



Evaluating the Effect of Zinc Supplement on Fecundity in Wistar Male Rats and Investigating its Mechanism (Biochemical and Molecular Docking)



Hussein M. Hamed¹, Mohamed R. Shehata² and Heba A. Abd El- Rahman^{3*}

¹ Biotechnology Department, Faculty of Science, Cairo University, Giza, Egypt.

² Chemistry Department, Faculty of Science, Cairo University, Giza, Egypt.

³ Zoology Department, Faculty of Science, Cairo University, Giza, Egypt.

Abstract

ZINC has a crucial role in maintaining reproductive health and fertility by preserving the structural integrity of sperm, boosting sperm parameters, and upgrading the quantity of reproductive hormones. The main aim of the current study was to evaluate the implications of zinc supplementation on the reproductive hormone and inspect the serum and testicular ions levels to know how zinc intake can affect testosterone levels through the LH-mediated ions pathway. Twenty-one fully-grown Wistar male rats weighing 180–200 g were used in this study. The rats were divided into three groups. The control group received saline orally for 56 days. Male rats in group 2 received 4.9 mg/kg zinc sulfate orally. Group 3 rats received 18.1 mg/kg zinc sulfate. This study showed that Zn treatment reduced animal body weight change without influencing reproductive organ weight. After Zn intake, testosterone, LH, and FSH levels were significantly higher than in the control group. Molecular docking showed a close association between zinc and two important biomolecules, the Steroidogenic Acute Regulatory (StAR) protein and the Hsd17b3 enzyme, which are necessary to the testosterone pathway. Zinc ions increased significantly in testicular and serum samples. Zinc supplements boost sperm count and reduce deformities. Sperm motility and germinal epithelium thickness decreased with high Zn intake. Our data indicate that 4.9 mg/day of zinc is better than 18.1 mg/day, the upper intake limit. Extreme consumption harms reproductive hormones, calcium ions, and sperm motility.

Keywords: Zinc Sulfate, Wistar Rats, Reproductive Health, Docking

Introduction

There were growing interest for using and adding Reproductive Health is not just the lack of illness; it means all the aspects of mental, physical, and social human health [1,2]. Reproductive health was and still is an important issue, especially after the pandemic of coronavirus which invaded the world in December 2019.

According to a study conducted by researchers [3], it has been proposed that the coronavirus had the potential to negatively impact reproductive health and influence fertility. Infertility can be described as the inability to achieve conception after engaging in consistent sexual activity for one year, without employing any contraceptive methods [4,5]. Different diseases always lead to infertility; it is not a distinct disease [6]. Between 1950 and 2017, the world's fertility rate decreased from 4.7 to 2.4 live births [7]. Approximately 48.5 million couples, constituting approximately 15% of

the total number of couples, have challenges related to infertility [8]. Male factors account for 55% of infertility causes, whereas female factors account for 35% and 10% of couples experience infertility of unknown cause [9]. Since the quality of men's sperm is declining globally, men's fertility may be declining [10-12].

Zinc (Zn) is the second most prominent trace element within the human body [13]. Adults generally contain between 1.4 and 2.3 g of zinc [14]. Only a small portion of the body's Zn²⁺ is circulated in the blood; most Zn²⁺ reserves are found in skeletal muscle, followed by bones [15]. Most of the body's zinc is attached to proteins [16, 17]. Zinc is a vital micronutrient necessary for maintaining optimal health [17]. It is crucial for human physiology, immunological processes mediated by cells, and intracellular signaling in

*Corresponding authors: Heba A. Abd El Rahman, E-mail: hebaabdelrahmana15@cu.edu.eg Tel.: +201127070059 (Received 18/03/2024, accepted 18/06/2024)

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oxidative stress. Also, for many chronic illnesses, it has an important therapeutic role [17].

Zn deficiency is implicated in several health issues [14], and because the body lacks a unique system for storing zinc, one needs a daily zinc dose supplementation [17,18,19]. According to the 2001 Dietary Reference Intakes established by the US Institute of Medicine/Food and Nutrition Board, the recommended daily intake of zinc is 11 mg/day for males and 8 mg/day for females. According to sources [20] and [17], the tolerated upper limit of consumption in humans is 40 mg per day.

Zinc has been shown to exert a considerable influence on the male reproductive system [21-23]. A notable observation was made regarding the greater quantity of zinc in fertile groups as compared to sterile groups [23,24]. Zinc is highly abundant in semen [25]. Zinc is essential for preserving testes and spermatogenesis [26,27]. By providing sperm structure with protection, zinc may increase male fertility. Also, sperm parameters were enhanced by zinc thereby [28]. Hypogonadism and oligospermia are linked to zinc deficiency [29,30]. It has been suggested that the occurrence of hypogonadism and development delay among teenage males in Egypt could perhaps be attributed to a low in zinc. The patients were administered zinc supplements for a duration of 12 to 24 months. Following the administration of supplements, hypogonadism and growth delay were eradicated, and subsequently, the development of secondary sexual features was observed in all instances [31,32].

In this research, we focused on studying different doses of zinc supplementation on spermatogenesis in rats, including the effect on the reproductive hormones testosterone, luteinizing hormone, and follicle-stimulating hormone, and evaluate the effect of zinc on serum and testicular ions, including calcium and potassium ions and finally, investigate the effect of Zn on sperm analysis.

Material and Methods

Chemicals

Zinc sulfate (ZnSO₄) was purchased from LOBA CHEMIE company for LABORATORY REAGENT & FINE CHEMICALS in Mumbai, INDIA. Formula: ZnSO₄·7H₂O. CAS-No.: 7446-20-0

Animals and experimental design:

The present investigation utilized a sample of twenty-one healthy adult male rats (*Rattus norvegicus*) with a weight range of 180-200 g. The animals were removed from the animal facility located within the Faculty of Veterinary at Cairo

University in Egypt. The rats were provided with ad libitum access to water and a conventional diet consisting of food pellets. They were housed and maintained under temperature (23±2) °C conditions and humidity (40-60%) on a 12:12 light/dark cycle. Food and water were available. Before the main experiment began, the animals completed 7-day acclimatization. The animals' weight was performed weekly during the experiment to record any sign of toxicity.

The Institutional Animal Care and Use Committee (IACUC) of Cairo University, Egypt, approved the study protocol under the IACUC Permit Number of CU/I/F/52/21. International standards for the care and use of laboratory animals were followed in all the experimental methods.

Zinc treatment in the form of Zinc sulfate (ZnSO₄) was administered orally once daily for 56 days (the duration of the spermatogenesis cycle in male rats). The daily recommended dose of Zn is 11 mg/day for men, according to the US Institute of Medicine/Food and Nutrition Board in the 2001 Dietary Reference Intakes. 40 mg/day is the tolerable upper limit of intake in adults. So, the zinc concentrations were obtained based on these concentrations [33].

