(S), spermatozoa, spermatogonia cells, and the lumen.

The Supplement group (S2) exhibited variable pathological effects of arginine on the testicular tissue across the three genetic lines.", where it exhibited a highly significant effect on the gray line represented by a thickening of basement membrane (TB), moderately increasing spermatogenesis (SPG) and in another field (B) there is infiltration of fat cells (f) among spermatogonic cells, and thickening of the basement membrane, (Figure 6). In addition Brown line presented in (Figure 5) moderately increases spermatogenesis process (SPG) and in another field (B) there is infiltration of fat cells (f) among spermatogonic cells. In (Figure 4) the effect of arginine on testes tissue in the white line was notably diminished, the figure revealing increasing spermatogenesis SPG and in another field (B) there is initiation of meiosis Brown arrow, and no infiltration of fat cells among the germinal stem cells. This led us to say the S2

5% of arginine treatment dose had a strong significant effect on the gray color and medium-intensity effect on the Brown line, while it had little effect on the white line. On the contrary, it was a good stimulant for the germinal stem cell of sperms.

The Supplement group (S3) displays a severe significant effect on the gray line as presented in Figure 9, which demonstrated the onset of lipoma in the testes of this line, as shown by the spermatogonia cells appearing in a condition of karyohexis SPG in conjunction with a severe drop in adult spermatozoa (SPZ), Sertoli cell (S), and basement membrane thickness (TB) hyperplasia of the cell lining. (figure 8) exhibited basement membrane thickness (TB) hyperplasia of cell lining, which may cause a start point of cancer as well as increasing spermatogenesis SPG with clear meiosis in line with increased in adult spermatozoa (SPZ), Sertoli cell (S), with little fat infiltration (F) can be seen in Brown line color. Meanwhile, in Figure 7 the figure 10% arginine –white line, showed a small thickness of basement membrane (B), highly increasing spermatogenesis SPG in line with highly increased in adult spermatozoa (SPZ), both occupied seminiferous tubule lumen, Sertoli cell (S), with little fat infiltration (F) can be seen. The study fiund that between the three-color line of the quail birds, the gray line color was the most sensitive to the arginine supplement in both doses and moderate effect for the Brown color line and no significant or a very low significant effect for both doses of arginine supplement on the white color line.

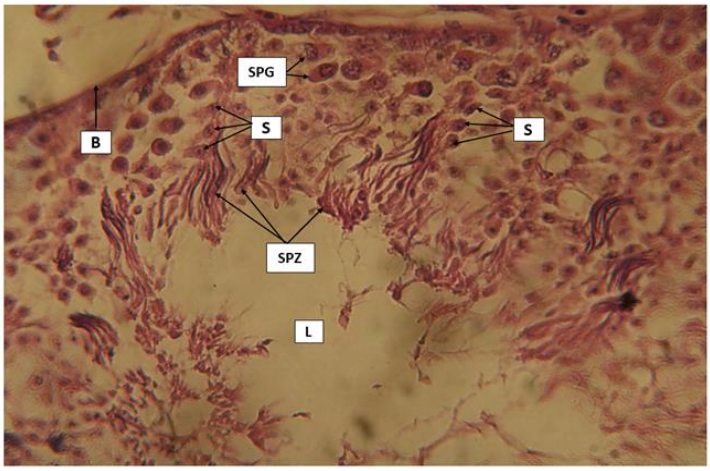

Figure (1) Control group-1-white, revealing normal histological architecture of seminiferous tubule. B-basement membrane, S-sertoli cells, SPZ spermatozoa, SPG-spermatogonia, L- lumen. (H&E) staining, 400X

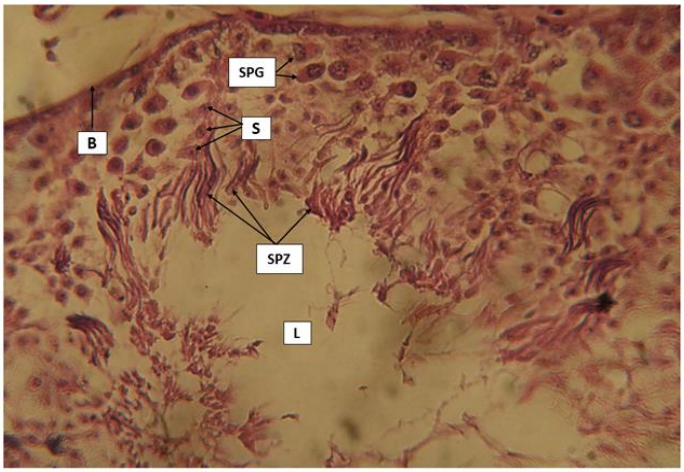

Figure (2) Control group-2-Brown, revealing normal histological architecture of seminiferous tubule.B-basement membrane, S-sertoli cells, SPZ spermatozoa, SPG-spermatogonia, L- lumen. (H&E) staining, 400X,

