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Effect of Sodium Selenite and Zinc Sulphate on the Weight, Size, Seminal Fluid Traits, and Histological Changes of the Testis and Epididymis in Kurdi Rams

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ABSTRACT

This study was conducted to investigate the effects of Sodium selenite (Se) and zinc sulfate (Zn) supplementation individually and blended between them on semen quality, testicle size, weight, and Histological changes of the testis and epididymis, in 12 Kurdi rams. The twelve rams were divided randomly into 4 equal groups. The animals were divided into four groups 3 rams per group. The first group was the normal diet control without the addition of selenium and zinc, the second group added selenium (sodium selenite) at a concentration of 0.5 mg/kg fed, and the third group added zinc (zinc sulfate) at a concentration of 100 mg/kg fed. The fourth group added selenium with zinc at a concentration of 0.5 + 100 mg/kg fed and was given gelatin capsules daily for 90 days. Semen was collected on the 90th day of the experiment. The results showed that the level of Selenium and Zinc in the blood serum significantly ($p \leq 0.05$) increased the groups that added Se, Zn, and a combination of them compared to the control group. Overall results revealed that the abnormal sperms were improved by dietary selenium supplementation, No significant differences ($P \geq 0.05$) were found in semen volume, color, PH, sperm motility, sperm concentration, and percent live spermatozoa. Our conclusion from this study confirmed that the supplementation of Sodium selenite and zinc sulfate can improve semen quality and has positive effects on Histological parameters in Kurdi rams.

INTRODUCTION

Some countries are under economic pressure calling for the development of agricultural production to meet the requirements of population growth, which led to the use of chemical fertilizers indiscriminately, which led to a large shortage of rare mineral elements in the soil moreover, there are some soils, naturally low levels of mineral elements (Singh et al., 2005) Mineral nutrient requirements in food are low and there is an interaction between minerals, nutrients and reproductive performance. They play an important role in the metabolism of the best increase in production. They are essential for maintaining health are involved in growth and reproduction and are also a catalyst for enzymes. (Princewill et al., 2015).

Mineral elements are necessary as require very few ratios in ruminants and are a chronic problem because most of the forage plants contain different proportions of rare mineral elements which are necessary to maintain health and to maintain the balance of mineral elements in animals are given a mixture of minerals to prevent the shortage that can cause some Diseases to maintain the highest productivity (Solaiman et al., 2006), it is necessary to add and provide minerals in sheep diets and that their deficiency causes changes in animal behavior,

blood components, and biochemistry that respond to treatment (Ebrahim et al., 2016).

Selenium (Se) and Zinc (Zn) are an important nutrient for animal health and hormonal regulators and improve the situation of antioxidants, and some serum biochemical indicators, Palani et al (2018a). Selenium (Se) is an essential ingredient in animal nutrition. It is made up of over 30 selenoproteins that have an important role in the body. It is necessary to protect cells from abnormal root damage and selenium participates in metabolism and reproductive functions and stimulates the immune system. It is complicated in that its properties are basic and toxic (Zurczynska et al., 2013). Selenium has a significant association between trace elements with zinc and copper in the blood serum has an important role in enzyme activity in the body and acts as a powerful antioxidant (Hassan et al., 2017).

The male reproductive organs, including both the testis and epididymis, require Se to regulate the synthesis of selenoproteins (Shalini, 2007). Selenium and zinc supplementation may decrease levels of other health-promoting elements in the serum of lambs and rams and may also improve some carcass characteristics of Kurdi male lambs The low levels of Selenium and Zinc in the blood of Kurdi sheep are due to its low level in plants and to its low concentration in the soil of Sulaimani governorate which is, in Kurdistan Region of Iraq (Palani et al., 2022; Palani et al., 2023). Enters selenium as part of an enzyme Glutathione peroxidase, selenium is a very specific agent in the early stages of growth, Zinc (Zn) is the second largest trace element in the animal's body and cannot be stored in the body. Therefore, it needs to be added to the diet, It promotes growth (Swain et al., 2016). It was indicated the addition of Selenium or Zinc supplements improves significantly the case of antioxidants and biochemical characteristics in the serum of Kurdi male lambs, Palani et al (2018b). The addition of selenium or zinc significantly improves the growth efficiency of the Kurdi lambs, Palani et al (2018c). Therefore this current work aims to address The effect of selenium(sodium selenite) and zinc (zinc sulfate) supplementation (individually or in combination) on some semen quality, size, weight, Histological changes of the testis and epididymis in the rams.

METHODS

The present study was conducted from May to August in the summer season at the University of Sulaimani, College of Agricultural Sciences Engineering, Animal Science Department in the Kurdistan Region of Iraq.