Adult Wistar male rats were separated casually into 3 groups (7 rats in each group): Group 1: The male rats in the control group were administered orally with saline solution. Group 2: male rats were administered zinc sulfate orally at a dosage of 4.9 mg/kg. The recommended dosage, as indicated by the guidelines for converting doses between animals and humans [34], is advised to be the appropriate intake dose. In Group 3, male rats were subjected to oral intake of zinc sulfate at a dosage of 18.1 mg/kg. The selection of this dosage was predicated upon the proposed upper limit of intake dose that is deemed tolerable, as indicated in the reference for the transformation of dosages between animal and human subjects [34].

Blood and Tissue sample collection:

The animals were subjected to weighing measures at the completion of the study, to record the body weight change, then euthanized using sodium pentobarbital intraperitoneal injection (dose at least 100 mg/kg) body weight. The blood samples were acquired using heart puncture and thereafter transferred into sterile tubes. The blood samples were carefully labeled and centrifugated at 3000 rpm for 15 minutes, and serum was separated and kept at -20°C for biochemical analysis (Ions, LH, FSH, and Testosterone analysis). The male rates were immediately dissected for the removal of reproductive organs (testes and epididymis). Phosphate-buffered solution (PBS) was used to

wash the organs before they were dried, weighed, and examined for morphological alterations. The tests were taken out for histological examinations, and some were preserved in sterile/clean Eppendorf tubes at -20°C for additional examinations.

Determination of sperm parameters:

Sperm concentration. Each animal's cauda epididymis was placed in 2 ml of normal saline and kept in a warmed medium at 37 °C. Small cuts were made in the epididymis to obtain the spermatozoa, suspended in a saline solution. Using a Pasteur pipette, 200 µl of the suspension was added to both chambers of a Neubauer hemocytometer slide for microscopic evaluation [35,36]. A light microscope with a 40X magnification was used for counting [37]. The total sperm number is expressed as million/ml of suspension according to the equation: Sperm concentration = (The total number of sperm)/ (4 × 10⁴ × 2).

Sperm motility. Into a warmed slide, two drops of epididymal sperm suspension were loaded and placed on a microscope stage warmed to 37°C and then examined with a light microscope. Motility was performed by counting at least 100 sperm from 5 different squares according to the Percentage of motile sperm. The sperm concentration and motility rate were calculated using the following equations: Sperm motility rate = (The number of motile sperm/ the total number of sperm) × 100.

Sperm morphology and viability

For morphological assessment, two slides were prepared from epididymal sperm suspension. A specimen of 10 µl was placed on a slide, smeared with another slide, and the preparation was left to dry in air and then stained with eosin-nigrosine stain (1% Eosin and 10% Nigrosine). This stain can pass through the sperm membrane. The stain penetration was prevented if the membrane was intact, as in the case of viable spermatozoa. At the same time, the dye could penetrate the sperm cytoplasm if the membrane was damaged, like dead spermatozoa [38,39]. By preparing an eosin-nigrosine smear at an optimum temperature of 37°C and assessing at least 100 sperm under a bright-field microscope using magnification 1000X, sperm viability was determined [38,40].

Determination of sera reproductive hormones concentration

Follicle-stimulating hormone (FSH), serum testosterone (T), and luteinizing hormone (LH) were measured by ELISA reader using commercial assay kits purchased from SUNLONG BIOTECH Company according to the manufacturer's protocols.

Determination of testicular and serum ions concentration

Zinc ions: The zinc assay kit was purchased from SIGMA-ALDRICH Company in Louis, USA, with Catalog Number MAK023.

Calcium ions: The calcium ions assay kit was purchased from the ELITech Group diagnostic company in France under CALA-0600.

Potassium ions: Potassium ions assay kits were purchased from LiNEAR company in Barcelona, Spain.

Histological analysis

The extracted testicular tissues were fixed in 10% neutral buffered formalin for 24 hours. After that, specimens underwent post-fixation treatment by being washed in distilled water and passing through an ascending series of alcohol. Dehydrated specimens were cleared in xylene and embedded in paraffin. Tissue blocks were prepared for sectioning at 5 µm thickness. Sections were rehydrated and stained with hematoxylin and Eosin for a routine examination. A light microscope was used for histological analysis [41].

Morphometric analysis

From each group, approximately 15 circular or roughly spherical seminiferous tubule sections were selected. Employing Image J (Version 1.53i), the tubular diameter, germinal epithelium height, and lumen diameter were determined.

Johnsen's score

Sections of testicular tissue were examined in 15 different inter-tubular regions under a 100x light microscope. The mean Johnsen's testicular biopsy score (MJTBS) was determined using a formerly authorized procedure.

Molecular Docking Interaction between Zn with 17βHSD and STAR protein

The probable binding modes for the receptor's most active site were determined by molecular docking research, employing the MOA2022 program [42]. The present study investigates the interaction modalities of the most highly active site of the 17βHSD protein, specifically focusing on the protein with the PDB ID: 6EMM [43]. The interaction mechanisms of the main active site of the STAR protein, identified by its Protein Data Bank (PDB) ID 7z8a, have been investigated [44]. The receptor crystal structures were obtained from the Protein Data Bank (PDB) website (<http://www.rcsb.org/pdb>).

Statistical Analysis

The data that was gathered was subjected to analysis and thereafter presented as the mean value accompanied by the standard error of the mean

(SEM). The statistical technique employed in this study involved conducting a one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons as a post hoc test. This approach was utilized to ascertain any significant variations among the different groups. Significance was determined using the IBM SPSS version 25 software package at a probability level of $P < 0.05$ [9].

Results

Effect of Zinc administration on body weight change

The absolute and relative weights of reproductive organs. The results indicated a statistically significant decrease ($P < 0.05$) in the body weight of male rats after receiving a dose of Zn, compared to the control group. No statistically significant differences were seen in the absolute and relative weights of the reproductive organs, namely the testes and cauda epididymis, among the three groups analyzed (refer to Tables 1, 2).

Effect of Zn supplementation on reproductive hormone after 56 days

The findings of the present study indicate that over a period of 56 days, the administration of oral zinc sulfate intake to albino Wistar rats resulted in a statistically significant increase in serum levels of Testosterone (T) and FSH hormones ($p < 0.05$) as compared to the control group. The study observed a significant increase in the level of luteinizing hormone (LH) after receiving a dose of zinc (4.9mg/kg) compared to the control group. On the other hand, the use of zinc sulfate at a higher dosage of 18.1 mg/kg led to a significant reduction in blood testosterone (T), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) levels as compared to the low-dose zinc group (4.9 mg/kg) (Table 3).