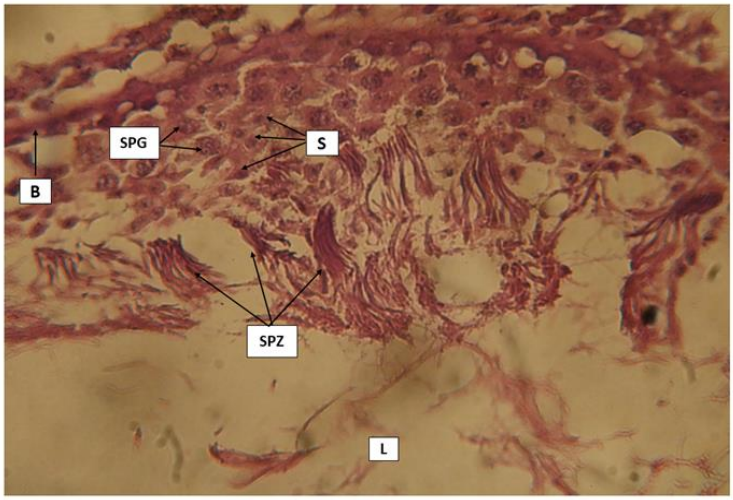

Figure (3) Control group-3-Ggray, revealing normal histological architecture of seminiferous tubule. B-basement membrane, S-sertoli cells, SPZ spermatozoa, SPG-spermatogonia, L- lumen. (H&E) staining, 400X.

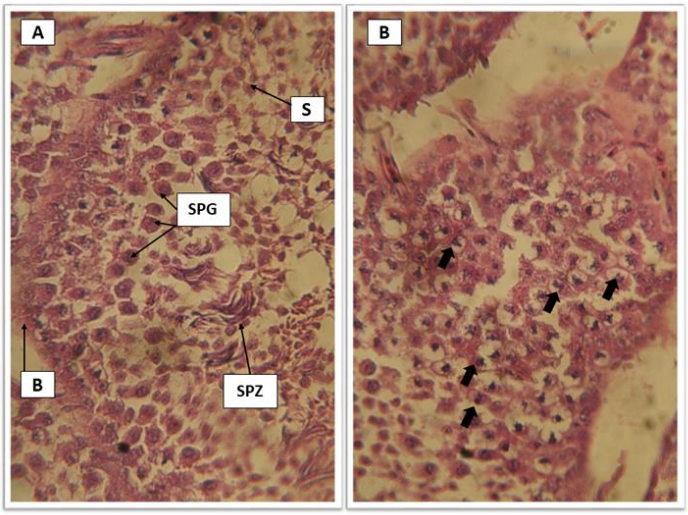

Figure (4) 5% arginine group-4-white, revealing increasing spermatogenesis SPG and in other field B there is initiation of meiosis Brown arrow. B-basement membrane, S-sertoli cells, SPZ spermatozoa, SPG-spermatogonia. (H&E) staining, 400X

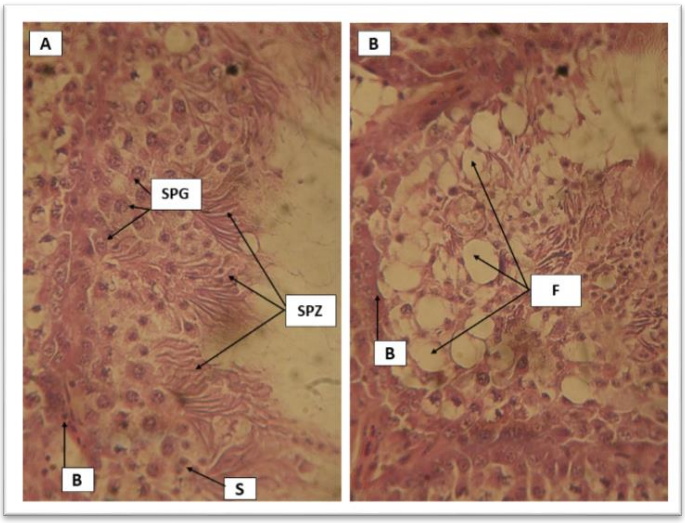

Figure (5) 5% arginine group-5-Brown, revealing moderately increasing spermatogenesis SPG and in other field B there is infiltration of fat cells (f) among spermatogonic cells.B-basement membrane, S-sertoli cells, SPZ spermatozoa, SPG-spermatogonia. (H&E) staining, 400X