Experimental Animal and Diet

Twelve Kurdi rams aged 16 to 18 months were divided randomly into four equal groups. Group 1 (control group) received a normal diet without supplements, the second group added selenium (sodium selenate) Na2SeO3 at a concentration of 0.5 mg/kg fed, the third group added zinc (zinc sulfate) ZnSO4 at a concentration of 100 mg/kg fed, the fourth group added (selenium with zinc) At a concentration of 0.5 + 100 mg/kg fed, the rams were randomly distributed The rams were fed 90 days before 14 days before the start of the experiment and were fed 2.5% of the animal body weight. The concentrations of the fodder concentrate were as follows: Crushed barley 60% bran Wheat 26% soybean 12% table salt 1% limestone 0,5% vitamins and minerals mixture 0.5% As for coarse fodder, barley hay was given free to eat from it to reduce saturation and to reach selenium and zinc to the animal was used empty gelatin capsules Weigh a minute amount of Sodium selenite(Se) and zinc sulfate (Zn) with a delicate sensitive scale and the weight of the diet consumed and then mixed in corn powder and fill in empty gelatin capsules and capsules were given to animals by mouth daily in the morning as soon as fed.

Collection of Blood Samples and Laboratory Analysis

Blood was withdrawn at the end of the experiment, ie on the 90th day of the experiment, where the feed and water were cut off from the experiment animals for 12 hours before the blood was withdrawn. The blood was collected through the jugular vein by a 5 ml medical syringe and the blood was emptied into sterile laboratory plastic tubes. To the laboratory and placed in the centrifuge at 3000/min for 15 minutes to separate the blood serum from the rest of the blood components then the blood serum was in sterile plastic tubes and sealed and stored at the temperature of -20°C in the frozen until the required analysis of the experiment, was determined Selenium and zinc in serum using JH Zatif Atomic Absorption Spectrophotometer AA-7000 from the Japanese company SHIMADZU

made after dilution of 1: 3 nitric acid, perchloric digested for 2 hours at a temperature of 120 C to completing deionized water 10 ml. Semen was collected on day 90 to analyze semen quality.

The rams were trained for semen collection and semen was collected in the morning hours using Electroejaculatro (Electro jac 6) by Anicam Enterprises Inc., Semen was collected into the graduatedcups with an accuracy of 0.10 ml attached, Color was evaluated by (Evan & Maxwell. 1987), PH estimated using pH paper, Color was evaluated by (Evan & Maxwell, 1987). Mass activity and Individual Motility were evaluated by (Avdi et al., 2004), sperm concentration (Salisbury et al. 1943), percentage of live spermatozoa, and morphological abnormalities (Hancock, 1952). Histomorphometric analysis After performing gross morphological studies the testes were divided into small pieces. These pieces were then immediately fixed by complete immersion in 10% neutral buffered formalin, labeled, and kept for two days, after 48 h the sample was washed with distilled water to remove salts and ions. Then the sample was fixed in Bouin's solution, for approximately 18 h. After fixation in Bouin's solution, the sample was washed in 70 % alcohol to remove picric acid from tissues. The histological slides were prepared and stained by hematoxylin and eosin. Slides of each group were arbitrarily selected and were observed by using the compound microscope. The micrographs were conducted by using a light microscope linked computer a related system, was programmed to take micrographs. The histological assemblies of the testes were noticed by using a light microscope.

Statistical Analysis

The snapshots of each sample were taken for better estimation of the results The study data were analyzed statistically using Complete Randomized Design (CRD) to determine the influence of additives (Sodium selenite and zinc sulfate separately and mixed between them) on the weight, size of testis and semen qualities. Data analysis was performed according to XLstat 2017 and then the significant differences were compared using the Duncan polynomial test (Duncan, 1955).

RESULTS AND DISCUSSION

The effect of selenium supplementation on rams serum is shown in Figure 1. The results showed a significant effect in the experimental treatments. The differences were significant ($P \leq 0.05$), where the highest treatment in the fourth treatment (selenium with zinc) was 35 ppb followed by the second treatment. Significant differences between the treatment (selenium with zinc) and the second treatment (selenium) compared with the control group were 14.667 ppb. These results are consistent with the results of the study of (Netto et al. 2014). The serum selenium level increased with the addition of sodium selenate for 90 days at a concentration of 0.5 mg/kg dry matter. The results of the present study are consistent with those of (Antunovic et al., 2013; Lee et al., 2007). The results also agree with the study of (Erdogan et al. 2017), where the level of selenium increased one week after the addition. Ml compared to the control group were 64.53 ng/ml were significant difference. The results were consistent with the study (Balick-Ramisi et al. 2006). The results of (Kumar et al. 2008), obtained when sodium was added at a concentration of 0.15 and 30.0 mg/kg feed, increased the level of selenium in the ram blood serum in both treatments.