Effect of Zn supplementation on serum ions after 56 days

The present investigation demonstrates a notable and statistically significant elevation in serum zinc ion concentrations following 56 days of oral zinc sulfate supplementation. This rise is observed in a dose-dependent manner, as compared to a control group. Furthermore, the consumption of zinc sulfate at a greater dosage of 18.1 mg/kg resulted in a substantial decrease in serum calcium ion levels as compared to the control group. No significant differences were seen in the potassium ion levels (Table 4).

Effect of Zn supplementation on testicular ions after 56 days

Our study displayed a significant rise in testicular Zinc ion levels compared to a control group. Also, data revealed that calcium ions

showed a significant decrease after Zn administration (18.1mg/kg). Potassium ions did not show any significant differences (Table 5).

Effect of Zn supplementation on sperm analysis after 56 days

The administration of a higher dosage of zinc supplementation results in a notable reduction in sperm motility in comparison to both the control group and the group receiving a lower dose of zinc (4.9 mg/kg). The data presented in the study demonstrated a statistically significant rise in sperm count following 56 days of oral zinc sulfate supplementation. This increase was observed in a dose-dependent manner, as compared to a control group. Moreover, the consumption of zinc resulted in a notable reduction in sperm abnormalities when compared to the control group (Table 6 & Fig. 1)

Histopathological observation

The control group demonstrates full spermatogenesis, characterized by the typical histological structure of seminiferous tubules. These tubules maintain their intact spherical shape and regular diameter and are filled with spermatogenic cells arranged in a regular pattern, tightly attached to the unaltered basement membrane. Within the tubules' lumens, one can observe the presence of spermatogonia, spermatocytes, spermatids, and mature spermatozoa. The Sertoli cells, which provide support, were observed to be located along the seminiferous tubules and situated amidst the spermatogenic cells. The interstitial tissue located among the tubules exhibited the presence of blood arteries and interstitial cells of Leydig (Fig. 2 a-b).

Administration of the zinc (4.9 mg/kg and 18.1mg/kg) doesn't induce any significant alterations in the histology of testicular tissue and showed standard, normal, and usual histology of tissue revealing complete set spermatogenesis, lumen filled with spermatozoa, adequate number of Leydig cells and the interstitial tissue between the tubules within normal size (Fig. 2 c-f).

Morphometric and Johnson score outcome

There was an absence of statistically significant change in the Johnson Score following the administration of Zn supplementation in comparison to the control group. The intake of zinc at a lower dosage of 4.9 mg/kg resulted in a notable elevation in the thickness of the germinal epithelium when compared to the control group (Table 7).

Docking result

In the current investigation, scientists determined the free energy binding of Zn to the active sites of the 17 β HSD receptor to be -135.5 kcal/mol, whereas the free energy binding of Zn to

the active sites of the STAR protein receptor was seen to be -193.8 kcal/mol. As indicated in Tables 8 and 9, and illustrated in Figs. 3&4, it can be observed that there exists an inverse relationship between the strength of the binding and the magnitude of the binding energy.

Discussion

Zinc is one of the essential elements for normal sperm formation and motility in the male reproductive system [45,46]. Males with zinc deficiency exhibit testicular disruption, decreased spermatogenesis, and poor semen parameters as a result. When rats were given diets lacking zinc, various degrees of maturation arrest in different stages of spermatogenesis were observed, along with smaller seminiferous tubule diameters [47,48]. Zn is essential for protein synthesis, cell differentiation, proliferation, and apoptosis [19,50,51].

Our study showed that after 56 days of oral zinc sulfate supplementation for albino Wistar rats, a significant decrease in the body weight of rats compared to the control group was observed. Zinc administration may interfere with neurotransmitters in the hypothalamus that influence food intake, affect appetite, and cause weight loss.

However, reproductive organs' absolute and relative weights were significantly unchanged. Many reports stated that Zn deficiency is associated with reduced testis volume and decreased testicular weight. Zinc deficiency is linked to a decrease in total germ cell mass, which accounts for 40-75% of testicular volume, which in turn causes testicular weight to decrease [19,26,53].

Previous research [54] gave Sprague Dawley rats oral zinc sulphate (3.2 mg/kg/d) for 4 weeks. The zinc sulfate-treated group had a significantly lower body weight than the obese group. There was no substantial weight loss compared to the control group. Zinc sulphate had no impact on testicular or epididymal weight compared to the control group.

In an 8-week Sprague Dawley rat study [55], Zn-treated rats had a substantial increase in body weight and testicular weight compared to Cisplatin-treated rats and a non-significant decrease compared to the control group. In research by [48], Wistar-Albino male rats fed 10 µL of zinc sulphate in the lateral ventricle daily for 20 days showed a significant decrease in food intake and weight gain.

Zinc can substantially restore testes and epididymis weight loss owing to obesity and cisplatin. Zinc (3 mg/kg, i.p.) reduces diabetes-induced germ cell damage in male Sprague Dawley rats [56]. Zinc may additionally restore smoking-induced testicular impairment [57]. Zn supports the development of testicles. Zinc improves testes, prostate, and epididymis function.

Our study found that zinc significantly increased reproductive hormones. Many researchers examined how zinc affects male reproductive hormones. Results showed that zinc supplementation raised serum testosterone, FSH, and LH hormone levels in patients with Prasad syndrome [58].

Our findings are consistent with [32] and [59] on 80 male Sprague-Dawley rats, which showed that 3 mg/kg/day of zinc for 4 weeks increased gonadotropic hormone levels, indicating that zinc affects male reproduction. The finding was consistent with [60], which found that 6 weeks of zinc supplementation on sedentary and athlete-healthy adult males (2.5–3 mg /kg /day) increased testosterone levels significantly. As indicated, zinc supplementation raised testosterone hormone levels in many trials. ZnSO₄ was orally-gavage to Sprague Dawley rats for 4 weeks in [54]. This study found a significant increase in serum testosterone, suggesting zinc sulphate may improve semen quality.

Zinc intake studies show different results for FSH and LH serum levels. The investigation gave Wistar rats zinc gluconate orally for 90 days, which revealed an increased blood testosterone but did not affect LH or FSH levels. Another distinct outcome was observed in the study conducted by [62], where various doses of zinc sulfate were administered to male albino Wistar rats over a period of 6 weeks. At 20g, 40g, and 80g zinc supplementation, blood testosterone levels increased. The group that received 80 g of zinc had a less testosterone increase than the others. FSH and LH levels were affected. This shows that the hypothalamus, impacted by testosterone's negative feedback effect, reduced FSH and LH. A study by [48] found that excessive zinc sulphate (10 µL) in the lateral ventricle of male Wistar-Albino rats significantly reduced blood LH hormone levels, while FSH and testosterone hormone levels remained unchanged. The authors proposed that the observed variations in the outcomes may be attributed to disparities in the method of zinc administration and variations in patients' responsiveness to zinc constituents.