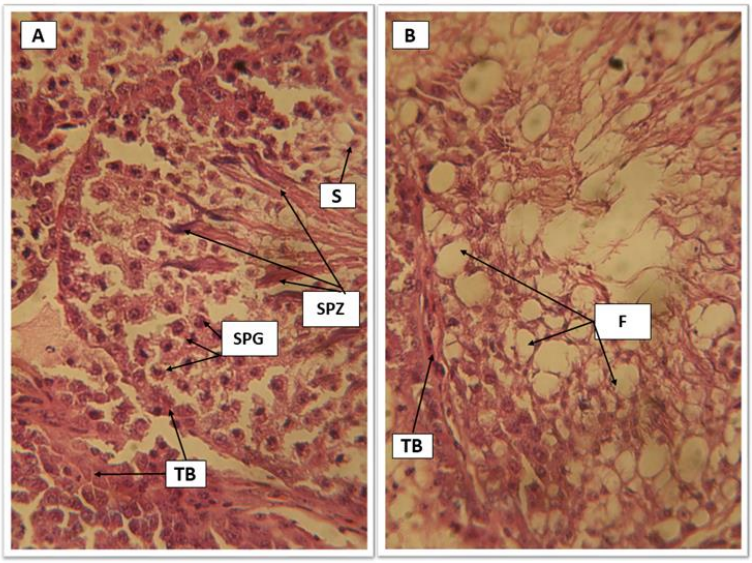

Figure (6) 5% arginine group-6-GRAY, revealing thickening of basement membrane (TB), moderately increasing spermatogenesis SPG and in other field B there is infiltration of fat cells (f) among spermatogonic cells, and thickening of basement membrane (TB). S-sertoli cells, SPZ spermatozoa, SPG-spermatogonia. (H&E) staining, 400X.

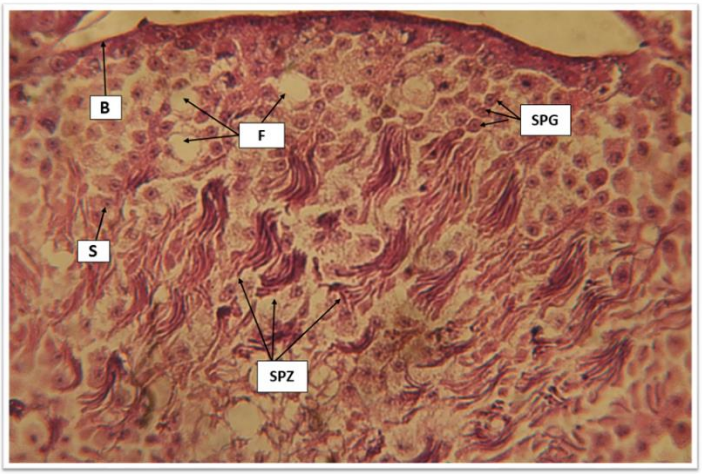

Figure (7) 10% arginine group-7-white, showed little thickness of basement membrane (B), highly increasing spermatogenesis SPG in line with highly increased in adult spermatozoa (SPZ), both occupied semniferous tubule lumen, sertoli cell (S), with little fat infiltration (F) can be seen, S-sertoli cells, SPZ spermatozoa, SPG-spermatogonia. (H&E) staining, 400X