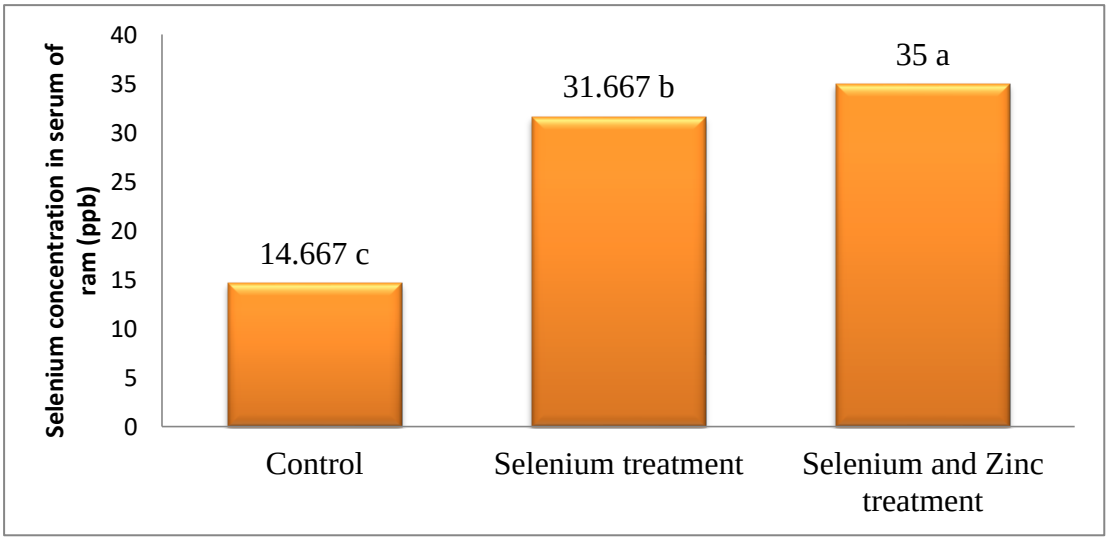


Figure 1. Effect of selenium addition for selenium level in blood serum of rams

Figure 2 shows the effect of zinc supplementation on the level of ram blood serum, the results were significant (P≤0.05) and the highest in the fourth treatment was 0.804 ppm followed by the third treatment was 0.775 ppm compared with control group at 0.434 ppm. These results were consistent with the results of (Garg et al., 2008). The zinc level in lamb serum increased by 20 mg/kg zinc for organic and inorganic fed. The differences were significant compared to the control group. The results were consistent with (Liu et al. 2015) Zinc sulfate concentrations (0, 20, 40, 80) mg/kg dry matter for males of goats for 90 days results showed significant differences in the increase of zinc level in blood plasma. The results were consistent with the study of (Kumar et al. 2013) who found serum zinc and serum zinc levels in goats. The results of selenium and zinc levels on day 90 were significant. The concentration of selenium and zinc varied from region to region. In sheep serum, 31 ng/ml (Pilarczyk et al., 2004) In an Iranian study the level of selenium in sheep serum was 28 ng/ml (Karimi – poor 2011), and in a German study, the selenium concentration in sheep blood was 45 ng/ml (Haumann Ziehank et al., 2013) who indicated that serum selenium concentration varies from region to region. The result data cannot be adopted for another area (Pamuku et al., 2001). This is also due to an increase in the level of selenium in the blood that increases after oral selenium intake (Karren et al., 2014). The reason, as (Haeeb et al., 2013) may have suggested, is that zinc concentration in the blood plasma is related to zinc levels in the diet. This may be due to the low level of selenium in the blood of the rams due to its low level in plants due to its content in the soil. Many regions, such as China, Iran, Poland, America, and New Zealand (FAO/WHO, 2004), decreased the level of zinc in sheep serum due to a decrease in the diet. He also noted that the low level of zinc is due to the low level in the diet, leading to a decrease in the level of serum and animal tissue (Paknahad et al., 2007). The low level of Selenium and Zinc in the blood of Kurdi sheep is due to its low level in plants and to its low concentration in the soil of Sulaimani governorate which is, in the Kurdistan Region of Iraq (Palani, 2019).