The first question we need to answer is why administering zinc sulfate increases LH and FSH hormone levels. Zinc affects the pituitary by stimulating gonadotropic hormone release. Zinc is a cofactor to peptidyl glycine α -amidating lyase in the amidation reaction to initiate active GnRH from a prohormone. Its deficiency causes hypothalamic-pituitary-gonadal axis suppression [63]. The anterior pituitary contains gonadotrophs, which release LH and FSH hormones upon stimulation of gonadotropin-releasing hormone (GnRH) [64].

That is why zinc administration results in increasing these hormones. The second question is

why a significant surge in testosterone accompanied the increase in LH hormone level. LH's principal role in the testis is stimulating testosterone synthesis by Leydig cells. LH binds to its target receptor on Leydig cells, luteinizing hormone-chorionic gonadotropin receptor (LHCGR). This results in gene expression stimulation of several key steroidogenic enzymes. As a result, it stimulates testosterone production through the Gs-adenylyl cyclase-cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA) pathway. LH binding with LHCGR results in three processes; stimulation of adenylyl cyclase, cAMP upregulation, and activation of protein kinase A (PKA) or protein kinase C (PKC) [64].

The question now is, what is the relation between zinc and the PKA signaling pathway, and what is the outcome of this pathway? [65] demonstrated that zinc promotes the PKA signaling pathway by increasing intracellular cAMP levels. cAMP-PKA pathway causes activation of transcription factors, which are important in steroidogenesis in Leydig cells, such as cAMP response element binding (CREB) protein, cAMP response element modulator (CREM) protein, and GATA4. cAMP production increases calcium ions influx in Leydig cells in response to LH activating a calcium-signaling pathway [64].

As a second messenger of the LH pathway, Ca²⁺ regulates steroidogenesis in Leydig cells. When LH stimulates proper androgen synthesis, cytoplasmic Ca²⁺ must rise. Leydig cells acquire intracellular free Ca²⁺ from external Ca²⁺ and organelles like endoplasmic reticulum and mitochondria through calcium outflow. T-type Ca²⁺ channels transport calcium from the endoplasmic reticulum to the cytoplasm. The principal calcium ion regulators in Leydig cells are IP₃ receptors (IP₃Rs) and RyRs in the endoplasmic reticulum. LH decoded PIP₂ into IP₃ and DAG28 via the PLC. IP₃ interacts to IP₃Rs to activate endoplasmic reticulum Ca²⁺ channels, raising cytoplasmic Ca²⁺. Thus, Leydig cell steroidogenesis is promoted. Calcium and DAG directly activate PKC, which phosphorylates its substrate, MARCKS, which drives CREB expression, StAR expression, and steroidogenesis [66].

That's why cAMP with Calcium ions regulates StAR gene expression together. Also, calcium ions influx induces Nur77 expression promoting StAR expression. Epidermal growth factor receptor (EGFR) activates ERK1/2 through LH-induced cAMP-PKA signaling, promoting StAR expression and proliferation of Leydig cells and, as a result, increasing testosterone production. The StAR protein regulates steroidogenesis by transferring cholesterol from the outer to the inner mitochondrial membrane. LH stimulates

testosterone biosynthesis from a cholesterol substrate by the Leydig cell [64].

After zinc (18.1 mg/kg) administration, testosterone and LH levels decreased significantly. Through a negative feedback loop, testosterone secretion regulates pituitary luteinizing hormone levels. The hypothalamus and pituitary gland recognize high testosterone levels in the bloodstream and inhibit LH synthesis through a negative feedback process. This reduces Leydig cell activation via the LHCGR, reducing testosterone production [64].

The next issue is zinc dose, specifically whether low or high is healthier. Zinc supplementation's effects on ion and sperm analysis must be examined to address this inquiry. The current study found that zinc supplementation increased serum and testicular zinc ions. Intake of 18.1 mg/kg zinc increased serum and testicular zinc ions and decreased serum and testicular calcium ions.

Enhancing calcium influx in the LH-mediated signalling pathway is crucial. High zinc ions can replace calcium in Ca²⁺ channels. Thus, calcium ions are removed from its binding site [67], causing insufficiency. This negatively impacts the LH-mediated signalling pathway. This means high zinc ions negatively effect calcium ions. The Zn-treated group had a considerable reduction in testosterone and LH (18.1 mg/kg). This suggests high zinc ion administration impairs steroid and spermatogenesis. Spermatozoa activation by calcium ion is crucial to spermatogenesis. Its insufficiency reduces sperm motility [62]. This supports a mouse study on high zinc consumption and testes and sperm motility [68]. Two groups were given 1.5 and 2.5 g/100 mL Zn. Sperm motility decreased significantly in the higher dosage group compared to the control group. High zinc supplementation causes seminiferous tubule degeneration, spermatogenic arrest, and interstitial tissue fibrosis, which reduces sperm motility [62,68].

The study used obese Sprague Dawley rats given zinc sulphate (3.2 mg/kg) orally for 4 weeks. Before and after zinc treatment, testosterone hormone levels increased and semen properties changed. Zinc supplementation enhances semen parameters and quality by increasing sperm motility and count in rats [54]. Zinc can impact testicular structure as testicular tissue histology shows considerable improvement, undisturbed spermatogenesis, and normal Leydig and Sertoli cells in the Zn-treated group [54].

Zinc increases LH, FSH, and testosterone, according to biochemical studies. Our research used molecular docking to confirm these findings. Zinc had a strong interaction with the Steroidogenic Acute Regulatory (StAR) protein

and the Hsd17b3 enzyme, according to molecular docking study. These biomolecules are essential to the testosterone pathway.

Cholesterol transfer from intracellular reserves to mitochondrial inner membranes starts steroid hormone synthesis [69]. The speed of steroid synthesis depends on the StAR protein transporting cholesterol to the mitochondrial inner membrane [70]. Cyp11a1 (P450scc) converts cholesterol into pregnenolone in the mitochondrial inner membrane. Consequently, Hsd17b3 is crucial to the enzymatic conversion of androstenedione into testosterone [71].

Zinc stimulates gonadotropin-releasing hormone in the pituitary gland. Gonadotrophs secrete LH and FSH from the anterior pituitary gland. These hormones are released via GnRH stimulation. Zinc increases LH and FSH hormones. LHCGR on Leydig cells interacts with luteinizing hormone. The cAMP-PKA pathway is activated by this interaction. This route produces a calcium-signaling cascade and activates transcription factors essential for Leydig cell steroidogenesis. This activates steroidogenic acute regulatory protein (StAR) expression. The StAR protein and Hsd17b3 enzyme help transport cholesterol from the outside to the inner mitochondrial membrane and convert it to testosterone, regulating steroidogenesis. Our molecular docking investigation shows that zinc and proteins interact strongly. The initiation of spermatogenesis depends on follicle-stimulating hormone (FSH), and a decrease in FSH levels reduces testicular volume and stereological characteristics.