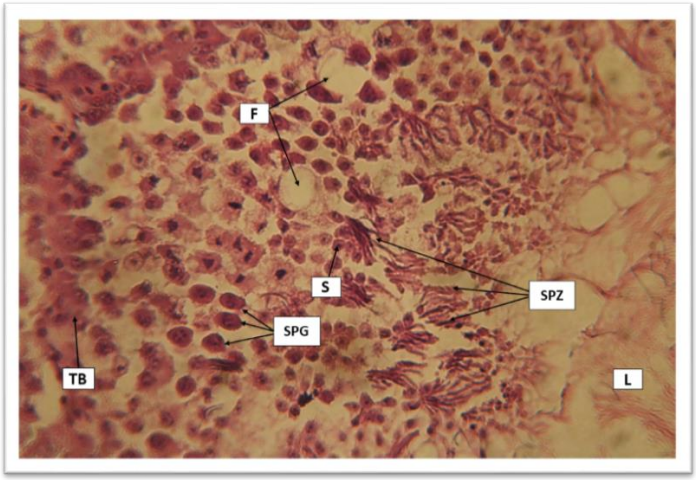

Figure (8) 10% arginine group-8-Brown, showed basement membrane thickness (TB) hyperplasia of cell lining, increasing spermatogenesis SPG with clear meosis in line with increased in adult spermatozoa (SPZ), sertoli cell (S), with little fat infiltration (F) can be seen, S-sertoli cells, SPZ spermatozoa, SPG-spermatogonia, L- lumen. (H&E) staining, 400X,

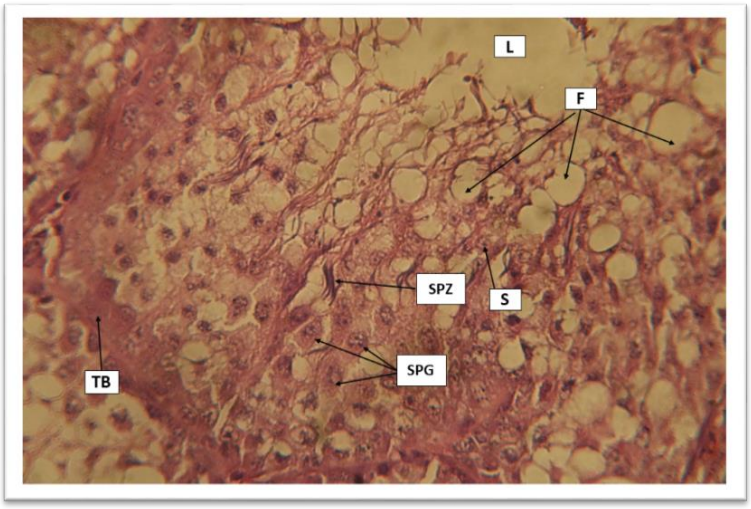

Figure (9) 10% arginine group-9-gray, showed basement membrane thickness (TB) hyperplasia of cell lining, spermatogonia cells are appear in state of karryohexis SPG in line with severe decrease in adult spermatozoa (SPZ), sertoli cell (S), and sever fat infiltration (F) can be seen, S-sertoli cells, SPZ spermatozoa, SPG-spermatogonia, L-lumen. (H&E) staining, 400X

Discussion

In this experiment, we test the effect of three different supplement doses of arginine (S1, S2, S3). S1(Control group 0%), S2 (5%), and S3 (10%) body weight on the testes of three genetic lines of quail birds white, Brown, and grey. Test slides showed a blunt contrast between the experimental groups and control groups. It was clear that long-term intake of 10% L-arginine supplementation for 12 weeks mixed with the feed in males of three genetic lines of quail birds were suffering from severe-to-moderate damage in testicular tissue and dysfunction of spermatogenesis process as well as increasing infiltration of fat tissues among the germinal stem cells of spermatozoa, and this predicts in the future on the formation of some fatty cancers such as lipoma which may develop from infiltration of adipocytes of the scrotum (14) (15). The results showed, that arginine had a progressive effect on all reproductive traits in the male white breed, which is agreed with the findings of (16); (17). It is commonly recognized that l-arginine increases the rate of the testosterone which plays a significant role in the process of spermatogenesis and in maintaining spermatogenesis in the testis. (18) (19).