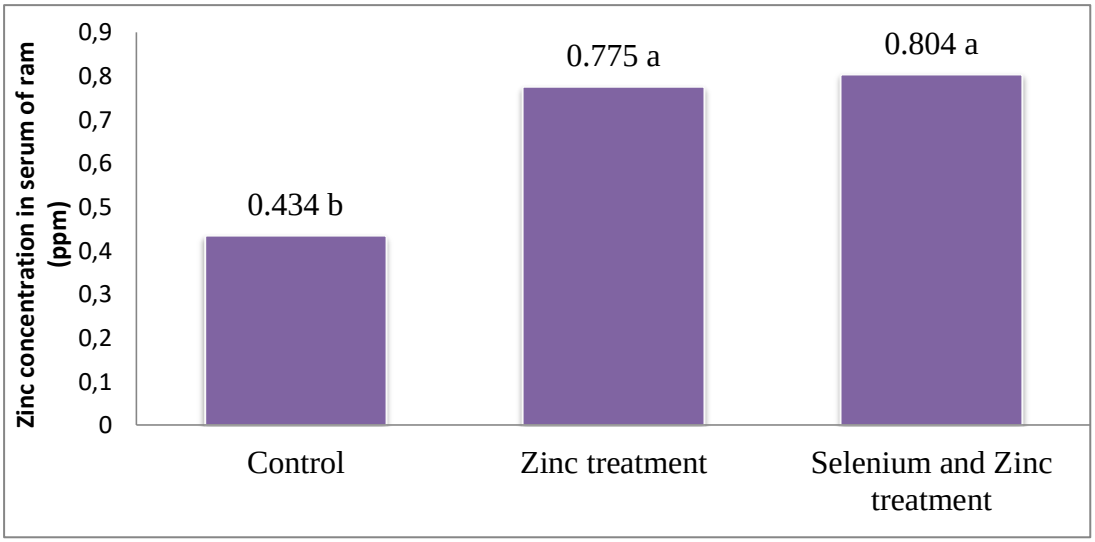


Figure 2. Effect of Zinc addition on Zinc level in blood serum of Rams

Table 1 showed no significant differences at the level of (P≥ 0.05) in the weight of the right and left testicle between all treatments compared to the control group and the differences were not significant at the level (P≥ 0.05) and the results of testicle size showed no significant differences at the level of (P≥ 0.05) in the statistical analysis to the right and did not show any significant differences at the level (P≥0.05). Arithmetic differences can not be relied on in extraction from this result.

Table 1. Effect of Sodium selenite and zinc sulfate on the weight and size of testis in Kurdi rams (Mean± SE).

Parameters	Testis Weight (g)		Testis Size (ml)	
Treatments	Right testicle	Left testicle	Right testicle	Left testicle
Treatment (Control)	179.3±41.2a	184.0±39.5a	185.3±37.0a	189.0±38.7a
Treatment (Se)	155.3±0.8a	164.3±0.3a	150.0±0.0a	156.3±0.8a
Treatment (Zn)	103.3±47.6a	219.0±9.8a	197.0±5.0a	211.6±7.2a
Treatment(Se+Zn)	192.3±36.3a	195.3±34.9a	218.6±31.8a	162.0±35.8a

Means with different letters within each column differ significantly (P≤0.05) according to Duncan's test.

Table 2 There was no significant difference (P≥ 0.05) in semen volume, semen color, and pH results, but there was an increase in the treatments compared to the control group and the concentration results. Statistical analysis No significant differences (P≥ 0.05) The results of statistical analysis in live sperm were improved for the treatments compared to the control group and the differences were not significant. Subliminal and individual movement is not significant, and can not rely on arithmetic differences in deriving from this result. Significant differences were Abnormal sperm observed between the second and the fourth treatment group compared to the third treatment group and the control group.