Furthermore, FSH is essential for Leydig cell testosterone secretion. Thus, FSH decrease affects spermatogenesis. The germinal epithelium, testis size, proportion of healthy and viable spermatozoa, and sexual lineage cell count decline throughout spermatogenesis [70].

The recent investigation found that Zn administrations minimize sperm abnormalities. Morphometric analysis showed no statistically significant difference in tubule and lumen diameter

between the two treatment groups and the control group. The 4.9 mg/kg zinc group had significantly increased germinal epithelial thickness.

Zinc at 4.9 mg/kg improves normal sperm morphology and germinal epithelial thickness. However, 18.1 mg/kg zinc adversely affected reproductive hormones, calcium ions, and sperm motility, reducing germinal epithelial thickness.

Zinc is essential for reproductive health and fertilization. Zinc affects reproductive hormones and ions, which trigger testosterone synthesis via the Gs-adenylyl cyclase-cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA) signaling pathway during spermatogenesis. The findings show that zinc at 4.9 mg/kg improves sperm quality. It increases sperm morphology and thickens the germinal epithelium. However, 18.1 mg/kg zinc had negative impacts on reproductive hormones, calcium ions, sperm motility, and germinal epithelial thickness.

Limitation:

Further research is required to establish the molecular underpinnings of zinc's involvement in the activation of genes associated with LH-mediated signaling pathways, such as the StAR gene.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

The protocol of the study was approved by the Institutional Animal Care and Use Committee (IACUC) of Cairo University, Egypt under the IACUC Permit Number of CU/I/F/52/21.

TABLE 1. Effect of Zn supplementation on body weight change and absolute of reproductive organs after 56 days.

Groups	Body Weight Gain (g)	Right Testis (g)	Left Testis (g)	Right Epididymis (g)	Left Epididymis (g)
Control	86.43 ± 2.83	1.48 ± 0.04	1.48 ± 0.04	0.21 ± 0.01	0.21 ± 0.01
Zn (4.9 mg/kg)	73.71 ^a ± 1.48	1.43 ± 0.02	1.42 ± 0.02	0.19 ± 0.01	0.20 ± 0.01
Zn (18.1 mg/kg)	76.29 ^a ± 2.10	1.48 ± 0.02	1.48 ± 0.02	0.19 ± 0.01	0.20 ± 0.01
P-value	0.002	0.39	0.27	0.14	0.83
F-value	9.29	0.97	1.39	2.11	0.17

This data represents as mean ± standard error of the mean. (n = 5). a P < 0.05 vs. the Control group; b P < 0.05 vs. Zn (4.9 mg/kg) group.

TABLE 2. Effect of Zinc administration on the relative weight of reproductive organs after 56 days.

Groups	Right Testis (%)	Left Testis (%)	Right Epididymis (%)	Left Epididymis (%)
Control	0.59 ± 0.03	0.59 ± 0.03	0.08 ± 0.00	0.08 ± 0.00
Zn (4.9 mg/kg)	0.57 ± 0.01	0.57 ± 0.01	0.08 ± 0.00	0.08 ± 0.00
Zn (18.1 mg/kg)	0.56 ± 0.03	0.56 ± 0.03	0.07 ± 0.00	0.08 ± 0.001
P-value	0.56	0.57	0.16	0.64
F-value	0.58	0.56	2.01	0.44

This data represents as mean ± standard error of the mean. (n = 5). a P < 0.05 vs. the Control group; b P < 0.05 vs. Zn (4.9 mg/kg) group.

TABLE 3. Effect of Zn supplementation on male reproductive hormone after 56 days.

Groups	Control	Zn	Zn	F	P Value
Parameters		4.9 mg/kg	18.1 mg/kg	Value	
T (ng/mL)	5.02±0.07	7.00±0.17 ^a	6.28±0.03 ^{ab}	84.16	0.00
FSH (mIU/mL)	2.54±0.04	4.56±0.06 ^a	3.58±0.05 ^{ab}	3555.95	0.00
LH (mIU/mL)	1.62±0.11	2.43±0.12 ^a	1.42±0.09 ^b	23.21	0.00

This data represents as mean ± standard error of the mean. (n = 5). a P < 0.05 vs. the Control group; b P < 0.05 vs. Zn (4.9 mg/kg) group.

TABLE 4. Effect of Zn supplementation on serum ions after 56 days.

Groups	Control	Zn	Zn	F Value	P Value
Parameters		4.9 mg/kg	18.1 mg/kg		
Zn (µg/dL)	33.30±0.65	43.22±0.88 ^a	48.2±0.46 ^{ab}	119.75	0.00
Ca (mg/dL)	9.08±0.25	9.38±0.07	8.79±0.01 ^b	3.97	0.04
K (mg/dL)	4.10±0.12	3.77±0.05	3.47±0.04	14.60	0.001

This data represents as mean ± standard error of the mean. (n = 5). a P < 0.05 vs. the Control group; b P < 0.05 vs. Zn (4.9 mg/kg) group.

TABLE 5. Effect of Zn supplementation on testicular ions after 56 days.

Groups	Control	Zn	Zn	F Value	P Value
Parameters		4.9 mg/kg	18.1 mg/kg		
Zn (µg/dL)	28.14±1.63	38.36±0.84 ^a	43.88±2.05 ^a	25.05	0.00
Ca (mg/dL)	9.69±0.16	9.10±0.22	8.94±0.20 ^a	3.99	0.04
K (mg/dL)	3.35±0.27	3.89±0.16	3.68±0.20	1.52	0.25

This data represents as mean ± standard error of the mean. (n = 5). a P < 0.05 vs. the Control group; b P < 0.05 vs. Zn (4.9 mg/kg) group.

TABLE 6. Effect of Zn supplementation on sperm analysis after 56 days.

Groups	Control	Zn	Zn	F	P
Parameters		4.9 mg/kg	18.1 mg/kg	Value	Value
Sperm Motility%	35.00±1.41	39.00±1.09	23.00±0.83 ^{ab}	53.3	0.00
Sperm count (million/ml)	25.20±0.63	50.20±0.80 ^a	71.8±0.86 ^{ab}	896.5	0.000
Sperm Abnormalities%	30.20±0.58	16.60±0.74 ^a	14.60±0.51 ^a	186.3	0.00
Without Hook Head	5.60±0.51	4.00±0.31 ^a	2.20±0.37 ^{ab}	17.4	0.00
Amorphous Shape Head	11.40±0.24	5.4±0.24 ^a	5.20±0.37 ^a	143.2	0.00
Banana Shape Head	3.00±0.31	2.00±0.83	1.20±0.37	2.6	0.10
Abnormal Tail	10.20±0.20	5.20±0.37 ^a	5.60±0.40 ^a	68.1	0.00

This data represents as mean ± standard error of the mean. (n = 5). a P < 0.05 vs. the Control group; b P < 0.05 vs. Zn (4.9 mg/kg) group.

TABLE 7. Morphometric result and Johnsen's mean testicular biopsy score.