The researcher (20) reported that a decrease in the proportion of spermatogenic tubules in testicular tissue and negative spermatogenesis coefficient might potentially cause disruptions to cell division, ultimately impairing spermatogenesis' ability to function. Given that spermatozoa release from sperm storage tubules is a prerequisite for fertilization in avian species, both active ciliary movement and the structural integrity of glandular cells are critical (20). Between the germ cells, vacuole development was seen (Fig. 9). Actually, these vacuoles are related to the accumulation of the fat cells and

can be one of the indicators of apoptosis as they can show a decrease in adhesion molecules like a loss of cell connections (21). The study was in the contract about the use of high doses of L-arginine or other types of amino acids such as L-valine in the diet was a principal cause of histopathological damage to testicular tissue in quails. In male Japanese quails, the amount of protein in the diet had an impact on testicular and body growth but not on reproductive efficiency(22);(23). Rapid body development throughout the growth period is closely linked to the development of reproductive organs (24). Thus, the availability of amino acids at this stage might impact the growth of birds.in our study, the effect of l-arginine level on the testicular basement membrane, epithelium cell thickness, and the increase of Sertoli cells and spermatogonia were not associated with increases in the sperm concentration of the birds. (23). In male Japanese quails, the amount of protein in the diet had an impact on testicular and body development but not on reproductive efficiency. Rapid body development throughout the growth period is closely linked to the development of reproductive organs (25). Thus, the availability of amino acids at this stage might impact the growth of birds. The data obtained suggest that breeder quails may require less protein in their diet between the ages of one and forty-two days. In addition to a diet low in protein attentive to the genetic line color because grey and Brown color lines were most sensitive to the high level of arginine meanwhile the white color revealed it was less sensitive and in contrast, this level of arginine could enhance of reproductive activity in this line.

Conclusion

The present trial demonstrated that long-term consumption of 5% and 10% doses of L-arginine for a long period of time in quail male birds will cause mild-to-sever testicular damage on Brown and gray line color respectively and no or low significant effects of L-arginine on the white line color, Therefore, our proposal is to determining the genetic line regarding the uses of the L-arginine supplement where it was deemed more useful in white line breed, and being very caution and avoiding of high doses of arginine supplement for other genetic lines such as gray and Brown or brown genetic line.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

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دراسة نسيجية مرضية للخصى في ثلاثة خطوط وراثية من السمان الياباني المعرضة لجرعات مختلفة من الارجنين

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الخلاصة

كان الغرض من الدراسة هو التحقيق في التأثير النسيجي لمكملات الأرجينين الغذائية بنسبة 0٪ و 5٪ و 10٪ حسب توصيات المجلس الوطني للبحوث على خصيتي 225 ذكرًا من السمان الياباني متكونة من ثلاثة خطوط وراثية (أبيض والبني ورمادي). أجريت الدراسة الحالية في الفترة من 6 ديسمبر 2022 إلى 1 يوليو 2023 في مزرعة الدواجن التابعة لقسم الإنتاج الحيواني بجامعة كركوك، كلية الزراعة. في البداية، مديرية البحوث الزراعية - بغداد. تم تقسيم الطيور حسب اللون إلى ثلاث مجموعات وتغذيتها على وجبات تحتوي على مستويات مختلفة من الأرجينين من عمر 30 إلى 90 يومًا. تم فحص الخصيتين من الطيور المختارة نسيجيًا بعد صبغها بصبغة الهيماتوكسلين والايوسين. حيث أظهرت النتائج أن نسبة 5٪ من الارجينين (S2) تسبب في تكوين نشاط مفرط في الحيوانات المنوية بشكل كبير لجميع المجموعات، ووقع التأثيرالاكبر والأكثر وضوحًا في السمان الرمادي حيث اظهرت (أغشية قاعدية سميكة، وزيادة تكوين الحيوانات المنوية، وتغلغل الدهون). في حين أظهر الخط البني تغيرات معتدلة، بينما أظهر الخط الأبيض تحفيزًا خفيفًا فقط دون أي تغلغل للدهون. عند استخدام 10% أرجينين(S3) ، أظهر الخط الرمادي تغيرات نسيجية مرضية حادة، بما في ذلك سماكة الغشاء القاعدي، واختلال خلايا سيرتولي، وتغلغل للدهون، وعلامات تكوّن ورم شحمي مبكر. أظهر الخط البني علامات فرط تنسج، بينما أظهر الخط الأبيض زيادة في تكوين الحيوانات المنوية ونضجها مع آثار جانبية طفيفة. في الختام، كان السمان الرمادي شديد الحساسية لمكملات الأرجينين، بينما أظهر السمان البني استجابة متوسطة، بينما أظهر السمان الأبيض زيادة في نشاط تكوين الحيوانات المنوية مع ضرر نسيجي طفيف.

الكلمات المفتاحية: طائر السمان,الارجنين,الخصى,التشريح المرضي,عملية تخلق الكائنات المنوية.