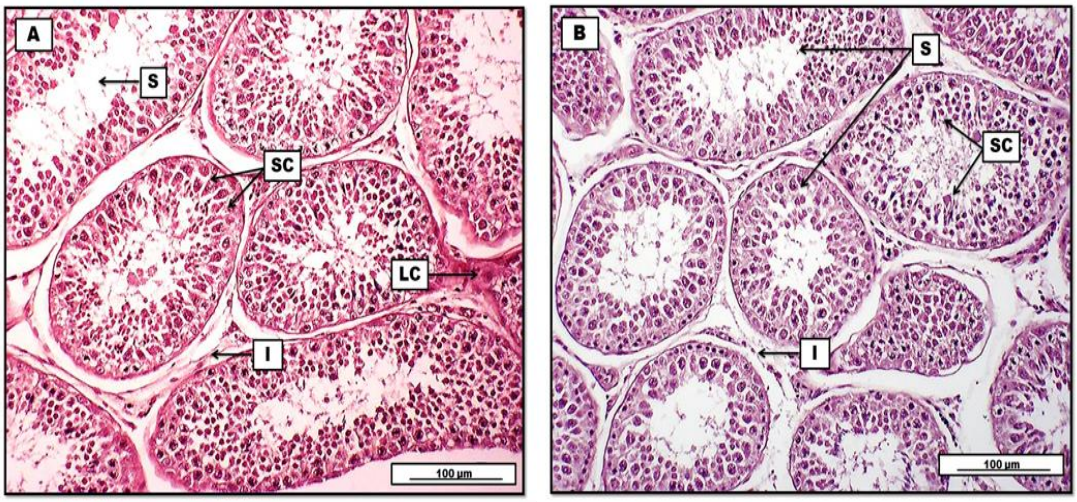
Table 2. Effect of Sodium selenite and Zinc sulfate on some seminal fluid traits in Kurdi rams (Mean± SE).

Treatments / Parameters	Treatment (Control)	Treatment (Se)	Treatment (Zn)	Treatment (Se+Zn)
Volume ml	0.40±0.0a	1.50±0.4a	0.70±0.2a	1.10±1.2a
Color	4.0±0.0a	3.0±0.5a	4.0±0.0a	3.6±0.3a
pH	6.5±0.3a	7.5±0.3a	6.6±0.3a	7.4±0.4a
Mass Activity (%)	4.3±0.3a	4.6±0.3a	5.0±0.0a	4.6±0.3a
Individual Motility (%)	3.6±0.6a	4.3±0.3a	4.3±0.3a	3.6±0.3a

Concentration X 10^7 / ml	142.6±39.2a	163.6±13.6a	160.0±20.0a	166.6±35.0a
Live sperm (%)	83.8±1.7a	87.2±0.9a	84.4±1.0a	86.3±1.8a
Abnormal sperm (%)	6.7±0.2a	5.6±0.1b	6.5±0.2a	5.9±0.1b

Means with different letters within each column differ significantly ($P \leq 0.05$) according to Duncan's test.

These results were consistent with the study of (Ghorbani et al., 2018) in the addition of Sodium selenate at a concentration of 0.3 mg/kg fed and Zinc at a concentration of 40 mg/kg fed with no significant differences in semen volume and live and dead animals. In terms of the percentage of deformed sperm, The results of this study are in agreement with the results of (Kumar et al., 2014) and the results of the Study (EL-Sheshtawy et al., 2014). The results were inconsistent with those of (Kumar et al., 2014) when Sodium selenate at 0.5 ppm and Zinc sulfate at 150 ppm were significant. (EL-Sheshtawy et al., 2014) in the addition of selenium at a concentration of 0.10 mg/kg and vitamin E were significant differences after 60 days in the proportion of live sperm and concentration and collective movement and individual movement and proportion of living sperm. Arithmetic differences can not be relied on in extraction from this result. However, the result of this research was disagreement with (Rahman et al., 2014) when Zinc sulfate was added at 100 mg/kg fed for goats significantly increased semen volume and concentration. And also with the results of (Marai et al., 2009) in the addition of Sodium selenate at a concentration of 0.1 mg/kg dry matter. And also with the results of the study of (Ghorbani et al., 2018) in terms of the proportion of deformed animals, collective and progressive movement, and concentration. The reason may be that Selenium and Zinc act as a cofactor for the synthesis of antioxidative enzymes, superoxide dismutase, and glutathione peroxidase. Thus, an increase in antioxidative status might be responsible for increased acrosomal integrity and HOST-responding spermatozoa as the reactive oxygen species that are continuously produced in spermatozoa membrane might have been neutralized by antioxidant enzymes (Bertelsmann et al., 2007). The reason may be that zinc stimulates growth and increases the secretion of sexual glands attached (Kumar et al., 2006). Zinc improves sperm characteristics. It affects Sertoli cells, which in turn have a function of the normal and abnormal shape of sperm. Zinc sulfate improves the properties of semen. Improved sperm characteristics are the cause of improved testicular function (Underwood, 1977). Selenium directly affects the testicle intercellular cells and affects the pituitary anterior lobe hormones and directly affects testicular secretion (Yousef, et al., 1990). The reason may be that Zinc is involved in enzymatic reactions of carbohydrates, protein, fat, DNA, and metabolism, which improves sperm. Zinc is also a bacterial antagonist in the prostate gland (McDonald, 2003).