Groups	Control	Zn		F Value	P Value
		4.9 mg/kg	18.1 mg/kg		
Tubule Diameter (μm)	18.68 \pm 0.11	18.91 \pm 0.11	18.56 \pm 0.07	2.87	0.067
Germinal epithelial thickness (μm)	5.77 \pm 0.10	6.51 \pm 0.09 ^a	5.60 \pm 0.06 ^b	29.61	0.000
Lumen diameter (μm)	12.88 \pm 0.28	12.38 \pm 0.15	12.93 \pm 0.07	2.47	0.096
Johnson Score	9.70 \pm 0.09	9.66 \pm 0.12	9.81 \pm 0.09	0.49	0.61

This data represents as mean \pm standard error of the mean. (n = 5). a P < 0.05 vs. the Control group; b P < 0.05 vs. Zn (4.9 mg/kg) group.

TABLE 8. The Docking interaction data calculations of Zn with 17 β HSD.

Ligand	Receptor	Interaction	Distance	E (kcal/mol)
O 2	O PHE 58	H-donor	3.16(1.86)	-7.5
O 5	OE2 GLU 43	H-donor	3.20(2.29)	-9.4
O 8	OE2 GLU 50	H-donor	2.79 (2.08)	-42.2
O 14	OE1 GLU 50	H-donor	2.85 (2.06)	-10.2
O 14	OE2 GLU 50	H-donor	3.03 (2.10)	-23.8
O 14	O PHE 58	H-donor	2.89 (1.89)	-22.5
O 5	OE2 GLU 43	Ionic	3.20	-3.3
O 8	OE2 GLU 43	Ionic	3.85	-0.8
O 8	OE2 GLU 50	Ionic	2.79	-6.0
O 14	OE1 GLU 50	Ionic	2.85	-5.5
O 14	OE2 GLU 50	Ionic	3.03	-4.3

This data represents as mean \pm standard error of the mean. (n = 5). a P < 0.05 vs. the Control group; b P < 0.05 vs. Zn (4.9 mg/kg) group.

TABLE 9. The Docking interaction data calculations of Zn with Star protein.

Ligand	Receptor	Interaction	Distance	E (kcal/mol)
O 2	OE1 GLU 250	H-donor	2.78 (1.76)	-40.0
O 5	OE1 GLU 250	H-donor	2.83 (1.82)	-29.2
O 8	OE1 GLU 190	H-donor	2.88 (2.08)	-9.4
O 11	OE1 GLU 190	H-donor	2.85 (1.91)	-27.2
O 11	OE2 GLU 190	H-donor	2.91 (1.95)	-12.2
O 14	OE1 GLU 190	H-donor	2.80 (1.89)	-45.9
O 2	OE1 GLU 250	Ionic	2.78	-6.2
O 5	OE1 GLU 250	Ionic	2.83	-5.7
O 8	OE1 GLU 190	Ionic	2.88	-5.4
O 8	OE1 GLU 250	Ionic	3.49	-1.9
O 11	OE1 GLU 190	Ionic	2.85	-5.6
O 11	OE2 GLU 190	Ionic	2.91	-5.1
O 14	OE1 GLU 190	Ionic	2.80	-6.0

This data represents as mean \pm standard error of the mean. (n = 5). a P < 0.05 vs. the Control group; b P < 0.05 vs. Zn (4.9 mg/kg) group.

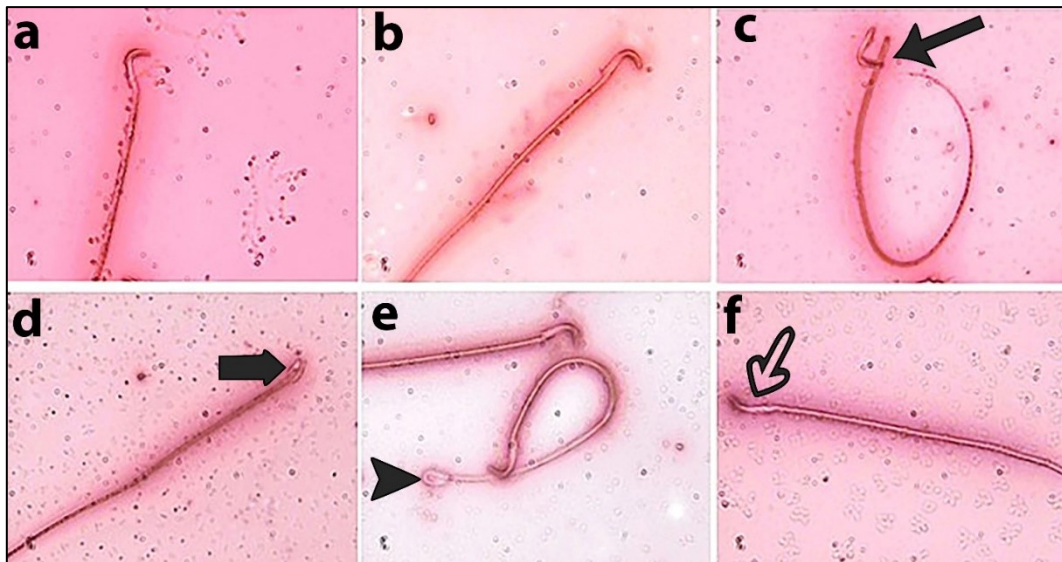


Fig. 1. Effect of Zn supplementation on sperm morphology after 56 days. (a-b): Representative photos of examined sperms with normal morphology and normal shape of head, neck, and tail regions. (c): sperm with detached Head (arrow). (d): Amorphous Shape Head (bold arrow). (e): Abnormal Tail (coiled Tail) (arrowhead). (f): Banana Shape Head (hollow arrow).