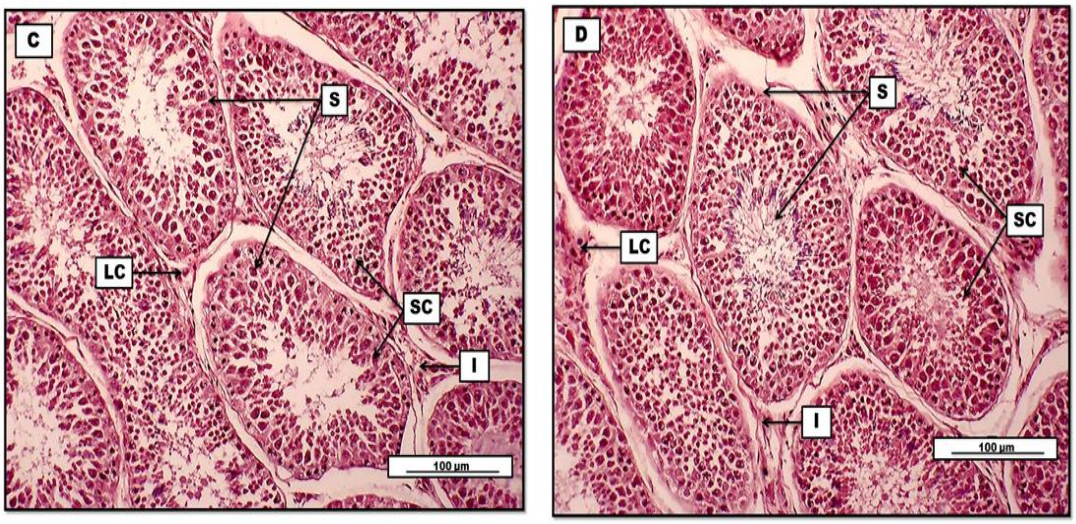
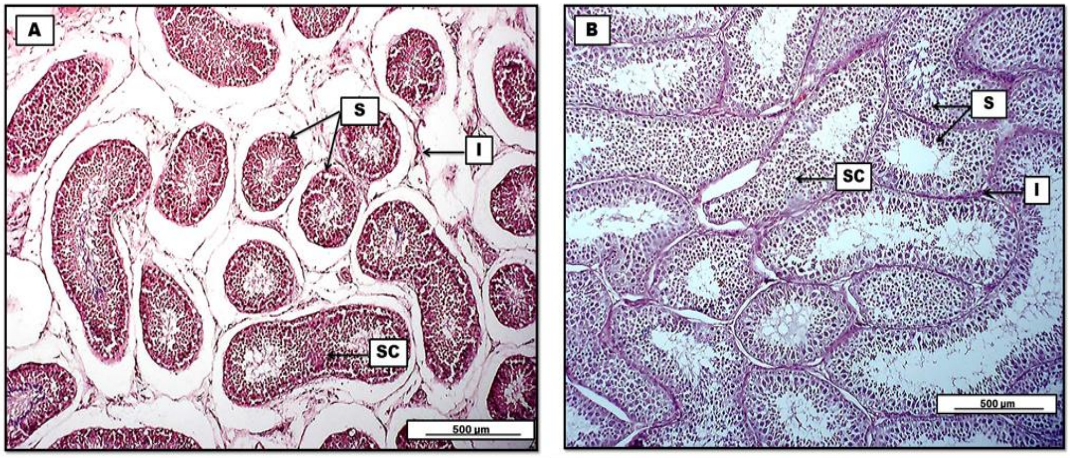


Figure 3. Effect of selenium (Se) and Zinc (Zn) and their combination on the histological of the testis of rams. Magnification: A, B, C, D, H&E. Scale bars: 100 µm.

Figure 3A: Photomicrograph of testis from group A (Control group), shows typical arrangement of spermatic cells particularly primary spermatocytes (SC) within the seminiferous tubules (S). Presence of Leydig cells (LC) with prominent acidophilic cytoplasm in the interstitial connective tissue. Figure B: Photomicrograph of testis from group B (Selenium group), demonstrates obvious increment in the seminiferous tubules diameter and size (S), in addition to an increase in the layers and numbers of spermatogenesis cells (SC) toward the lumen, the tubules are interlaced with interstitial connective tissue space (I). Figure C: Photomicrograph of testis from group C (Zinc group), shows apparent augmentation in seminiferous tubules diameter and size (S), together with an increase in the layers and numbers of spermatogenesis cells (SC) toward the lumen, different stages of cells can be seen in a given section. Tubules are interweaved with interstitial connective tissue space (I). Presence of Leydig cells (LC) with prominent acidophilic cytoplasm. Figure D: Photomicrograph of testis from group D (Selenium and Zinc group), illustrate visible augmentation in seminiferous tubules diameter and size (S), increase in the layers and numbers of spermatogenesis cells (SC) toward the lumen, different stages of spermatogenesis cells (SC) can be seen in a given section together with immature spermatids within the lumen. Large primary and secondary spermatocytes can be seen as well (SC). Tubules are separated with thin interstitial connective tissue (I), with the presence of acidophilic cytoplasm Leydig cells (LC).



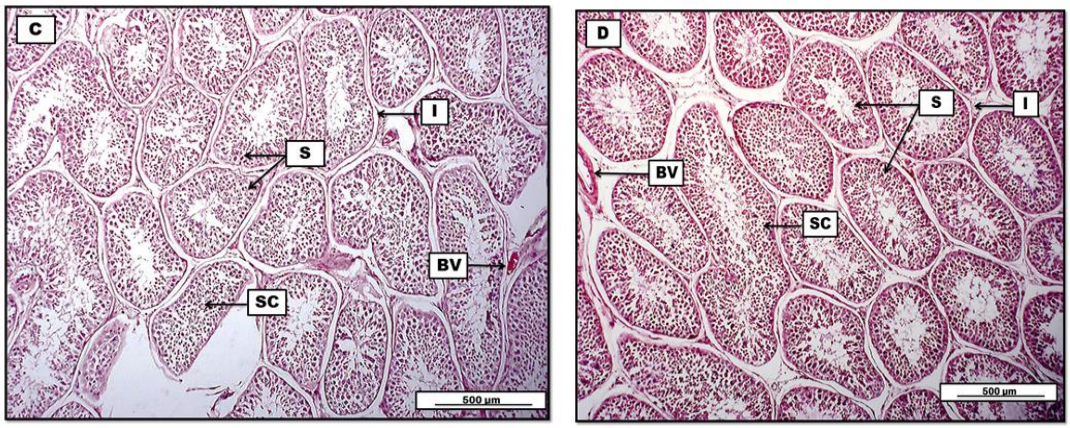
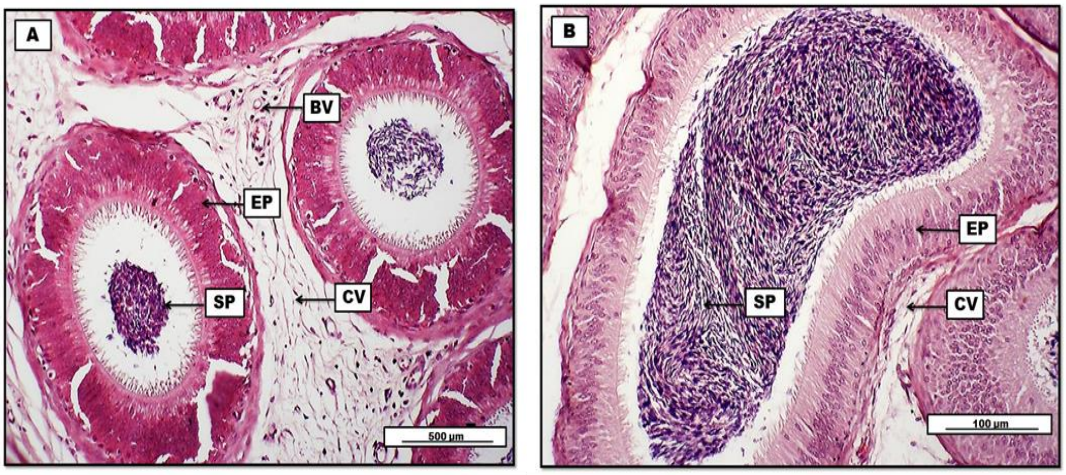


Figure 4. Effect of selenium (Se) and Zinc (Zn) and their combination on the histological of the testis of rams. Magnification: A, B, C, D, H&E. Scale bars: 500 µm.

Figure 4 A: Photomicrograph of testis from group A (Control group), displays consistent numbers of seminiferous tubules (S), lined by steady spermatogenic cells (SC), the seminiferous tubules separated by interstitial connective tissue (I). Figure B: Photomicrograph of testis from group B (Selenium group), displays the profound increase in the spermatogenesis cells (SC), together with the increase in seminiferous tubules diameter (S) which clearly reduces the interstitial connective tissue space (I). Figure C: Photomicrograph of testis from group C (Zinc group), demonstrates a considerate increase in the number and diameter of seminiferous tubules (S) in a given section. Moreover, there is marked growth in the spermatogenesis cells (SC) within the seminiferous tubules evident by the increased number of these cells. (I) represents interstitial connective tissue space which is clearly narrowed, and contains blood vessels (BV). Figure D: Photomicrograph of testis from group D (Selenium and Zinc group), shows a substantial increase in the number of seminiferous tubules (S) in a given section, besides their clear increment in diameter, which almost occupy the section. Obvious growth in spermatogenesis cells (SC) is evident by increased cell numbers toward the lumen. Narrowing of interstitial connective tissue (I). Presence of large blood vessels (BV).