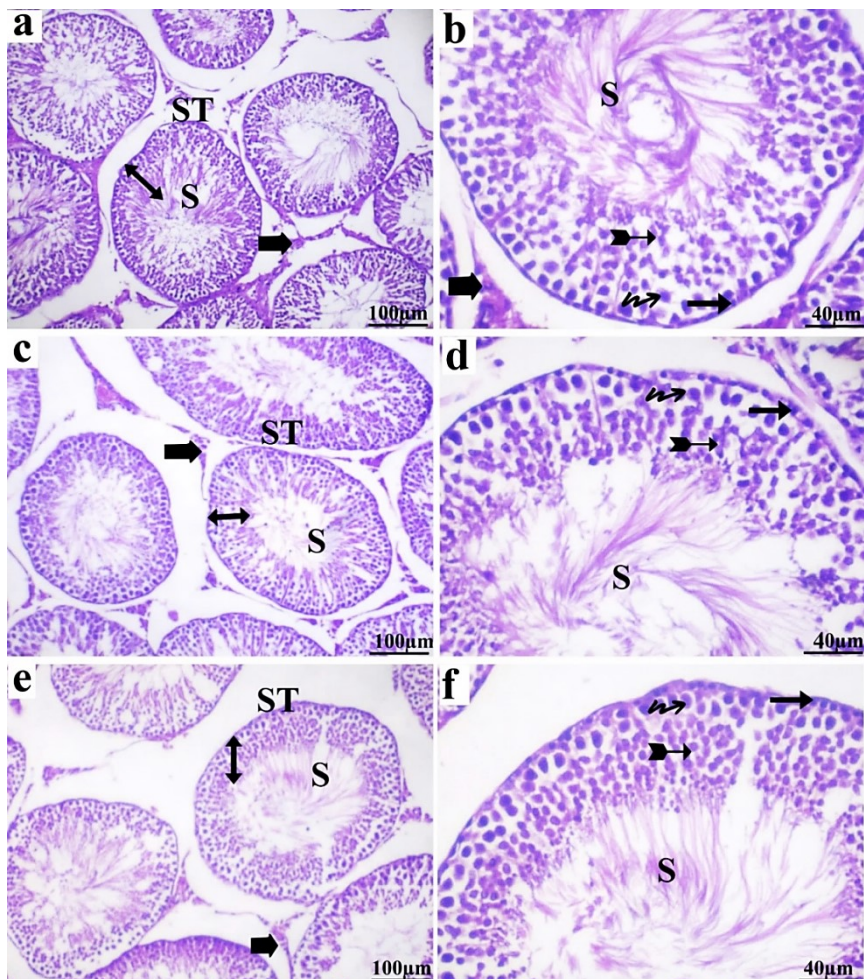


Fig. 2. Effect of Zn supplementation on testicular histology after 56 days. Showing normal histology of testicular tissue from Control (a-b), Zn (4.9 mg/kg) group (c-d), and Zn (18.1mg/kg) group (e-f). seminiferous tubule (ST), germinal epithelial layer (double head arrow), interstitial cell (bold arrow), spermatozoa (S), spermatid (bifid arrow), spermatocyte (wavy arrow) and spermatogonia (thin arrow).

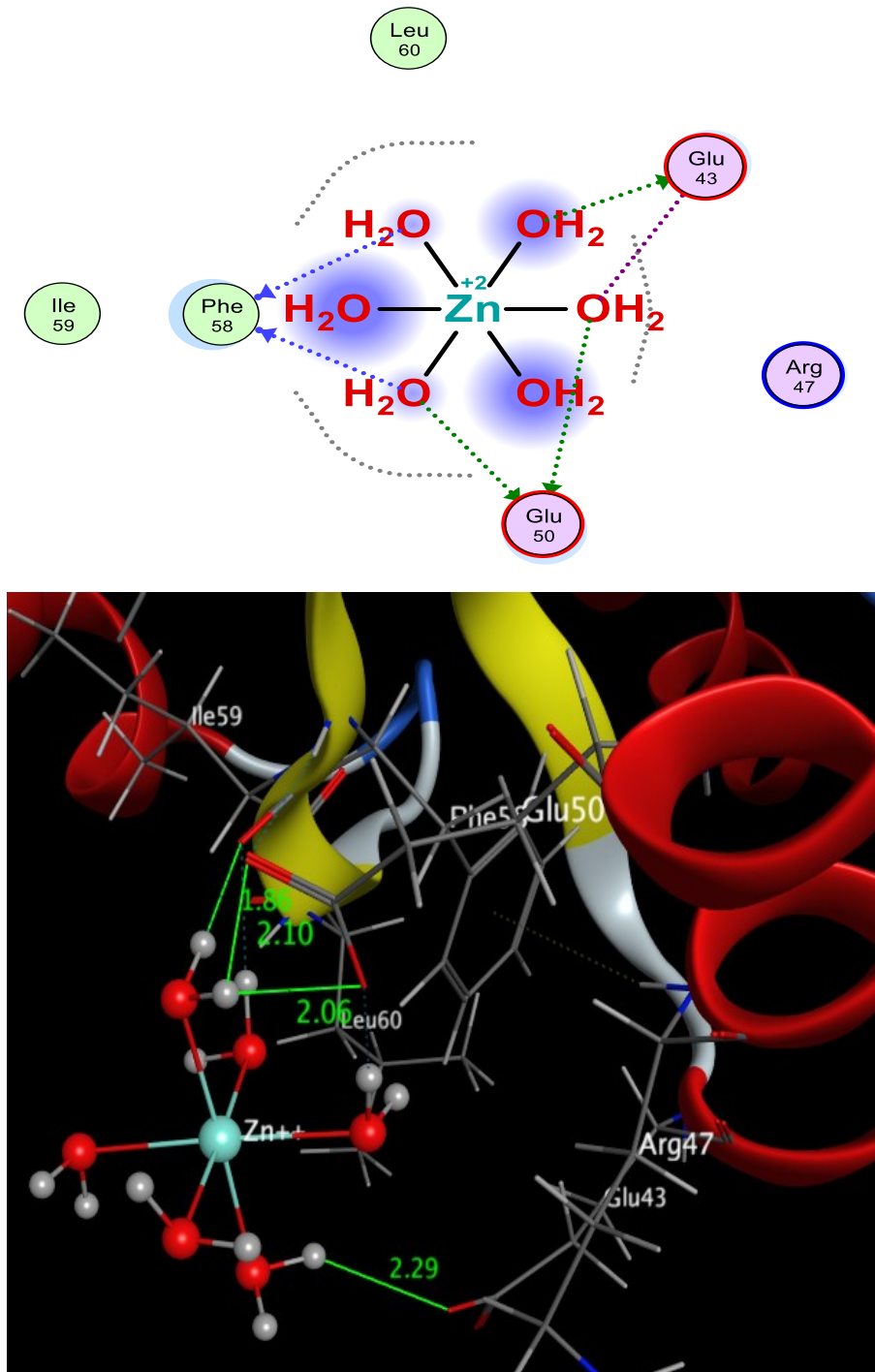


Fig. 3. The 2D and 3D molecular docking simulation studies of the interaction between Zn with 17 β HSD.

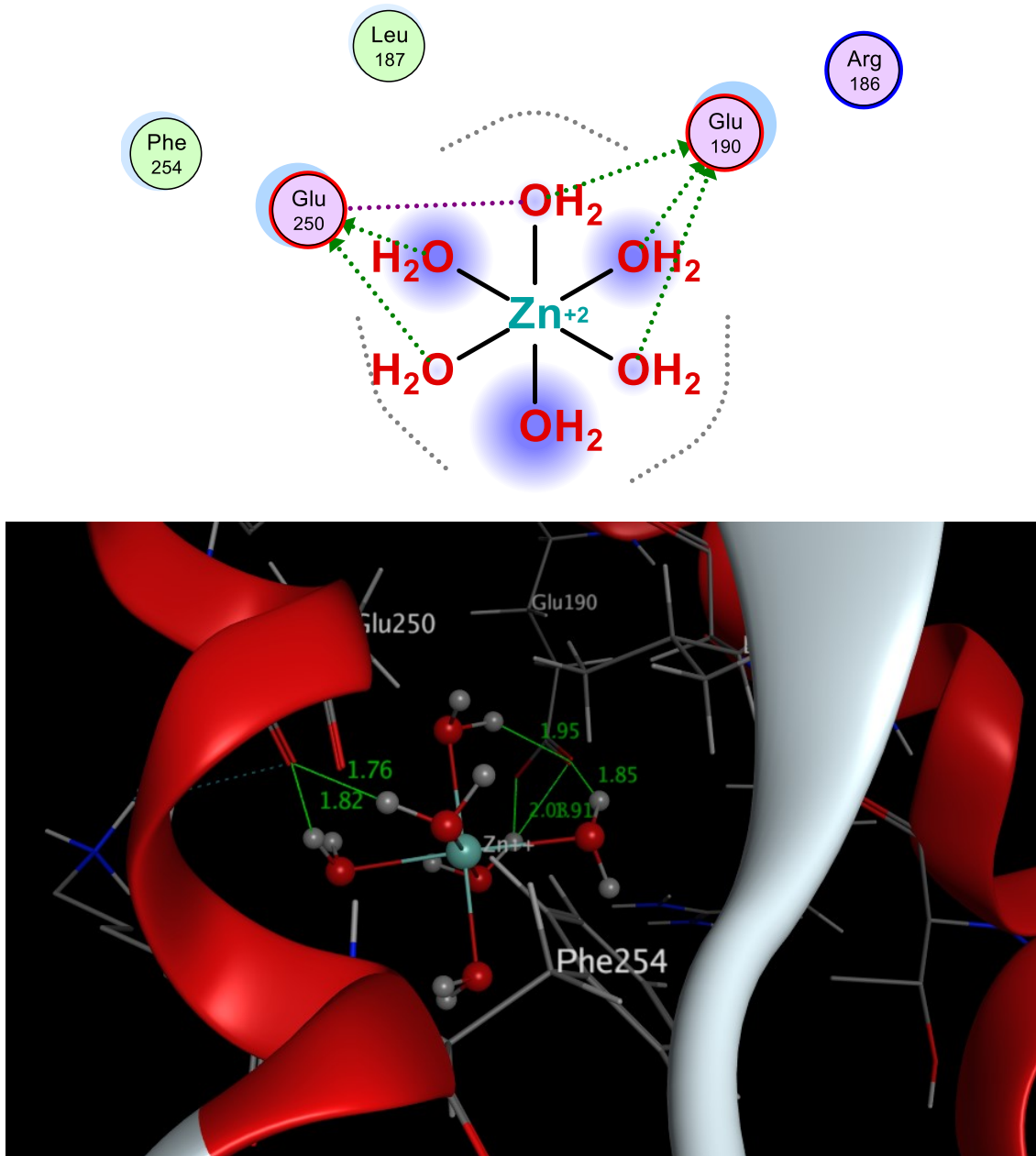


Fig. 4. The 2D and 3D molecular docking simulation studies of the interaction between Zn with Star protein.

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تقييم تأثير مكمل الزنك على الخصوبة في ذكور الجرذان ويستار والتحقيق في آلياتها (دراسة بيوكيميائية وإقحام جزيئي)

حسين محمود حامد¹، محمد رفعت شحاتة² و هبة علي عبد الرحمن³

¹ قسم البيوتكنولوجيا - كلية العلوم - جامعة القاهرة - الجيزة - مصر.

² قسم الكيمياء - كلية العلوم - جامعة القاهرة - الجيزة - مصر.

³ قسم علم الحيوان - كلية العلوم - جامعة القاهرة - الجيزة - مصر.

الملخص

يلعب الزنك دورا حاسما في الحفاظ على الصحة الإنجابية والخصوبة من خلال الحفاظ على السلامة الهيكلية للحيوانات المنوية، وتعزيز معايير الحيوانات المنوية، ورفع مستوى كمية الهرمونات التناسلية، وأيونات الخصية. كان الهدف الرئيسي من الدراسة الحالية هو تقييم آثار مكملات الزنك على الهرمون التناسلي وفحص مستويات الأيونات في المصل والخصية لمعرفة كيف يمكن أن يؤثر تناول الزنك على مستويات هرمون التستوستيرون من خلال مسار الأيونات بواسطة الهرمون المنشط للجسم الأصفر (هرمون ملوتن). استخدمت الدراسة الحالية عينة من واحد وعشرين من ذكور الجرذان البيضاء الناضجة، والتي تتميز بمتوسط وزن يتراوح بين 180 و 200 جرام. تم تقسيم هذه الفئران إلى ثلاث مجموعات. تلقت المجموعة الضابطة عن طريق الفم محلول ملحي لمدة 56 يوما. في المجموعة 2، تم إعطاء الجرذان عن طريق الفم بجرعة 4.9 مغ / كغ من كبريتات الزنك. في المجموعة 3، أعطيت الجرذان جرعة 18.1 مغ / كغ من كبريتات الزنك. أظهرت الدراسة الحالية أن إعطاء الزنك أدى إلى انخفاض في الوزن الجسم للحيوانات مع عدم وجود تأثير على الوزن المطلق والنسبي للأعضاء التناسلية. أظهر فحص الهرمونات التناسلية ارتفاعا ملحوظا في مستويات هرمون التستوستيرون هرمون ملوتن والهرمون المنشط للحوصلة بعد إعطاء الزنك مقارنة بالمجموعة الضابطة. أظهر الإقحام الجزيئي وجود صلة وثيقة بين الزنك وجزيئين حيويين رئيسيين، بروتين التنظيم الحاد الستيرويدي (StAR) وإنزيم Hsd17b3 اللذان يعتبران حاسمين لتقدم مسار التستوستيرون. لوحظت زيادة ملحوظة في كميات أيونات الزنك في كل من عينات الخصية والمصل. مكملات الزنك يؤدي إلى ارتفاع كبير في عدد الحيوانات المنوية وانخفاض كبير في تشوهات الحيوانات المنوية. تسبب تناول الزنك بجرعة عالية في انخفاض كبير في نسبة حركة الحيوانات المنوية. في الختام، أشارت نتائجنا إلى أن الجرعة اليومية الموصى بها من الزنك 4.9 مجم / يوم أفضل من الحد الأعلى المسموح به لجرعة المدخول البالغة 18.1 مجم / يوم، مما يؤثر سلبا على الهرمونات التناسلية وأيونات الكالسيوم وحركة الحيوانات المنوية.

الكلمات الدالة: كبريتات الزنك ، الجرذان ، الصحة الإنجابية ، الخصوبة ، الإقحام الجزيئي.