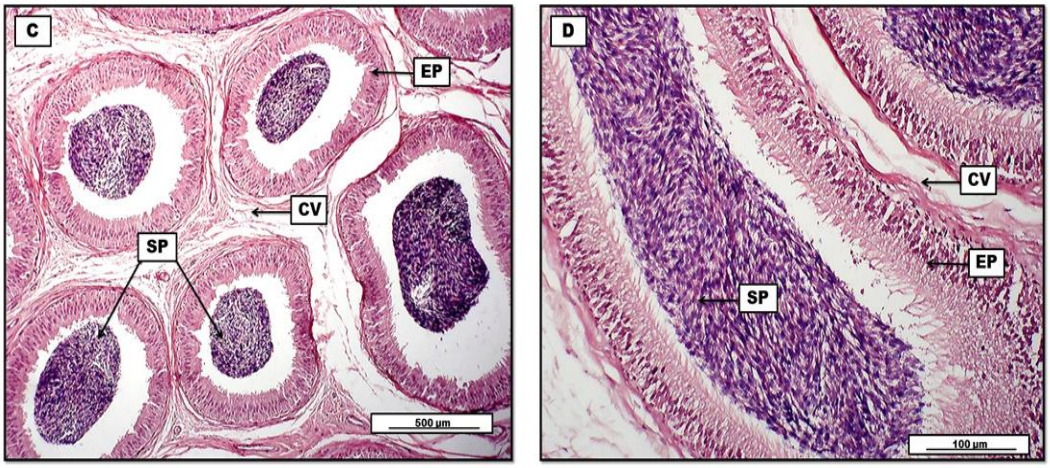


Fig. 5. Effect of selenium (Se) and Zinc (Zn) and their combination on the histological of the epididymis of rams. Magnification: A, B, C, D, H&E. Scale bars: 500&100 µm.

Figure 5A: Photomicrograph of epididymis from group A (Control group), shows epididymis (EP) lined with ciliated epithelial cells. Presence of sperm cells within the epididymis (SP). The tubules separated with fibrous connective tissue (CV), contain fibroblasts some inflammatory cells, and blood vessels (BV). Figure 3(B): Photomicrograph of epididymis from group B (Selenium group), reveals an apparent increase in the size of the ciliated epithelial lining of epididymis (EP), together with the presence of enormous numbers of sperm cells within the epididymal lumen (SP). The tubules separated with fibrous connective tissue (CV), contain fibroblast some adipocytes. Figure C: Photomicrograph of epididymis from group C (Zinc group), reveals an obvious increase in the size and numbers of epididymis (EP), the tubules in a given section, together with the presence of vast numbers of sperm cells within the epididymal lumen (SP). The tubules separated with fibrous connective tissue (CV), contain fibroblast some adipocytes. Figure D: Photomicrograph of epididymis from group D (Selenium and Zinc group), exposes a noticeable increase in the size of the epididymis (EP), evident by the increased number of tall columnar ciliated cells that lined the tubes, together with the presence of massive numbers of sperm cells within the epididymis lumen (SP), clearly stained purple with routine stain. The tubules are separated with pinkish fibrous connective tissue (CV), rich in fibroblast.

The apparent improvement in histological sections of the selenium and zinc addition group and selenium supplementation group with zinc may be due to the role of selenium and zinc as a powerful antioxidant and thus increase antioxidant states that are responsible against reactive oxygen species and their role in maintaining testicular oxidation enzymes (Bertelsmann et al., 2007). The improvement may be due to the fact that selenium directly affects the testicular interstitial cells and affects the secretion of testosterone (Yousef et al., 1990). Zinc contains enzymes, that essentially stimulate the hormone testosterone and affect reproductive characteristics by activating the epithelial epithelium (Wong et al., 2002).

CONCLUSION

The study concluded that adding sodium selenite and zinc sulfate or a combination of them for 90 days in Kurdi rams led to an increase in the level of selenium and zinc in the blood and an improvement in the testicle weight in the selenium and zinc mixture addition treatments and an improvement in some semen characteristics. It had a positive effect on the histological parameters of the testicle and epididymis, especially when adding the selenium and zinc mixture in Kurdi rams.

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